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Gastrointestinal helminths of raccoons (*Procyon lotor*) in western Poland (Lubuskie province) - with particular regard to *Baylisascaris procyonis*

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Abstract

The aim of the study was to estimate the prevalence of gastrointestinal parasites in raccoons with particular regard to zoonotic parasites. Fifty-five raccoons, hunted or found dead on roads, were examined. The small and large intestines were collected from all raccoons and, additionally, the stomach was collected from 43 animals. The samples were examined with the use of sedimentation and counting technique. The intestines and stomach were examined separately. Samples of raccoon faeces were collected from their environment localised in Słubice district, Lubuskie province (Poland). The samples were collected once a month in 2012. In total, 154 faecal samples were obtained and examined with the use of McMaster flotation technique. The following parasites were detected in the intestinal and stomach contents: tapeworms *Mesocestoides* spp. (67.3%), Echinostomatidae flukes (34.5%), and nematodes *Capillaria* spp. (25.5%). Moreover, Acanthocephala were found in the intestines of three raccoons. The highest intensity of infection were observed in case of *Mesocestoides* spp. *Mesocestoides* spp. and Echinostomatidae were found statistically more often in the intestines than in the stomach. In the case of these two parasites, there was positive correlation between the intensity of infection in the intestines and the presence of the same parasites in the stomach. Moreover, significantly higher prevalence and intensity of *Mesocestoides* spp. in males than in females were also observed. Faecal samples contained *Baylisascaris procyonis* eggs (mean 60 epg). These eggs were found in three samples collected in November and December. Furthermore, in some faecal samples eggs of flukes, *Capillaria* spp., *Mesocystoides* spp., and coccidian oocysts were found. It is one of rare reports concerning *Baylisascaris procyonis* in Poland confirming the presence of this dangerous parasite in Polish raccoon population.

Keywords: raccoon, intestines, stomach, faeces, parasites, *Baylisascaris procyonis*, Poland.

Introduction

Raccoons (*Procyon lotor*) are the carnivore animals native to North and Central America. In the first half of 20th century, raccoons were introduced into Europe, where they developed the free-living population. Nowadays, this species is widespread especially in Germany and also observed in other European countries (1, 3, 9, 13). In Poland, the noticeable expansion of the wild raccoons from western border was observed after 1990. From that time, raccoons are observed more often, especially in the western regions of the country (Lubuskie and Zachodnio-Pomorskie provinces) where, in some locations, they occur in the high density (1). Raccoons expansion carries along some problems connected with appearance of the new parasites to synanthropic and sylvatic cycles. Especially important parasite in zoonotic aspects is raccoon roundworm - *Baylisascaris procyonis*. This nematode is known as a reason of larva migrans in many animal species, also in humans, causing fatal neurological or ocular syndrome (12, 16, 24).

There are some papers describing intestinal helminths occurring in raccoons, most of them from North America (4, 5, 22, 23), Japan (15), and some from Europe (2, 6, 9, 26). Most of investigations indicated the *B. procyonis* as a dangerous element of

parasitic fauna of raccoons. However, only single investigations concerning parasites in wild population of raccoons in Poland were described. Besides the first report describing *B. procyonis* in raccoon in 1951 by Stefański and Żarnowski, (25) only a few papers were published. Firstly, Bartoszewicz *et al.* (1) confirmed the presence of *B. procyonis* in free-living raccoons. Then, some interesting data about intestinal parasites of Polish population of these animals was supplied by Popiołek *et al*. (20), who detected, among other parasites, also eggs of *B. procyonis*.

The aim of the present study was to determine the helminth fauna of free-living raccoons in Lubuskie province, in western Poland (area with one of the highest density of raccoon population in) with special regard to the presence of *B. procyonis*, the parasite, which is dangerous for human health.

Material and Methods

Examination of the intestines and stomach. The study was conducted on 55 raccoons, which were shot by hunters (in the frame of cooperation with the Polish Hunting Association) or found dead on roads. There were 30 males, 21 females, and sex of four animals was not determined due to partial damage of the carcasses.

All raccoons were necropsied. The small and large intestines were collected from all animals, and the stomach only from 43 ones. The samples were put in plastic bags and frozen at least for 7 d at -80°C before examination. All samples of the gastro-intestinal tract were examined with the use of sedimentation and counting techniques (SCT) (8, 17). Sediment was examined on Petri dishes under stereomicroscope at 15x-40x magnification to detect and count parasites present in the intestinal and stomach contents. The number of parasites was estimated separately: per intestine and per stomach.

Moreover, during necropsy, samples of faeces (1-5 g) were collected from a distal part of the rectum of 53 animals and examined with the use of quantitative McMaster flotation method with Raynaud modification (21). Samples were examined under a light microscope at 100x-400x magnification to detect parasitic developmental stages (eggs, oocysts). The number of eggs (oocysts) was calculated per 1 g of the sample (epg/opg).

Examination of the faecal samples from the environment. Samples of raccoons faeces were collected from the ground in places of raccoons' defecation (latrines). Sampling was conducted in locations near the right bank of the Odra river on the range of Górzyca commune, Słubice district, Lubuskie province (latitude 52º31'32''North, longitude 14º36'39''East). Samples were obtained 10 times during 2012 (in January, February, March, April, May, June, September, October, November, December) (Fig. 1). In total, 154 samples were collected (in each month from 12 to 18 samples) and examined by quantitative McMaster flotation method with Raynaud modification (21).

Statistical analysis. The prevalence was presented as the percentage of the total number of animals, which were infected. Intensity of the infections was presented as the mean number of parasites, coefficient of variation (CV%), and range. The distribution of data was estimated by Shapiro-Wilk test as non-normal. Mann-Whitney test was used for statistical analysis of differences in prevalence and intensity of parasites between males and females, results in SCT and flotation, and also between two locations (stomach and intestines). Kruskal-Wallis test was used for comparison of prevalence and intensity of different parasitic infections. However, the analysis of differences between all individual parasite types was made by Bonferroni *post hoc* test. Correlation between intensity of infection in the intestines and the presence of the parasite in the stomach was analysed by Spearman's rank correlation test. Moreover, the correlations between the presence of different parasites in the same animals were analysed by this test. Statistical significance was accepted at the $P < 0.05$. All analyses were made with the use of SPSS Statistic 22 (IBM) package.

Results

Examination of the intestines and stomach. Forty-six (83.6%) raccoons were infected with parasites, including 27 (90.0%) males and 16 (76.2%) females. A mean intensity of all parasites detected in gastro-intestinal content was 379.5 (ranging from 1 to 8088). Four different types of parasitic helminths were found: *Mesocestoides* spp. *Capillaria* spp., Echinostomatidae flukes, and Acanthocephala worms. The prevalence and intensity of infections of the gastro-intestinal tract (with the use of SCT) are presented in Table 1.

The most often detected parasite was *Mesocestoides* spp. (in 60.0% of animals). The highest prevalence of this parasites was confirmed statistically (it differed significantly from other parasites). *Capillaria* spp. roundworms and flukes form Echinostomatidae family were detected relatively often (34.5% and 25.5%) – showing statistically similar prevalence. Worms from the order of Acanthocephala, detected only in three raccoons, were the rarest. The differences between intensities of parasite infections were also observed. The highest intensity was noted in the case of *Mesocestoides* tapeworms (mean: 510.5, range: 1-8088), and it differed significantly from the intensities of infections with other parasites. Comparison concerning intensity between other parasites did not show any differences.

Analysis of correlation between the presence of different parasites in the same raccoon showed only

low negative correlation between the occurrence of *Mesocestoides* spp. and Acanthocephala (R_s = -0.294).

Results of SCT examination (separately for the stomach and intestines) are presented on Fig. 1. Tapeworms *Mesocestoides* spp. and Echinostomatidae flukes were found more often in the intestines than in the stomach (differences were statistically significant). There were no such differences in the case of *Capillaria* spp. However, Acanthocephala worms were detected only in the intestines. Similarly, the intensity of tapeworms and flukes infections was significantly higher in the intestines than in the stomach. Moreover, statistical analysis showed the low positive correlations between the intensity of infection in the intestines and the presence of the same parasites in the stomach in the case of *Mesocestoides* spp. (R_s = 0.288) and Echinostomatidae (R_s = 0.397).

Statistical estimation of parasitic infection data between sex of raccoons demonstrated significantly higher prevalence and intensity of *Mesocestoides* spp. in males than in females. There were no significant differences in the prevalence and intensity for other parasite species.

Microscopic examination of faecal samples (McMaster flotation) collected from distal part of the rectum indicated lower number of positive samples than by SCT. Namely, *Mesocestoides* spp. eggs were found only in one (1.9%) animal (30 epg) and *Capillaria* spp. eggs in 7.5% of raccoons (mean 59.3 epg, SD = 48.0, CV% = 82.0). The prevalence of these two parasites estimated by detection of eggs in faeces was significantly lower than SCT results (Mann-Whitney test, $P < 0.05$). Additionally, coccidia oocysts were detected in 11.3% of samples (mean 362.5 opg, SD = 500.7, CV% = 138.1).

Examination of the faecal samples from the environment. The detailed results of faecal sample examination are presented in Table 2.

Among parasitic forms, oocysts of coccidia were detected in the highest percentage of faecal samples (16.9%). However, *Mesocestoides* spp. eggs were detected only in one (0.6%) sample. Detection of eggs of zoonotic roundworm – *B. procyonis* (Fig. 2) was the most interesting finding of this study. These eggs were found in three samples collected in November and December of 2012.

Table 1. Parasites detected in the raccoons' gastro-intestinal tract by sedimentation and counting techniques

	Prevalence (%)	Intensity	
		Mean (range)	SD (CV%)
Acanthocephala	5.5 [c]	2.0 (1-4) [b]	1.7 (86.6)
Capillaria spp.	25.5 [b,c]	5.5 (1-18) [b]	5.9 (107.9)
Echinostomatidae	34.5 [b]	27.7 (1-153) [b]	41.3 (149.0)
Mesocestoides spp.	60.0 [a]	510.5 (1-8088) [a]	1413.5 (276.9)
Total	83.6	379.5 (1-8089)	1129.8 (297.7)

Values in the same column with differing superscripts ([a,b,c]) are significantly different (Mann-Whitney test, $P < 0.05$)

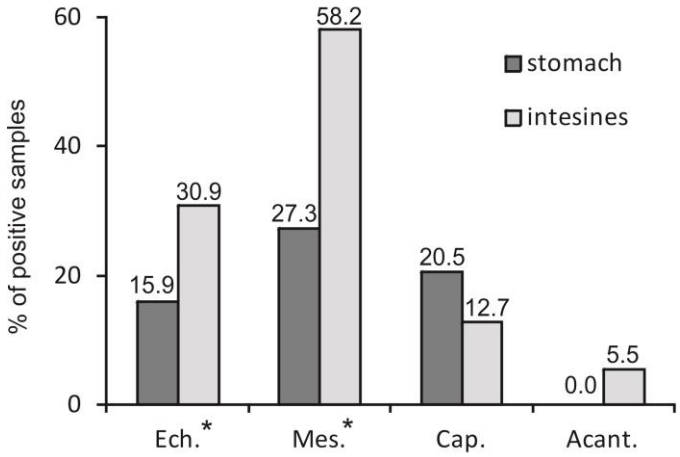


Fig. 1. Prevalence of helminths in raccoons with taking into consideration the location (stomach and intestines) - examined by sedimentation and counting technique (SCT). (Ech. – Echinostomatidae, Mes. – *Mesocestoides* spp., Cap. - *Capillaria* spp., Acant. – Acanthocephala). * means statistically significant differences between prevalence in the stomach and intestines (Mann-Whitney test, $P < 0.05$)

Table 2. Results of microscopic examination of raccoon faeces collected from the natural environment (epg/opg: eggs per 1 g/oocysts per 1 g)

	Positive samples (%)	epg/opg	
		Mean (range)	SD (CV%)
Baylisascaris procyonis	1.9	60.0 (15-150)	77.9 (129.9)
Capillaria spp.	3.2	11.8 (7-15)	4.4 (37.1)
Coccidia	16.9	12 341.0 (7-210 000)	4 3443.8 (351.9)
Mesocestoides spp.	0.6	50.0	-
Trematoda	2.6	23.3 (7-50)	18.7 (80.6)

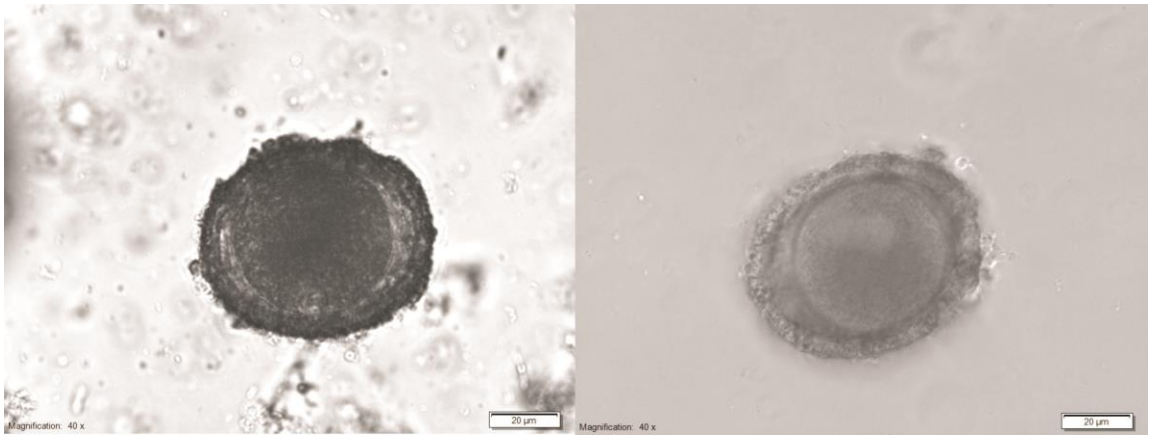


Fig. 2. Eggs of *Baylisascaris procyonis* isolated from raccoon's faeces

Discussion

Results of the present study strongly confirmed the presence of *B. procyonis*, parasite very dangerous for humans, in Polish population of raccoons. However, a relatively low rate of this infection was indicated. In comparison to the earlier studies (1, 20) the percentage of *B. procyonis* positive faecal samples was similar. Our investigation revealed the presence of the eggs of these parasites in 1.9% of samples, and earlier study in 3.7% and 3.3%. Additionally, we did not find the adult worms in the intestines of the examined raccoons. It probably resulted from a relatively low number of examined animals but also pointed at the low prevalence of this parasite in the region.

Introduction of raccoons in Germany took place in two independent locations (9, 13). *B. procyonis* occurs relatively often (from 39% to 71%) in population of raccoons in the middle Germany (7, 26). However, in the eastern region, Brandenburgia, bordering with Poland, raccoons' roundworm was not found or found only sporadically (14). Because raccoons in western Poland originated mainly from Germany, it is probable that they also carried *B. procyonis* from this country. This could explain rare occurrence of *B. procyonis* in Poland.

The highest prevalence (more than 60%) was noted in the case of *Mesocestoides* spp. Statistically, in comparison to other parasites, this tapeworm occurred most often in the examined raccoons. Additionally, *Mesocestoides* spp. was significantly more prevalent in males than in females. The second intermediate host of these tapeworms, in which third-stage larva (tetrathyridium) develops, includes small vertebrates, which are the most important source of feed for raccoons (1). Therefore, higher prevalence of *Mesocestoides* spp. in males could suggest higher preying activity of the parasites. However, another explanation of this difference could be lower immunity of males in parasitic infections. It is worth noting that *Mesocestoides* was not observed in previous studies in Poland (1, 20). This was probably caused by the use of a different diagnostic method - flotation. Eggs of this tapeworm are difficult to detect by coproscopy, which was also confirmed in our investigation: among 33 raccoons with detected *Mesocestoides* worms in the gastro-intestinal tract only one was positive in flotation. *Mesocestoides* occurrence was noted relatively rarely also in raccoon's native location - North America. The investigation conducted in western Canada (5) and in the southern New York (27) did not detect any mature tapeworms. Other studies performed in Arkansas (22) and south-eastern United States (23) demonstrated *Mesocestoides* spp. only in 10% and 14% of raccoons, respectively. Higher prevalence of *Mesocestoides* found in our study might be the effect of assimilation of common parasites of local carnivores (especially foxes) to the new host. On the other hand, as many as

95% of Canadian raccoons were infected with hookworms (5) - in our study these nematodes were absent. However, previous investigation described by Popiołek *et al.* (20) confirmed that hookworms can exist in Polish raccoons; the authors detected *Ancylostoma* spp. eggs in single faecal samples. Authors of previous Polish investigation also described detection of eggs of other parasites, among others: *Strongyloides* and *Spirocerca,* which were not found in our study (20).

Acanthocephala worms were found only in three animals. Previously, the presence of this type of parasites in raccoons in Poland was described only once by Piróg and Popiołek (19). In North America Acanthocephala worms were detected in different percentage, for example: more than 40% of infected raccoons were reported by Richardson *et al*. (22) and Wright and Gompper (27), and about 10% (only cystacanth stages originating from the preyed intermediated hosts) by Ching *et al.* (5).

In our study, there was a relatively high prevalence of Echinostomatidae trematodes (34.5%) and *Capillaria* spp. (25.5%). It is worth noting, that the results published earlier (20) revealed much lower prevalence of *Echinostoma* sp. and Capillariidae (2.25% and 3.3% respectively), probably because of the great difference in the sensitivity of used methods (SCT and flotation) (10, 11). However, our results obtained during examination of faeces from the environment showed similar percentages of positive samples: 3.2% - fluke eggs, 2.6% - *Capillaria* spp. eggs. The differences in results between examination of gastro-intestinal content and detection of eggs in the faeces were shown very clearly by microscopic examination of faeces collected from the rectum of raccoons examined previously by SCT. It indicated that coproscopy could confirm the presence of these parasites only in the very limited range. *Capillaria* eggs were detected about four times more rare (*Mesocestoides* - 30 times) than adult worms in the intestines. Of course, such differences can change depending on seasonal variability in egg production by the parasites.

Moreover, some significant differences were observed between the occurrence of some parasites in the stomach and intestines. Namely, *Mesocestoides* spp. and Echinostomatidae were detected more often in the intestines. These results are strongly connected with the common location of these parasites in the small intestine of the final host and only sporadic occurrence in other parts of gastro-intestinal tract. The results suggest that the presence of the parasites in the stomach relates to the worm migration during more intensive infections in the intestine - the presence of these parasites in the stomach depended on the higher intensity of infection in the intestine.

Our investigation has shown that in contrast to raccoons from other regions (especially native locations) the dominant intestinal helminth in western Poland is *Mesocestoides* spp., tapeworm (common for local carnivores), which probably colonises the new host. The results strongly confirmed the presence of the dangerous roundworm *B. procyonis* in population of Polish raccoons. However, a relatively low degree of this infection was revealed. Nevertheless, it can be assumed that in the future, the intensity of *B. procyonis* could increase, especially in areas with high raccoon density (18). This has to be taken into account as a risk factor of the occurrence of a new zoonotic disease in Poland.

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