Susceptibility of *Nectomys rattus* (Pelzen, 1883) to Experimental Infection with *Schistosoma mansoni* (Sambon, 1907): a Potential Reservoir in Brazil

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The aim of the present research was to evaluate the potential of Nectomys rattus, *the "water rat", to develop* Schistosoma mansoni *infection. Comparison with* N. squamipes *was carried out. Both species of rodents were submitted to transcutaneous infection using different infective cercariae loads: 50, 100 or 500.* N. rattus *showed high susceptibility to* S. mansoni, *with an infection rate of 71%. Rodents were able to excrete viable eggs of* S. mansoni *in the feaces during all infection period. For both species, the small intestine, followed by the liver and the large intestine, presented the highest concentration of eggs among the surveyed organs. Infection caused no animal death. Moreover,* N. rattus *accomplished the parasite's life cycle, by infecting the snails* Biomphalaria glabrata *and later* Mus musculus. *These evidences indicate that both* N. rattus, *as for* N. squamipes *are potential reservoirs for schistosomiasis in Brazil. Considering the fact that* N. rattus *and* N. squamipes *exist in the same natural ecosystems of* S. mansoni, *we suggest that these rodents must be regarded as influential factors in epidemiology surveys.*

Key words: *Nectomys rattus* - *Nectomys squamipes* - wild rodent - *Schistosoma mansoni* - experimental infection

The occurrence of wild populations of small rodent naturally infected in endemic schistosomiasis areas had been considered as an additional complication factor for the control of the disease (Rey 1993).

The species *Nectomys squamipes*, the "water rat", is one of the most important non-human hosts for *Schistosoma mansoni* in Brazil (Antunes et al. 1973, Picot 1992). This species excretes viable eggs of the parasite during its hole life-span (Rodrigues-Silva 1988) and shows strong fitness to parasitism (Machado-Silva et al. 1994). Furthermore, *N. squamipes* has a high susceptibility to experimental infection (Souza et al. 1992), reinfection (Maldonado Jr. et al. 1994), and with a moderate pathogenic response (Silva & Andrade 1989).

Recent taxonomic revision of *Nectomys* genus (Bonvicino 1994) resulted in a new designation for last named *N. squamipes* found in the Paraná-Paraguai basin, Amazônia basin and small basins at the eastern Brazilian coast, from north of the municipality of São Lourenço da Mata (State of Pernambuco) to the Amazon river. That new given designation is *N. rattus* (2n=52).

N. squamipes (2n=56) distribution has been restricted to the Atlantic coast of Brazil, in the basins of São Francisco river and Paraná river, as well as in the small independent basins of southern São Lourenço da Mata, in eastern Brazil.

The broad distribution of *N. rattus* includes endemic areas of schistosomiasis apart from those where *N. squamipes* are found. However, there have been only two reports where *N. rattus* (formerly named *N. squamipes amazonicus*) were found naturally infected (Bastos et al. 1982, 1984).

The aim of this research was to evaluate the potential of *N. rattus* to develop *S. mansoni* infection, and its ability to complete the parasite's life cycle under experimental conditions. All results were compared to the *N. squamipes* relation with *S. mansoni* infection.

MATERIALS AND METHODS

Experimental groups - Thirty seven specimens of *N. rattus* and 58 specimens of *N. squamipes* of both sexes, aged 3 to 5 months and weighing 250 to 300 g were used. All experimental animals were raised in our laboratory colonies (D'Andrea et al. 1996). The colony of the *N. rattus* derived from animals captured in the State of Goiás

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(Central Brazil) and the *N. squamipes* derived from animals captured in the State Rio de Janeiro (south-eastern Brazil). *Mus musculus* Swiss Webster were used to test *S. mansoni* cercariae infective after *N. rattus* and *Biomphalaria glabrata* passage.

The rodents were allocated in six experimental subgroups according to cercariae loads used (50, 100 or 500): R-50 (n=12), R-100 (n=10) and R-500 (n=9) for *N. rattus* and S-50 (n=24), S-100 (n=11) and S-500 (n=17) for *N. squamipes*.

Experimental infection - The animals were exposed to transcutaneous infection through their tails during thirty minutes. *S. mansoni* cercariae used in this study belonged to the Belo Horizonte (BH) strain maintained in *B. glabrata* snails and Swiss Webster mice (Paraense & Corrêa 1989).

After 16 weeks of infection, the rodents were sacrificed and submitted to portal-hepatic perfusion by Pellegrino and Siqueira (1956) technique, followed by a collection of worms from mesenteric veins with a pointed tool.

Coprologic examinations - Examinations were carried out daily by the Kato-Katz technique (Katz et al. 1972), from the 35th to the 48th day, in order to define the pre-patent period. In the next step, examination was carried out twice a week until the 16th week of infection (two slides per sample). The total amount of eggs excreted in the feaces was compared among the subgroups. Five especimens of *B. glabrata* were exposed to all miracidia hatched from 1.5 g of feaces per rodent from the subgroups R-500 and S-500, totaling 30 snails per subgroup. Cercariae released were tested for its infecting capacity for *M. musculus* (50 larvae per animal).

C*ounting of eggs lodged in the different organs* - The lungs, spleen, liver, small and large intestines were weighted prior to digestion in a 4% aqueous KOH solution (Cheever 1968) and the number of eggs were estimated per gram of tissue.

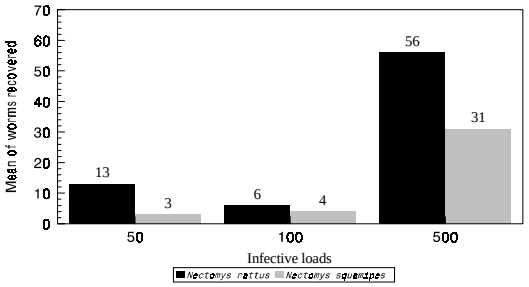
Quantitative oogram - The small intestine were removed from the pyloric sphincter to the ileo-cecal valve and divided into three equal sections, where samples of 1 cm length were taken for an oogram performance (Pellegrino & Faria 1965)

Statistical analysis - Data obtained were analyzed by the Kruskal-Wallis and/or Mann-Withney tests. Differences in sex-ratio of recovered worms were tested using chi-square test. The relationship between the total number of eggs obtained from the oogram performance and the number of recovered couple worms was tested by the Spearman's correlation coefficient. Values of $p<0.05$ were considered statistically significant (Siegel 1975).

RESULTS

Cercariae penetration sucess was greater than 90% in all experimental subgroups. Regardless of the cercariae loads used, infection rates were 71% and 80% for *N. rattus* and *N. squamipes*, respectively. Independent analysis by subgroups revealed higher susceptibility for R-500 and S-500 with infection rates of 100% and 94%, respectively. All subgroups always presented more than 70% except for R-100, which presented an infection rate of 50%, and what is significantly different from other subgroups ($p<0.05$).

Regarding the amount of worms recovered, subgroup R-50 showed a significant greater number than subgroup S-50 ($p<0.01$), (Figure). There was no difference in the number of worms recovered between the subgroups exposured to 50 and 100 cercariae loads for a single species. However, it was observed a significant difference among the subgroups exposed to loads of 50 and 100 cercariae, and the subgroup exposed to 500 cercariae when both species were compared. In all of the subgroups the sex-ratio was biased to male: 2.5:1 (R-50); 5.0:1 (R-100); 4.6:1 (R-500); 3.4:1 (S-50); 3.3:1 (S-100) and 2.6:1 (S-500) ($0.001<p<0.01$ for all subgroups).



Total of worms recovered in *Nectomys rattus* and *N. squamipes* experimentally infected with different infective loads of *Schistosoma mansoni*.

The pre-patent period varied from 40 to 42 days to both *Nectomys* species in all experimental subgroups.

The amount of *S. mansoni*'s eggs excreted in the feaces of *Nectomys* varied greatly among individuals of the same experimental subgroup, and for the same individual throughout the infection period. Values varied from 0 to 264 eggs per gram of feaces, characterizing an assyncronic egg excretion pattern for either *Nectomys* species. Despite this variation, viable eggs were observed during the hole period of observation in all subgroups.

B. glabrata snails were successfully infected by miracidia from eggs from feaces of both sub-

groups (R-500 and S-500). These snails released cercariae which were infective to *M. musculus*. Adult worms of both sexes were recovered from those animals at nine week after infection.

The amount of eggs found in different organs after KOH digestion and in the quantitative oogram revealed a greater number for *N. rattus* than for *N. squamipes* (Table I). However a significant difference was observed only at the 50 cercariae subgroup ($p < 0.05$).

TABLE I

Comparison among the total amount of eggs of *Schistosoma mansoni* placed on tissues by digestion by KOH and oogram methods of *Nectomys rattus* and *N. squamipes* (Mean values ± Standard Desviation)

Subgroup	Eggs found in tissues Digestion Quantitative oogram	
R-50	475 ± 182.3	14977 ± 4079.3
S-50	261 ± 109.1	5664 ± 1302.0
R-100	370 ± 161.9	10349 ± 3056.2
S-100	257 ± 123.7	8801 ± 3753.3
R-500	1386 ± 365.7	83684 ± 3665.8
S-500	878 ± 257.3	74467 ± 13013

R-50: *N. rattus* exposed to 50 cercariae; R-100: *N. rattus* exposed to 100 cercariae; R-500: *N. rattus* exposed to 500 cercariae; S-50: *N. squamipes* exposed to 50 cercariae; S-100: *N. squamipes* exposed to 100 cercariae; S-500: *N. squamipes* exposed to 500 cercariae.

Through tissue digestion it was possible to determine that the greater number of eggs were placed in the small intestine, followed by the liver and the large intestine. Few eggs were found in the lungs and spleen (Table II). These results were common to both species.

Egg's distribution along the segments of the small intestine had similar amounts for *N. rattus* and *N. squamipes*. For both species, 90% of eggs were found in the small intestine, and a small number of eggs in the large intestine (Table III). There was no significant difference in eggs distribution among intestines in both species.

There was a positive correlation between the number of eggs found in the intestine and the number of couple worms recovered ($p<0.02$) in the different subgroups of both species.

DISCUSSION

Although *S. mansoni* shows a low specificity in relation to its vertebrate host choice (Combes 1990), only few species, including rodents, are capable of developing the infection and allowing the parasite to complete its biological cycle in natural environment (Rey 1993).

The rodent *N. rattus* is the second most widely distributed *Nectomys* species over the Brazilian schistosomiasis endemic area. Despite of that, there is no data concerning its potential to act as a reservoir.

TABLE II

Mean number of eggs of *Schistosoma mansoni* found in one gram of tissue in the analyzed organs of *Nectomys rattus* (R) and *N. squamipes* (S), after KOH digestion. The results presented the variance values (SD)

Subgroups	Lungs	Spleen	Large intestine	Liver	Small intestine
R-50	0	0	4.1 ± 2.7	20.3 ± 8.7	75.6 ± 12.4
S-50	0	5.9 ± 2.9	2.2 ± 1.5	35.9 ± 9.9	56.0 ± 10.1
R-100	0	0	9.7 ± 4.5	26.2 ± 7.8	63.1 ± 13.2
S-100	1.0 ± 0.2	0	7.3 ± 3.5	19.8 ± 5.5	71.9 ± 10.9
R-500	0	0	15.8 ± 6.8	16.2 ± 7.3	68.0 ± 20.1
S-500	0	3.0 ± 1.8	10.0 ± 3.9	23.7 ± 9.8	59.2 ± 17.4

TABLE III

Quantification of the number of eggs (oogram) in percentage and variance values (SD) on the proximal, medial and distal sections of small intestine, as well as large intestine of *Nectomys rattus* (R) and *N. squamipes* (S) infected with 50, 100 or 500 cercariae of *Schistosoma mansoni*

	Small intestine (Proximal)	Small intestine (Medial)	Small intestine (Distal)	Large intestine
R-50	38.9 ± 13.7	29.4 ± 14.4	25.7 ± 9.8	6.0 ± 2.3
S-50	27.9 ± 10.8	31.9 ± 12.4	38.0 ± 17.0	2.2 ± 0.7
R-100	29.6 ± 11.2	38.0 ± 19.9	27.1 ± 10.2	5.3 ± 2.1
S-100	24.6 ± 9.3	37.0 ± 19.5	36.7 ± 15.8	1.7 ± 0.9
R-500	38.0 ± 17.9	36.3 ± 15.8	20.6 ± 8.8	5.1 ± 2.0
S-500	28.7 ± 9.8	34.5 ± 13.2	30.6 ± 11.7	6.2 ± 2.3

N. rattus revealed high experimental infection rates, presenting similar levels of susceptibility compared to *N. squamipes* (Maldonado et al. 1993). Also, both species presented similar results for the pre-patent period and the number of recovered worms in equivalent experimental groups.

Despite a larger number of recovered worms in the *N. rattus* subgroup (R-50), both species presented similar responses to infection by *S. mansoni*, when exposed to the same number of cercariae.

The sex-ratio biased to male found in this study is in agreement with the results of natural infection in *N. squamipes* observed by Rodrigues-Silva et al. (1992). On the other hand, Souza et al. (1992) verified that in a long-term infection period (between 12th and 16th post infection) there is a decrease toward females worms. However, these results differed from those found by Maldonado Jr. et al. (1994) for *N. squamipes* under experimental conditions, where the sex ratio was well-balanced. Moné (1997) pointed out that sex-ratio among adult worms from vertebrate hosts are usually male-biased. However, the working mechanisms for the existing gap between male and female still requires a better explanation.

The great variation in the number of excreted eggs observed in this study was similar to results found in naturally infected *N. squamipes* (Carvalho 1982), *Holochilus brasiliensis leucogaster* (Dias et al. 1978) and in wild African rodents (Sène et al. 1996).

In both, tissue digestion and quantitative oogram, coincident results were found in regard to the predominance of eggs in the small intestine. However, these results differed from those demonstrated by Rodrigues-Silva (1988) for *N. squamipes*, where no significant difference among segments of the intestines were found. Yet, the studies by Imbert-Establet et al. (1997), analyzing two African wild rodents infected with *S. intercalatum*, verified that eggs concentrate mainly in the small intestine of *Mastomys huberti*. As for *Arvicanthis niloticus*, eggs distribution changes with the development of the infection, when eggs start to concentrate in the large intestine.

The positive correlation found between the number of eggs in the intestines and the number of couple worms, in both species, revealed that there was not fecundity down regulation dependent of the parasite load in *S. mansoni-Nectomys* model. Also, Coyne and Smith (1991) observed similar results for *S. matthei*.

Based on the results of this study and considering the fact that *N. squamipes* and *N. rattus* have semi-aquatic habits (Ernest & Mares 1986), and broad geographic distribution coincident with *S. mansoni* endemic areas, we suggest that these rodents must be regarded as influential factors in the maintenance of the schistosomiasis transmission, and therefore they should be taken into account in epidemiology surveys.

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