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Original paper

Flatworm (Platyhelminthes: Cestoda, Trematoda) parasites of three mouse-like rodents in Serbia

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Summary. A total of 231 individual mouse-like rodents (striped field mice *Apodemus agrarius*, yellow-necked mice *A. flavicollis* and common voles *Microtus arvalis*) from five localities in Serbia were subjected to parasitological research. Seven helminth species were identified based on morphological and morphometric criteria: two trematodes (*Brachylaimus recurvus* and *B. apodemi*) and five cestodes (*Hymenolepis fraterna*, *H. diminuta*, *H. straminea*, *Paranoplocephala omphalodes* and *Skrjabinotaenia lobata*). Total sample prevalence was 15.1% (14.9% in female and 15.4% in male hosts) with a significantly higher number of infected *A. agrarius* specimens than infected *A. flavicollis* specimens. Furthermore, the studied *Apodemus* host species differed in parasite species richness. Yellow-necked mice served as hosts for five flatworm species, while in striped field mice only three species were found. The species that infected the highest number of hosts was *H. fraterna*. The trematodes *B. recurvus* and *B. apodemi* are reported in Serbia for the first time. We highlight the presence of two species with zoonotic potential, *H. fraterna* and *H. diminuta,* in the sample of *Apodemus* hosts, pointing to a need for future monitoring.

Keywords: flatworm, helminth fauna, rodent, Serbia, zoonoses.

INTRODUCTION

Parasites are ubiquitous in the lives of wild animals and represent a major component of global biodiversity. More than 50% of known species are parasites or pathogens by some definition, and 60% of all known human pathogens are of zoonotic origin (Froeschke and Matthee 2014). Helminths are one of the most diverse and widespread parasites of animals and man. While they represent different phyla or classes (acanthocephalans, roundworms, tapeworms and flukes), their transmission, infection and pathogenesis follow a typical pattern (Islam et al. 2020). About a third of the global human population is infected by at least one helminth species, while diseases caused by these parasites are largely neglected (Islam et al. 2020; Paladsing et al. 2020). Members of the phylum Platyhelminthes, flukes (Trematoda) and tapeworms (Cestoda), are parasites characterized by a complex life cycle that often involves mammals as definitive hosts. The trematode and cestode fauna of Europe, northern Asia and North America has been relatively well studied, and research indicates that adult tapeworms are widely distributed and abundant in small mammals (Morand et al. 2006).

Rodents constitute the largest group of mammals, containing 2277 species, or approximately 42% of global mammal diversity (Vlasov et al. 2015; Kirillova et al. 2020). They act as hosts to numerous helminth species, some of which have zoonotic potential (Bjelić-Čabrilo et al. 2013; Vlasov et al. 2015; Islam et al. 2020; Kirillova et al. 2020; Paladsing et al. 2020; Spickett et al. 2020). Mice of the genus *Apodemus* are the most wide-ranging small rodents in natural habitats such as mixed forests and open areas of the Palearctic. Although the striped field mouse, *Apodemus agrarius* (Pallas, 1771) and the yellow-

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necked mouse, *A. flavicollis* (Melchior, 1834) differ in their geographic range, in areas where these two species coexist they often partly overlap due to their similar ecological needs (Ondríková et al. 2010). The yellow-necked mouse is strongly dependent on forest environments, exhibiting a preference for mature deciduous forest, whereas the striped field mouse inhabits mosaic habitats consisting of agricultural land and forest, with a preference for wetland habitats near the forest edge (Ondríková et al. 2010; Dwużnik et al. 2017). The majority of small mammal helminth fauna studies in Europe have focused on the wood mouse *A. sylvaticus*, and data on *A. agrarius* and *A. flavicollis* remains scant (Ondríková et al. 2010). The common vole, *Microtus arvalis*, is widely distributed in Eurasia. It prefers habitats with a well-developed vegetation cover, but is ecologically plastic and inhabits arable land, floodplains, meadows, scrubland, forest edges, pastures and gardens. Common voles are typically herbivorous, feeding on approximately 80 plant species (Kirillova et al. 2020). Flatworms that are parasites in *M. arvalis* have often been studied in Europe, mainly because of the significance of the species as an intermediate host for *Echinococcus multilocularis* (Führer et al. 2010; Guerra et al. 2014; Beerli et al. 2017).

Among small European mammals, the eulipotyphlans – shrews, hedgehogs and moles – have a higher prevalence of fluke and tapeworm infection, owing to the higher proportion of invertebrates in their diet (Lewis 1987). Various biotic and abiotic factors may shape parasites' spatial and temporal dynamics, affecting their survival and/or transmission. The yellow-necked and striped mice are granivores, their diet consisting primarily of seeds and fruits. Additionally, *A. flavicollis* is a more specialized species, with invertebrates making up 20% of its diet, compared to 40% in *A. agrarius* (Ondríková et al. 2010).

Studies of rodent parasites that are of medical and veterinary importance are conducted with the aim of preventing their transmission to humans and domestic animals (Sohn et al. 2014). Various rodent species are studied for this very reason in different regions (Sohn et al. 2014), with the majority of helminthological research of small mammals localized in the Palearctic region (Spickett et al. 2020), in countries such as Spain, Poland, Slovakia, Lithuania and Belarus. Data from Serbia are still lacking (Bjelić-Čabrilo et al. 2013). During the nineteen-eighties, studies of small mammal helminth fauna in Serbia laid a solid foundation for further work, but not all aspects of helminthological research received equal attention in the years that followed. The emphasis was on intestinal nematode parasites, with parasitic flatworms being comparatively under-studied. Considering the important role of rodents in the transmission and maintenance of these parasites in natural and urban environments, this lack of data is particularly conspicuous (Bjelić-Čabrilo et al. 2015). Therefore, the present study aimed to report on the fauna of parasitic flatworms of three mouse-like rodents (*A. agrarius*, *A. flavicollis* and *M. arvalis*) in Serbia, especially those species with zoonotic potential.

MATERIALS AND METHODS

The host sample consisted of 231 individual rodents: 79 *A. agrarius*, 145 *A. flavicollis* and 7 *M. arvalis*. The animals were collected from five localities in Serbia (Ruski Krstur, Košutnjak, Misača, Milošev Do, Župa). All but two of the sample sites are hilly or mountainous areas with a temperate continental climate. The landscape of Ruski Krstur is dominated by loess, with a lowland moderate continental climate.

The intestinal tract of the rodents was examined for the presence of parasites. Following dissection, intestines were placed in Petri dishes in tapwater and cut lengthwise to liberate their contents. The contents of each Petri dish were then transferred into 250 ml conical glasses filled with tapwater for suspension. When the intestinal contents, including parasites, settled at the bottom of the glass, excess water was removed and more tapwater was added. This process was repeated until the water in the glass became clear. Once the excess water was removed for the last time, a small amount of liquid containing intestinal contents and parasites was transferred to a Petri dish and examined under a stereomicroscope. All flatworms were stored in 70% ethanol until preparation and identification.

To prepare for the staining process, flukes and tapeworms were taken out of the ethanol and transferred into distilled water where they remained for 24 hours. The following day the parasites were stained by dipping in carmine solution for a few seconds, and then dehydrated in a series of ethanol solutions of increasing concentration (15 minutes each in 70%, 80% and 90% ethanol followed by 5 minutes in 96% ethanol). Stained and dehydrated parasites were then placed in cedarwood oil for 24 hours. On the last day of preparation, the parasites were mounted in Canada balsam as permanent microscopic slides. Slides were observed under a microscope (40× and 100× magnification). Flatworm species were identified based on keys by Ryzhikov et al. (1979) and Genov (1984). Prevalence (P%) was determined according to Bush et al. (1997). Helminth species diversity at the component community level, apart from species richness, was also calculated using the unbiased Simpson diversity index and Berger-Parker dominance index (Behnke et al. 2001). Infection prevalence between species was analysed using Two by Two Contingency Tables and McNemar's chi-squared test in Statistica 7.0 software.

RESULTS

A total of seven flatworm species, two trematodes and five cestodes, were recovered from three host species. Total flatworm prevalence was 15.1%; trematodes infected 1.7% and cestodes 12.1% of the examined hosts. The highest prevalence of parasitic flatworms was noted for the common vole (42.9%); in contrast, only 8.3% of examined yellow-necked mice were infected (Table 1). Infection prevalence was statisticaly significantly higher in *A. agrarius* than in *A. flavicollis* ($\chi^2 = 13.31$, df = 1, $P < 0.001$). In the sample of *A. agrarius*, the most prevalent parasite was *Hymenolepis fraterna* (16.2%) (Table 2), whereas *H. diminuta* infected the highest percentage of *A. flavicollis* individuals (4.1%) (Table 3). Only one flatworm species, the tapeworm *Paranoplocephala omphalodes*, was found in the common vole.

The most abundant parasite species was *H. fraterna*, which represented 71 individuals among the total number of collected parasites. None of the remaining species were nearly as abundant, with the second (*B. recurvus*) and third (*H. straminea*) most numerous species numbering 10 and 9 individuals, respectively. Of the seven flatworm species, *H. fraterna* had the highest prevalence, infecting 14 hosts (6%) (Table 4). The host sample consisted of 114 females and 117 males (the sex of one animal could not be determined). Infection prevalence was 14.9% for females and 15.4% for males (Table 5).

Regarding helminth diversity, species richness ranged from 1 to 5 (Table 6). *Hymenolepis straminea* and *H. fraterna* were found in two host species, the striped and yellow-necked mouse, whereas the remaining flatworms were found to infect a single host species (Table 6). The Berger-Parker dominance index for the total/overall host sample was 0.66, indicating the dominance of *H. fraterna*. Total flatworm species diversity according to the Simpson index was 0.54. The dominant species in the yellow-necked (Berger-Parker index 0.53) and striped field mouse sample (Berger-Parker index 0.83) were *B. recurvus* and *H. fraterna* respectively. The Simpson index value was higher for the component community in *A. flavicollis* (0.65) compared to *A. agrarius* (0.30). A majority of rodents were infected with a single parasite species, with four individuals carrying two parasite species (Tables 2 and 3). Of these four hosts, two were infected with *H. straminea* and *B. apodemi*, and two with *H. diminuta* and *B. recurvus*.

DISCUSSION

Brachylaimus recurvus and *B. apodemi* belong to the family Brachylaimidae and use land snails as their intermediate hosts (Nakao et al. 2018). They are considered to be typical parasites of rodents, which act as their definitive hosts

Table 1. Quantitative parameters of flatworm infection for the total host sample.

Host species	n	I	P%
Apodemus agrarius	79	20	25.3
Apodemus flavicollis	145	12	8.3
Microtus arvalis	7	3	42.9
Σ	231	35	15.1

n – total number of examined hosts; I – number of infected hosts; P% – prevalence.

Table 2. Quantitative parameters of flatworm infection for the striped field mouse (*Apodemus agrarius*) host sample.

Helminth species	n	N	P%
Hymenolepis fraterna	13	70	16.2
Hymenolepis straminea	6	8	7.5
Brachylaimus apodemi	3	4	3.8
All flatworms	20	82	25.3

n – total number of infected hosts; N – number of individual parasites; P% – prevalence.

Table 3. Quantitative parameters of flatworm infection for the yellow-necked mouse (*Apodemus flavicollis*) host sample.

Helminth species	n	N	P%
Hymenolepis fraterna	1	1	0.7
Hymenolepis straminea	1	1	0.7
Hymenolepis diminuta	6	6	4.1
Skrjabinotaenia lobata	1	1	0.7
Brachylaimus recurvus	5	10	3.4
All flatworms	12	19	8.3

n – total number of infected hosts; N – number of individual parasites; P% – prevalence.

Table 4. Quantitative parameters of infection for specific helminth species for the total host sample.

Helminth species	I/Σ	N	P%
Hymenolepis fraterna	14/231	71	6.0
Hymenolepis straminea	7/231	9	3.0
Hymenolepis diminuta	6/231	6	2.6
Paranoplocephala omphalodes	3/231	4	1.3
Skrjabinotaenia lobata	1/231	1	0.4
Brachylaimus apodemi	3/231	4	1.3
Brachylaimus recurvus	5/231	10	2.2
All flatworms	35/231	105	15.1

I – number of infected hosts; Σ – total number of examined hosts; N – number of collected helminths of the given species; P% – prevalence.

Table 5. Infection parameters for male and female rodents for the total host sample.

	♂			♀		
Host species	n	I	P%	n	I	P%
Apodemus agrarius	40	10	25	39	10	25.6
Apodemus flavicollis	74	5	6.8	71	7	9.9
Microtus arvalis	3	3	100	4	0	0
Σ	117	18	15.4	114	17	14.9

n – total number of examined hosts; I – number of infected hosts; P% – prevalence.

Table 6. A checklist of helminth species found in mouse-like rodents in Serbia.

	Aa	Af	Ma
Cestoda			
Hymenolepis fraterna	+	+	-
Hymenolepis straminea	+	+	-
Hymenolepis diminuta	-	+	-
Paranoplocephala omphalodes	-	-	+
Skrjabinotaenia lobata	-	+	-
Trematoda			
Brachylaimus apodemi	+	-	-
Brachylaimus recurvus	-	+	-
Species richness	3	5	1

Aa – *Apodemus agrarius;* Af – *Apodemus flavicollis;* Ma – *Microtus arvalis.*

(Kirillova et al. 2020). *Brachylaimus apodemi* was previously found in the striped field mouse in Russia (Nadtochii 1970), whereas *B. recurvus* is mentioned in rodents of northern Europe and northern Africa by Mas-Coma and Montoliu (1986). It has also been registered in the wood mouse in France (Gracenea and González-Moreno 2002) and Scotland (Kruidenier and Gallicchio 1959), and the common vole in Russia (Kirillova et al. 2020). The first finding of the fluke species *Brachylaima* sp. in Serbia was previously reported from *Apodemus agrarius* (Bjelić-Čabrilo et al. 2013).

Three of the tapeworm species reported in the present study belong to the family Hymenolepididae: *Hymenolepis fraterna*, *H. straminea* and *H. diminuta*, with *H. fraterna* being the most prevalent and abundant. This high number of individuals may be explained by the ability of *H. fraterna* to reproduce and complete its life cycle without an intermediate host, in contrast to *H. diminuta* which requires one (Foronda et al. 2011). *Hymenolepis fraterna* is frequently placed in the genus *Rodentolepis*, but there is no clear consensus on its taxonomic position. Indeed, while *H. nana* and *H. fraterna* are sometimes considered to be conspecific, alternatively, they have been proposed to represent two different species: one parasitizing humans (*H. nana*) and the other rodents (*H. fraterna*) (Foronda et al. 2011). Whatever their true identity, both of these species possess zoonotic potential (Ondríková et al. 2010; Foronda et al. 2011; Kataranovski et al. 2011). In the current study, *H. fraterna* and *H. straminea* were found in both the striped and yellow-necked mouse, whereas *H. diminuta* was only found in the yellow-necked mouse. Hughes (1940) lists various voles and mice as frequent hosts of *H. fraterna* and *H. diminuta*, and the hamster (*Cricetus cricetus*) as the host of *H. straminea*. *Hymenolepis fraterna* and *H. straminea* were found in Serbia for the first time in *Apodemus* mice (Bjelić-Čabrilo et al. 2013), and all three *Hymenolepis* species reported in the current study were later found in *C. cricetus* (Bjelić-Čabrilo et al. 2015).

Paranoplocephala omphalodes was only registered in the common vole at Ruski Krstur. Genov (1984) lists the species as a common parasite of *M. arvalis* in montane areas, and less commonly in *M. guentheri* and *M. arvalis* in lowland habitats. In Vojvodina (northern Serbia), where our Ruski Krstur site is also located, *P. omphalodes* was found in the water vole (Bjelić-Čabrilo et al. 2014) and hamster (Bjelić-Čabrilo et al. 2015). This tapeworm is considered a Holarctic species that ranges from western Europe to Alaska. While it has been reported in a wide spectrum of hosts, it is foremostly a parasite of *Microtus* species (Haukisalmi et al. 2004; Vlasenko et al. 2019). *Skrjabinotaenia lobata* was found in *A. flavicollis* in a single study site (Milošev Do). Its first report in Serbia is in the wood mouse from Zasavica locality (Bjelić-Čabrilo et al. 2013). In the rest of Europe, it has been found in the striped and yellow-necked mouse in Slovakia (Ondríková et al. 2010) and the house mouse in Spain (Hidalgo et al. 2000). This tapeworm can absorb heavy metals such as lead, marking it as a potential bioindicator of heavy metal pollution (Torres et al. 2006).

It is important to note that studies of the flatworm fauna of mouse-like rodents are still relatively rare in Serbia. Consequently, there is no reference point for comparison with the results of the current study. Previous research indicates low levels of flatworm infection in rodents (Kataranovski et al. 2011; Bjelić-Čabrilo et al. 2013; Bjelić-Čabrilo et al. 2015). The dominant group of intestinal parasites in rodents are monoxenous roundworms, which is easily explained by their direct life cycle, as opposed to the heteroxenous trematodes and cestodes, which generally require an intermediate host to complete their development.

Considering the higher proportion of invertebrates in its diet, the lower diversity of helminth species observed in the striped field mouse is unexpected; on the other hand, the statisticaly significant higher prevalence of infection in *A. agrarius* is in line with its feeding habits. Another possible explanation for the higher infection prevalence in *A. agrarius* lies in habitat selection. Striped field mice frequently inhabit agricultural land, which can be expected to be contaminated with the feces of other mammal species (Lee et al. 2018); this would imply a greater presence of helminth infective stages. While differences in parasite prevalence or load between host sexes were not addressed in the present study, females of *A. flavicollis* had a slightly higher infection prevalence, in agreement with results from Ondríková et al. (2010) in Slovakia. Our sample of *M. arvalis* is small, but it is important to note that all males were infected, while no infected individuals were detected among females. A higher infection prevalence in male common voles was noted by Gubányi et al. (1992). In contrast, other authors report a higher prevalence of infection in females (Sanchez et al. 2011; Beerli et al. 2017).

The significance of parasitological surveys of small mammals, and rodents in particular, stems from their role as natural reservoirs of zoonoses, many of which are caused by helminths. Considering the high abundance of rodents and their ability to come into contact with humans and domestic animals, the importance of such research is even more pronounced. Studies of this type are necessary in Serbia to provide better insight into pathogen presence and helminth biodiversity. The results presented in the present study add to a relatively small pool of knowledge and raise awareness of potential parasitic hotspots, which is an important early step in maintaining the health of humans, domestic and wild animals.

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