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HELMINTH FAUNA OF THE BANK VOLE *MYODES GLAREOLUS* (RODENTIA, ARVICOLINAE) ON THE TERRITORY OF FRUSKA GORA MOUNTAIN (SERBIA) – A POTENTIAL SOURCE OF ZOONOSES

O. BJELIC-CABRILO[1], D. KOSTIC[1], E. POPOVIC[1], M. CIRKOVIC[2], N. ALEKSIC[3] and J. LUJIC[1]
[1] *University of Novi Sad, Faculty of Sciences, Department of Biology and Ecology, 2100 Novi Sad, Serbia*
[2] *University of Novi Sad, Faculty of Agriculture, 2100 Novi Sad, Serbia*
[3] *University of Belgrade, Faculty of Veterinary Medicine, 11000 Belgrade, Serbia*

Abstract

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The quantitative and qualitative composition of the helminth fauna of 588 bank voles (*Myodes glareolus*) from the territory of Fruska Gora Mountain (Serbia) were analysed with the aim to determine if the species is a natural reservoir of any zoonotic infection. The presence of nine nematode and five cestodes species was detected. The following roundworm species were revealed: *Capillaria murissylvatici* (Dieseng, 1851, Nematoda: Capillariidae), *Trichocephalus muris* (Schrank, 1788, Nematoda: Trichuriidae), *Heligmosomoides glareoli* (Baylis, 1928, Nematoda: Heligmosomidae), *H. polygirus* (Dujardin, 1845), *Heligmosomum mixtum* (Schulz, 1954, Nematoda: Heligmosomidae), *Aspiculuris tetraptera* (Nitzsch, 1821, Nematoda: Oxyuridae), *Syphacia petrusewiczi* (Bernard, 1996, Nematoda: Oxyuridae)*, S. stroma* (Linstow, 1884, Nematoda: Oxyuridae) and *Rictularia proni* (Seurat, 1915, Nematoda: Rictulariidae). Two species of adult tapeworms parasitized the bank vole: *Catenotaenia henttoneni* (Kirschenblatt, 1949, Cestoda: Catenotaeniidae) and *Hymenolepis asymmetrica* (Janicki, 1904, Cestoda: Hymenolepididae), and three in the larval stage: *Taenia martes* (Zeder, 1803, Cestoda: Taeniidae), *Taenia polyacantha* (Leuckart, 1856, Cestoda: Taeniidae) and *Mesocestoides lineatus* (Goeze, 1782, Cestoda: Mesocestoididae).

The largest number of hosts was infected with *Heligmosomoides glareoli* and *Catenotaenia henttoneni*. In addition, three larval tapeworms important for veterinary science and medicine were recovered: *Taenia martes*, *Tatratirotaeina polyacantha* and *Mesocestoides lineatus.* For these, the bank vole is an important intermediate host. Given the fact that, up until now, the bank vole has not been researched for parasite fauna, it is a new host for all determined helminth species. The results obtained contribute to the knowledge on helminth fauna of rodents in Serbia, which was not thoroughly studied so far.

Key words: cestoda, nematode, natural reservoir, Vojvodina
Abreviations: P - prevalence; MI - mean intensity; AB - abundance; n - the number of invaded hosts; N - the number of isolated parasite individuals; min– minimum number of isolated parasite individuals; max – maximum number of isolated parasite individuals

E-mails: olivera.bjelic-cabrilo@dbe.uns.ac.rs; desanka.kostic@dbe.uns.ac.rs; ester.popovic@dbe.uns.ac.rs; jelena.lujic@dbe.uns.ac.rs; miroslavcirkovic@yahoo.com; nenaaleksic@vet.bg.ac.rs

Introduction

Lately, the increase in the incidence of zoonoses, diseases transmissible from animals to humans, has been registered. The causative agents can be transferred by direct contact with infected animals, their excreta, by parasites or indirectly through contaminated environment (soil, food and water). It is believed that there are more than 200 registered zoonoses (Klimpek et al., 2007).

Distribution of the bank vole *Myodes glareolus* (Schreber, 1780) covers forest habitats of the Western Palaearctic ecozone, from France and Scandinavia in north-west to Lake Baikal in the east. It spreads to northern Spain and northern Italy, including the Balkan Peninsula, western Turkey, northern Kazakhstan and the Altai and Sayan Mountains (at the Russian-Mongolian border), including Great Britain and south-western part of Ireland (Musser et Carleton, 1993). In addition to the yellow-necked mouse, the bank vole is a dominant species in habitats which it occupies (deciduous and mixed forests with well developed level of herbaceous vegetation) (Mikes et al., 1979; Mikes and Habijan-Mikes, 1986; Gerasimov and Minkova, 1999; Anikanova and Bugmyrin, 2003; Paunovic et al., 2005). Its population density and cathemeral activity pattern render it an easy prey for birds of prey and carnivorous mammals. This enables the infection of predators with parasites for which the bank vole is an intermediate host, as well as with diseases of other aetiology, some of which are dangerous for humans. Certain tapeworm species can be transferred from small mammals via cats to humans (Mazeika et al., 2003). It has been established that the appearance of the Hantavirus which causes nephropathy in humans is in positive correlation with the population density of its rodent host (Olsson et al., 2003). Niklasson et al. (1998) indicated that the appearances of Guillain-Barre syndrome and diabetes mellitus, as well as death caused by myocarditis are linked to the fluctuations in the population density of the rodents which is the carrier of the Puumala virus (genus Hantavirus, fam. Bunyaviridae). Niklasson et al. (1999) isolated a new Picornavirus from the bank vole. In spite of their importance, there are few data on the possible transmissible agents in the bank vole in Vojvodina Province and Serbia. Meszaros et al. (1983) gave an overview of nematodes in agrobiocenosis rodents in Vojvodina having described 11 species which are common in those hosts. Mikes et al. (1986) described the infection with helminths in mammals in the valley of the Tisa River. Habijan-Mikes et al. (1998) provided data on helminths of small mammals in mountainous regions of former Yugoslavia. The nematode fauna composition of the yellow-necked mouse in highland areas of Serbia was given by Habijan-Mikes et al. (1989), who described five species. In addition, Habijan-Mikes (1990) registered eight nematode species in *Apodemus flavicolis* on the territory of Fruska Gora Mountain. Recently, the published papers analyse parasite fauna of the house mouse (*Mus musculus*) in the suburban area of Belgrade (Vukicevic-Radic et al., 2007 and Kataranovski et al., 2008).

The goal of the paper is to show parasite fauna of the bank vole from the researched area and to determine to what extent is the given species natural reservoir of zoonoses.

Fruska Gora Mountain is located between 45° 0' and 45° 15' N and between 16° 37' and 18° 01' E, and thus is in the area of temperate continental climate. It lies at the south of the Panonian Plain, between the Danube and Sava rivers. It is 80 km long, and has the maximum width of 15 km. It is a low mountain with the highest peak 539 m above mean sea level (http://www.npfruskgora.com).

Fruska Gora has a great diversity of forest populations in different habitats, which is a result of complex ecological factors (Stojsic et al. 2004). The basic identifications of its forests are: the sessile oak (*Quercus petraea*), the European hornbeam (*Carpinus betulus*), the lime (*Tilia* sp) and the beech (*Fagus moesiaca*). With other species of forest trees these build pure or mixed phytocenosis, among which the most widely spread is the mixed sessile oak+European hornbeam

forests (*Aculeato-Querco-Carpinetum serbicum* B. Jov.1977), while pure sessile oak forests (*Festuco montanae-Quercum petraea* M. Jank.) are widely spread forest population of Fruska Gora (Jankovic et al., 1980).

Regarding small mammals, dominant terrestrial species of forest habitats in Fruska Gora are the yellow-necked mouse (*Apodemus flavicolis*) and the bank vole (*Clethrionomys glareolus*), while other, less abundant species are the striped field mouse (*A. agrarius*), and insectivora: the common (*Sorex araneus*) and bicoloured shrew (*Crocidura leucodon*) (Habijan-Mikes, 1990).

Fruska Gora was declared a national park in 1960.

Material and Methods

The individuals of the bank vole were collected in the Fruska Gora National Park between 1985 and 2005. The total of 588 animals was caught: 312 males and 276 females.

The animals were caught with two types of traps: spring-loaded traps set in a line, 5 m apart from each other, and Longworth cage traps put in the same manner. Usually, in each catch 3 lines consisting of 50 traps each were set.

Parasitological analysis was performed on the small and the large intestine of the voles. The bowel content was rinsed in 0.9% saline solution and the extracted helminths conserved in 70% ethanol until species determination.

Afterwards, the nematodes were immersed in lactophenol or lactic acid. Species identification was based on morphological and morphometric characteristics of adult parasites.

Isolated cestodes were rinsed in distilled water, pressed and dehydrated in increasing concentrations of ethanol (70%, 80%, 90% and 96%). In the first three alcohol solutions, the parasites were dehydrated for 15 minutes, and in the last one for 5 minutes. Afterwards, the tapeworms were stained with carmine (Carmine powder, Sigma-Aldrich) and immersed in cedar oil for 24 hours. Finally, the material was mounted in Canada balsam, thus completing the process of permanent microscopic slides preparation.

The identification of species was done using the keys provided by Genov (1984) and Skrjabin et al. (1954, 1957, 1960) and the Key for rodent helminth fauna of the USSR (1978, 1979).

Parasitological terms and quantitative parameters were taken from Buch et al. (1997): prevalence (P %), mean intensity (MI) and abundance (AB).

Modelled on Behnke et al. (2001) the following diversity indices were calculated: Berger-Parker dominance index and Sampson's diversity index.

The data were statistically processed with Statistica Version 8.0 software (Statsoft Inc., Tulsa, USA).

Results

On the territory of Fruska Gora, 588 bank voles were caught and examined for endoparasites. Nematode infections were diagnosed in 60.2% hosts, whilst cestode infections in only 20.75%.

The presence of the following nine nematode species were revealed: *Capillaria murissylvatici* (Dieseng, 1851, Nematoda: Capillariidae), *Trichocephalus muris* (Schrank, 1788, Nematoda: Trichuriidae), *Heligmosomoides glareoli* (Baylis, 1928, Nematoda: Heligmosomidae), *H. polygirus* (Dujardin, 1845), *Heligmosomum mixtum* (Schulz, 1954, Nematoda: Heligmosomidae), *Aspiculuris tetraptera* (Nitzsch, 1821, Nematoda: Oxyuridae), *Syphacia petrusewiczi* (Bernard, 1996, Nematoda: Oxyuridae), *S. stroma* (Linstow, 1884, Nematoda: Oxyuridae) and *Rictularia proni* (Seurat, 1915, Nematoda: Rictulariidae). In addition, five cestode species were found to infect the bank vole, two of them being adult *Catenotaenia henttoneni* (Kirschenblatt, 1949, Cestoda: Catenotaeniidae), *Hymenolepis asymmetrica* (Janicki, 1904, Cestoda: Hymenolepididae), and the rest larval: *Taenia martes* (Zeder, 1803, Cestoda: Taeniidae), *Taenia polyacantha* (Leuckart, 1856, Cestoda: Taeniidae) and *Mesocestoides lineatus* (Goeze, 1782, Ces-

Table 1
Qualitative indicators of presence of certain nematode species in the sample

	n	P%	N	MI	AB	Min	Max
C. murisylvatici	94	16.38	1152	12.26	2.01	1	271
T. muris	3	0.52	3	1	0.005	1	1
H. glareolig	189	32.93	986	5.21	1.72	1	43
H. p. polygirus	2	0.35	4	2	0.01	1	3
H. mixtum	26	4.53	100	3.85	0.17	1	18
A. tetraptera	1	0.17	5	5	0.01	5	5
S. Petrusewiczi	123	21.43	5074	41	8.79	1	434
S. stroma	4	0.70	31	7.75	0.05	1	22
R. proni	1	0.17	3	3	0.005	3	3
Total	354		7358			-	-

toda: Mesocestoididae). Most hosts were infected with only one nematode species (75.54%), but the maximum number of species found per host was four. The largest number of isolated nematodes belonged to the species *S. petrusewiczi* (5043) and it showed the highest mean intensity (MI-41) of infection, whilst the species *H. glareoli* infected the highest number of hosts (32.93%). The minimum number of individual helminths per hosts in almost all species was one, while the maximum ranged between 3 and as much as 434. The majority of bank voles were infected with one or two parasite specimens (Table 1).

Among the registered cestode species, *C. hentoneni* showed the highest prevalence (85.24%), while the species *T. polyacantha* showed the highest mean intensity - 28.25. Only one cestode species was registered in each host. The minimum number of cestode individuals per host was 1, and the maximum was 72. Similarly to nematodes, the greatest number of hosts was invaded with one or two individuals (Table 2).

Discussion

According to our research, the bank vole was infected with two groups of endoparasites: nematodes – 60.2% and cestodes – 20.74%. The domination of nematodes is a phenomenon which had already been noticed in the bank vole by other authors. Thus, at all sites analysed in Poland, Benhke et al. (2001) found significantly smaller infection level of cestodes in comparison to nematodes, which was confirmed by Bernard et al. (2002), while Klimpel et al. (2007) found that only nematodes invaded the abovementioned host, thus concluding that the given rodent species is not the natural reservoir of zoonoses in Germany. The same authors, as well as Milazzo et al. (2003) discovered the presence of flukes, although in a small percentage of hosts, which was not comfirmed in our research. The obtained data are quite expected and result from the bank vole's diet: it is an herbivorous animal which feeds on food of animal origin only in traces (Kesmanovic, 1979; Lewis, 1987). Thus, intermediate hosts of biohelminths, mainly cestodes and flukes, are ingested in small numbers by the vole, rendering the chance of infection by nematodes which do not require an intermediate host higher. This is further supported by the results presented by Lewis (2008), who compared the helminth fauna of rodents and insectivorous animals in Great Britain, and found cestodes to be more frequently present in insectivo-

Table 2
Quantitative indicators of host infection with cestodes

	n	P%	N	MI	AB	Min	Max
M. lineatus-larva	3	2.46	5	1.67	0.008	1	4
T. polyacantha-larva	4	3.28	113	28.25	0.192	7	72
C. henttoneni	104	85.24	279	2.68	2.68	1	9
*H. asimetri**ca***	6	4.92	11	1.83	0.019	1	2
T. martes-larva	5	4.1	42	8.4	0.07	1	21
Total	122		450				

n - the number of invaded hosts; P - prevalence; N - the number of isolated parasite individuals;MI - mean intensity; AB - abundance; min - minimum number of isolated parasite individuals; max - maximum number of isolated parasite individuals

rous animals, and nematodes in rodents, which is also the consequence of their eating habits.

In most cases (75.54%), bank voles in Fruska Gora were infected with only one nematode species, 24.46% were infected with two, 0.93% with three, whilst only one single individual was infected with four nematode species. Similar results were reported by Habijan-Mikes (1990) in the yellow-necked mouse. Benhke et al. (2001) stated that the number of species per host was either 2 or 3, and Ribas et al. (2009) that it ranged between 0 and 3.

The prevalence of nematode infection was moderately high, 61.67%, being less than the lowest registered in Poland, which ranged between 68.3% and 95% (Behnke et al., 2001, Bernard et al., 2002, 2003), but somewhat higher than the one in Spain, according to Ribas et al. (2009) between 73.80% and 51.42%. However, in Lithuania, the prevalence of roundworm infection was significantly lower than in Fruska Gora: 18.3% (Mazeika et al., 2003). Most of the authors underline that the quantitative and qualitative composition of parasitic fauna are strongly influenced, and thus may be explained, by the density of the host's population and its behaviour (Feilu et al., 1997; Behnke et al., 2001). Bernard et al. (2003), for example, pointed to the significant differences in the behaviour of the bank vole in ecologically very similar and geographically close habitats, which resulted in differences in nematode composition.

The species *H. glareoli* infected the highest number of hosts (32.93%), whilst the total largest number of nematodes belonged to the species *S. petrusewiczi* (5074), which infected 21.43% of the voles. *H. glareoli* was also the most prevalent species in Italy, whilst the number of *S. petrusewiczi* per host varied from two to eight (Milazzo et al., 2003). Genov (1984) explained the predominance of *H. glareoli*, *H. mixtum* and *S. petrusewiczi* with the fact that they are typical parasites of the bank vole. Lewis (1987) stated that the species of the *Syphacia* genus have rapid development cycle, and may easily be transferred among hosts, which explains the reason why they are widespread. According to the claims of authors across Europe on the nematode fauna composition of the bank vole, the commonly present species are *H. glareoli*, *H. mixtum* and *S. petrusewiczi*, followed by *T. muris* and *C. murissylvatici* (Meszaros et al., 1979; Feilu et al., 1997; Meszaros, 2001; Mazeika et al., 2003; Grikieniene, 2005), which is in accordance with our results.

The number of individual worms per host varied. The greatest number of voles was infected with 1 or 2 individuals of both nematodes and

cestodes. However, the widest range in the number of specimens was registered in *Syphacia petrusewiczi*: between 1 and 434. These results were in accordance with the findings in Europe reported by Benhke et al. (2001) and Bernard et al. (2003), who had also observed the greatest range in *S. petrusewiczi*. The reason for this disposition of parasites lays in their life cycle and routes of infection. *S. petrusewiczi* infects the specific group of hosts and accumulates in it, while *H. glareoli* and *H. mixtum* infect a variety of hosts but in smaller and balanced numbers.

In the bank voles in Fruska Gora, five species of the Cestoda class were registered: the adults of *Catenotaenia cricetorum* and *Hymenolepis asymmetrica,* and the larvae of *Taenia martes*, *Tatratirotaeina polyacantha* and *Mesocestoides lineatus*. The most common species was *Catenotaenia henttoneni* (85.24%).

T. martis larvae are relatively common in the bank vole (Genov, 1984; Feilu et al., 1997; Mazeika, 2004), while mature forms infect Mustelidae, more precisely the Martes genus (Ribas et al., 2004). Kirillova et al. (2008) pointed to the importance of the bank vole as an intermediate host of *T. polyacantha* and *M. lineatus*. In adult stage the former infects Canidae (Gicik et al., 2009), and the latter beasts, more precisely Felidae (Torres et al., 1998), thus all researchers emphasize the importance of these species from the point of view of the veterinary and medical sciences. The presence of the larvae of *T. polyacantha* and *M. lineatus* may serve to confirm the presence of definite hosts of these tapeworms in the researched area (Ribas et al., 2009).

The highest prevalence of *H. glareoli* and *C. hentonneni* are in accordance with the claims of Ribas et al. (2009), and may be explained with the fact that they are typical parasites of the *Myodes* genus. Hauksilami et al. (1993), as well as Romashova (2003) pointed out the differences between the species *H. glareoli* and *C. hentonneni*, the parasites of small intestine. According to them, *C. hentonneni* prefers the duodenum, and *H. glareoli* other parts of the small intestine; therefore although parasitizing the same host, they avoid overlapping of niches and competition. According to the given results, the largest percentage of *C. hentonneni* parasitized the same host as *H. glareoli*. They were isolated from Different segments of the small intestine, supporting the claim about avoiding competition. Some authors state that *H. assymetrica* is typical of bank voles (Genov, 1984; Kirillova and Kirillov, 2008). Nevertheless, its presence was insignificant in the bank vole in Fruska Gora.

Conclusion

The importance of the obtained results lies in the fact that, up to now, there were no data on helminth fauna of the bank vole in Serbia, therefore it can be considered a new host for all the following nematode species: *C. murissylvatici*, *T. muris*, *H. polygirus*, *A. tetraptera*, *S. stroma* and *R. proni.* The last species, *R. proni,* is first reported in the bank vole in Europe. Nematodes *Heligmosomoides glareoli*, *Heligmosomum mixtum* and *Syphacia petrusewiczi* were registered for the first time in Serbia. *Myodes glareolus* is a new host for all the named cestode species in Serbia.

Although no species with high tendency of infecting humans, for example *Hymenolepis diminuta* or *H. nana*, or the very dangerous *Alveococcus multilocularis*, were recovered from the bank vole in Fruska Gora, it was diagnosed with infection with certain species of veterinary and medical importance, such as larvae of *Taenia martes*, *Taeina polyacantha* and *Mesocestoides lineatus.* For this reason *Myodes glareolus* should be considered a potential natural reservoir of zoonoses and requires further monitoring.

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