SHORT COMMUNICATION

Resistance of *Biomphalaria occidentalis* from Varzea das Flores Dam, Minas Gerais, to *Schistosoma mansoni* Infection Detected by Low Stringency Polymerase Chain Reaction

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Biomphalaria occidentalis *Paraense, 1981 from Varzea das Flores dam, MG, Brazil, was exposed to infection with* Schistosoma mansoni. *Individual infection was performed with 140* B. occidentalis *and 100* B. glabrata *snails using LE and SJ strains. Two groups of* B. occidentalis *were killed after seven day-miracidia exposure to detect* S. mansoni *DNA, through the low stringency polymerase chain reaction (LS-PCR), and were negative. The infection rates were 69.2% (LE strain) and 96.7% (SJ strain) for* B. glabrata *and 0% for* B. occidentalis. *LS-PCR enabled early resistance diagnosis.*

Key words: *Schistosoma mansoni* - *Biomphalaria occidentalis* - resistance - Minas Gerais

Among the ten mollusc species and one subspecies of the *Biomphalaria* genus (Pulmonata: Planorbidae), which are present in Brazil, three are recognized as *Schistosoma mansoni* intermediate hosts: *B. glabrata*, *B. tenagophila* and *B. straminea*. *B. amazonica* and *B. peregrina* are experimentally susceptible to the trematode (Corrêa & Paraense 1971, Paraense 1973). Among the five resistant species to *S. mansoni* infection, it was included *B. occidentalis* Paraense, 1981, very similar to *B. tenagophila* by shell morphology.

B. occidentalis occurrence in Varzea das Flores dam has been recently reported (Lima et al. 1993). The Varzea das Flores dam, located in Contagem municipality, in Belo Horizonte microregion, MG, Brazil, is a leisure area used by bathers and for sporting competitions, mainly swimming tournaments. Malacological surveys were undertaken in the tributary streams and dam of this region in 1996 and 1997 by the Laboratório de Malacologia of Centro de Pesquisas René Rachou with the collaboration of the technicians from National Health Foundation. The study was performed to detect the presence of schistosomiasis vector molluscs in the dam region. The total of collected snails was: 180 specimens of *B. glabrata*, 35 of *B. occidentalis*, 17 of *Melania tuberculata* and 3 of *Pomacea* sp. The occurrence of these mollusc genus in Varzea das Flores dam was previously reported by Lima et al. (1993). Ten *B. occidentalis* snails, after the identification of the species (Paraense 1981), were placed into an aquarium for rearing. Afterwards, a hundred specimens, with 8-10 mm diameter, were individually exposed to 50 miracidia of the LE *S. mansoni* strain, from Belo Horizonte, MG and to 50 miracidia of the SJ strain, from São José dos Campos, SP, more adapted to *B. tenagophila* (Paraense & Corrêa 1978). Simultaneously, two *B. glabrata* groups with 50 specimens each were individually exposed to 20 miracidia, of each strain, per mollusc.

After 30 days, the molluscs from each group were individually exposed to artificial light and examined under stereomicroscope, to detect snails which were shedding cercariae. They were then examined weekly until 52 days after exposure, when negative snails were submitted to squeezing. In June 1999, a new collect of *B. occidentalis* was undertaken in the same place, and after species identification they were placed into the aquarium for rearing. From the descendants, two groups of 20 specimens each, were individually exposed to 50 miracidia of the LE and SJ strains. After seven

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day-exposure to miracidia, snails were killed for detection of *S. mansoni* DNA through the low stringency polymerase chain reaction (LS-PCR), described by Dias Neto et al. (1993) and used for diagnosing *S. mansoni* infection in *B. glabrata* (Jannotti Passos et al. 1997). The results of the first and second experiments are shown in the Table and in the Figure. The gel was silver stained as described by Sanguinetti et al. (1994).

The miracidia of both *S. mansoni* strains penetrated in the snails of the two *Biomphalaria* species. *B. glabrata* were infected with both strains of the trematode, shedding cercariae (Table). *B. occidentalis* did not shed cercariae showing resistance to the infection with the trematode strains (Table). The parasite DNA profile was not detected in the molluscs after seven day-exposure (Figure). The characteristic *S. mansoni* DNA profile is observed by the presence of a band ladder separated by approximately 62 bp (Figure). This pattern was just observed in the lanes two and three where *S. mansoni* DNA was present. In the lane three there is an infected *B. glabrata* and just *S. mansoni* DNA can be observed, as the primer was specifically designed for this trematode. In the lanes, where there was not *S. mansoni* DNA in the snails, a band complex was observed as the primers aligned less complementarily due to the low stringency alignment conditions of the reaction (Figure). The lack of parasite DNA indicates that although the miracidia had penetrated through the tentacles of the snails under the stereomicroscope, they did not develop and after seven days they had already been destroyed by the internal defence system of the mollusc.

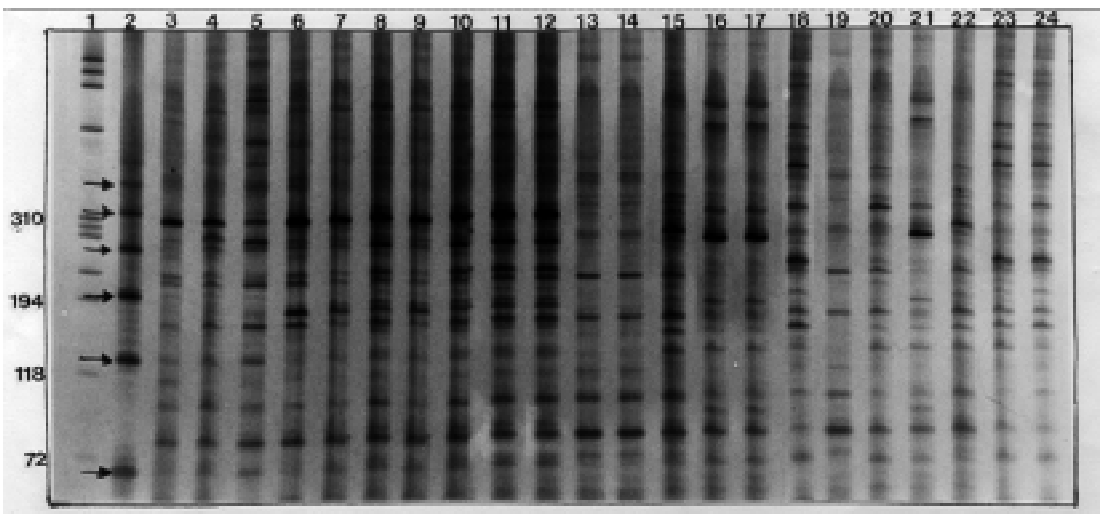
These results are in accordance with those observed for *B. occidentalis* from ten localities in the states of Mato Grosso do Sul, Paraná and São Paulo

TABLE

Experimental infection of *Biomphalaria occidentalis* from Várzea das Flores dam, Contagem, MG, and *B. glabrata* group with two *Schistosoma mansoni* strains

	Mollusc					
Genus and species	Number exposed	Diameter (mm)	Number of infected	Infection rate (%) [a]	Mortality rate (%) [a]	*S. mansoni* strain
B. occidentalis	50	8-10	0	0	38	LE
B. glabrata	50	8-10	27	69.2	22	LE
B. occidentalis	50	8-10	0	0	32	SJ
B. glabrata	50	8-10	30	96.7	38	SJ

a: mortality and infection rates after 52 day-exposure to miracidia



Silver stained 6% polyacrylamide gel showing low stringency polymerase chain reaction (LS-PCR) products obtained with specific primers ER(5'ACCTACCGTACTATGACG) and EF(5'GGTTTCTTAGTGTTATAGCC) for the mtDNA minisatellite and DNA. Lane 2: *Schistosoma mansoni* cercariae; lane 3: infected *Biomphalaria glabrata;* lanes 4 to 13: negative *B. occidentalis* seven days after *S. mansoni* exposure (strain LE); lanes 14 to 23: negative *B. occidentalis* seven days after *S. mansoni* exposure (strain SJ); lanes 24 and 25: negative *B. occidentalis* that were not exposed to *S. mansoni*. Lane 1: DNA size markers (f X174 restriction enzyme *Hae* III digestion)

and also for specimens from Acre and Mato Grosso, which did not shed cercariae after experimental infection (Paraense & Corrêa 1982, Coimbra Jr & Engel 1982). All these populations showed to be resistant to infection with Brazilian *S. mansoni* strains. Hence, the LS-PCR technique performed parallelly to snails light exposure, have confirmed the resistance of *B. occidentalis* from Minas Gerais to two *S. mansoni* strains. This method showed that the parasite was destroyed at the first week after its penetration in the snail, enabling the early diagnose of the resistance.

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