Degree of Host-parasite Compatibility between *Schistosoma mansoni* and their Intermediate Molluscan Hosts in Brazil

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The compatibility of Biomphalaria tenagophila, B. straminea *and* B. glabrata *from Minas Gerais with different strains of* Schistosoma mansoni *was evaluated using the method of Frandsen (1979b) in standardized experiments. One hundred and fifty of each species of snail were individually exposed in the laboratory to 50 miracidia of* S. mansoni *lines LE, SJ and AL. The cercariae from the infected snails were counted and used to calculate TCP/100 indices, which were compared with those of Frandsen (1979b). For* B. tenagophila *the TCP/100 indices varied from 37,996 to 74,266 (class II and III). The snail was poorly compatible with LE (class II) and compatible with SJ and AL (class III). For* B. straminea *the indices varied from 9,484 to 20,508. The snail was not very compatible with SJ (class I) and poorly compatible with LE and AL (class II). For* B. glabrata *the indices varied from 588,828 to 1,039,065. The snails was extremely compatible (class VI) with the three lines of* S. mansoni. *These results confirm the epidemiological importance of* B. glabrata *in Brazil followed by* B. tenagophila *and* B. straminea.

Key words: degree compatibility - molluscan - hosts - *Schistosoma mansoni* - Brazil

According to Cheng (1968) the concept of host-parasite compatibility, involving snails and trematodes is defined by the physical and physiological state of both the host and parasite that allows the latter to penetrate and develop guaranteeing the perpetuation of the species. Incompatibility is the result of factors that partially or totally inhibit the establishment and normal development of the parasite.

Frandsen (1979a,b) developed a quantitative method to express the degree of compatibility between *Schistosoma* and its intermediate molluscan hosts in standardized experiments using the index TCP/100. The author compared the compatibility of diverse species of *Biomphalaria* from various geographical regions for *S. mansoni* and also snails of the genus *Bulinus* for African lines of the *Schistosoma* species/strains.

In Brazil the geographical distribution of the three species of *Biomphalaria* that are hosts for *S. mansoni* is extremely wide (Paraense 1972, 1983, 1986, Paraense et al. 1983). In the state of Minas Gerais the presence of *B. glabrata* has been determined in 167 municipalities, *B. tenagophila* in 31 and *B. straminea* in 69 (Paraense 1972, 1986, Souza & Lima 1990). There are few studies, however, that compare the cercarial production of each species of snail (Barbosa 1975).

In the present paper, experimental infections were undertaken under standardized conditions to determine the degree of compatibility of each species with different lines of the trematode with the objective of evaluating the epidemiological importance of *B. tenagophila*, *B. straminea* and *B. glabrata* as host for *S. mansoni* in Minas Gerais.

MATERIALS AND METHODS

The following laboratory reared snails were used: (a)*B. glabrata* a line descendant from specimens collected in Barreiro de Cima, Belo Horizonte (MG), maintained in the laboratory for more than 20 years. The snails used had a mean diameter of 8-10 mm; (b)*B. tenagophila* descendants of specimens collected in the lake of Pampulha, Belo Horizonte in 1988, with diameter of 8-10 mm; (c)*B. straminea* descendants of snails captured in Paracatu (MG) in 1989, with diameter of 4-7 mm.

The lines of *S. mansoni* are maintained in this laboratory by passage through *B. glabrata* and mice or hamsters. LE was isolated from a patient resident in Belo Horizonte and has been maintained in the laboratory for more than 20 years (Pellegrino & Katz 1968); SJ derived from snails

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from São José dos Campos (SP), provided by the Interdepartmental Schistosomiasis Study Group of the UFMG in 1975 and adapted to *B. tenagophila* (Paraense & Correa 1963, 1978, 1981); AL, derived from *B. glabrata* from the state of Alagoas, isolated in 1980.

In each experiment, 50 specimens of each species were exposed individually to 50 miracidia per snail in parallel infections under identical conditions. Colorless plastic plates with 24 x 2.5 ml cylindrical wells and fitted with lids (as used for tissue culture) wells were used for exposure. A single snail was placed in each well together with 2.5 ml of a suspension containing 50 miracidia of each line being tested. The time of exposure to the miracidia was 6 hr at a mean temperature of 27±1°C under artificial illumination.

Following exposure, the snails were maintained in separate aquaria in the same room at a temperature of 25±1°C. Thirty days after infection the snails were exposed to light for 40 min and examined using a stereo microscope. The positive specimens were separated and the negative specimens were submitted to weekly examinations for 10 weeks. Following this period the negative snails were squashed between glass plates in order to detect the possible presence of sporocysts. Specimens which died before the prepatent period were also squashed and examined.

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. The date of death of each positive snail was noted.

The compatibility of each snail species with *S. mansoni* was evaluated using the method of Frandsen (1979b). The index TCP/100 (total number of cercariae produced multiplied by 100 and divided by the number of snails exposed) was calculated. The indices obtained for each species with the three lines were compared with the data of Frandsen (1979b) who described six classes of compatibility, based on the number of cercariae shed, in addition to class zero that were resistant.

Statistical analysis - The numerical differences obtained in the experiments were evaluated using the χ^2 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 the standard deviations were proportional to the means (Snedecor & Cochran 1968). In all calculations the level of significance was taken as 5% ($p</0.05$).

RESULTS

The susceptibility of the three species of snails infected with the *S. mansoni* lines LE, SJ and AL were found to be different. The differences between the levels of infection were statistically significant ($p<0.05$) (Tables I, II, III). The lowest levels of infection were obtained with *B. straminea*, 8.0 to 11.3% (Table II). The snail exhibited similar levels of susceptibility to the three *S. mansoni* lines. *B. tenagophila* presented an intermediate level of susceptibility of between 24.0 and 38.6%. The level of infection with the line LE was significantly lower than with SJ ($p<0.05$), between LE and AL, SJ and AL the differences were not significant ($p>0.05$) (Table I). *B. glabrata* was the most susceptible species to the three lines of the trematode and no statistical difference between the levels of infection was obtained (Table III).

The means of the daily numbers of cercariae shed per snail were similar for *B. straminea* and *B. tenagophila* (Tables I, II, Figs 1, 2) and signif-

TABLE I

Percentage infection and mortality of *Biomphalaria tenagophila* infected with three lines of *Schistosoma mansoni* and the means and totals of cercariae shed

	Snails				Cercariae shed	
S. mansoni origin	Number exposed	Number infected	Percentage infected	Percentage mortality[b]	Mean ± SD	Total
LE	150	36	24.0[a]	13.3	48 ± 29	56,955
SJ	150	58	38.6[a]	13.3	75 ± 73	111,400
AL	150	49	32.6	20.6	79 ± 90	78,416
Total	450	143	31.8	15.7	67 ± 18.7	246,771

a: differences statistically significant ($p<0.05$)
X^2 = 7.53, the level of infection with SJ was higher than LE
The differences between LE and AL, SJ and AL were not statistically significant ($p>0.05$)
b : snail death during the pre-patent period
SD: standard deviation

TABLE II

Percentage infection and mortality of *Biomphalaria straminea* infected with three lines of *Schistosoma mansoni* and the means and totals of cercariae shed

	Snails				Cercariae shed	
S. mansoni line	Number exposed	Number infected	Percentage infected	Percentage mortality[a]	Mean ± SD	Total
LE	150	12	8.0	16.6	119 ± 99	27,136
SJ	150	15	10.0	12.6	91 ± 82	14,227
AL	150	17	11.3	9.3	112 ± 100	30,763
Total	450	44	9.7	12.8	107 ± 18	72,126

a: snail death during the pre-patent period
SD: standard deviation

TABLE III

Percentage infection and mortality of *Biomphalaria globrata* infected with three lines of *Schistosoma mansoni* and the means and totals of cercariae shed

	Snails				Cercariae shed	
S. mansoni line	Number xposed	Number infected	Percentage infected	Percentage mortality[b]	Mean ± SD	Total
LE	150	38	75.3	22.0	504 ± 323	505,736
SJ	150	39	73.3	26.0	707 ± 343	552,594
AL	150	40	75.3	9.3	432 ± 435	309,910
Total	450	117[a]	74.6	19.3	548 ± 141	1,368,240

a: for the control was used only 117 snails between 336 infected
b: snail death during the pre-patent period
SD: standard deviation

TABLE IV

Compatibility of *Biomphalaria tenagophila, B. straminea* and *B. glabrata* from state of Minas Gerais to different *Schistosoma mansoni* lines

Snail		*S. mansoni*	TCP/100	Compatibility	
Species	Origin	Line	Index	Class	Description
B. tenagophila	Pampulha	LE	37,996	II	Poorly compatible
	Pampulha	SJ	74,226	III	Compatible
	Pampulha	AL	52,277	III	Compatible
B. straminea	Paracatu	LE	18,090	II	Poorly compatible
	Paracatu	SJ	9,484	I	Not very compatible
	Paracatu	AL	20,508	II	Poorly compatible
B. glabrata	Laboratory	LE	1,002,599	VI	Extremely compatible
	Laboratory	SJ	1,039,065	VI	Extremely compatible
	Laboratory	AL	588,828	VI	Extremely compatible

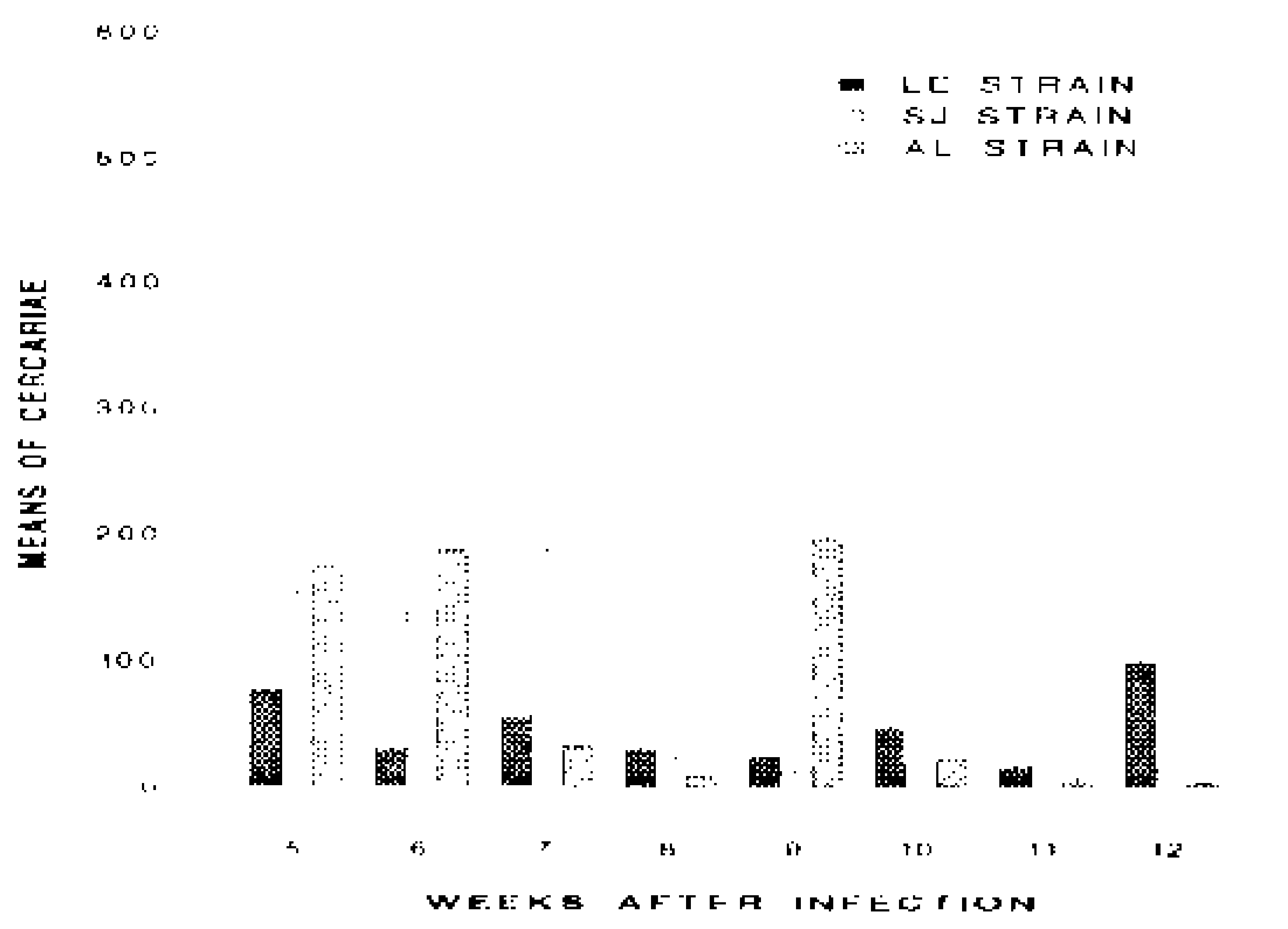

Fig. 1: means of cercariae produced per day by *Biomphalaria tenagophila* infected with the LE, SJ and AL strains of *Schistosoma mansoni*.

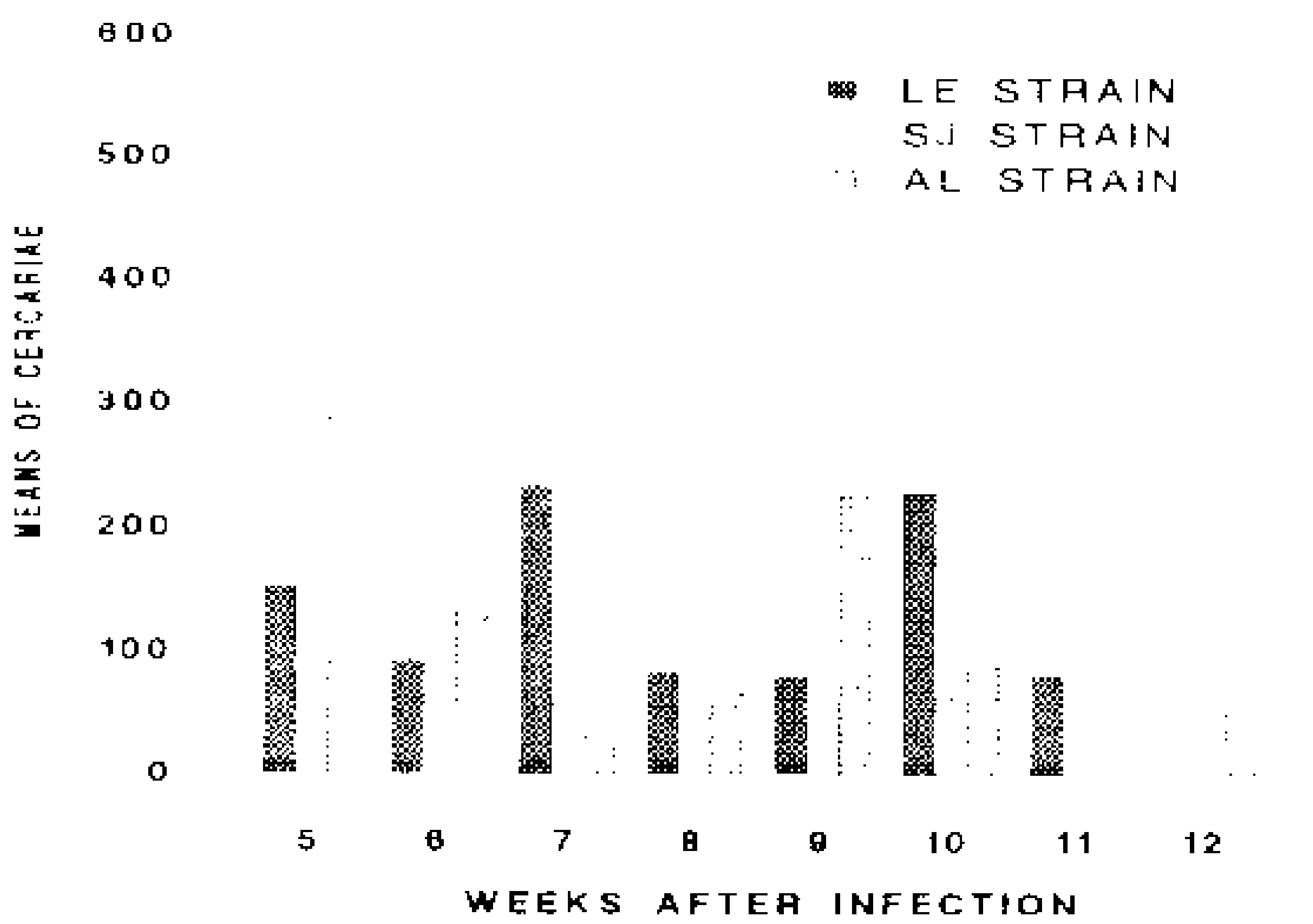

Fig. 2: means of cercariae produced per day by *Biomphalaria straminea* infected with the LE, SJ, and AL strains of *Schistosoma mansoni*.

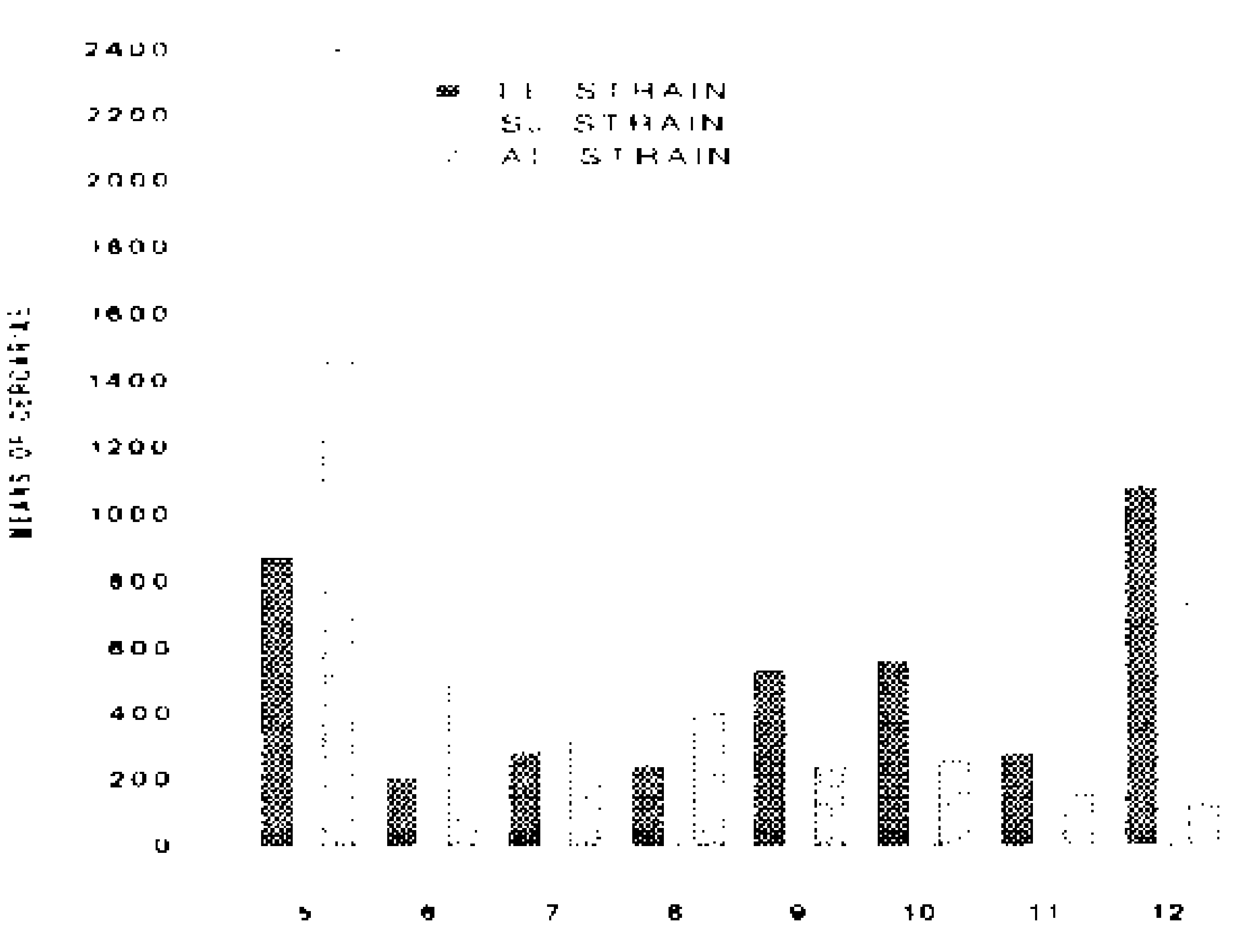

Fig. 3 : means of cercariae produced per day by *Biomphalaria glabrata* infected with the LE, SJ, and AL strains of *Schistosoma mansoni*.

icantly higher for *B. glabrata* (Table III, Fig. 3) (Student's t-test). The total lifetime number of cercariae shed by the infected snails (Tables I, II, III) were used to calculate the TCP/100 indices (Frandsen 1979b) which are shown in Table IV. Since for *B. glabrata* only a fraction of the snails were used as a control, a correction for the number of cercariae eliminated was made by extrapolation. We took the mean number of cercariae shed per snail per day and multiplied this by the total number of specimens infected with LE, SJ or AL in order to obtain the total number of cercariae. The index calculated for the LE line was 1,503,899 x 100/150=1,002,599 - Class VI.

The pre-patent period of *B. tenagophila* infected with *S. mansoni* lines was of 30-71 days with LE (mean 41.7±28.6 SD); 30-58 days with SJ (mean 37.0±11.7 SD) and 30-65 days with AL (mean 44.0±15.9 SD); for *B. straminea* the pre-patent period was of 30-58 days with LE (mean 43.8±15.1 SD); 30-71 days with SJ (mean 43.8±16.8 SD) and 30-58 days with AL (mean 37.0±11.7 SD); for *B. glabrata* the pre-patent period was of 30-44 days with LE (mean 34.6±7.2 SD); 30-37 days with SJ (mean 31.1±2.8 SD) and 30-51 days with AL (mean 34.6±8.5 SD).

The medians for mortality of the infected snails were: for *B. tenagophila* 71 days with LE, 43 days with SJ and 57 with AL and the mean 57±7 days. For 44 specimens of *B. straminea* the medians were 57 days (LE), 50 (SJ) and 64 (AL) and the mean 57±14 days. The median of mortality of 117 specimens of *B. glabrata* were 43 days (LE), 50 (SJ) and 50 (AL) and the mean 47.6±5.1 days.

The percentage survival of infected snails for more than 90 days was similar for *B. glabrata* and *B. straminea*, 6.0% and 6.8%, respectively. The highest longevity was registered for specimens with bisexual infection, 154 days for a *B. glabrata* specimen and 134 days for a *B. straminea* specimen both infected with AL. The percentage survival for more than 90 days of *B. tenagophila* was 12.6%. Amongst 18 surviving specimens (44.4%) were infected with male larvae, 5.5% with females and 38.9% with both sexes. It was not possible to determine the sex of the cercariae in two instances due to the premature death of the mice in one case and the absence of worms at perfusion in the other. The greatest longevity, of 134 days, was registered for a snail with a unisexual male infection (LE).

DISCUSSION

In the experiments reported here the total number of cercariae shed by 117 *B. glabrata* specimens was around 1,368,240 which was 5.5 times more than the number shed by 143 *B. tenagophila* specimens (246,771) and 19.0 times more than the number shed by 44 *B. straminea* specimens (72,126) (Tables I, II, III). The mean numbers of cercariae produced per snail during their lifetime were 11,694 for *B. glabrata*, 1,725 for *B. tenagophila* and 1,639 for *B. straminea*.

The study of host-parasite combinations using the index TCP/100 (Frandsen 1979b) showed that *B. glabrata* was extremely compatible with the three lines of trematodes exhibiting indices greater than 500,00 (Table IV). The SJ line was the most adapted to *B. tenagophila*; in our laboratory however this line has been maintained in *B. glabrata* which explains the similar results to those obtained with LE and AL.

Biomphalaria tenagophila from Pampulha was shown to be poorly compatible with the autoctonous LE, with an index less than 50,000 (class II) and compatible with SJ and AL with indices of more than 50,000 (class III). In fact the level of experimental infection of this species with LE has risen in relation to earlier measurements of 0.0 and 35.0% with LE and SJ respectively (Correa et al. 1979) and of 40.0, 30.0 and 6.0% with SJ, AL and LE respectively (Souza et al. 1987). The natural level of infection of this species recorded by Carvalho et al. (1985) in the lake at Pampulha was 0.03%. The 90 days survival of 44.4% of the infected specimens with male *S. mansoni* larvae may explain the earlier finding that two specimens captured in the lake had this type of unisexual infection (Souza et al. 1987).

Biomphalaria straminea from Paracatu was not very compatible with the SJ line with an index of less than 10,000 (class I). The snail was poorly compatible with the LE and AL lines with indices of less than 50,000 (class II) (Table IV). The compatibility of this species with the SJ line (class I) was less than that reported by Paraense and Correa (1989), for a snail similar to *B. straminea* from Espinillar, Uruguay, which showed class III compatibility. *B. straminea* was not captured with a natural *S. mansoni* infection in Paracatu although, nine autoctonous cases of schistosomiasis in children were recorded in the region where the only snail host that is found is of this species (Carvalho et al. 1987).

The results shown confirm the greater epidemiological importance of *B. glabrata* in this country as compared with *B. tenagophila* and *B. straminea*. As the geographical distribution of the latter two species is very wide in this country (Paraense 1972, 1983, 1986, Paraense et al. 1983) and the density of *B. straminea* in the breeding sites is very high, principally in the northeast (Barbosa & Figueiredo 1970, Figueiredo 1989) they are perfectly able to maintain the parasite cycle in regions where they are found. Thus, there is a need for more research in order to quantify the number of cercariae shed by the three species of snail host for *S. mansoni* in different geographical regions of Brazil. The determination of the degree of compatibility allows a better evaluation of the epidemiological importance of each species as transmitters of schistosomiasis in this country.

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REFERENCES

Barbosa FS 1975. Survival and cercaria production of brazilian *Biomphalaria glabrata* and *B. straminea* infected with *Schistosoma mansoni*. *J Parasitol 61*:151-152.

Barbosa FS, Figueiredo T 1970. Susceptibility of the snails intermediate hosts of schistosomiasis from northeastern Brazil to the infection with *Schistosoma mansoni*. *Rev Inst Med Trop São Paulo 12*: 198-206.

Carvalho OS, Guimarães CT, Massara CL, Bonésio JER 1985. Situação atual da esquistossomose mansoni no Lago da Pampulha, Belo Horizonte, MG, Brasil. *Rev Saúde Públ São Paulo 19*: 270-277.

Carvalho OS, Rocha RS, Massara CL, Katz N 1987. Expansão da esquistossomose mansoni em Minas Gerais. *Mem Inst Oswaldo Cruz 82*: 295-298.

Cheng T 1968. The compatibility and incompatibility concept as related to trematodes and molluscs. *Pacific Science 22*: 141-150

Correa MCR, Coelho PMZ, Freitas JR 1979. Susceptibilidade de linhagens de *Biomphalaria tenagophila* e *Biomphalaria glabrata* a duas cepas de *Schistosoma mansoni* (LE - Belo Horizonte, MG e SJ - São José dos Campos, SP). *Rev Inst Med trop São Paulo 21*: 72-76.

Figueiredo CCSB 1989. Dispersão de *Biomphalaria straminea* no Estado de Sergipe: um estudo comparativo com 19 anos de intervalo. *Mem Inst Oswaldo Cruz 84*: 383-387.

Frandsen F 1979a. Studies of the relationships between *Schistosoma* and their intermediate hosts. The genus *Bulinus* and *Schistosoma haematobium* from Egypt. *J Helminthol 53*: 15-29.

Frandsen F 1979b. Discussion of the relatioships between *Schistosoma* and their intermediate hosts, assessment of the degree of host-parasite compatibility and evaluation of schistosome taxonomy. *Z Parasitenk 58*: 275-296.

Paraense WL 1972. Fauna planorbídica do Brasil, p. 213-239. In CS Lacaz, RG Baruzzi, JW Siqueira, *Introdução à Geografia Médica do Brasil*. Edgard Blucher & USP, São Paulo.

Paraense WL 1983. A survey of planorbid molluscs in the Amazonian Region of Brazil. *Mem Inst Oswaldo Cruz 78*: 343-361.

Paraense WL 1986. Distribuição dos moluscos no Brasil p. 117-127. In FA Reis, I Faria , N Katz, *Modernos conhecimentos sobre a Esquistossomose mansônica*. Suplemento dos Anais de 1983/84 da Academia Mineira de Medicina, Belo Horizonte, vol. 14.

Paraense WL, Correa LR 1963. Sobre a ocorrência de duas raças biológicas do *Schistosoma mansoni* no Brasil. XV Reunião Anual da Sociedade Brasileira para o Progresso da Ciência, Resumos, Campinas, SP.

Paraense WL, Correa LR 1978. Differencial susceptibility of *Biomphalaria tenagophila* populations to infection with a strain of *Schistosoma mansoni*. *J Parasitol 64*: 822-826.

Paraense WL, Correa LR 1981. Observations of two biological races of *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz 76:* 287-291.

Paraense WL, Correa LR 1989. A potencial vector of *Schistosoma mansoni* in Uruguay. *Mem Inst Oswaldo Cruz 84:* 281-288.

Paraense WL, Alencar AJT, Correa LR 1983. Distribuição dos planorbídeos e prevalência da xistosomose mansoni no Estado do Espírito Santo. *Mem Inst Oswaldo Cruz 78:* 373-384.

Pellegrino J, Katz N 1968. Experimental chemotherapy of schistosomiasis mansoni p. 233-290. In Ben and Dawes (ed.) *Adv Parasitol* London, Acad Press.

Snedecor GW, Cochran WG 1968. *Statistical Methods.* Iowa state University press Ames, 6th ed.

Souza CP, Lima LC 1990. *Moluscos de interesse parasitológico do Brasil.* Série Esquistossomose nº1, FIOCRUZ-CPqRR, Belo Horizonte, MG, 76pp.

Souza CP, Araujo N, Carvalho OS, Freitas JR 1987. Potencialidade de *Biomphalaria tenagophila* do Lago da Pampulha, Belo Horizonte, MG, como hospedeira do *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz 82:* 67-70.