HELMINTH FAUNA OF *RATTUS NORVEGICUS* BERKENHOUT, 1769 FROM THE BELGRADE AREA, SERBIA

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Abstract - The aims of this study were to provide baseline knowledge about intestinal parasites in Norway rats (*Rattus norvegicus*) that inhabit the Belgrade area, and to analyze the associations among helminths. Of 302 trapped rats, 52% were females and 48% males, with 39% and approx. 37% of juvenile-subadult individuals, per sex, respectively. The following parasites were detected (with their respective prevalence): Cestoda – *Hymenolepis diminuta* (30.46%) and *Rodentolepis fraterna* (12.58%); Nematoda - *Heterakis spumosa* (36.75%), *Nippostrongylus brasiliensis* (16.22%), *Capillaria* sp. (5.96%), *Trichuris muris* (5.96%), *Syphacia muris* (4.30%) and *Strongylus* sp. larvae (0.33%). Flukes (Trematoda) were not recorded. Of all examined rats, 68.54% were found to harbor at least one parasite species, with higher prevalence in male hosts and in adult individuals. There were no age-related differences in the prevalence of infection with individual helminth species. Multiple infections occurred with up to four species per rat showing different combinations of parasite infections. These are the first records of the gastrointestinal helminth fauna of Norway rats in Serbia.

Key words: *Rattus norvegicus*, gastrointestinal helminths, Belgrade area, Serbia

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INTRODUCTION

Extreme adaptation to life under the most distinct habitat conditions and types, euryvalency toward numerous environmental factors, tendency to synanthropy, high reproduction potential, expressed genetic polymorphism and the ability to acquire resistance to some rodenticide preparations represent the main characteristics of representatives from the genera *Rattus* and *Mus*. These characteristics enable their optimal existence in the immediate human environment, at the same time making the problem of their population abundance much more complex than it seems at the first sight (Kataranovski, 1988). Investigations in the field of biology and the population ecology of pest synantropous rodents are of multiple interest and significance because of economic and hygienic-epidemiological reasons; they pose a threat to public health, both through the consummation of different food and by serving as reservoir for pathogens that can be transmitted to humans and may cause outbreaks of diseases often with high morbidity and some mortality (Robinson and Olsen, 1960; Marchette, 1966; Cabrera, 1976; Jones and Twigg, 1976; Hoogstraal, 1979; Kaplan et al., 1980; Dick, 1983; Meehan, 1984; Kumar and Bubare, 1986; Gratz, 1988, 2005; Horgović et al., 1991; Kliks and Palumbo, 1992; Pozio et al., 1992, 2009; Gustafson, 1994; Young et al., 1998; Crompton, 1999; Markell et al., 1999; Serbezov et al., 1999; Salazar-Bravo et al., 2002; Garber et al., 2003; Petavy et al., 2003; Kumar et al., 2003; Calisher et al., 2005; Giraldo-Gomez et al., 2005; Satoh et al., 2005; Klimpel et al., 2006; Landais et al., 2007; Kijlstra et al., 2008; Samardzic et al., 2008; Cook et al., 2009; Salkeld and Lane, 2010).

The Norway (brown, sewer) rat, *Rattus norvegicus* Berk., 1769, is very prevalent and represents a dominant rodent pest species on the territory of former Yugoslavia (Kataranovski, 1999) and present Serbia. Given its wide distribution and its close contact with different animals and humans, the Norway rat plays an important role as a reservoir host for vector-borne disease agents. The helminths of the Norway rat have been studied in many countries in Europe (Feliu et al., 1985, 1997; Webster and Macdonald, 1995; Webster, 1996; Ceruti et al., 2001; Mažeika et al., 2003; Stojčević et al., 2004; Redrobe and Patterson-Kane, 2005, Pozio et al., 2009), and in various parts of the world: Canada (Firlotte, 1948), the USA (Wallace and Rosen, 1965; Farhang-Azad, 1977; Bailey and Schantz, 1990; Easterbrook, 2007), South Korea (Seo et al., 1968; Seong et al., 1995), Brazil (Galvao, 1976), India (Biswas, 1978), Guadeloupe (Combes and Delattre, 1981), New Zealand (McKenna, 1997), Qatar (Abu-Madi et al., 2001; 2005), Peru (Iannacone and Alvarino, 2002), Taiwan (Siritantikorn et al., 2003), Argentina (Gomez Villafañe et al., 2008), Malesia (Paramasvaran et al., 2009). However, the diversity of the helminths in *Rattus norvegicus* in the Belgrade area and the whole of Serbia is relatively unknown. Recently data on *Calodium hepaticum* (Bancroft, 1893) (syn. *Capillaria hepatica*) and *Taenia taeniaeformis* (Batsch, 1786) (syn. *Hydatigera taeniaeformis*) liver infections in *R. norvegicus* were obtained by Kataranovski et al. (2010) and this is the first record of these parasites in Norway rats in Serbia.

The aims of this study were to determinate the parasitic fauna of *R. norvegicus* in the Belgrade area and to analyze the associations among helminths.

MATERIALS AND METHODS

Rat samplings were conducted during the period of four consecutive years, from May 2005 to July 2009. A total of 302 rats were collected in the Belgrade area (44^{0}N, 20^{0}E; approximate geometric center of Belgrade 44^{0}49'14"N, 20^{0}27`44"E) using snap live traps (31 x 16.5 x 14 cm). The traps were baited with pieces of smoked bacon and/or fresh-water fish. The captured rats were euthanized and necropsied in the laboratory of the Institute for Biological Research "Siniša Stanković", Belgrade.

For each rat examined, the data of trapping locality, body length (head and body), weight and sex were noted. The rats were separated into two age groups: juveniles-subadults (< 2.5 months old) and adults (>2.5 months old). The following criteria were used for separating adults from juvenile-subadult animals 1) the body weight (borderline value approx. 200 g, according to Podloucky, 1977; Savić and Kataranovski, 1981; Kataranovski et al., 1991), and 2) the weight of the dry eye lens pairs (14.04-14.30 mg, according to Savić and Kataranovski, 1981; Kataranovski, 1988; Kataranovski et al., 1994). The material was analyzed using the standard parasitological procedure according to Kataranovski et al. (2008). The identification of helminths was based on the Key for Helminths of Rodents of the Fauna of the USSR (1978,1979), and taxonomic keys to cestode and nematode parasites given by Anderson et al. (1974), Genov (1984) and Khalil et al. (1994).. The parasitological terminology and quantitative parameters are according to Buch et al. (1997). The quantitative descriptors of parasite infection were calculated, including prevalence (P), mean intensity of infection (MI) and mean abundance of infection (MA). Also, an index of infection (I) was calculated according to Kisielewska (1970). Statistical analysis was performed using the statistical software package STATISTICA 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

RESULTS

Of the 302 rats sampled during the study, 48.01% were males and 51.99% were females. Two hundred and seven rats (68.54%) were infected with at least one parasite or more. A higher prevalence of infection was noted in the males compared to the female adult rats (Table 1). Similar numbers of male and female juvenile-subadult rats were infected.

The total number of eight parasite species was found, namely: six Nematoda species - *Heterakis*

Table 1. Prevalence of intestinal helminth infection in rats of different sex and age. Significantly different from females at * p<0.01; from juvenile-subadults at # p<0.05.

	Males (%)	**Females (%)**	**Combined (%)**
Number of rats	145/302 (48.01)	157/302 (51.99)	302/302 (100)
Infected	111/145 (76.55)*	96/157 (61.15)	207/302 (68.54)
Non-infected	34/145 (23.45)	61/157 (38.85)	95/302 (31.46)
Juvenile-subadults	53/145 (36.55)	61/157 (38.85)	114/302 (37.75)
Infected	35/53 (66.04)	34/61 (55.74)	69/114 (60.53)
Non-infected	18/53 (33.96)	27/61 (44.26)	45/114 (39.47)
Adults	92/145 (63.45)	96/157 (61.15)	188/302 (62.25)
Infected	76/92 (82.61)*#	62/96 (64.58)	138/188 (73.4)
Non-infected	16/92 (17.39)	34/96 (35.42)	50/188 (26.6)

spumosa (Schneider, 1866), *Nippostrongylus brasiliensis* (Travassos, 1914), *Capillaria sp* (Zeder, 1800), *Syphacia muris* (Yamaguti, 1935), *Trichuris muris* (Schrank, 1788) and *Strongylus sp. larvae*, and two Cestoda species - *Hymenolepis diminuta* (Rudolphi, 1819) and *Rodentolepis fraterna* (Stilles, 1906). Data on the prevalence, index of infection, mean infection intensity and mean abundance of gastrointestinal cestodes and nematodes in male and female Norway rats are presented in Table 2a and Table 2b. The most prevalent parasites were *H. spumosa* (36.75%), *H. diminuta* (30.46%), *N. brasiliensis* (16.22%) and *R. fraterna* (12.58%). There were no significant differences in the prevalence for individual helminth species with the exception of *Capillaria* sp. where there was a higher prevalence in males compared to females. *Strongylus sp.* larvae was found in only one host (0.33%) and was not taken into further statistical analysis.

Multiple infections were noted with up to four helminth species per host. Parasitism involving only one species was found in 51.21% of the infected rats. Multiple infections including two species of parasites were found in 32.37% of the infected rats. Three helminth species were found in 14% and four parasite species were found in 2.42% of the hosts (Table 3) Types of polyparasitism were assessed for all rats with multiple infections (Table 4). They were divided into classes with two, three and four species, respectively, representing the number of parasite species and specific combinations of parasites. The most frequent infection type was the *H. spumosa/H. diminuta* combination. Other infection types presented combined infection with different species. Fig. 1 shows types of polyparasitism among all the rats. *S. muris* were negatively associated with *N. brasiliensis* and *T. muris*, and *T. muris* is also negatively associated with *Capillaria sp.*

DISCUSSION

Urbanization and the growth of towns have resulted in an increase in the accumulation of garbage and refuse which create favorable conditions for the proliferation of rats, and they rarely remain uninfected or harbor just a single species' infections in nature (Behnke et al., 2001). As *R. norvegicus* is a synanthropic species, the potential for spillover of zoonotic agents poses a threat to public health. This study records the first data on

Table 2a. Quantitative indices of helminth infection of *Rattus norvegicus* (n = number of rats infected, Z = total number of rats, P% = prevalence, * = p<0.05).

Helminth species	n	Z	P%
Heterakis spumosa	111	302	36.75
juvenile-subadult	34	114	29.82
adult	77	188	40.96*
Hymenolepis diminuta	92	302	30.46
juvenile-subadult	27	114	23.68
adult	65	188	34.57*
Nippostrongylus brasiliensis	49	302	16.22
juvenile-subadult	13	114	11.4
adult	36	188	19.15*
Rodentolepis fraterna	38	302	12.58
juvenile-subadult	10	114	8.77
adult	28	188	14.89
Capillaria sp.	18	302	5.96
juvenile-subadult	8	114	7.02
adult	10	188	5.32
Trichuris muris	18	302	5.96
juvenile-subadult	4	114	3.51
adult	14	188	7.45
Syphacia muris	13	302	4.30
juvenile-subadult	8	114	7.02
adult	5	188	2.66
Strongylus sp. larvae	1	302	0.33
juvenile-subadult	0	114	0
adult	1	188	0.53

intestinal helminth parasites in the Norway rat from the Belgrade area along with recent data on *Calodium hepaticum* and *Taenia taeniaeformis* liver infections in this rodent (Kataranovski et al., 2010). This study shows that *R. norvegicus* from this area is host to two cestode species and six nematode species. The monoxenous nature of the life cycle of nematodes may be responsible for this parasitic group dominating the helminth community of small mammals worldwide, especially rodents. Parasites with simple and direct life cycles may have more chance of following the dispersion of their hosts than parasites with indirect life cycles (Bellocq, 2003). The longevity of *H. diminuta* in its normal mammalian host can cause a high prevalence of infections with this parasite. Once established, it can live as long as the host (Read, 1967).

The moderate prevalence of *H. spumosa* is in accordance with the results of Seo et al. (1968) in South Korea and Stojčević et al. (2004) in Croatia. However, research results of Firlotte (1948) in Canada, Tscherner (1996) in Germany and in Buenos Aires, Argentina (Gomez Villafañe et al., 2008) show a high prevalence of *H. spumosa* in the Norway rat.

A moderate to high prevalence of *H. diminuta* is in accordance with data obtained in different parts of the world; in Kuwait (Zakaria and Zaghloul, 1982), Great Britain (Webster and Macdonald, 1995), Qatar (Abu-Madi et al. 2001, 2005), Croatia (Stojčević et al., 2004), Argentina (Gomez Villafañe et al., 2008), and Southeastern Asia - Kuala Lumpur, Malesia (Paramasvaran et al., 2009). Paramasvaran et al. (2009) included in their study several rat species in addition to *R. norvegicus*: *Rattus exulans* (Peale, 1848) and *Rattus rattus diardii* (Jentink 1879).

Results concerning *N. brasiliensis* are in accordance with results from other studies (Stojčević et al., 2004; Gomez Villafañe et al., 2008; Paramasvaran et al., 2009).

All parasite species showed a higher prevalence in male rats than in females. This could be explained by the fact that infected males have larger territories than uninfected males, and that the home range of males tends to overlap which could increase their exposure to infection while reproductive females show a stronger site-specific organi-

Table 2b. Quantitative indices of individual intestinal helminth infection of *R. norvegicus*. n-number of animals infected, N-total number of parasites, P-prevalence, I-index of infection, MI-mean intensity, MA-mean abundance. Significantly different from females at * p<0.05.

Helminth species		n	N	P%	I	MI	MA
Cestoda							
Hymenolepis diminuta	total	92	580	30.46	0.59	6.30	1.92
	♂♂	50	319	34.48	0.76	6.38	2.20
	♀♀	42	261	26.75	0.44	6.21	1.66
Rodentolepis fraterna	total	38	219	12.58	0.09	5.76	0.73
	♂♂	22	132	15.17	0.14	6.00	0.91
	♀♀	16	87	10.19	0.06	5.44	0.55
Nematoda							
Heterakis spumosa	total	111	897	36.75	1.09	8.08	2.97
	♂♂	54	437	37.24	1.12	8.09	3.03
	♀♀	57	460	36.31	1.06	8.07	2.93
Nippostrongylus brasiliensis	total	49	677	16.22	0.36	13.82	2.24
	♂♂	27	370	18.62	0.47	13.70	2.55
	♀♀	22	307	14.01	0.27	13.95	1.96
Capillaria sp.	total	18	181	5.96	0.04	10.06	0.60
	♂♂	14	126	9.66*	0.08	9.00	0.87
	♀♀	4	55	2.55	0.01	13.75	0.35
Trichuris muris	total	18	86	5.96	0.02	4.78	0.28
	♂♂	11	49	7.59	0.03	4.45	0.34
	♀♀	7	37	4.46	0.01	8.29	0.24
Syphacia muris	total	13	97	4.30	0.01	7.46	0.32
	♂♂	6	50	4.14	0.01	8.33	0.34
	♀♀	7	47	4.46	0.01	6.71	0.30
Strongylus sp. larvae	total	1	1	0.33	0.00	1.00	0.00
	♂♂	1	1	0.69	0.00	1.00	0.01
	♀♀	0	0	0.00	0.00	0.00	0.00

Table 3. Prevalence of intestinal helminth infection in rats of different sex and age. Significantly different from females at * p<0.01; from juvenile-subadults at # p<0.05.

Total (302)	Non-infected	Infected	Number of parasite species per host			
			1	2	3	4
Number of rats	95	207	106	67	29	5
%	31.46	68.54	51.21	32.37	14.00	2.42
Juvenile-subadults (114)						
Number of rats	45	69	41	20	8	0
%	39.48	60.52	59.42	28.99	11.59	0
Adults (188)						
Number of rats	50	138	65	47	21	5
%	26.60	73.40	47.10	34.06	15.22	3.62

zation (Davis et al., 1948; Pisano and Storer, 1948; Calhoun, 1962). Also, the male hormone testosterone has a negative effect on the immune function (Grossman, 1989; Folstad et al., 1992). Another hypothesis assumes that among mammals the larger bodies of males are easier targets for parasites (Arneberg, 2002).

The prevalence and abundance of helminths may also be influenced by various types of associations between species of parasites, which can be both synergistic (positive) and antagonistic (negative). Such associations can arise for ecological/behavioral reasons, although there remains the possibility that the host immune system may also be involved. In this study, the most frequent infection type was the *H. spumosa/H. diminuta* combination, which is probably a consequence of the fact that these are the two most prevalent parasites in this study, and does not imply a connection between these two species. It is possible that these parasites are in positive associations because they are located in different parts of the host; *H. spumosa* is localized in the lower intestine and/or caecum-rectum and *H. diminuta* is localized in the upper intestine, so the interaction is minimized and competition is reduced. The negative associations between *Capillaria sp.* and *T. muris*, *N. brasiliensis* and *S. muris*, *S. muris* and *T. muris* may be the result of competition between them or the different times of year when infection occurs. For the *N. brasiliensis* and *S. muris* association the first alternative is less probable because these species are located in different parts of the host's intestine.

The eight helminth species reported in this study is a new record for the Belgrade area. Norway rats represent some very important links in the food chains of the ecosystems they inhabit and, on the other hand, they often come into contact with people and may act as transmitters of various pathogens. The sparse knowledge of their parasites indicates the necessity of further parasitological studies.

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Table 4. Types of polyparasitism among all rats with multiple parasite infections. Hs = *Heterakis spumosa*, Hd = *Hymenolepis diminuta*, Rf = *Rodentolepis fraterna*, Nb = *Nippostrongylus brasiliensis*, Sm = *Syphacia muris*, Cap = *Capillaria* sp., Tm = *Trichuris muris*, Str = *Strongylus sp. larvae*.

Single infections			
	total 106	♂♂ 54	♀♀ 52
Hs	36	16	20
Hd	33	20	13
Rf	19	9	10
Nb	9	5	4
Sm	7	3	4
Cap	1	1	0
Tm	1	1	0
Str	1		
Multiple parasite infections			
2 species	total 67	♂♂ 39	♀♀ 28
Hs+Hd	24	13	11
Hs+Nb	10	5	5
Hd+Tm	6	5	1
Cap+Hs	4	4	0
Hs+Rf	4	3	1
Hd+Nb	4	0	4
Cap+Hd	3	2	1
Rf+Tm	3	1	2
Hs+Sm	2	1	1
Nb+Tm	2	2	0
Cap+Rf	1	1	0
Cap+Str	1	1	0
Hs+Tm	1	0	1
Hd+Sm	1	0	1
Nb+Rf	1	1	0
3 species	total 29	♂♂ 17	♀♀ 12
Hs+Hd+Nb	9	5	4
Hs+Hd+Rf	4	1	3
Cap+Hs+Hd	3	2	1
Hs+Hd+Sm	3	1	2
Hs+Hd+Tm	2	0	2
Cap+Hs+Rf	1	1	0
Cap+Nb+Rf	1	1	0
Cap+Rf+Sm	1	1	0
Hs+Nb+Rf	1	1	0
Hs+Nb+Tm	1	1	0
Hs+Rf+Sm	1	1	0
Hd+Nb+Tm	1	1	0
Nb+Rf+Tm	1	1	0
4 species	total 5	♂♂ 2	♀♀ 3
Hs+Hd+Nb+Sm	2	1	1
Cap+Hs+Nb+Rf	1	0	1
Cap+Hs+Hd+Nb	1	0	1
Hs+Nb+Rf+Tm	1	1	0

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