

BIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF *Schistosoma mansoni* FROM RIBEIRA VALLEY, STATE OF SÃO PAULO, BRAZIL. I — SUSCEPTIBILITY OF *Biomphalaria tenagophila* SNAIL TO SYMPATRIC *S. mansoni* STRAIN (1)

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SUMMARY

In the São Paulo State, Brazil, where the *Biomphalaria tenagophila* is the intermediate host, the Ribeira Valley is an important endemic schistosomiasis mansoni area. During last eleven years there has been intense control measures focusing on schistosomiasis. The efforts have been concentrated in the municipalities of Pedro de Toledo and Itariri. We determined the susceptibility of *B. tenagophila* to sympatric strain of *S. mansoni*, both recently isolated from Itariri field. In 1988, this strain was isolated and maintained in the experimental model: Swiss mice — sympatric *B. tenagophila*. The second generation of the worm was evaluated. The snails were divided in the three groups of 60 snails each. One group was exposed to 1 miracidium and other to 10. The third group was the control. The mortality and the shedding of cercariae were checked during 78 days. After that, the positive snails were observed until they ceased to shed cercariae. The exposed molluscs showed mortality rates of 23% and 31% and infection indexes were of 8% and 60% to 1 and 10 miracidia respectively. The mortality was of 22% in the control group. The periods of shedding cercariae in the two groups were 82 and 104 days. We can conclude that *B. tenagophila* is an effective intermediate host to the sympatric strain of *S. mansoni* sympatric strain.

KEY WORDS: *Schistosoma mansoni*; *Biomphalaria tenagophila*; Experimental susceptibility.

INTRODUCTION

The major intermediate host of *S. mansoni* in the State of São Paulo is *B. tenagophila*. Another species, *B. glabrata*, is the most important intermediate host in endemic areas of schistosomiasis mansoni in Brazil⁶ and, in the São Paulo State it is responsible only for transmission in Paranapanema Valley^{15, 17, 18, 19, 20}. *B. straminea* is found in 27 municipalities of São Paulo but,

as of yet, does not have any significant role in the transmission of *S. mansoni*¹⁶.

During the last eleven years, there has been intense control of schistosomiasis mansoni in the valley of "Ribeira de Iguape" river, where the *B. tenagophila* is the sole intermediate host. The main efforts have been concentrated in the

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municipalities of Pedro de Toledo and Itariri^{4, 5, 8}. However, schistosomiasis transmission still occurs in those municipalities, and 47.9% of the cases are considered autochthonous¹⁴. The risk of acquiring the infection is high because there are active foci, and patterns of intense human contact with water. Furthermore, the biological characteristics of *S. mansoni* strain from this area are unknown. Three papers on this subject are under preparation. The present work deals with the snail susceptibility to *S. mansoni* sympatric strain.

Effective transmission depends upon the degree of compatibility between the snail host and the local strain of the parasite. FILES & CRAM⁷ showed the existence of physiological differences in parasites of different geographical areas. It is necessary to occur a physiological adjustment between the mollusc and the sympatric strain of *S. mansoni*^{10, 12}. MINCHELLA & LOVERDE⁹ indicated that snails insusceptible to *S. mansoni* were affected negatively in the presence of either susceptible snails or schistosome parasites. The insusceptible snails were not predominant in natural populations responsible for transmission of the disease, because they are selectively disadvantaged. The parasite-host relationship has epidemiological importance, as showed in Minas Gerais State, Brazil, with *Biomphalaria* snails¹³. SOUZA¹³ calls the attention to the importance of *B. tenagophila* and *B. straminea* in areas where *B. glabrata* was the species responsible for transmission of schistosomiasis.

In this paper, we will study the susceptibility of *B. tenagophila* to a strain of *S. mansoni*, both from the municipality of Itariri, SP.

MATERIALS AND METHODS

In 1988, the strain was isolated from naturally infected *B. tenagophila* snails, collected in an active focus of *S. mansoni* in the district of Ana Dias, municipality of Itariri, Valley of Ribeira de Iguape river, State of São Paulo, Brazil. Eggs from these snails were used to obtain the first generation in the laboratory. The trematode strain was maintained in the laboratory through Swiss albino mice and sympatric *B. tenagophila* snails. Our experiment was performed with the

second generation in the laboratory of this *S. mansoni* strain.

Experimental design: Three groups of 60 snails each were used in the experiments: Group S1 was exposed to 10 miracidia; group S2 was exposed to 1 miracidium; and group S3, used as control, was not exposed.

Snail infection: The miracidia were obtained from feces of infected mice³. Snails with 5-8 mm in size were exposed individually. They were placed in glass vials (15 x 17 mm) containing 0.2 — 0.4 ml of aerated tap water and exposed to either one or ten miracidia, at a temperature of 25 — 27°C. The snails were exposed for two hours under artificial illumination. After exposure, snails were maintained in groups of 30, in glass containers with 1.5 l of aerated tap water and supplied with lettuce and lab animal ration. The control group was submitted to the same physical conditions as of the exposed groups.

Mollusc examination: daily observations were made for mortality throughout the experimental period of 78 days. Twenty-one days after exposure, the snails were examined weekly to check for cercariae shedding³. After 78 days, those snails shedding cercariae were kept isolated and observed weekly until they ceased to shed cercariae. The molluscs were maintained at room temperature, around 25°C, during the experimental period.

RESULTS

The infected snails with 1 and 10 miracidia showed the same pre-patent period of 55 days even though the infection rates were different (8.3% and 60.0% respectively) (table 1 and figure 1). The cercariae-shedding period in both infected groups was long. The snails infected with 1 miracida shed cercariae during 82 days after the pre-patent period, and those infected with 10 larvae shed for 104 days (figure 1).

The mortality rates of infected and control groups during the 78 days of follow-up were not different (table 1). The χ^2 test for multiple proportions showed not statistically significant ($\chi^2 = 5.99; = 0.05$).

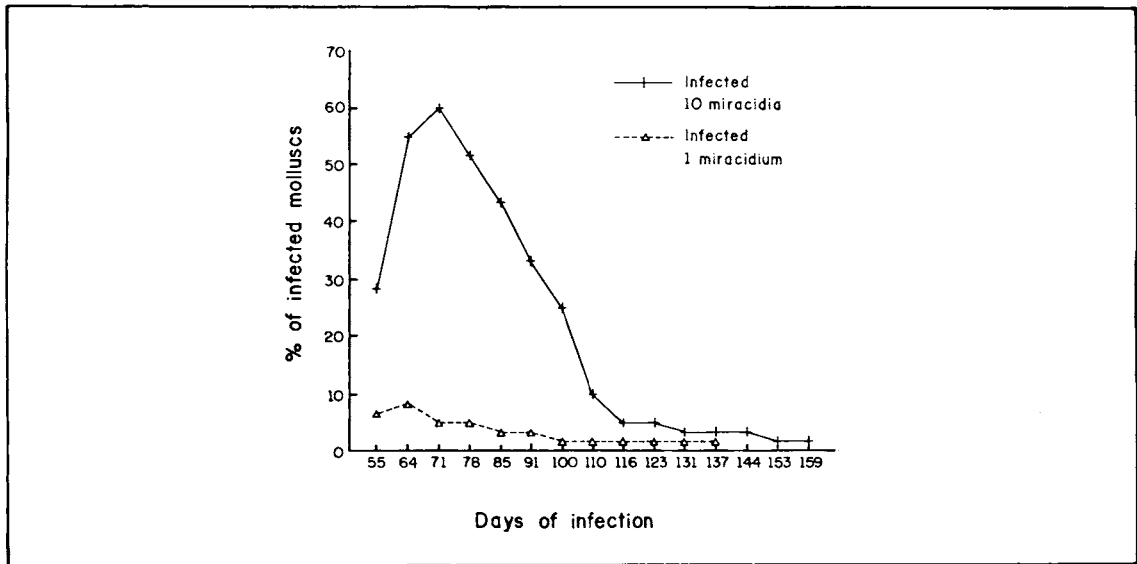


Figure 1 — Kinetics of shedding cercariae of *Biomphalaria tenagophila* infected with 1 and 10 miracidia of progeny 2 of *Schistosoma mansoni* strain from municipality of Itariri, São Paulo State, Brazil in 1988.

TABLE 1
Susceptibility of *Biomphalaria tenagophila* to 10 and 1 miracidium of progeny 2 of *Schistosoma mansoni* strain, isolated from municipality of Itariri, São Paulo State, Brazil, in 1988.

| Days of follow-up after infection | Infected molluscs | | | | | | Control | | | |
|-----------------------------------|-------------------|--------|--------------------|-------------------|--------|--------------------|-------------|--------|----|------|
| | With 10 miracidia | | | With 1 miracidium | | | No infected | | | |
| | Alives | Deaths | Shedding cercariae | Alives | Deaths | Shedding cercariae | Alives | Deaths | | |
| | n | % | n | % | n | % | n | % | | |
| 21 | 60 | 0,0 | 0 | 0,0 | 58 | 3,3 | 0 | 0,0 | 60 | 0,0 |
| 28 | 60 | 0,0 | 0 | 0,0 | 56 | 6,7 | 0 | 0,0 | 59 | 1,7 |
| 35 | 58 | 3,3 | 0 | 0,0 | 55 | 8,3 | 0 | 0,0 | 57 | 5,0 |
| 42 | 58 | 3,3 | 0 | 0,0 | 53 | 11,7 | 0 | 0,0 | 56 | 6,7 |
| 48 | 53 | 11,7 | 0 | 0,0 | 53 | 11,7 | 0 | 0,0 | 56 | 6,7 |
| 55 | 52 | 13,3 | 17 | 28,3 | 51 | 15,0 | 4 | 6,7 | 53 | 11,7 |
| 64 | 47 | 21,7 | 33 | 55,0 | 48 | 20,0 | 5 | 8,3 | 51 | 15,0 |
| 71 | 46 | 23,3 | 36 | 60,0 | 46 | 23,3 | 3 | 5,0 | 49 | 18,3 |
| 78 | 41 | 31,7 | 31 | 51,7 | 46 | 23,3 | 3 | 5,0 | 47 | 21,7 |

DISCUSSION

According to several authors^{1, 2, 3, 13}, susceptibility of *B. glabrata* and *B. tenagophila* to strains of *S. mansoni* shows different infection

rates. In general *B. glabrata* shows infections indexes higher than those observed for *B. tenagophila*. Nevertheless, even though the snail studied here was *B. tenagophila*, the infection rates of the molluscs exposed to 10 miracidia was high,

reaching 60.0%. However, PARAENSE & CORREA¹¹ verified that 91.5% of *B. tenagophila* from Ana Dias, Itariri, shed cercariae when infected by a strain of *S. mansoni* from São José dos Campos in the Paraíba River Valley, an other endemic region of São Paulo State. Earlier, DIAS et al² demonstrated that susceptibility of *B. tenagophila* to human and wild sympatric strains of *S. mansoni* from Pedro de Toledo, a municipality adjacent to Itariri, was low. Exposure to 10 miracidia caused only 4% and 2% of the snails to shed cercariae from their respective strains.

Our findings of 8.3% of infected snails that were exposed to one miracidium was higher than those observed by PARAENSE & CORREA¹², who found 3.98% of snails shedding cercariae and having sporocysts, when exposed to one miracidium from sympatric SJ strain of *S. mansoni*.

The kinetics of shedding cercariae revealed that the highest survival time for positive snails was of 137 days, and 159 days for molluscs exposed to 1 and 10 miracidia respectively (figure 1). ZANOTTI-MAGALHÃES et al²¹ verified higher survival time for *B. glabrata* infected with the BH sympatric strain of *S. mansoni*.

Another important point was the low mortality rate in the infected snails. A comparison of mortality indexes of exposed molluscs to 1 and 10 miracidia and the control group, showed no statistical differences in proportions. It means that the infection was not able to produce significant mortality amongst the exposed snails, when compared to that verified in the same period for the control snails (table 1). DIAS et al² followed the SJ *B. tenagophila* from São José dos Campos for 70 days after exposure to human and wild rodents strains of *S. mansoni* showed mortality rates of 56% and 52% respectively, whereas the mortality in the control group was 28%.

Our results demonstrate that *B. tenagophila* from Itariri, São Paulo State, is susceptible to a newly field isolated sympatric strain of *S. mansoni*. By carrying out experiments with the second progeny of the trematode, the infection rate was high with low mortality, and the period of cercariae shedding was long. Therefore, the host-parasite relationship can be considered suc-

cessful. This effective host-parasite relationship associated with the endemic area of Ribeira Valley, represents an important aspect to be considered in the epidemiological study of schistosomiasis in this region, where several active transmission foci and intense pattern of water contact by humans occurs^{8, 14}. The degree of susceptibility of the snail host could influence the production of cercariae and determine the epidemiological pattern of the disease.

RESUMO

Características biológicas e morfológicas de cepa paulista de *Schistosoma mansoni* do vale do Ribeira. I — Suscetibilidade de *Biomphalaria tenagophila* do Vale do Ribeira, SP, a cepa simpátrica de *Schistosoma mansoni*.

Dentre as regiões do Estado de São Paulo, onde *Biomphalaria tenagophila* é hospedeira intermediária, o vale do Ribeira é área endêmica da esquistossomose. Nos últimos onze anos a endemia vem sendo intensamente controlada no vale, principalmente, nos municípios de Pedro de Toledo e Itariri. Estudamos a suscetibilidade de *B. tenagophila* de Itariri à linhagem simpátrica de *Schistosoma mansoni*, isolada do campo em 1988 e mantida no laboratório em camundongos suíço e *B. tenagophila*. Estudou-se a 2ª geração do trematódeo. Constituíram-se 3 grupos de 60 moluscos cada, sendo um grupo exposto a um miracídio e outro a 10 e um grupo controle não infectado. Observou-se mortalidade e eliminação de cercárias durante 78 dias. Findo este período, foram seguidos apenas moluscos que liberaram larvas até cessar sua eliminação. Nos moluscos expostos a 1 e 10 miracídios as mortalidades foram de 23% e 31% e os índices de infecção de 8% e 60% respectivamente, assim como a eliminação de cercárias foi de 82 e 104 dias. No controle a mortalidade foi de 22%. Assim, podemos concluir que *B. tenagophila* apresenta boa adaptação a linhagem simpátrica de *S. mansoni*.

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REFERENCES

1. CHIEFFI, P. P. — Resistência de cepa de *Biomphalaria tenagophila*, originária de Londrina (Paraná, Brasil) à infecção por duas cepas de *Schistosoma mansoni*. *Rev. Soc. bras. Med. trop.*, 4: 209-212, 1975.
2. DIAS, L. C. de S.; UETA, M. T. & GUARALDO, A. M. A. — Suscetibilidade de *Biomphalaria glabrata*, *B. straminea* e *B. tenagophila* a diferentes cepas de *Schistosoma mansoni*. *Rev. Inst. Med. trop. S. Paulo*, 29: 205-212, 1987.
3. DIAS, L. C. de S.; BRUCE, J. I. & COLES, G. C. — Strain variation in the infectivity of *Schistosoma mansoni* for *Biomphalaria glabrata*. *Rev. Inst. Med. trop. S. Paulo*, 30: 86-90, 1988.
4. DIAS, L. C. de S.; GLASSER, C. M.; ETZEL, A.; KAWAZOE, U.; HOSHINO-SHIMIZU, S.; KANAMURA, H. Y.; CORDEIRO, J. A.; MARÇAL JR, O.; CARVALHO, J. F.; GONÇALVES JR, F. L. & PATUCCI, R. — The epidemiology and control of schistosomiasis mansoni where *Biomphalaria tenagophila* is the snail host. *Rev. Saúde públ. (S. Paulo)*, 22: 462-463, 1988.
5. DIAS, L. C. de S.; KAWAZOE, U.; GLASSER, C. M.; HOSHINO-SHIMIZU, S.; KANAMURA, H. Y.; CORDEIRO, J. A.; GUARITA, O. F. & ISHIHATA, G. J. — Schistosomiasis mansoni in the Municipality of Pedro de Toledo (São Paulo, Brazil) where the *Biomphalaria tenagophila* is the snail host. I — Prevalence in human population. *Rev. Inst. Med. trop. S. Paulo*, 31: 110-118, 1989.
6. DOUMENGE, J. P.; MOTT, K. E.; CHEUNG, C.; VILLENAVE, D.; CHAPUIS, O.; PERRIN, M. F. & REAUD-THOMAS, G. — Atlas de la repartition mondiale des schistosomiasis. Talence, CEGET-CNRS; GENEVE, OMS/WHO; TALENCE, PUB, 1987.
7. FILES, V. S. & CRAM, E. B. — A study on comparative susceptibility of snails vectors to strains of *Schistosoma mansoni*. *J. Parasit.*, 35: 555-560, 1949.
8. MARÇAL JR., O. — Fatores ligados ao homem na transmissão da esquistossomose mansônica no Município de Pedro de Toledo, São Paulo, 1987. Campinas, 1989. [Tese de mestrado — Instituto de Biologia da Universidade Estadual de Campinas].
9. MINCHELLA, D. J. & LOVERDE, P. T. — Laboratory comparison of the relative success of *Biomphalaria glabrata* stocks which are susceptible and insusceptible to infection with *Schistosoma mansoni*. *Parasitology*, 86: 335-344, 1983.
10. PARAENSE, W. L. & CORREA, L. R. — Susceptibility of *Australorbis tenagophilus* to infection with *Schistosoma mansoni*. *Rev. Inst. Med. trop. S. Paulo*, 5: 23-29, 1963.
11. PARAENSE, W. L. & CORREA, L. R. — Differential susceptibility of *Biomphalaria tenagophila* populations to infection with a strain of *Schistosoma mansoni*. *J. Parasit.*, 64: 822-826, 1978.
12. PARAENSE, W. L. & CORREA, L. R. — Observations on two biological races of *Schistosoma mansoni*. *Mem. Inst. Oswaldo Cruz*, 76: 287-291, 1981.
13. SOUZA, C. P. de — Estudo de moluscos do gênero *Biomphalaria* de Minas Gerais, com relação a adaptação parasito hospedeiro e importância na epidemiologia da esquistossomose. *Rev. Inst. Med. trop. S. Paulo*, 28: 287-291, 1986.
14. SUCEN — Avaliação do Programa de Controle de Esquistossomose — Serviço Regional 2 da Superintendência de Controle de Endemias, São Vicente, SP, 1989. (Relatório Interno)
15. TELES, H. M. S. & VAZ, J. F. — Distribuição de *Biomphalaria glabrata* (Say 1818) (Pulmonata, Planorbidae), no Estado de São Paulo, Brasil. *Rev. Saúde públ. (S. Paulo)*, 21: 508-512, 1987.
16. TELES, H. M. S. & VAZ, J. F. — Distribuição de *Biomphalaria straminea* (Dunker, 1848) (Pulmonata, Planorbidae) no Estado de São Paulo, Brasil. *Ciênc. e Cult.*, 40: 173-176, 1988.
17. VAZ, J. F.; ELMOR, M. R. D.; GONÇALVES, L. M. C. & ISHIHATA, G. K. — Resultados do levantamento planorbídico da área de Presidente Prudente — Estado de São Paulo. *Rev. Inst. Med. trop. S. Paulo*, 25: 120-126, 1983.
18. VAZ, J. F.; TELES, H. M. S. & TAKAKU, L. — Levantamento planorbídico do Estado de São Paulo: 7ª região administrativa. *Ciênc. e Cult.*, 37: 2057-2062, 1985.
19. VAZ, J. F.; TELES, H. M. S.; LEITE, S. P. S.; CORREA, M. A.; FABBRO, A. L. D. & ROSA, W. S. — Levantamento planorbídico do Estado de São Paulo. *Rev. Saúde públ. (S. Paulo)*, 20: 352-361, 1986.
20. VAZ, J. F.; MANTEGAZZA, E.; TELES, H. M. S.; LEITE, S. P. S. & MORAIS, L. V. C. — Levantamento planorbídico do Estado de São Paulo (Brasil): 4ª região administrativa. *Rev. Saúde públ. (S. Paulo)*, 21: 371-379, 1987.
21. ZANOTTI-MAGALHÃES, E.; PAIVA, S. M. de; MAGALHÃES, L. A. & CARVALHO, J. F. — Viabilidade de mirácidos de *Schistosoma mansoni*, obtidos de fezes e de granulomas hepáticos de camundongos experimentalmente infectados com a linhagem BH. *Rev. Saúde públ. (S. Paulo)*, 22: 479-483, 1988.

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