Preliminary studies on the zoonotic importance of rodents as a reservoir of toxocariasis from recreation grounds in Wrocław (Poland)

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Summary

Due to their specific biology and behaviour, rodents could play a role as an intermediate, definitive or paratenic host for many helminth species, as well as for species of zoonotic significance such as *Toxocara* spp. or *Echinococcus multilocularis*. The aim of our preliminary study was to investigate the nematode fauna of rodents collected from recreation grounds located in the vicinity of Wrocław, and to determine their role in the transmission of toxocariasis in this area. During a one-year period, 90 individuals belonging to three rodent species, i.e. *Apodemus agrarius*, *A. flavicollis* and *Myodes glareolus*, were collected. The overall prevalence of infection with nematodes amounted to 63.33 ± 10.15 % and differed between hosts. *Toxocara* spp. larvae were located in livers and brains of *A. agrarius* (12.9 %). Our results indicate a role of rodents in the circulation of toxocariasis in sub-urban areas, which serve as recreation grounds for the city of Wrocław.

Keywords: nematodes; *Toxocara*; rodents

Introduction

Rodents could play a role as an intermediate, definitive or paratenic host for many helminth species on account of their specific biology and behaviour. According to Pojmanska *et al.* (2007), 15 species of digenean trematodes, 30 species of cestodes and 40 species of nematodes were reported in small rodents across Poland. In the helminth fauna of rodents we can find species of zoonotic significance such as *Toxocara* spp. or *Echinococcus multilocularis* that use small mammals as paratenic hosts. Toxocariasis is a serious zoonosis reported in humans, in which case the main sources of infection are domestic animals (e.g. dogs and cats). Simultaneously, *Toxocara* spp. is also detected in wild carnivores (e.g. foxes). The recent growth in the size of the fox population and their synanthropization has increased the risk of transmission of *Toxocara* spp.

between urban and sylvatic cycles. This phenomenon is especially associated with suburban and rural areas (Antolova *et al.*, 2004) as they could function as recreation grounds. Rodents can transmit the infectious agents to pets or domestic animals, which could facilitate the further transmission to humans. They can also play an important role as reservoir hosts for vector-borne diseases such as babesiosis, borreliosis or bartonellosis. In recent years, issues concerning the role of small mammals as the aetiological factor of parasitic zoonosis have been discussed widely (Dubinský *et al.*, 1995; Eckert & Deplazes, 2004; Deplazes *et al.*, 2004; Pawelczyk *et al.*, 2004; Antolová *et al.*, 2005; Welc-Falęciak *et al.*, 2008). The growth of interest in epizootiological studies on micromammals has contributed to the development of methods used in diagnosis of zoonotic agents within this group of hosts. It was also observed in the instance of methods applying to recovery of *Toxocara* spp. in paratenic hosts both experimentally (Prokopic & Figallova, 1982; Lescano *et al.*, 2004; Borecka *et al.*, 2008) and in field studies (Dubinský *et al.*, 1995; Antolová *et al.*, 2005).

The aims of our study were investigations of the nematode fauna of rodents collected from recreation grounds located in the environs of Wrocław and an attempt to determine the role of rodents in the transmission of *Toxocara* spp. in this area.

Materials and methods

Study area and sampling of hosts

In our preliminary studies, rodents were trapped in the Redzin forest (51°10'19"N/16°57'02"E), which functions as a recreation ground for the inhabitants of Wrocław. The forest is located in the north-western part of the city. It is one of the biggest wooded areas within Wrocław, distinguished by rich flora and fauna including many carnivorous species (e.g. foxes, martens and badgers).

Table 1. The structure of collected rodents

	males	females	total
<i>A. agrarius</i>	10	21	31
<i>A. flavicollis</i>	14	22	36
<i>M. glareolus</i>	11	12	23
Total	35	55	90

Wooden traps were placed in the afternoon and collected early in the morning on the next day. Collection of rodents was conducted during a one-year period in 2007. In the laboratory, after anesthesia, mice were killed by cervical dislocation, and their alimentary canals and organs were subjected to parasitological examination. In order to find *Toxocara* spp. larvae, brains and livers were studied under a light microscope by the compression method (Prokopic & Figallova, 1982). Organs were cut into small pieces and squeezed between glass plates and examined for presence of larval stages. Although during our parasitological examination helminths representing Digenea, Cestoda and Nematoda were collected, this study was limited to the nematode parasites only. The material obtained was maintained in 70 % ethanol, fixed and cleared for examination with glycerol, and identified using available literature data.

Ecological analysis

The use of descriptive ecological terms follows Bush *et al.* (1997). The total number of nematode species and prevalence of infection (with 95 % confidence intervals) for particular host species were calculated. According to

Behnke *et al.* (2008) the infracommunity structure was assessed by the mean (with standard error of the mean – S.E.M) and maximum number of nematode species per host, the mean (with S.E.M) number of nematode individuals, and the mean abundance and prevalence of particular species. The differences between prevalence of infection within particular host species were tested by the chi-square test. In order to estimate the degree of parasite aggregation, the index of discrepancy (*D*) (Poulin, 1993) was calculated using Quantitative Parasitology 3.0 implemented by Rozsa *et al.* (2000).

Results

In our preliminary studies 90 individuals belonging to three species, i.e. *Apodemus agrarius*, *A. flavicollis* and *Myodes glareolus*, were collected. The sex and number of trapped rodents are given in Table 1. The overall prevalence of infection with nematodes amounted to 63.33 ± 10.15 % and differed between species of hosts ($\chi^2 = 19.133$, *df* = 2, *p* < 0.000). The highest prevalence was observed in *A. agrarius* ($93.5\% \pm 5.7$), the lowest in *M. glareolus* ($39.1\% \pm 22.3$) while the level of infection of *A. flavicollis* equaled $55.6\% \pm 16.5$. We described the component community structure of nematodes for particular host species by prevalence, mean/median intensity and index of discrepancy; the data are summarized in Table 2. Rodents belonging to the genus *Apodemus* harboured four (*A. agrarius*) and five (*A. flavicollis*) nematode species while *M. glareolus* was infected only with *H. mixtum* and *A. annulosa*. Accurate data on the infracommunity structure are given in Table 3.

Table 2. The prevalence, mean and median intensity of helminths by host

Species	No. of hosts		Prevalence $\pm$ 95% confidence limit	Intensity		Index of discrepancy
	Total	Infected		Mean	Median	
<i>Apodemus agrarius</i>						
<i>Heligmosomoides polygyrus</i>	31	27	87.1 $\pm$ 12.6	17.41	10.00	0.546
<i>Syphacia agraria</i>	31	3	9.7 $\pm$ 11.8	42.33	18.0	0.918
<i>Heterakis spumosa</i>	31	26	83.9 $\pm$ 14.1	34.77	22.5	0.556
<i>Toxocara</i> spp.	31	4	12.9 $\pm$ 13.1	2.25	1.00	0.896
Overall	31	29	93.5 $\pm$ 9.85	52.07	38.0	0.493
<i>Apodemus flavicollis</i>						
<i>Heligmosomoides polygyrus</i>	36	16	44.4 $\pm$ 16.9	10.30	5.5	0.760
<i>Syphacia frederici</i>	36	1	2.8	3.00	3.00	
<i>Heterakis spumosa</i>	36	7	19.4 $\pm$ 13.9	2.14	1.00	0.859
<i>Aonchotheca annulosa</i>	36	4	11.1 $\pm$ 11.3	7.75	8.50	0.892
<i>Angiostrongylus</i> sp.	36	1	2.8	4.00	4.00	
Overall	36	20	55.6 $\pm$ 17.0	11.00	3.50	0.737
<i>Myodes glareolus</i>						
<i>Heligmosomum mixtum</i>	23	9	39.1 $\pm$ 20.8	1.44	1.0	0.686
<i>Aonchotheca annulosa</i>	23	2	8.7 $\pm$ 11.3	8.50	8.50	0.892
Overall	23	9	39.1 $\pm$ 20.8	3.33	1.0	0.792

Table 3. Measures of infracommunity structure by host

	Host		
	<i>A. agrarius</i>	<i>A. flavicollis</i>	<i>M. glareolus</i>
Maximum number of nematode species per host	3	3	2
Mean number of nematode species $\pm$ S.E.M	1.81 $\pm$ 0.12	0.88 $\pm$ 0.16	0.48 $\pm$ 0.14
Mean number of nematodes $\pm$ S.E.M	48.71 $\pm$ 9.09	6.11 $\pm$ 1.77	1.30 $\pm$ 0.68

During microscopic examination of livers and brains *Toxocara* spp. larvae were reported only for *A. agrarius* (Fig. 1). Larvae were found in four individuals; in three cases larvae were located in the liver and once in the brain. Intensity ranged between 3 to 6 specimens per host; the prevalence and mean intensity are given in Table 2.

Fig. 1. *Toxocara* spp. larva in the liver (scale bar = 100 μ m)

Discussion

Numerous studies on the parasite fauna of rodents concerning various aspects, such as the impact of extrinsic and intrinsic factors on helminth interactions, have been carried out in recent years (Bajer *et al.*, 2005; Behnke *et al.*, 2005; Eira *et al.*, 2006; Fuentes *et al.*, 2007). Apart from ecological aspects, relevant information about the taxonomical status or host-parasite relationship became apparent during investigations on the helminth fauna of rodents (Feliu *et al.*, 2000; Hildebrand *et al.*, 2004; Hildebrand *et al.*, 2007a). Moreover, recent studies show that the possible parasite spectrum has not yet been completely described (Haukisalmi & Henttonen, 2001, 2005; Hildebrand *et al.*, 2007b).

The number of nematode species reported in murid or arvicolid rodents is inconstant, differing between sites and depending on climatic changes (Abu-Madi *et al.*, 2000; Bajer *et al.*, 2005; Behnke *et al.*, 2008). According to Pojmanska (1998), the nematode fauna of *M. glareolus* in Poland is represented by 13 species, though some statements are still debatable (Tenora & Meszaros, 1975; Hildebrand *et al.*, 2007a). In our study, only two species, *H. mixtum* and *A. annulosa*, were found. It is a rather poor result compared with other research carried out in Poland or Europe. During their long-term studies on the helminth structure of bank voles in the Mazury Lake District, Bajer *et al.* (2005) recorded five nematode species. Similar nematode species richness was also reported by Hauki-

salmi *et al.* (1988). In comparison with these cyclic and long-term investigations, our results take on a preliminary character.

Most papers describing the parasite fauna of rodents representing the genus *Apodemus* pertain to *A. sylvaticus* (Behnke *et al.*, 1999; Abu-Madi *et al.*, 2000; Fuentes *et al.*, 2007). So far only a study from Germany (Klimpel *et al.*, 2007) refers to the parasites of *A. flavicollis*. Our results indicate the presence of five nematode species harbouring *A. flavicollis*. The same number of species was observed by Klimpel *et al.* (2007). The remarkable similarities observed in the nematode fauna of these mouse species can be associated with their similar pattern of living (Klimpel *et al.*, 2007). The only difference in the nematode community of rodents from the Redzin forest is the high prevalence of *H. spumosa*. This species has been reported mainly from *A. agrarius* or *Rattus* sp. and its presence in the field mouse species (*A. flavicollis* and *A. sylvaticus*) seems occasional (Klimpel *et al.*, 2007). During our parasitological examination of *A. flavicollis*, nematodes belonging to the genus *Angiostrongylus* were detected but the prevalence was low as compared with data presented by Eira *et al.* (2006) and Gouy de Bellocq *et al.* (2003).

Another species of the genus *Apodemus*, i.e. *A. agrarius*, does not constitute a frequent subject of parasitological investigations. During long-term studies on the helminth fauna of the striped field mouse in the Belorussian Polesie region, Shimalov (2002) reported six nematode species. In our preliminary study, four species were reported and *A. agrarius* was the most frequently infected host species; the prevalence and mean number of nematode species were two times higher than in *A. flavicollis*. Besides the typical species such as *H. polygyrus*, *H. spumosa* and *S. agraria*, we found larvae of *Toxocara* spp. located in the brains and livers. Research on the zoonotic importance of rodents in the circulation of *Toxocara* spp. has been pursued only in Slovakia (Dubinský *et al.*, 1995 and Antolová *et al.*, 2004). The most frequent seropositive species was *A. agrarius* (15.9 %) and the seropositivity of small mammals was higher at sub-urban localities (Antolová *et al.*, 2004). In our study the prevalence reached 12.9 % and was reported only for *A. agrarius*. Small mammals as paratenic hosts are a significant reservoir of *Toxocara* larvae. These results may suggest the risk of the transmission of pathogens into red foxes as main disseminators of *Toxocara* spp. eggs in sub-urban areas, which constitute recreation grounds for Wrocław inhabitants.

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