

Prevalence of intestinal parasite infection in dogs from selected rural areas of central and southern Poland

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The main aim of the study was to compare parasites of dogs living in rural areas of selected counties of the Masovian and Malopolska Voivodeships. Furthermore, the prevalence of intestinal parasite infection was determined in relation to selected factors (sex and season). The research was carried out from October 2015 to May 2016. A total of 207 faecal samples were analysed by the flotation method and by direct smears. Infection by protozoa and nematodes was observed. The mean prevalence was 17.8% for *Giardia intestinalis*, 0.9% for coccidia of the genus *Cystoisospora*, 7.2% for *Toxocara canis*, 2.4% for *Toxascaris leonina*, 20.2% for *Trichuris vulpis* and 22.2% for nematodes of the family Ancylostomatidae. Infection with particular parasites was more common in males than in females. The occurrence of *Giardia intestinalis* and hookworms was statistically significantly correlated with the sex of the host and the season.

KEY WORDS: internal parasites / dogs / rural areas

The report 'Animals in Polish homes', prepared by TNS Poland in October 2014, reveals that more than 48% of Polish people have a pet. Among these, 83% have dogs and 44% have cats. Pets can be found mainly in the countryside. It is estimated that as many as 92% of residents of rural areas have a dog. While dogs play a very important role in human life, they can be also a reservoir of parasites that can pose a threat to human health and life. The risk of zoonotic disease is greater in rural environments, where people have more frequent contact with the soil (during work in the fields), which contains potentially dangerous eggs of roundworms or cysts of *Giardia intestinalis*. Rural dogs are a distinctive group of animals, with living conditions differing from those of their urban counterparts. They are much less often dewormed (both for economic reasons and due to a lack of awareness

among dog owners of the potential threat of parasitic infections). Extensive areas are penetrated by packs of dogs, which at least theoretically have owners to whom they return from time to time. There is also a large group of dogs that remain exclusively in one backyard or even in a stall. These dogs defecate in a small area and may have much less contact with infective stages of parasitic species as compared to urban dogs, which are taken to parks or green areas in housing estates.

The aim of this study was to compare the internal parasites of rural dogs from selected counties of the Masovian and Małopolska Voivodeships and to determine the relationship between the prevalence of intestinal parasite infection and selected factors, i.e. the sex of the animals and the season when the samples were collected.

Material and methods

The material for the study was collected from October 2015 to May 2016. In total 207 faecal samples from dogs from rural areas were analysed (158 from the Masovian Voivodeship – Wołomin, Mińsk, Otwock, Grodzisk Mazowiecki, Żyrardów and Szydłowiec counties – and 49 from the Małopolska Voivodeship – Proszowice and Miechów counties). The samples were placed in sterile plastic containers and stored at 4°C for a maximum of 5 days. The owners were asked about the sex and age of their dogs, whether they were kept in the house or outdoors, and if they had been dewormed. The faeces were examined by direct smear and flotation. Each faecal sample (about 2 g) was placed in a test tube and mixed with saturated NaCl solution to obtain a uniform suspension. Then the tube was filled with the solution until a convex meniscus was obtained, and a cover glass was placed on the meniscus. After 20 minutes the cover glass was transferred to a microscope slide and observed under a microscope at 100 x magnification [10].

Data on the frequency of occurrence of internal parasites was analysed using the SPSS 21.0 statistics package. Pearson's chi-square test and Fisher's exact test were used to estimate the effect of selected factors on the prevalence of intestinal parasites in dogs in 2015 and 2016. The following factors were analysed:

- sampling site (rural areas of the Masovian and Małopolska Voivodeships)
- sex (male or female)
- sampling season (autumn/winter, i.e. October to February, and spring, i.e. March to May)

Two significance levels were used: $P \leq 0.01$ – highly significant and $P \leq 0.05$ – significant.

Results and discussion

Analysis of the interviews with pet owners indicates that only a small percentage of dogs are dewormed. In the rural areas of the Masovian Voivodeship ten dogs (6.3% of collected samples) had been dewormed, and in the Małopolska Voivodeship only three (6.1%). In most cases the faeces samples were collected from dogs that had never been

tested for intestinal parasites, and their owners often confused deworming with vaccination. This suggests the importance of monitoring deworming in dogs from rural areas and the need to educate pet owners of the necessity of regular deworming and the consequences of zoonotic diseases. Many authors of research on infection of dogs and cats with intestinal parasites and soil contamination with the eggs of geohelminths suggest the need for preventive measures at many levels to keep the environment free of contamination, including systematic check-ups for animals, removal of faeces by their owners, regular deworming of female dogs and puppies by their owners, and reduction of the number of stray cats [5, 13, 17].

A significant factor in the persistence of parasites in the environment is the faeces of infected animals. Tests of dog faeces for the presence of parasite eggs have found wide variation in parasites. Analysis of samples from rural areas of the Masovian and Małopolska Voivodeships revealed that the most common parasites in dogs were hookworms Ancylostomatidae (22.2%), whipworms *Trichuris vulpis* (20.2%) and *Giardia intestinalis* (17.8%) – Table. Infection with coccidia (the genus *Cystoisospora*) was observed sporadically (0.9%), and only in the Masovian Voivodeship. The prevalence of the roundworms *Toxocara canis* (7.2%) and *Toxascaris leonina* (2.4%) in dogs from rural areas of the Masovian and Małopolska Voivodeships was much lower than in the case of other parasites or groups of parasites (Table). The prevalence of individual intestinal parasites in dogs from the Masovian Voivodeship ranged from 3.2% to 25.9%, and except for *Giardia intestinalis* was much higher than in the Małopolska Voivodeship (0-20.4%). The differences in the prevalence of hookworms (Ancylostomatidae) in the two voivodeships were significant ($P=0.029$). As shown in the table, each of the factors investigated had a highly significant effect on Ancylostomatidae. The prevalence of infection with hookworms was 22.2% (Table), and was much higher in the Masovian Voivodeship (25.9%) than in the Małopolska Voivodeship (10.2%).

Michalczyk and Sokół [15] noted much higher prevalence of coccidia in dogs (7.7%). Oocysts of coccidia were found in 4.4% of samples collected from dogs in the Czech Republic and in 3.9% of samples in Greece [7, 23]. Prevalence of hookworms was found to be 6.7% in dogs from Olsztyn [8] and 4.61% in the West Pomeranian Voivodeship [26]. It is worth noting that the prevalence of these parasites is much higher in dogs in rural areas than in urban areas. Tylkowska et al. [26], in a study carried out in 2006-2007 in the West Pomeranian Voivodeship, found that the total prevalence of *Toxocara canis* was 20.62%, and was highest in Mieszkowice (40%). The authors reported that the average prevalence of *Toxocara canis* was higher in autumn and winter than in spring, which is consistent with the results of the present study, in which eggs of *Toxascaris leonina* were found only in samples from the Masovian Voivodeship (3.2%), only during the spring (5.6%), and only in males (5.4%) – Table. In Olsztyn, Gaca et al. [8] reported an equally low rate of infection with *T. leonina* (1%). In the West Pomeranian Voivodeship the overall prevalence of this nematode was comparable to that observed in the present study, at 2.9%. It should be emphasized that this parasite was also noted only in the spring (March–May) [26].

Table
Comparison of prevalence of intestinal parasite infection in dogs from rural areas depending on selected factors (voivodeship: Masovian and Malopolska), season and sex

Factors	Number of samples (N)	Parasite species/group											
		<i>Giardia intestinalis</i>		<i>Cystoisospora</i>		<i>Toxocara canis</i>		<i>Toxascaris leonina</i>		<i>Ancylostomatidae</i>		<i>Trichuris vulpis</i>	
		positive samples % (N)	P	positive samples % (N)	P	positive samples % (N)	P	positive samples % (N)	P	positive samples % (N)	P	positive samples % (N)	P
Voivodeship:													
Masovian	158	17.1 (27)	0.670 ^a	1.3 (2)	1.0 ^b	8.9 (14)	0.202 ^b	3.2 (5)	0.594 ^b	25.9 (41)	0.029 ^a	21.5 (34)	0.543 ^a
Malopolska	49	20.4 (10)		0 (0)		2.0 (1)		0 (0)		10.2 (5)		16.3 (8)	
Season:													
autumn/winter	117	23.1 (27)	0.029 ^a	0 (0)	0.188 ^b	7.7 (9)	0.796 ^a	0 (0)	0.015 ^b	34.2 (40)	0.000 ^a	24.8 (29)	0.082 ^a
spring	90	11.1 (10)		2.2 (2)		6.7 (6)		5.9 (5)		6.7 (6)		14.4 (13)	
Sex:													
male	115	13.0 (12)	0.001 ^a	1.1 (1)	1.0 ^b	8.7 (8)	0.184 ^b	5.4 (5)	0.054 ^b	15.2 (14)	0.000 ^a	19.6 (18)	0.010 ^a
female	92	11.6 (8)		1.4 (1)		2.9 (2)		0 (0)		17.4 (12)		11.6 (8)	
TOTAL	207	17.8 (37)		0.9 (2)		7.2 (15)		2.4 (5)		22.2 (46)		20.2 (42)	

Significance level: highly significant at $P \leq 0.01$, significant at $P \leq 0.05$

a – results of Pearson's *chi*-squared test

b – results of Fisher's test

Much lower prevalence of *T. vulpis* than in our study was observed in patients of veterinary clinics in Olsztyn (1%) [8] and in the West Pomeranian Voivodeship (0.27%) [26], where whipworm eggs were found more often in samples collected in the period from June to August, which is consistent with our results.

The table presents the results of analysis of the effect of selected factors on the prevalence of internal parasite infection in dogs. Prevalence was found to be most dependent on sex. The effect of this factor was found to be highly significant, significant, or bordering on significant in the case of four of the six parasites or parasite groups tested: *Giardia intestinalis*, hookworms, whipworms and *Toxascaris leonina*. The frequency of infection by each of the parasites was higher in males; only in the case of Ancylostomatidae was it higher in females, while prevalence of *Cystoisospora* spp. was similar in both sexes. Mizgajska and Luty [17] reported that in dogs in Poznań and in the small nearby town of Murowana Goślina detected *T. canis* several times more often in males than in females. Similarly, Gaca et al. [8], in a study on intestinal parasites in domestic dogs in Olsztyn, found prevalence of *Toxocara canis* at a level of 41% in males and 7% in females—a pattern similar to that noted in our results (Table). According to the authors [8], the higher prevalence of infection in males is explained by their tendency to roam.

Another factor significantly affecting the incidence of parasitic infections was the season when the material was acquired (Table). A statistically highly significant or significant effect was observed for three of the six types of infection (giardiasis, hookworm infections and trichuriasis). Parasitic invasions were much more frequent in dogs in the autumn and winter than in the spring. Coccidian oocysts and eggs of the roundworm *Toxascaris leonina* were found only in faecal samples collected from dogs in the spring. On the other hand, eggs of nematodes of the family Ancylostomatidae were detected more frequently in samples collected in the autumn and winter (34.2%) than in spring (6.7%). In contrast, in the West Pomeranian Voivodeship Tylkowska et al. [26] detected these nematodes more frequently in the spring. The authors also showed that whipworm eggs occurred more often in samples collected between June and August, which is consistent with our results.

The climate warming, mild winters and hot summers which have been observed in Poland for several years prolong the season during which hosts may become infected. In a two-year study conducted in Łębork, an increase in the incidence of parasite eggs was observed in the spring and summer months [24]. According to the authors, the seasonality of occurrence of geohelminth eggs in both sand and animal faeces is explained by the longer days, owing to which hosts spend more time outdoors, but also by the appearance of puppies and kittens, which at the age of a few weeks are chiefly responsible for the spread eggs of *Toxocara* spp. This factor makes it more difficult to combat toxocariasis, as pointed out by Mizgajska and Luty [17], who emphasize the possibility of intrauterine and lactogenic infection as well as infection via consumption of paratenic hosts, i.e. rodents and birds. Ecological determinants in the transmission of *Toxascaris leonina* or *Toxocara* spp. are also discussed by Okulewicz [20]. In zoos, with unnatural conditions for hosts and their parasites, deworming and disinfection are carried out periodically, and transmission

takes place almost exclusively via paratenic hosts (rodents). Because in the natural environment the circulation of nematodes of the Ascarididae family largely involves rodents, regular extermination of rodents reduces their numbers and thus parasitic infections in animals [13].

Brochocka et al. [6] observed that in developed countries dog and cat faeces are one of the main causes of biological contamination of the soil with roundworm eggs in urban areas. A dog infected with adult forms of nematodes sheds thousands of eggs per day with its faeces [12]. As soil contaminated with infectious eggs of geohelminths is a potential source of zoonotic diseases, in many regions of Poland tests have been carried out for the presence of helminth eggs not only in pet faeces but in the environment (mainly recreational areas, playgrounds, sandboxes, and backyards). This research has been conducted mainly in urban areas: Warsaw [3], Poznan [17], Lublin [2, 25], Łęborg [24], Cracow [16], Szczecin [26] and the Tri-City [13]. This type of research has been carried out less often in rural areas [11, 16, 18, 25], and yet rural dogs can be a reservoir of numerous parasite infections posing a threat to the health of animals and humans; a high percentage of soil contamination has been observed in both rural and urban areas [6]. Mizgajska and Luty [17] reported that the frequency of toxocariasis in dogs in Poznan and the small town of Murowana Goślina was not proportional to the degree of soil contamination with the eggs of this parasite. They pointed out other factors, not considered in our study, which affected the prevalence of infection with *T. canis*. Egg-producing *T. canis* was detected in 21.4% of dogs tested: 32.3% of those up to the age of one year and only 3.6% of older dogs. The frequency of *T. canis* infection in dogs was markedly varied between the two areas; 11.8% in the large metropolis and 35.2% in the small town. In both sites *T. canis* eggs were mainly spread by young dogs, up to the age of 12 months, in which the parasite was detected 6-8 times more often than in older dogs. Relatively few eggs of *Toxocara* spp. were found in soil from Murowana Goślina in relation to the high level of infection of dogs with *T. canis*. The authors suggest that this could be explained by the low density of hosts over the large area of the town and the fairly common practice there of keeping dogs on a tether. However, it is possible that in Poznan, where the level of contamination was highest, cats played a significant role in contamination of the environment with eggs of *Toxocara* spp. Here eggs of *Toxocara* spp. were found in soil samples much less often (12%) [17] than in Lublin and its vicinity (36%) [11] or in Lublin itself (58%) [25]. In a study conducted later on the occurrence of parasite eggs in recreational areas and sandboxes in three Lublin neighbourhoods, the most frequently detected species were *Toxocara* spp. (7% of soil samples and 20% of sand samples) and *Toxascaris leonina* (11% of soil samples and 11.6% of sand samples) [2]. Eggs of *Trichuris* spp. and sporadically eggs of nematodes from the family Ancylostomatidae were detected as well. In the Łódź Voivodeship 30.4% of rural backyards and 23.3% of urban courtyards were contaminated with roundworm eggs [4]. Eggs of *Toxocara* spp. were also found in 30% of soil samples collected in Cracow (especially in courtyards and town squares), and in 16% of samples in neigh-

bouring villages [16]. In the years 2000-2005 *Toxocara* spp. eggs were found in 19.8% of soil samples collected in Poznan and in 15.6% of samples from three nearby villages [18]. In the Masovian Voivodeship 29.3% of rural areas and 26.4% of urban areas were contaminated with the eggs of this parasite [9]. Mizgajska and Luty [17] reported the highest level of contamination with *Toxocara* spp. eggs in urban courtyards (19% of samples in Murowana Goślina and 27% in Poznan). Urban courtyards are a distinctive environment where natural processes of self-purification of soil are slower [14]. This is a cause for particular concern, as children play in these areas.

The results of the study indicate the need to introduce monitoring of parasites by faeces examination in villages. Administration of antihelminthic drugs without a diagnosis may lead to drug resistance. Analysis of the prevalence of intestinal parasite infection in dogs should be carried out throughout Poland and continually repeated due to the emergence of new environmental factors and potential drug resistance. It is especially important to monitor giardiasis in pets. It is estimated that *Giardia intestinalis* is the most common parasitic protozoan in the world [1, 22]. Infections with *Giardia intestinalis* of various genotypes (A to G, depending on host specificity) are widespread not only in humans but also in dogs, cats, cattle and other mammals [27]. In various parts of the world the prevalence of *Giardia intestinalis* infection among dogs ranges from 1.6% to 53% [19]. Zygnier et al. [27] performed a microscopic analysis of faecal samples from dogs supplied to Warsaw laboratories and found 5.14% prevalence of *G. intestinalis*. Using PCR methods these authors determined the frequency of the D, A-I and C genotypes to be 6.28%, 1.71% and 1.14%, respectively. It should be emphasized that the A-I genotype is also found in humans. The role of dogs in the spread of giardiasis is not yet fully understood, but they must be considered potential zoonotic agents because of their frequent contact with humans. For this reason greater attention should be given to the need to combat this disease in dogs. It is currently fought on a large scale only in the United States, where commercial vaccines against *Giardia intestinalis* are used for dogs and cats [21]. *Giardia intestinalis* undoubtedly has epidemiological potential of importance to public health. To fully elucidate this question, research using PCR techniques should be conducted in order to differentiate individual genotypes of *Giardia intestinalis* functioning as zoonotic agents.

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