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 pre-patent 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 $\chi^2$ 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

<table>
<thead>
<tr>
<th><em>S. mansoni</em> origin</th>
<th>Number exposed</th>
<th>Number infected</th>
<th>Percentage infected</th>
<th>Percentage mortality&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean ± SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>150</td>
<td>36</td>
<td>24.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.3</td>
<td>48 ± 29</td>
<td>56,955</td>
</tr>
<tr>
<td>SJ</td>
<td>150</td>
<td>58</td>
<td>38.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.3</td>
<td>75 ± 73</td>
<td>111,400</td>
</tr>
<tr>
<td>AL</td>
<td>150</td>
<td>49</td>
<td>32.6</td>
<td>20.6</td>
<td>79 ± 90</td>
<td>78,416</td>
</tr>
<tr>
<td>Total</td>
<td>450</td>
<td>143</td>
<td>31.8</td>
<td>15.7</td>
<td>67 ± 18.7</td>
<td>246,771</td>
</tr>
</tbody>
</table>

<sup>a</sup>: 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 ($p>0.05$)

SD: standard deviation

b: snail death during the pre-patent period
### TABLE II

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

<table>
<thead>
<tr>
<th>S. mansoni line</th>
<th>Number exposed</th>
<th>Number infected</th>
<th>Percentage infected</th>
<th>Percentage mortality&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean ± SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>150</td>
<td>12</td>
<td>8.0</td>
<td>16.6</td>
<td>119 ± 99</td>
<td>27,136</td>
</tr>
<tr>
<td>SJ</td>
<td>150</td>
<td>15</td>
<td>10.0</td>
<td>12.6</td>
<td>91 ± 82</td>
<td>14,227</td>
</tr>
<tr>
<td>AL</td>
<td>150</td>
<td>17</td>
<td>11.3</td>
<td>9.3</td>
<td>112 ± 100</td>
<td>30,763</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>450</strong></td>
<td><strong>44</strong></td>
<td><strong>9.7</strong></td>
<td><strong>12.8</strong></td>
<td><strong>107 ± 18</strong></td>
<td><strong>72,126</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup>: snail death during the pre-patent period  
SD: standard deviation

### TABLE III

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

<table>
<thead>
<tr>
<th>S. mansoni line</th>
<th>Number exposed&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number infected</th>
<th>Percentage infected</th>
<th>Percentage mortality&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean ± SD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>150</td>
<td>38</td>
<td>75.3</td>
<td>22.0</td>
<td>504 ± 323</td>
<td>505,736</td>
</tr>
<tr>
<td>SJ</td>
<td>150</td>
<td>39</td>
<td>73.3</td>
<td>26.0</td>
<td>707 ± 343</td>
<td>552,594</td>
</tr>
<tr>
<td>AL</td>
<td>150</td>
<td>40</td>
<td>75.3</td>
<td>9.3</td>
<td>432 ± 435</td>
<td>309,910</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>450</strong></td>
<td><strong>117&lt;sup&gt;a&lt;/sup&gt;</strong></td>
<td><strong>74.6</strong></td>
<td><strong>19.3</strong></td>
<td><strong>548 ± 141</strong></td>
<td><strong>1,368,240</strong></td>
</tr>
</tbody>
</table>

<sup>a</sup>: for the control was used only 117 snails between 336 infected  
<sup>b</sup>: 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

<table>
<thead>
<tr>
<th>Snail</th>
<th>Species</th>
<th>Origin</th>
<th>S. mansoni</th>
<th>TCP/100</th>
<th>Compatibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Line</td>
<td>Index</td>
<td>Class</td>
</tr>
<tr>
<td><em>B. tenagophila</em></td>
<td>Pampulha</td>
<td>LE</td>
<td>LE</td>
<td>37,996</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Pampulha</td>
<td>SJ</td>
<td>SJ</td>
<td>74,226</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Pampulha</td>
<td>AL</td>
<td>AL</td>
<td>52,277</td>
<td>III</td>
</tr>
<tr>
<td><em>B. straminea</em></td>
<td>Panacatu</td>
<td>LE</td>
<td>LE</td>
<td>18,090</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Panacatu</td>
<td>SJ</td>
<td>SJ</td>
<td>9,484</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Panacatu</td>
<td>AL</td>
<td>AL</td>
<td>20,508</td>
<td>II</td>
</tr>
<tr>
<td><em>B. glabrata</em></td>
<td>Laboratory</td>
<td>LE</td>
<td>LE</td>
<td>1,002,599</td>
<td>VI</td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td>SJ</td>
<td>SJ</td>
<td>1,039,065</td>
<td>VI</td>
</tr>
<tr>
<td></td>
<td>Laboratory</td>
<td>AL</td>
<td>AL</td>
<td>588,828</td>
<td>VI</td>
</tr>
</tbody>
</table>
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 $1,503,899 \times 100/150 = 1,002,599$ - 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 33.8±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); 30-77 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±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 some 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 autochthonous 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 autochthonous 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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