Biomphalaria molluscs (Gastropoda: Planorbidae) in Rio Grande do Sul, Brazil

Michele Soares Pepe1,2, Roberta Lima Caldeira2, Omar dos Santos Carvalho2, Gertrud Muller1, Liana Konoaloff Jannotti-Passos2,3, Alice Pozza Rodrigues1, Hugo Leonardo Amaral1, Maria Elisabeth Aires Berne1

1Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, CP 354, 96010-900 Pelotas, RS, Brasil 2Laboratório de Helminthologia e Malacologia Médica 3Moluscário Lobato Paraense, Instituto de Pesquisas René Rachou-Fiocruz, Belo Horizonte, MG, Brasil

The present study was aimed at characterising Biomphalaria species using both morphological and molecular (PCR-RFLP) approaches. The specimens were collected in 15 localities in 12 municipalities of the southern region of the state of Rio Grande do Sul, Brazil. The following species were found and identified: Biomphalaria tenagophila guaibensis, Biomphalaria oligoza and Biomphalaria peregrina. Specimens of the latter species were experimentally challenged with the LE Schistosoma mansoni strain, which showed to be refractory to infection.

Key words: Biomphalaria sp - Southern Brazil - experimental infection

Freshwater snails belonging to the genus Biomphalaria are intermediate hosts of Schistosoma mansoni, the etiological agent of schistosomiasis. Among the Biomphalaria species that occur in Brazil, three are regarded as intermediate hosts of S. mansoni, namely, Biomphalaria glabrata, Biomphalaria tenagophila and Biomphalaria straminea. Investigations on experimental infection using Biomphalaria peregrina and Biomphalaria amazonica have shown that they are potential hosts for the trematode (Corrêa & Parãae 1971, Parãae & Corrêa 1973).

In Rio Grande do Sul (RS), Brazil, there have been reports on the occurrence of B. tenagophila (Parãae & Deslandes 1959), B. straminea (Cunha Neto 1972), Biomphalaria oligoza (Parãae 1974), B. peregrina (Parãae 1966), Biomphalaria tenagophila guaibensis (Parãae 1984) and B. glabrata (Carvalho et al. 1998). The presence of S. mansoni in the state has been associated with the occurrence of B. glabrata in water collections surrounding Rio dos Sinos, in the municipality of Esteio (Graeff-Teixeira et al. 1999).

Considering the fact that little is known on freshwater snail diversity in the Southern Region of Brazil, the aim of the present study was to identify the Biomphalaria species in this region and assess their susceptibility to S. mansoni infection, either as intermediate hosts and/or as potential hosts. Molluscs were collected in 15 localities of 12 municipalities of RS during 2005. The collection areas included: Arroio Grande, Bagé, Camaquã, Cançu, Capão do Leão, Dom Pedrito, Jaguarão, Pelotas, Rio Grande, Rosário do Sul, Santa Vitória do Palmar and São Gabriel, between the 30-34° parallels and the 51-55° meridians. The molluscs collected were sent to our laboratory to obtain their F1 progeny. Morphological and molecular identification of Biomphalaria was undertaken according to Parãae (1975, 1981, 1984) and Vidigal et al. (2000), respectively.

Specimens of B. peregrina (São João Batista do Glória/MG), B. oligoza (Eldorado do Sul/RS), B. t. guaibensis (Esteio/RS) and Biomphalaria occidentalis (Belo Horizonte, Minas Gerais (MG)), previously identified by both morphological and molecular means, were included for comparison. The snails were identified as B. oligoza, B. peregrina and B. t. guaibensis both through their morphology and the use of molecular techniques (Fig. 1). Since the genetic profiles of B. t. guaibensis and B. occidentalis appeared to be similar when digested with the restriction enzyme Ddel, the amplified fragments that had been previously identified as B. t. guaibensis were then digested with Alul since it allows differentiation between these species (Fig. 2). Table shows the field-collected snails, B. oligoza, B. peregrina and B. t. guaibensis in RS.

Groups of 50 F1- snails (4-6 mm) from two B. peregrina populations collected in the municipalities of Rio Grande and Dom Pedrito (Estrada do Meio) were individually challenged with 100 miracidia/mollusc of the LE S. mansoni strain. As an infection control, we included 50 B. glabrata specimens from Barreiro de Cima, Belo Horizonte (MG), infected with 20 miracidia/mollusc of the same strain, which had been kept in the Moluscário Lobato Paraense at Instituto de Pesquisas René-Rachou-Fiocruz (MG). The susceptibility levels in these populations were assessed by light exposure and squeezing (Souza & Lima 1997). All field-collected molluscs were determined to be S. mansoni-negative.

+ Corresponding author: micpepe2@yahoo.com.br
Received 5 August 2008
Accepted 3 March 2009

online | memorias.ioc.fiocruz.br
Light exposure examinations of F1 progeny from two *B. peregrina* populations that had been challenged with *S. mansoni* miracidia showed no evidence of either cercariae or sporocysts. The mortality rate of the snails ranged from 0-16%.

The present investigation constitutes the first report on the occurrence of *B. t. guaibensis* in the municipalities of Camaquã, Dom Pedrito and Santa Vitória do Palmar. Although a survey by Teles et al. (1991) showed no evidence of such species in RS, Paraense (1984) had reported its occurrence in Arroio Grande, Jaguarão and Pelotas (RS).

*Olzoa* is also being reported for the first time in Canguçu, Capão do Leão and São Gabriel and was previously reported by Paraense (1974), Frões and Lima (1975) and Pereira et al. (2000) in the municipalities of Filipson, Gravataí, Guaíba, Lagoa Vermelha, Mariana Pimentel, Morro Reuter, Nova Petrópolis, Porto Alegre, Santo Antônio, Seival, Tramandai, Tramandai, Tupanciretã and Viamão. In Bagé, Dom Pedrito, Porto Alegre and Rosário do Sul, previous reports have described the presence of *B. peregrina* (Paraense 1966, Frões & Lima 1975). In addition to these municipalities, the authors also reported the occurrence of *B. peregrina* in Camaquã, Jaguarão, Pelotas and São Gabriel. The results of our study, however, showed no occurrence of *B. peregrina*, which may be due to differences in the collection areas.

Early studies of shell morphology and the internal anatomy of planorbids were conducted to identify suitable traits for accurate and reliable species identification (Paraense 1972, 1975). In some *B. t. guaibensis* specimens from different populations than those analysed in our study, a dark pigmentation was observed on the renal tube, which had not been reported by Paraense (1984). Among the planorbid species described in Brazil, the only species known to have a pigmented renal tube is *B. glabrata*, the main *S. mansoni* transmitter species in Brazil (Paraense 1975). According to Paraense (1972), the specific trait found in *B. glabrata* and the main feature used to differentiate it from *B. tenagophila* is the pigmented crest that extends along the renal tube. This pigmentation found in *B. tenagophila guaibensis* might be due to either intraspecific variation or contact with staining substances present in the water collections (Paraense 1972).

Specific morphological characteristics described by Paraense (1966, 1974) were observed in the *B. peregrina* and *Oligoza* populations analysed in our present study.
TABLE

Biomphalaria species identified through morphological and molecular techniques collected in municipalities in the state of Rio Grande do Sul

<table>
<thead>
<tr>
<th>Municipalities</th>
<th>Origin of snails</th>
<th>species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arroio Grande</td>
<td>Barragem Chasqueiro</td>
<td><strong>Biomphalaria tenagophila guaibensis</strong></td>
</tr>
<tr>
<td>Bagé</td>
<td>Bagé</td>
<td><strong>Biomphalaria peregrina</strong></td>
</tr>
<tr>
<td>Camaquã</td>
<td>Pacheca</td>
<td><strong>Biomphalaria oligoza</strong></td>
</tr>
<tr>
<td>Canguçu</td>
<td>Cabanha Cafundó</td>
<td></td>
</tr>
<tr>
<td>Capão do Leão</td>
<td>Fazenda Bela Vista</td>
<td></td>
</tr>
<tr>
<td>Dom Pedroito</td>
<td>Passinho do Amor</td>
<td><strong>B. p. guaibensis</strong></td>
</tr>
<tr>
<td>Dom Pedroito</td>
<td>Chácara do Cedro</td>
<td><strong>B. p. guaibensis</strong></td>
</tr>
<tr>
<td>Dom Pedroito</td>
<td>Estrada do Meio</td>
<td><strong>B. p. guaibensis</strong></td>
</tr>
<tr>
<td>Jaguarão</td>
<td>Granja Bretanha</td>
<td><strong>B. t. guaibensis</strong></td>
</tr>
<tr>
<td>Pelotas</td>
<td>Barragem SANEP</td>
<td><strong>B. t. guaibensis</strong></td>
</tr>
<tr>
<td>Rio Grande</td>
<td>Rio Grande</td>
<td><strong>B. peregrina</strong></td>
</tr>
<tr>
<td>Rosário do Sul</td>
<td>Estância Santa Ambrosina</td>
<td><strong>B. peregrina</strong></td>
</tr>
<tr>
<td>Santa Vitória do Palmar</td>
<td>Estância Ipiranga</td>
<td><strong>B. t. guaibensis</strong></td>
</tr>
<tr>
<td>Santa Vitória do Palmar</td>
<td>Granja Figueira</td>
<td><strong>B. t. guaibensis</strong></td>
</tr>
<tr>
<td>São Gabriel</td>
<td>São Gabriel</td>
<td><strong>B. oligoza</strong></td>
</tr>
</tbody>
</table>

The rates of infection and of mortality in the control group, *B. glabrata* from Barreiro de Cima, were 52% and 16%, respectively.

The susceptibility level of these different *Biomphalaria* species to infection by the same strain of *S. mansoni* varies greatly (Paraense & Corrêa 1973, 1978). Besides the difference in susceptibility observed between *Biomphalaria* species compatible with the parasite, some strain or geographical isolates of the same species of *Biomphalaria* also presented great variation in susceptibility to the parasite.

Given that *S. mansoni* is highly specific to its intermediate host, a survey of the areas where *Biomphalaria* occurs is useful, as it provides invaluable information to help design schistosomiasis control measures.

REFERENCES


Graeff-Teixeira C, Anjos CB, Oliveira VC, Velloso CFP, Fonseca MBS, Valar C, Moraes C, Garrido CT, Amaral RS 1999. Identification of a transmission focus of *Schistosoma mansoni* in


