Rev. Inst. Med. trop. São Paulo
38 (2): 141-145, março/abril, 1996

SCHISTOSOMA MANSONI: THE SEX RATIOS OF WORMS IN ANIMALS INFECTED WITH CERCARIAE FROM THREE SPECIES OF *BIOMPHALARIA*.

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SUMMARY

The ratios of male to female worms of *Schistosoma mansoni* were determined in mice infected with cercariae from LE, SJ and AL strains shed by mollusc hosts of the parasite in Brazil.

The sex ratios of worms in the animals were similar with cercariae from *Biomphalaria glabrata* and *B. tenagophila* varying from 1.1:1 to 1.6:1 with LE and AL strains and 1:1.1 with SJ. In the animals infected with cercariae from *B. straminea* the ratio of male to female worms was similar to those obtained using cercariae shed from the other two species of molluscs, 1.5:1 with LE strain. Inoculations by AL and SJ cercariae resulted in sex ratios of 3.1:1 and 6:1 respectively. The normal sex ratios of worms established in Brazil in animals inoculated with cercariae from *B. glabrata* and *B. tenagophila* is from 1:1 to 2:1.

The higher number of male worms that developed from cercariae of the AL and SJ strains obtained from *B. straminea* indicate a lower compatibility of the snail concerning these strains of *S. mansoni*.

KEYWORDS: *Schistosoma mansoni*; *Biomphalaria*; Cercariae; Worms; Ratio male female.

INTRODUCTION

The goal of researches to determine the rate of natural infection in non-human hosts of *Schistosoma mansoni* in different regions of Brazil has been effective; however, the elucidation of the relationship between male and female worms in these populations has not been determined[1,14,16]. One of the factors related to the epidemiological importance of non-human vertebrate hosts is the number of eggs from *S. mansoni* found in faeces of these animals, and to the number of female worms derived from them. The major factors controlling development of female cercariae in *B. glabrata* are related to infectious ability of female miracidium[2,5] and physical factors like temperature[19].

The ratios of male to female worms of *S. mansoni* using uni-sexual and bi-sexual infection of animals in Brazil and Venezuela were 1:1 and 2:1[6,8,15].

According to LIBERATOS[5] the ratio of male to female worms in *Schistosoma* can vary between 1:5 to 5:0, and this variation is related to immunological processes of the vertebrate host, where can occur a selective destruction of eggs with female larvae.

In studies on *Rattus norvegicus* with natural infection with *Schistosoma japonicum* in the Philippines, MITCHELL et al.[7] found that the distribution of male and female worms was 1:7. Infecting of mice with different strains of *S. mansoni*, the same authors found a sex ratio between 1:6 to 5:7, demonstrating a predominance of females[7].

In the present study, parallel infections were carried out in groups of mice with three strains of *S. mansoni* cercariae derived from three different species

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of mollusc hosts, *Biomphalaria tenagophila, B. straminea* and *B. glabrata*. The objective of these studies was to determine the number of male and female worms recovered from animals infected with cercariae derived from each of the intermediate mollusc hosts in Brazil.

MATERIAL AND METHODS

Molluscs collected in Minas Gerais: *B. glabrata* from Belo Horizonte, *B. straminea* from Paracatu and *B. tenagophila* from Pampulha, Belo Horizonte, were raised in the laboratory for individual infection with larvae from the LE, SJ and AL strains of *S. mansoni*. The origin of the strains of *S. mansoni* are: LE from Belo Horizonte, SJ from São José dos Campos, SP and AL from the state of Alagoas. All the strains were maintained in the laboratory for more than 10 years. Each mollusc were exposed individually to 50 miracidia. The exposed molluscs, 50 of each species were maintained in identical conditions. In three experiments 150 molluscs of each species, were used for infection with the three strains of *S. mansoni*. The experiments with the three species of molluscs were carried out in the parallel. The snails were examined 30 days after infection. The positive specimens were separated and the negative specimens were submitted to weekly examination for 10 weeks. The cercariae eliminated into the aquaria containing infected snails were counted in 2 ml volumes which were collected three times per week before changing the water. 40 days old Swiss albino male mice bred in the facilities at the Centro de Pesquisas "René Rachou" were used . Three mice were inoculated with 30 to 100 cercariae of one mollusc of each species. After 50 to 55 days, the mice were perfused using the technique of PELLEGRINO & SIQUEIRA[12] with modifications[13]. Mice inoculated with cercariae from each mollusc were perfused separately and the number of male and female worms was determined. The percentage of worms recovered in mice inoculated with cercariae from each species of snail was calculated using the total number of larvae inoculated and the number of worms recovered.

Statistical analysis – The numerical differences obtained from the various experiments were evaluated using the chi-square test. Quantitative variables were submitted to analysis of variance and the means compared by Student's t-test. The variable mean number of cercariae (x) was submitted to logarithmic transformation log (x + 1) so that standard deviations were proportional to the means (SNEDECOR & COCHRAN, 1968)[17]. In all of the calculations, a level of significance of 5% ($p < / 0.05$) was used.

RESULTS

The lowest level of infection of the three species of molluscs were obtained with *B. straminea*, 8.0% (LE), 10.0% (SJ) and 11.3% (AL). The snail *B. tenagophila* presented intermediate level of susceptibility, 24.0% (LE), 38.6% (SJ) and 32.6% (AL). The level of infection of *B. tenagophila* with the strain LE was significantly lower than with SJ ($p < 0.05$).

The means and standard deviations of daily number of cercariae shed per snail infected with each strain of *S. mansoni* were for *B. straminea*, infected with LE were (119±99 SD), with SJ (91±82 SD) with AL (79±90 SD); for *B. tenagophila* infected with LE were (48±29 SD), with SJ (75±73 SD) and with AL (79±90 SD); for *B. glabrata* infected with LE were (504±323 SD), with SJ (707±343 SD) and (432±435 SD) with AL. The means of the daily numbers of cercariae shed per snail were similar for *B. straminea* and *B. tenagophila*, and significantly higher for *B. glabrata* (Student's t-test).

The results obtained with the perfusion of the mice inoculated with cercariae from the LE, SJ and AL strains of *S. mansoni* derived from each species of molluscs are shown in Tables 1, 2, and 3.

The percentage of male and female worms recovered from the studied host-parasite combinations: in the inoculations using cercariae derived from *B. tenagophila* were 58.3% of males and 41.7% of females, with strain LE, (1.4:1); 48.8% of males and 51.2% of females, with strain SJ, (1:1.1); 52,8% of male and 47.2% of females with strain AL (1.1:1). These differences were statistically significant ($p > 0.05$) (Table 1). With the strain SJ there was little predominance of females. The inoculations with cercariae derived from *B. straminea* gave rise to 60.9% of male worms and 39.1% of females with strain LE, (1.5:1); 85.8% of male and 14.2% of female, with strain SJ, (6:1); 75,8% of male and 24.2 % of female with strain AL, (3.1:1) (Table 2). Inoculations with cercariae derived from *B. glabrata* gave rise to 56.6% of male and 43.3% of female with the strain LE, (1.3:1); 56.2% of males and 43.8% of females with strain SJ, (1.3:1); and 62.4% of males and 37.6% of females with strain AL, (1.6:1) (Table 3).

The percentage of worms recovered from mice infected with cercariae derived from *B. tenagophila* and *B. glabrata* infected with LE, SJ and AL strains (Tables 1 and 3) were similar. The differences were statistically significant in mice inoculated with cercariae from strain SJ, derived from *B. straminea* (Table 2).

TABLE 1

Number and percentage of worms recovered from mice inoculated with cercariae derived from *Biomphalaria tenagophila* infected with different strains of *Schistosoma mansoni*.

Strain of *S. mansoni*	Number of molluscs	Mean of cercariae inoculated	Number of mice	Worms recovered			
				Male	Female	Total	Percentage
LE	31	38.0	79	528*	377*	905	30.1
SJ	36	39.0	92	519*	545*	1,064	29.6
AL	35	59.0	93	900*	802*	1,702	31.0

* Differences statistically significant ($p < 0.05$), $p = 5.99$
$x^2 = 13.0$, the number of male worms was greater than female, with LE.
$x^2 = 6.98$, the number of female worms was greater than male, with SJ.
$x^2 = 54.52$, the number of male worms was greater than female, with AL.

TABLE 2

Number and percentage of worms recovered from mice inoculated with cercariae derived from *Biomphalaria straminea* infected with different strains of *Schistosoma mansoni*.

Strain of *S. mansoni*	Number of molluscs	Mean of cercariae inoculated	Number of mice	Worms recovered			
				Male	Female	Total	Percentage
LE	11	43.0	29	245*	157*	402	32.2 **
SJ	9	31.0	23	145*	24*	169	23.7 **
AL	15	59.0	41	557*	178*	735	30.4 **

* Differences statistically significant ($p < 0.05$), $p = 5.99$
$x^2 = 46.05$, the number of male worms was greater than female, with LE.
$x^2 = 12.52$, the number of male worms was greater than female, with SJ.
$x^2 = 67.72$, the number of male worms was greater than female, with AL.
** $x^2 = 39.7$, the percentage of worms recovered was less with strain SJ.

TABLE 3

Number and percentage of worms recovered from mice inoculated with cercariae derived from *Biomphalaria glabrata* infected with different strains of *Schistosoma mansoni*.

Strain of *S. mansoni*	Number of molluscs	Mean of cercariae inoculated	Number of mice	Worms recovered			
				Male	Female	Total	Percentage
LE	38	98.0	96	1,613*	1,234*	2,847	30.2
SJ	33	100.0	71	1,135*	883*	2,018	28.4
AL	38	100.0	92	2,284*	1,375*	3,659	39.7

* Differences statistically significant ($p < 0.05$), $p = 5.99$
$x^2 = 82.81$, the number of male worms was greater than female, with LE.
$x^2 = 19.37$, the number of male worms was greater than female, with SJ.
$x^2 = 176.43$, the number of male worms was greater than female, with AL.

DISCUSSION

Studies by MAGALHÃES & CARVALHO[6] using bisexual infection of laboratory animals with cercariae derived from *B. tenagophila* and similarly from *B. glabrata*[8] detected a ratio of male to female worms of around 2:1.

By using *B. straminea*, FREITAS[3] found a ratio of male to female worms of 2.2:1 in mice submerged in water with *B. straminea* from the area of Lagoa Santa, MG and of 1.5:1 for those submerged in water that contained *B. glabrata* from the same region.

According to EVANS & STIREWALT[2] and earlier, LIBERATOS[5], a major reason for the high proportion of male worms could be the low infectious ability of female miracidium for the mollusc hosts.

In Guadeloup IMBERT-ESTABLET[4] found the average number of *S. mansoni* worms of 119±73 in *Rattus rattus* and of 21±14 woms in *Rattus norvegicus* with natural infection. The ratio of male to female worms from these wild rats was 1:08 and the differences in the percentages were not statistically significant at 51.89% males and 48.11% females. In the Philippines, MITCHELL et al.[7] found the ratio of 1:6 to 5:7 for male to female worms in mice infected with different strains of *S. mansoni*.

There did not exist information concerning the distribution of male and female worms in non-human hosts with natural *S. mansoni* infection in Brazil. However, there are informations concerning the natural infection rate of animals and the egg number found in the faeces[1,14,16].

About the distribution of male and female worms in non human host infected with *S. mansoni* in the northeast region of Brazil, where *B. straminea* is an important host of schistosomiasis there did not exist information in the literature.

How the number of cercariae shed per day by the snails *B. straminea* and *B. tenagophila* were low the means of larvae used to infect the mice were less than those of *B. glabrata* used to infect the animals.

In the present experiment inoculation of mice with LE, SJ, and AL cercariae derived from *B. tenagophila* demonstrated a predominance of male worms with strains LE and AL and of females using strain SJ. The results suggest that in strains of *S. mansoni* more adapted to distinct species of molluscs[9,10,11] the destruction of female larvae is small. The inoculations of a small number of mice, due to the low number of infected molluscs, with cercariae derived from *B. straminea* from Minas Gerais, led to a predominance of male worms, similar to that seen using *B. glabrata* and *B. tenagophila* with strain LE from Belo Horizonte, with a ratio of 1.5:1. With the strains AL and SJ (from state of Alagoas and from São Paulo, respectively) the number of male worms increased 3 to 6 times more than the female. The higher number of male worms that developed from cercariae of the AL and SJ strains derived from *B. straminea* probably is related with a lower compatibility of the snail concerning these strains of *S. mansoni*[18].

These data demonstrate the variability of the results of experimental animal infection using different strains of *S. mansoni* obtained from the three species of mollusc vectors in Brazil. Besides the non-permissive vertebrate hosts, where no *S. mansoni* eggs were found, there exist invertebrate hosts that are of low compatibility where the female larvae develop less efficiently. However, more research is necessary to a better understanding of the distribution of male and female worms of *S. mansoni* in non-human hosts in Brazil and what are the relationships between the species of mollusc vectors in the studied regions.

RESUMO

Schistosoma mansoni: relação de vermes machos e fêmeas em animais inoculados com cercárias de três espécies de _Biomphalaria_.

A relação de vermes machos e fêmeas de *Schistosoma mansoni* foi determinada em camundongos infectados com cercárias das cepas LE, SJ e AL, eliminadas pelos moluscos hospedeiros do parasita no Brasil.

Os números de vermes machos e fêmeas recuperados em inoculações de cercárias provenientes de *Biomphalaria glabrata* e *B. tenagophila*, foram semelhantes, variando de 1,1:1 a 1,6:1 com LE e AL e de 1:1,1 com SJ. Nos animais inoculados com cercárias provenientes de *B. straminea* a relação de vermes macho e fêmea foi semelhante à obtida com cercárias provenientes das outras duas espécies com a cepa LE, relação de 1,5:1. Nas inoculações de cercárias de AL e SJ provenientes de *B. straminea* a relação foi de 3,1:1 e 6:1, respectivamente. A relação normal de vermes macho para fêmea registrada no Brasil, em inoculações de cercárias de *B. glabrata* e *B. tenagophila*, é de 1:1 a 2:1. O grande número de vermes machos provenientes de cercárias das cepas AL e SJ eliminadas por *B. straminea* mostrou a compatibilidade menor do molusco com essas cepas de *S. mansoni*.

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Recebido para publicação em 21/09/1995
Aceito para publicação em 06/02/1996