

Research Note

Is there a host sex bias in intestinal nematode parasitism of the yellow-necked mouse (*Apodemus flavicollis*) at Obedska bara pond, Serbia?

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Article info

Received February 2, 2018
Accepted April 24, 2018

Summary

Fifty-one yellow-necked mice from the Obedska bara locality were analysed for the presence of intestinal nematode parasites in order to assert whether there was a host sex bias in infection. Previous research indicated that males would be the more infected sex, either due to the immunosuppressive effect of testosterone or their different allocation of resources towards immune defence. Quantitative infection parameters were compared between host sexes for all nematode species and nematodes in general. In addition, the influence of host sex, age, total body length, body mass and presence of other nematode species on parasite abundance was analysed. No statistically significant differences between males and females were noted for any of the studied quantitative parameters, leading to an absence of sex-biased parasitism in this study.

Keywords: nematodes; sex-biased parasitism; immunocompetence; host behaviour; Serbia

Introduction

Studies of sex-biased parasitism (SBP) date as far back as the beginning of the 20th century (Zuk & McKean, 1996), but the full extent of the phenomenon and the factors that underlie it remain open questions. In parasitological literature, studies generally report a male-biased infection (Poulin, 1996; Zuk & McKean, 1996; Krasnov *et al.*, 2005). The two main groups of hypotheses explaining this refer to the immunosuppressive effect of the male sex hormone testosterone (Zuk & McKean, 1996; Schalk & Forbes, 1997; Zuk, 2009; Krasnov *et al.*, 2012) and, more broadly, the differential effect of natural selection on males and females of the same host species (Zuk & McKean, 1996; Rolff, 2002; Nunn *et al.*, 2009; Zuk, 2009). The main takeaway from both groups of hypotheses is that males trade off immunity for reproductive success, and thus become more susceptible to parasite infections. The yellow-necked mouse, *Apodemus flavicollis*, is a rodent species that is a host of a number of ectoparasites and helminths.

Experimental studies and mathematical modelling have shown that males play a crucial role in the transmission and maintenance of the nematode *Heligmosomoides polygyrus* in its populations (Ferrari *et al.*, 2004, 2007), but to this date no prior study found evidence of sex-biased parasitism in this rodent species (Krasnov *et al.*, 2012). The aim of the present study was to determine whether a host sex bias in intestinal nematode infection exists in a population of yellow-necked mice at Obedska bara pond. This preliminary analysis is the first study of sex-biased nematode parasitism to be carried out on this host species in Serbia.

Materials and Methods

A total of 51 individuals (30 males, 21 females) of the yellow-necked mouse were captured at Obedska bara locality, near the Sava river (44.746° N, 20.006° E) in October 2011. Obedska bara is a protected wetland and forest habitat situated on the Sava river, in the Srem (Syrmia) region of the northern autonomous

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province of Vojvodina, Serbia. Sampling of hosts was carried out in a mixed deciduous forest, consisting of trees such as English oak (*Quercus robur*), manna ash (*Fraxinus ornus*), black poplar (*Populus nigra*) and common hornbeam (*Carpinus betulus*). Trapping was carried out over three nights using 200 Longworth traps with bait and bedding supplied. Biometric data (body and tail length, body mass) were collected for all captured mice. In addition, host age was determined based on dry eye lens weight (Nabaglio & Pachinger, 1979).

All of the captured animals were sacrificed and kept in a freezer until dissection. Part of the intestinal tract (small intestine, caecum, colon and rectum) was removed from each animal and cut longitudinally in order to release its content into a Petri dish that was then emptied into a larger conical glass filled with tap water. Water was refilled and decanted in the conical glass until the supernatant was clear. All helminths were fished out under a stereomicroscope and conserved in 70 % ethanol. Identification was carried out based on morphological and morphometric characters using keys by Ryzhikov *et al.* (1979) and Genov (1984). The parasites were deposited in the collection of the Laboratory for animal ecology at the University of Novi Sad, protocol numbers 3391-3438, 3450-3451 and 3457.

Prevalence, mean and median intensity, and mean abundance for each nematode species and nematode parasites in total were calculated in accordance to Bush *et al.* (1997) for each host sex and for the total sample. Comparison of prevalence between host sexes was carried out via the exact unconditional test. Mean abundances and intensities were compared with a bootstrap test with 20000 replications, and median intensities were compared with Mood's median test. The null hypothesis was that there were no statistically significant differences between sexes with regards to the analysed parameters. All calculations and statistical tests were performed in Quantitative Parasitology software, version 3.0 (Rózsa *et al.*, 2000).

Parasite abundance was further analysed by fitting abundance data in a generalized linear model (GLM). For the total sample, host age (with dry eye lens weight as a proxy), sex, total length and body mass were used as terms predicting the numerical response. Abundance data of each nematode species was also fitted, with host age, host sex, total length, body mass and abundances of

the remaining species used as factors. The full models incorporated all of the terms and their two-way interactions. Factors and interactions below significance level ($p=0.05$) were excluded step by step until a minimal model was obtained. Calculations were performed in R software, version 3.2.1 (R Core Team, 2015) in standard packages.

Ethical Approval and/or Informed Consent

The research was conducted under permits issued by the Ministry of Natural Resources, Mining and Spatial Planning, Republic of Serbia (number: 353-03-250/2010-04). The mice were treated according to Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. All animal procedures were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

Results and Discussion

Of the fifty-one examined mice, forty (78.4 %) were infected with intestinal nematodes. Nematode prevalence was higher in male (83.3 %) than in female mice (71.4 %). Five roundworm species were recovered from the sample: *Aonchotheca annulosa*, *Heligmosomoides polygyrus*, *Syphacia frederici*, *S. stroma* and *Trichuris muris* (Table 1). Three of them were present in both host sexes, with *H. polygyrus* only recorded from a single female and *S. frederici* from a single male. Thus, the species richness of nematode parasitic community was identical, with four species found in each host sex. The average number of nematode species carried by infected individuals was 1.35. Infections with one nematode species were most common (70 % of infected hosts), with two (25 %) and three (5 %) species infections less frequently observed. While raw data seemed to suggest that males were more heavily infected (Table 2), statistical tests found no significant differences in infection between sexes. Comparisons of prevalence, mean intensity, mean abundance and median intensity between male and female total samples yielded no evidence of sex-biased parasitism. Furthermore, abundance data fitting showed no influence of host sex

Table 1. Parasitological parameters of infection for individual nematode species and intestinal nematodes as a group in the total sample of fifty-one mice from Obedska bara. 95 % confidence intervals in brackets, where applicable.

	I	P%	MI	MA
<i>A. annulosa</i>	12	23.5 (13.4 – 37.2)	8.1 (1.8 – 30.3)	1.9 (0.5 – 8.2)
<i>H. polygyrus</i>	1	2 (0.1 – 10.4)	1	0.02 (0 – 0.06)
<i>S. frederici</i>	1	2 (0.1 – 10.4)	13	0.3 (0 – 0.8)
<i>S. stroma</i>	30	58.8 (45.1 – 71.7)	29.2 (18.2 – 54.2)	17.2 (10.2 – 33.3)
<i>T. muris</i>	10	19.6 (10.5 – 33.2)	2.7 (1.5 – 4.9)	0.5 (0.2 – 1.2)
Nematodes	40	78.4 (64.8 – 87.9)	25.4 (16.4 – 44.8)	19.9 (12.4 – 35.5)

I – number of infected hosts, P% – prevalence, MI – mean intensity, MA – mean abundance

Table 2. Values of prevalence (P%), mean intensity (MI) and mean abundance (MA) for all nematode species and total intestinal nematodes in male and female yellow-necked mice from Obedska bara, Serbia. 95 % confidence intervals in brackets, where applicable.

	Males (30)			Females (21)		
	P%	MI	MA	P%	MI	MA
Aa	30 (16.3 – 48.3)	10.33 (1.7 – 34.1)	3.1 (0.5 – 12.7)	14.3 (4 – 35.4)	1.3 (1 – 1.7)	0.2 (0 – 0.5)
Hp	–	–	–	4.8 (0.3 – 23.3)	1	0.1 (0 – 0.14)
Sf	3.3 (0.2 – 17.7)	13	0.4 (0 – 1.3)	–	–	–
Ss	63.3 (45 – 78.6)	32.2 (16.8 – 73.1)	20.4 (10.1 – 47.1)	52.4 (30.5 – 72.4)	24.1 (11.9 – 42.6)	12.6 (5.5 – 25.8)
Tm	23.3 (11.2 – 41.6)	3.3 (1.6 – 5.9)	0.8 (0.3 – 1.8)	14.3 (4 – 35.4)	1.3 (1 – 1.7)	0.2 (0 – 0.5)
nem	83.3 (65.3 – 93.2)	29.6 (16.8 – 58.4)	24.7 (13.7 – 50.8)	71.4 (49.4 – 86.8)	18.3 (8.8 – 34.4)	13.1 (5.9 – 25.5)

Aa – *Aonchotheca annulosa*, Hp – *Heligmosomoides polygyrus*, Sf – *Syphacia frederici*, Ss – *S. stroma*, Tm – *Trichuris muris*, nem – nematodes

or any other analysed factor. The results of these analyses aren't shown but are available from the authors upon request.

Sex-biased parasitism in *A. flavicollis* has not been sufficiently addressed in the past and to the best of our knowledge no study that deals with it specifically exists for this rodent species. When such studies were performed on its close relative, the wood mouse (*A. sylvaticus*), the general conclusion was an absence of SBP (Goüy de Bellocq *et al.*, 2003; Fuentes *et al.*, 2004, 2007, 2010; Milazzo *et al.*, 2010). Abu-Madi *et al.* (2000) state that intrinsic factors such as host sex, as well as host age, have a comparatively weak influence on the helminth fauna of *A. sylvaticus*. In light of previous data for the wood mouse, it may be possible that sex-biased parasitism as a whole does not occur, or is not common, in European *Apodemus* mice. However, an important conclusion reached by Ferrari *et al.* (2007) is that the sexes may differ in their relative contribution to parasite release and dispersal even in the absence of a bias in parasite levels, males and females having different roles in parasite dynamics. Males, due to being less immunocompetent and having wider ranges, generate a higher number of successful infective stages of parasites than females.

The present study found no evidence for sex-biased parasitism in the yellow-necked mouse at the Obedska bara locality, but it must be emphasized that this is a complicated and still incompletely understood phenomenon driven by a large assemblage of factors. More localities and more host/parasite combinations need to be studied, with the host sample structured with respect to age, habitat, trapping season and other relevant factors aside from sex. This preliminary study could possibly mark the beginning of such efforts, and lead to a better understanding of the mechanisms behind sex-biased parasitism not only in Serbia but also in general.

Conflict of Interest

Authors state no conflict of interest.

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