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BRIEF COMMUNICATION

SUSCEPTIBILITY OF *Biomphalaria tenagophila* AND *Biomphalaria straminea* TO *Schistosoma mansoni* INFECTION DETECTED BY LOW STRINGENCY POLYMERASE CHAIN REACTION

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SUMMARY

In order to determine *Schistosoma mansoni* infection rates in *Biomphalaria tenagophila* and *B. straminea*, low stringency polymerase chain reaction (LS-PCR) technique was used as a complementary method to light exposure technique. LS-PCR has already been standardized in our laboratory to detect the trematode DNA in *B. glabrata*. Higher *S. mansoni* infection rates were detected using conventional method and LS-PCR. The parasite DNA profile was detected in both species after 7-day exposure to miracidia, using LS-PCR. This technique enables early detection of schistosomiasis transmission focuses, in endemic areas, before the beginning of cercariae shedding.

KEYWORDS: *Schistosoma mansoni*; *Biomphalaria tenagophila*; *Biomphalaria straminea*; Susceptibility; LS-PCR technique

INTRODUCTION

In Brazil, the three *Biomphalaria* species found naturally infected by *Schistosoma mansoni* are: *Biomphalaria glabrata, B. straminea* and *B. tenagophila*. *Biomphalaria tenagophila* plays an important role in the transmission of schistosomiasis through the south and southeastern regions of Brazil. *Biomphalaria straminea* is present from the north to the south of the country, being an important schistosomiasis vector in the northeastern region, in spite of the low natural infection rates in these snails[8,9].

The current methods to detect *S. mansoni* infection in *Biomphalaria* are: exposition of the snails to light and squeezing them between two glass slides. The first method does not detect the infection during the prepatent period and it is also necessary snails manipulation during an indefinite time in the laboratory. In *B. straminea* and *B. tenagophila* species the prepatent period is longer, once it naturally occurs a delay on *S. mansoni* development. Thus, the conventional techniques are not suitable for detecting *S. mansoni* in these less susceptible species, due to the long time spent and the mortality of the snails[10]. *Biomphalaria straminea* and *B. tenagophila* infection rates, in experimental infections, according to several authors, are normally lower[1,2,3,13,14,16], probably due to the snails mortality before eliminating cercariae. Facing the limitations of the traditional diagnosis methods, other faster and more efficient techniques have been developed based on the detection of the parasite DNA profile in *B. glabrata*[5,6,7].

The aim of the current work was to verify whether the low stringency polymerase chain reaction (LS-PCR) technique, already standardized in our laboratory[7] to detect *S. mansoni* DNA in *B. glabrata*, would also detect the parasite in other *Biomphalaria* species, once their profile are different from *B. glabrata*[17]. Another objective was to detect the total *S. mansoni* infection rate in *B. tenagophila* and *B. straminea* using light exposure and LS-PCR.

MATERIAL AND METHODS

A hundred specimens of *B. straminea* from Paracatu, MG, and a hundred of *B. tenagophila* from Pampulha lake Belo Horizonte, MG, reared in laboratory, were individually exposed to 50 *S. mansoni* miracidia of the LE strain, from Belo Horizonte. After 7 days of exposure, twenty snails of each species were killed and used for DNA extraction. After days 30, 37 and 42 post exposition, the snails were examined after light exposure and the ones shedding cercariae were separated. The DNA was extracted from snails after 7 days of exposure to miracidia, positive and negative snails, snails 42-day after exposure and control group. The DNA extraction was performed using Wizard genomic DNA purification kit (Promega). For polymerase chain reaction (PCR), one ng of template DNA was amplified using 0.8 units of *taq* polymerase (Cenbiot, RS, Brazil), 200 mM dNTP's, 1.5 mM $MgCl_2$, 50 mM KCL, 10mM Tris-HCl, pH 8.5, and 5 pmol of each primer in a final volume of 10 µl. The pair of primers used in these reactions was designed to amplify across adjacent tandem minisatellite units from *S. mansoni* mtDNA[11]. DNA

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Table 1
Infection rates (No. and percentages) of *Biomphalaria straminea* and *B. tenagophila* with *Schistosoma mansoni* detected by light exposure and LS-PCR techniques

Genus Species	Snails exposed to Miracidia	Snails alive after 42 days of miracidia exposure	Infection rate (%)		
			Light exposure	LS-PCR	Total
B. straminea	80	20	4 (20.0)	7(35.0)	11 (55.0)
B. tenagophila	80	71	32 (45.0)	16 (22.5)	48 (67.6)

amplification was performed using a MJR thermocycler with the same program described in our previous work[7].

RESULTS AND DISCUSSION

The infections rate using LS-PCR in 20 snails of each species, killed 7 days after miracidia exposition, were 15.0% for *B. straminea* and 50.0% for *B. tenagophila*.

The infection rates obtained by exposing 80 *B. straminea* and 80 *B. tenagophila* to light until 42 days after miracidia exposure were 20.0 and 45.0%, respectively (Table 1). When the LS-PCR technique was performed using the specimens, which have not been shedding cercariae until 42 days of exposure, the infection rates increased for 55.0 and 67.6%, respectively (Table 1). The Figs. 1A and B represent the DNA profiles of *B. straminea* and *B. tenagophila* infected with *S. mansoni* after 7 days of exposure to miracidia (lanes 5 to 7), snail shedding cercariae (lane 1), negative snail (lane 2), snails exposed to miracidia that were not infected (lanes 3 and 4), snails after a 42- day exposure that did not shed cercariae, but showed the presence of *S. mansoni* (lanes 8 to 10), and *S. mansoni* cercariae (lane 11). The obtained PCR gel electrophoresis profiles (Figs. 1A and B: lanes 1 and 5 to 11) allowed the detection of the parasite by the presence of bands far from each other approximately 62 bp, correspondent to *S. mansoni* mtDNA amplification (indicated by arrow). The characteristic band standard of the amplified *S. mansoni* mtDNA was not observed in *B. straminea* and *B. tenagophila*, exposed to miracidia and did not shed cercariae (Figs. 1A and B lane 3 and 4), and in negative snails (Figs. 1A and B lane 2). In these lanes we can observe the band complex correspondent to the random annealing of the primers due to the low stringency conditions of the LS-PCR reactions. Those low stringency conditions allow an intern control of the reactions avoiding the necessity of a second pair of primer, which enables the standardization of the technique.

SOUZA *et al*.[15] studied *B. straminea* and *B. tenagophila* exposed to miracidia and could observe inflammatory diffused reactions with encapsulated sporocysts by amebocytes. This cellular reaction suggests a low susceptibility and a delay on the parasite development into the snail, which increases the prepatent period making the diagnosis through light exposure very difficult. LS-PCR technique was able to detect *S. mansoni* infection in *B. straminea* and *B. tenagophila* in the prepatent period, 7 days after exposure to miracidia. When both detecting methods were used, it was possible to obtain a higher *S. mansoni* infection rate (Table 1). In susceptible *B. glabrata* specimens the infection rate was approximately 90.0% using light exposure technique to detect *S. mansoni*. However, in partially resistant *B. glabrata*, small sporocysts in ectopic regions[12] were observed as well as a delay on *S. mansoni* development, with late elimination of the cercariae until seven months after miracidia exposition[4], similar to *B. tenagophila* and *B. straminea* snails.

Therefore, LS-PCR technique can be used as a complementary tool to the light exposition method in field snail studies, for the three host species, once it detects *S. mansoni* DNA presence, enabling to distinguish the infected specimens from other trematode infections. The presence of a background parasite's DNA derived bands are detectable clearly in the three species of infected *Biomphalaria* using LS-PCR after seven day exposure, despite the extensive interspecific genomic variation in these species[17] .

This technique may also be used for early detection of schistosomiasis transmission focuses in the field before cercariae elimination, avoiding larvae contamination of the watercourses and the transmission to man, rodent and other hosts in endemic regions.

RESUMO

Suscetibilidade de *Biomphalaria tenagophila* e *Biomphalaria straminea* a infecção por S*chistosoma mansoni* detectada pela reação em cadeia da polimerase em baixa estringência

Para determinar a taxa de infecção pelo *Schistosoma mansoni* em *Biomphalaria tenagophila* e em *B. straminea* foi utilizada a reação em cadeia da polimerase em baixa estringência (LS-PCR), como técnica complementar ao método de exposição à luz. A LS-PCR já foi padronizada no nosso laboratório para detectar o DNA do trematódeo em *B. glabrata*. A taxa de infecção pelo *S. mansoni* foi maior quando utilizou-se a técnica convencional e a LS-PCR. O perfil do DNA do parasita foi detectado após 7 dias de exposição a miracídios em ambas as espécies, quando utilizou-se a LS-PCR. Esta técnica possibilita a detecção precoce de focos de transmissão, em áreas endêmicas, antes do início da eliminação de cercárias.

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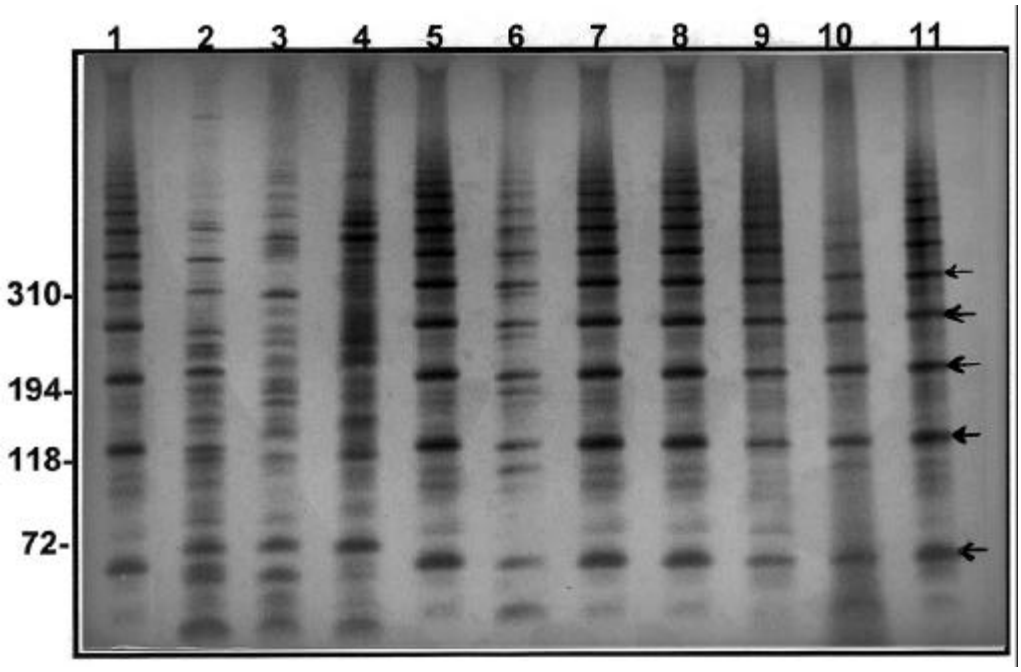

Fig. 1A - Silver stained 6% polyacrylamide gel showing Low Stringency Polymerase Chain Reaction (LS-PCR) products obtained with specific primers ER(5'ACCTACCGTACTATGACG) and EF(5'GGTTTCTTAGTGTTATAGCC) for the mtDNA minisatellite and DNA from: lane 1: *Biomphalaria straminea* shedding *Schistosoma mansoni* cercariae; lane 2: negative *B. straminea*; lanes 3 and 4: negative *B. straminea* to miracidia exposure; lanes 5 to 7: positive *B. straminea* 7 days after *S. mansoni* exposure; lanes 8 to 10: positive *B. straminea* 42 days after *S. mansoni* exposure; lane 11: *S. mansoni* cercariae. Molecular size markers are shown on the left of the gel.

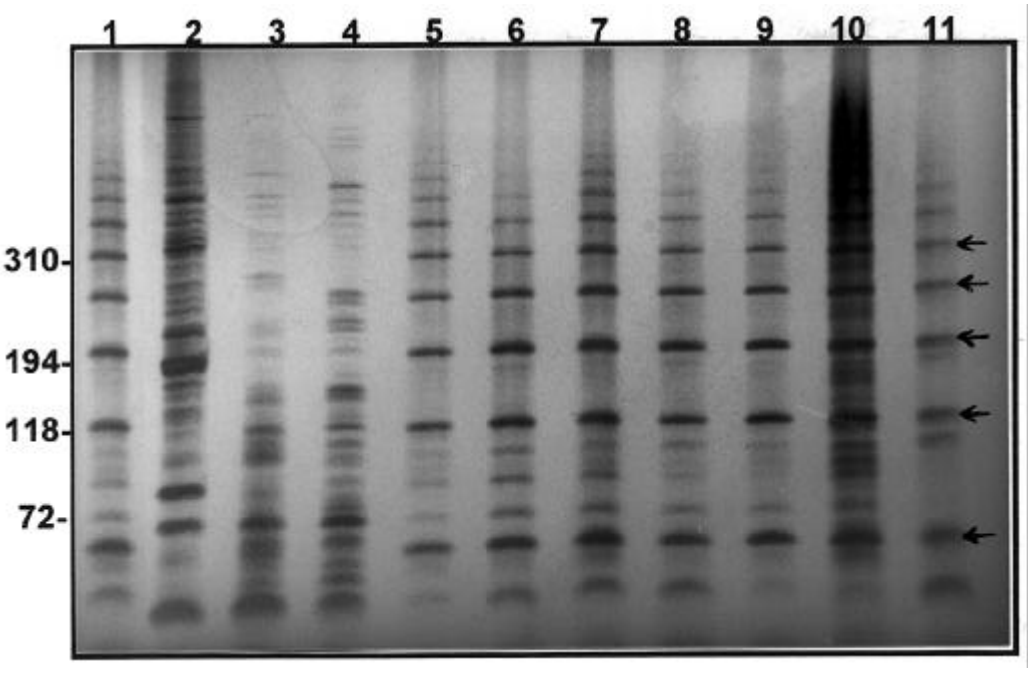

Fig. 1B - Silver stained 6% polyacrylamide gel showing LS-PCR products obtained with specific primers ER (5'ACCTACCGTACTATGACG) and EF (5'GGTTTCTTAGTGTTATAGCC) for the mtDNA minisatellite and DNA from lane 1: *B. tenagophila* shedding *S. mansoni* cercariae; lane 2: negative snail; lanes 3 and 4: negative *B. tenagophila* after miracidia exposure; lanes 5 to 7: positive *B. tenagophila* 7 days after *S. mansoni* exposure; lanes 8 to 10: positive *B. tenagophila* 42 days after *S. mansoni* exposure; lane 11: *S. mansoni* cercariae. Molecular size markers are shown on the left of the gel.

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