

# Susceptibility of Argentinean Biomphalaria tenagophila and Biomphalaria straminea to infection by Schistosoma mansoni and the possibility of geographic expansion of mansoni schistosomiasis

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### **ABSTRACT**

**Introduction:** Human migration and the presence of natural vectors (mollusks) of *Schistosoma mansoni* are the primary causes of the expansion of mansoni schistosomiasis into southern areas of South America. Water conditions are favorable for the expansion of this disease because of the extensive hydrographic network, which includes the basins of the Paraná and Uruguay rivers and favors mollusk reproduction. These rivers also aid agriculture and tourism in the area. Despite these favorable conditions, natural infection by *S. mansoni* has not yet been reported in Argentina, Uruguay, or Paraguay. **Methods:** Two species of planorbid from Argentina, *Biomphalaria straminea* and *B. tenagophila*, were exposed to the miracidia of five Brazilian strains of *S. mansoni*. **Results:** *Biomphalaria tenagophila* (Atalaya, Buenos Aires province) was infected with the SJS strain (infection rate 3.3%), confirming the experimental susceptibility of this Argentinian species. *Biomphalaria straminea* (Rio Santa Lucía, Corrientes province) was susceptible to two Brazilian strains: SJS (infection rate 6.7%) and Sergipe (infection rate 6.7%). **Conclusions:** These results demonstrate that species from Argentina have the potential to be natural hosts of *S. mansoni* and that the appearance of foci of mansoni schistosomiasis in Argentina is possible.

Keywords: Schistosomiasis. Biomphalaria. Susceptibility.

# INTRODUCTION

Mansoni schistosomiasis has expanded in recent decades, primarily because of the migration of individuals who have been parasitized by *Schistosoma mansoni*, live in inadequate sanitary conditions in peripheral areas of large cities, and contaminate the water supply with their waste. These water supplies contain trematode vectors such as freshwater mollusks, permitting the establishment of disease foci. In recent decades in Brazil, the disease has spread toward the southern states, with autochthonous cases of this parasitosis reported in Paraná, Santa Catarina, and Rio Grande do Sul<sup>1-4</sup>. Ecosystem changes in northeastern Argentina caused by the construction of dams have led to an increase in infectious diseases due to an increase in temperature<sup>5</sup>. Of the planorbid vector species of *S. mansoni* found in the southernmost states of Brazil (Rio Grande do Sul and Santa Catarina), *Biomphalaria tenagophila* is the most

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Phone: 55 19 3521-6303 e-mail: emzm@unicamp.br Received 26 June 2013 Accepted 27 September 2013 in São Francisco do Sul, Santa Catarina. Among the species described in Rio Grande do Sul (Brazil), the primary vector of S. mansoni in the South American continent, B. glabrata, is responsible for the occurrence of the disease in the municipality of Esteio<sup>4</sup>. In Uruguay, Paraguay, and Argentina, numerous planorbid breeders have been described in association with B. tenagophila and B. straminea<sup>8-12</sup>, which are known to be natural vectors of S. mansoni. Breeding conditions are favorable for these mollusks in the flooded fields used for plantations and in irrigation canals and reservoir dams. A study was conducted in Argentina to investigate the growth of natural populations of Biomphalaria in the La Plata river basin. A greater steady growth was confirmed for B. tenagophila compared with B. peregrina<sup>13</sup>. Despite the existence of schistosomiasis foci in the Brazilian states bordering Argentina, Uruguay, and Paraguay, there have been no reports of the natural transmission of schistosomiasis in the basin of the La Plata river in these countries<sup>12,14</sup>. However, experimental studies with populations of B. tenagophila from Argentina, Uruguay, and Paraguay have demonstrated that this species is susceptible to the Brazilian strain of S. mansoni<sup>11,12,14-16</sup>. Paraense & Corrêa<sup>17</sup> assessed the susceptibility of a planorbid population from Uruguay that was morphologically similar to B. straminea and confirmed an infection rate of 23% after exposure to miracidia of the SJ strain of S. mansoni. The susceptibility exhibited by the mollusks

common<sup>6,7</sup>. This species is responsible for schistosomiasis foci

depends on the degree of physiological adaptation between the parasite and its intermediary host, as suggested by Magalhães<sup>18</sup>, thus explaining the different infection rates observed among populations of the same species of mollusk when infected with different strains of the parasite. Physiological adjustment was also demonstrated by Paraense & Corrêa, <sup>19</sup> who reported that *S. mansoni* that were adapted to *B. glabrata* from Belo Horizonte (State of Minas Gerais (MG), Brazil) resisted infection when challenged by *B. tenagophila* from São José dos Campos (State of São Paulo (SP), Brazil) and vice versa. Paraense & Corrêa<sup>19</sup> demonstrated the existence of two strains of the trematode in Brazil: one from Belo Horizonte (BH) that uses *B. glabrata* as an intermediary host and one from São José dos Campos (SJ) that uses sympatric *B. tenagophila* as an intermediary host.

The great rivers that form the La Plata river basin are the most likely dispersion routes of schistosomiasis toward Argentina, Paraguay, and Uruguay. In the Mesopotamia region of Argentina in which these rivers are located, numerous *B. tenagophila* and *B. straminea* breeders are known vectors of *S. mansoni. Biomphalaria peregrina*, a mollusk that has not yet been found to be naturally infected by the trematode but has high infection rates in the laboratory, is also present in this region. The aim of the present study was to perform susceptibility tests on the planorbid mollusks *B. tenagophila* and *B. straminea* from Argentina after exposure to a number of Brazilian strains of *S. mansoni*. Studies of the association between mollusks and *S. mansoni* have epidemiological significance because they facilitate the prediction of areas that become endemic regions for schistosomiasis.

### **METHODS**

The specimens of B. straminea and B. tenagophila used in the present study were isolated in the laboratory from specimens collected in Argentina from the Santa Lucía River, in the province of Corrientes, and in Atalaya on the La Plata river, in the province of Buenos Aires, respectively. These specimens were provided by Dr. Alejandra Rumi. Five strains of S. mansoni were used for the susceptibility tests: the BH strain (Belo Horizonte, MG, Brazil), which was maintained in sympatric B. glabrata; the SJ strain (São José dos Campos, SP, Brazil), which was maintained in sympatric B. tenagophila; the SJS strain (São José dos Campos, SP, Brazil), which was genetically selected for susceptibility and maintained in sympatric B. tenagophila that were genetically selected for susceptibility to S. mansoni<sup>20</sup>; the BA strain (Bahia, Brazil), which was isolated from the feces of a tourist from Barra Grande, Bahia, Brazil, and maintained in B. glabrata from Minas Gerais (MG, Brazil); and the SE strain (Sergipe (SE), Brazil), which was isolated from B. glabrata from Ilha das Flores (SE, Brazil) and maintained in sympatric B. glabrata. Five groups of B. tenagophila (5-7mm) and B. straminea (2-3mm), each containing 30 specimens, were individually exposed to 10 miracidia of each of the specified strains of S. mansoni. The miracidia were obtained from eggs retrieved from the feces of Swiss mice (Mus musculus) that were experimentally infected with the respective strains of

Schistosoma mansoni. After exposure, the groups of mollusks were kept in aquariums containing dechlorinated water at room temperature (±25°C) and fed lettuce ad libitum. The water was changed periodically, and from the fourth to the 16th week after exposure to the miracidia, the mollusks were examined to confirm the liberation of cercariae. The aquariums were maintained under artificial light at 28°C. At the end of the experiment, the surviving mollusks were compressed between two glass plates and examined under a stereoscopic microscope to confirm the presence of sporocysts in the tissue. Statistical analysis of survival was performed by comparing the 10 survival curves obtained from the combinations of species of mollusk (B. tenagophila and B. straminea) and trematode strain (BH, SJ, BA, SE, and SJS). Alternative analysis was also performed to investigate the species effect and the strain effect on survival. The survival curves were adjusted using the Kaplan-Meier method and compared with log-rank statistics, for which the significance probability allowed an assessment of the differences between the curves.

# **RESULTS**

**Table 1** displays the number of mollusks that were infected and the mortality rate. Figure 1 and Figure 2 display the infectivity of mollusks from the fourth week of infection to the end of the experiment. Only four of the specimens of B. straminea that were exposed to infection exhibited elimination of cercariae: two that were exposed to the SJS strain and two that were exposed to the SE strain. The prepatent period was six weeks for the SJS strain and eight weeks for the SE strain. The specimens of B. straminea that were exposed to the SE strain and eliminated cercariae died between the eighth and ninth weeks of infection. The specimens of B. straminea that were exposed to the SJS strain and eliminated cercariae died between the eighth and ninth weeks of infection: one survived for one week and the other for two weeks. Only one of the specimens of B. tenagophila that were exposed to infection eliminated cercariae: the SJS strain of S. mansoni. The prepatent period was seven weeks, and this mollusk died between the seventh and eighth weeks of infection.

Analysis of the survival curves (**Figure 3**, **Figure 4A**, and **Figure 4B**), which were softened by the Weibull distribution, revealed a highly significant difference between the curves (p<0.0001). The shortest survival times were associated with the SE strain (Sergipe). No significant difference (p=0.32580) was observed for the effect of the mollusk species (**Figure 4A**). A significant difference (p<0.0001) was observed for the effect of the strain (**Figure 4B**), which disappeared (p=0.7018) when the SE strain was removed from the data set (Sergipe).

# **DISCUSSION**

The susceptibility of mollusks that are vectors of *S. mansoni* is controlled genetically and is heritable over generations<sup>21-23</sup>. The different degrees of susceptibility exhibited by the vectors of the trematode are the result of the frequency of the phenotypes of the parasite that are pre-adapted to the population of mollusks.

TABLE 1 - Infection and mortality rates of *Biomphalaria straminea* (n=30) and *Biomphalaria tenagophila* (n=30) from Argentina after exposure to 10 miracidia from five different strains of *Schistosoma mansoni*.

Species of Biomphalaria	Strain of Schistosoma mansoni	Number of mollusks that eliminated cercariae	Infection rate (%)	Mortality rate	
				n	%
Biomphalaria straminea	ВН	0	0.0	15	50.0
	SE	2	6.7	24	80.0
	SJS	2	6.7	24	80.0
	SJ	0	0.0	14	46.6
	BA	0	0.0	25	83.3
Biomphalaria tenagophila	ВН	0	0.0	20	66.6
	SE	0	0.0	20	66.6
	SJS	1	3.3	10	33.3
	SJ	0	0.0	13	43.3
	BA	0	0.0	9	30.0

BH strain (Belo Horizonte, State of Minas Gerais, Brazil); SE strain (Sergipe, Brazil); SJS strain (São José dos Campos, genetically selected, State of São Paulo, Brazil); SJ strain (São José dos Campos, State of São Paulo, Brazil); BA strain (Bahia, Brazil).

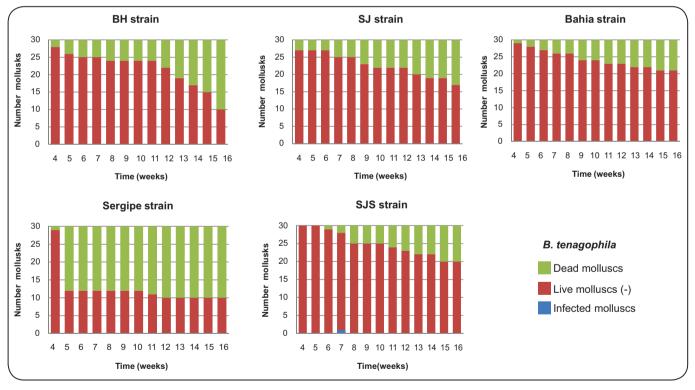


FIGURE 1 - Prepatent period and infectivity of *Biomphalaria tenagophila* from Argentina after exposure to five different strains of *Schistosoma mansoni*. BH: Belo Horizonte; SJ: São José dos Campos; SJS: São José dos Campos, genetically selected.

The results of the present study demonstrated that, of the five strains of *S. mansoni* used, only the SJS and SE strains were able to develop in populations of Argentinean mollusks. The SJS strain originated from a population of *B. tenagophila* that is sympatric to the SJ strain<sup>19</sup>, which was genetically selected for

its susceptibility<sup>20</sup>. Although *B. glabrata* from Belo Horizonte was resistant to infection by the SJ strain, this species of mollusk was susceptible to infection by the SJS strain<sup>24</sup>. In the present study, *B. tenagophila* and *B. straminea* from Argentina released cercariae after exposure to miracidia of the SJS strain of

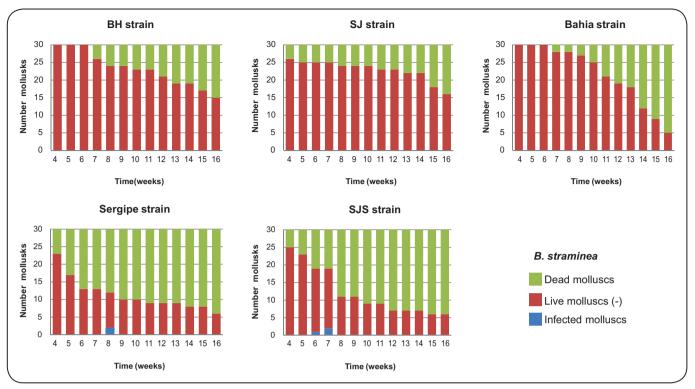


FIGURE 2 - Prepatent period and infectivity of *Biomphalaria straminea* from Argentina after exposure to five different strains of *Schistosoma mansoni*. BH: Belo Horizonte; SJ: São José dos Campos; SJS: São José dos Campos, genetically selected.

Schistosoma mansoni. Among the five Brazilian strains of S. mansoni used in the susceptibility tests, B. tenagophila specimens from the province of Buenos Aires (Argentina) were susceptible only to the SJS strain, with liberation of cercariae and an infection rate of 3.3% (Table 1). This result demonstrates that B. tenagophila is only susceptible to parasites that originate in mollusks of the same species, corroborating the results of Borda & Rea<sup>12,14,16</sup>, who found that liberation of cercariae only occurred in B. tenagophila from Corrientes (Argentina) after exposure to the SJ strain of S. mansoni. The infection rate confirmed by the authors was between 2% and 22%<sup>14</sup>, with a prepatent period of 31 to 54 days. In the present study, the prepatent period was seven weeks. The longer prepatent periods and lower infection rates indicate that the development of the trematode was hampered in the mollusk. However, subsequent generations of B. tenagophila were more susceptible to S. mansoni, as reported by Borda & Rea $^{14}$ , who found a higher infection rate in the F1 generation of B. tenagophila. Susceptibility is easier to obtain than an increase in resistance<sup>25</sup>. Bernadini & Machado<sup>1</sup> confirmed that *B. tenagophila* was the vector species in schistosomiasis foci in Santa Catarina (Brazil). Along the coast of Rio Grande do Sul (Brazil), the population of B. tenagophila in the Taim Ecological Station has been shown to be resistant to infection by S. mansoni<sup>26</sup>. These Brazilian states share a border with the Argentinean province of Corrientes. Paraense<sup>27</sup> reported morphological differences in the penile complex of planorbids in Rio Grande do Sul (Brazil) and Uruguay, suggesting the existence of a subspecies called B. tenagophila guaibensis, which had previously<sup>28</sup> been shown to

be resistant to infection by the BH and SJ strains of *S. mansoni*. However, specimens of *B. tenagophila tenagophila* from Tramandaí (on the northern coast of the same state) exhibited an infection rate of 2.08% after exposure to SJ miracidia.

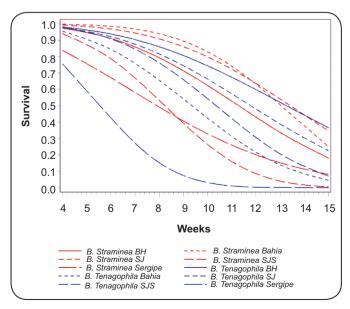


FIGURE 3 - Survival curves adjusted for the Weibull distribution with respect to the experimental groups. B: *Biomphalaria*; BH: Belo Horizonte; SJS: São José dos Campos; SJS: São José dos Campos, genetically selected.

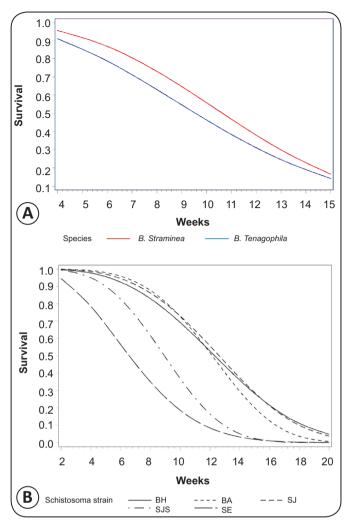


FIGURE 4 - Survival curves adjusted for the Weibull distribution with respect to the two species of mollusks, *B. straminea* and *B. tenagophila* (A), and the strains of *Schistosoma mansoni* used in the experiments, BA, BH, SE, SJ, and SJS (B). B: *Biomphalaria*; BH: Belo Horizonte; SJS: São José dos Campos, genetically selected; BA: Bahia, SE: Sergipe; SJ: São José dos Campos.

Biomphalaria straminea specimens from the province of Corrientes (Argentina) were infected by the SJS and SE strains (Table 1), an unprecedented result for this species from Argentina. Paraense & Corrêa<sup>17</sup> observed a morphologically similar species infection of B. straminea from Uruguay after exposure to the SJ strain. Biomphalaria straminea specimens from Argentina were notably susceptible to the Sergipe strain (SE), which was isolated from naturally infected B. glabrata. Infection by this strain, which has a high degree of endemicity for schistosomiasis, caused a higher mortality rate in the mollusks (Figure 3, Figure 4A and Figure 4B). This increased mortality was attributed to the parasite; the larval stages of the trematode compromise the tissues and organs of mollusks infected with S. mansoni<sup>29</sup>. The preparent period ranged from six to eight weeks, indicating restricted S. mansoni development, although one of the specimens survived for two weeks. Figueiredo<sup>30</sup> observed a natural infection rate of 2.5% for B. straminea from Laranjeiras,

Sergipe (Brazil), which the author considered to be relatively high. This species is a significant transmitter of S. mansoni in this Brazilian state. The SE strain used in the present study was obtained from naturally infected B. glabrata and collected from Ilha das Flores in Sergipe (SE), Brazil. The significance of B. straminea as a vector of S. mansoni is associated with greater endemicity in northeastern Brazil, Furthermore, an extended geographical distribution of B. straminea in South America has been noticed in recent years<sup>6,10</sup>. Michelson & Dubois<sup>31</sup> defined B. straminea as a species of competitive superiority due to its capacity to invade territory occupied by other species. A number of authors<sup>30,32,33</sup> have commented on the displacement of B. glabrata by B. straminea in northeastern Brazil and the high capacity of B. straminea to resist periods of drought, which are characteristic of this region. Naturally infected B. straminea<sup>34</sup> have been found in Cruzeiro, in the valley of the Paraíba do Sul River (SP, Brazil). In experimental tests<sup>35</sup>, specimens from this municipality were susceptible to human and wild strains of S. mansoni. This species was reported<sup>6</sup> in a pisciculture aquarium in Porto Alegre (RS, Brazil). B. straminea and B. tenagophila have been identified in rice fields in Corrientes (Argentina); B. straminea was more abundant than B. tenagophila<sup>10</sup>.

The results of the present study confirm the susceptibility of *B. tenagophila* from Argentina to a *S. mansoni* strain that originated in sympatric *B. tenagophila*. The susceptibility of *B. straminea* to two Brazilian strains demonstrates the potential of this species to establish schistosomiasis foci in Argentina.

# **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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