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Molecular detection of prepatent *Schistosoma mansoni* infection in *Biomphalaria glabrata* snail vectors

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ABSTRACT

Approximately 240 million people worldwide are infected by *Schistosoma.* In Brazil, one of the main intermediate hosts of this parasite is *Biomphalaria glabrata* snails. The early detection of larval stages in intermediate hosts is an important challenge to public health, but it also represents an opportunity as a new alternative to indicate earlier natural infections before cercariae differentiation and emergence. In this context, we demonstrated that PCR amplification of a 28S gene fragment from the parasite does demonstrate *S. mansoni* infection in snails 14 days post infection. This conventional polymerase chain reaction amplified clear bands and was able to detect parasitic infection in the intermediate host *B. glabrata* under experimental conditions. However, we reinforce that this approach requires deeper investigations and further comparisons to confirm its specificity and sensitivity in earlier time points after miracidia infection. This approach has relevant potential as an effective molecular-based strategy for the monitoring of schistosomiasis transmission.

KEYWORDS: *Schistosoma mansoni*. *Biomphalaria glabrata.* Molecular diagnosis. Surveillance. Snail. Vector. Molecular diagnosis. Polymerase Chain Reaction.

INTRODUCTION

Approximately 240 million people worldwide are infected by *Schistosoma* trematodes. In Brazil, one of the main intermediate hosts of this parasite is the *Biomphalaria glabrata* snail[1-3]. The early detection of larval stages in intermediate hosts is an important challenge to public health, but it has the potential to detect positive snail hosts before the emergence of cercariae, thus becoming a new alternative to detect intramollusc forms[4,5], revealing reliable parasite prevalence rates. The objective of this study is to evaluate the prepatent detection of *S. mansoni* DNA in *B. glabrata* snails using the polymerase chain reaction (PCR) 14 days after snails exposed to miracidia. Furthermore, we aimed to standardize the entire process, evaluating the specificity of the primers in two groups of snails.

MATERIAL AND METHODS

Laboratory maintenance of the *S. mansoni* experimental cycle

The laboratory cycle of *S. mansoni* has been implemented more than 30 years ago at the Instituto de Medicina Tropical de Sao Paulo, using a *S. mansoni* strain

obtained in Belo Horizonte, Minas Gerais, Brazil (BH strain), as well as a *B. glabrata* strain (GIDE), collected in Campinas, Sao Paulo, Brazil.

Since the beginning, the *S. mansoni* cycle was maintained through periodic infections of hamsters (*Mesocricetus auratus*) and *B. glabrata* snails at the Laboratory of Schistosomiasis Immunopathology (LIM-06). Hamsters were kept in the animal house of of Instituto de Medicina Tropical de Sao Paulo, Universidade de Sao Paulo, Brazil, and all the experiments followed the Ethical Principles in Animal Experimentation of the Brazilian College of Animal Experimentation (CEP IMT-2011/096).

Infection of *B. glabrata* by *S. mansoni*

Approximately 30 miracidia were obtained from a laboratory cycle of *S. mansoni* and were counted on Kline plates. Ten *B. glabrata* (BH) snails were infected and compared to ten non-infected snails to evaluate the primers' specificity to *S. mansoni* DNA, and the presence of spurious non-specific bands derived from the snail host. A total of ten *B. glabrata* snails were exposed to approximately 30 miracidia and had their head-foot portions removed 14 days after this exposure. DNA was extracted soon after removing the snail's head and foot, using the QIAamp® DNA Mini Kit (QIAGEN Inc., Hilden, USA), following the manufacturer's instructions. DNA was quantified in a Nanodrop DR 1000 Spectrophotometer V3.8® (Thermo Fisher Scientific, Delaware,USA).

Amplification of *S. mansoni* DNA from primary sporocysts

PCR amplification was performed with the following *S. mansoni*-specific primers: SmF (5'-GAGATCAAGTGTGACAGTTTTGC-3') and SmR (5'-25ACAGTGCGCGC GTCGTAAGC-3'), targeting the parasite 28S rDNA , yielding a 350-base pairs amplicon, as previously described by Sandoval *et al*.[6].

PCR amplifications were performed in a final volume of 25 µL containing 2.5 µL 10 X reaction buffer, 3 mM $MgCl_2$, 0.5 mM of each dNTP, 0.2 mM of each primer, 2.5 U of Platinum Taq DNA polymerase (Invitrogen TM Life Technologies, Carlsbad, CA, USA) and 1 µL of DNA. The amplification consisted of 35 cycles, as follows: 94 °C for 20 s, 65 °C for 20 s, and 72 °C for 30 s. Amplicons were evaluated in 2% SYBR-safe-stained agarose gels and visualized under UV lights. DNA derived from adult *S. mansoni* worms (90 ng) and ultrapure Milli-Q water were used as positive and negative controls, respectively.

The possibility of samples contamination was minimized by the physical separation of rooms for DNA extraction and DNA amplification and by using laminar flow hoods, ultraviolet light chambers, and sterile, disposable laboratory materials including pipette filter tips.

RESULTS AND DISCUSSION

This study evaluated infection in the intermediate host by using a molecular biology technique.The visualization of the expected size amplicons, corresponding to *Schistosoma mansoni* 28S rDNA gene showed single amplicons for the positive control as well as for the infected snails, suggesting its specificity to the parasite DNA; in addition, no bands were seen when DNA from the non-infected snails or water were used as templates.

The amplification products of *S. mansoni* DNA extracted together with DNA from the snail host showed clear and specific bands 14 days after miracidia infection, indicating that the lower mass of sporocyst-derived DNA was enough to provide positive results (Figure 1).

Detection of *Schistosoma* DNA in the prepatent period, with different parasite loads, was first described by Jannotti-Passos *et al*. in 1997[5], using a tandem repeated sequence of the parasite genome as the amplification target region. Subsequently, the same group reported the molecular identification of the parasite, targeting a mitochondrial DNA repeat in *S. mansoni* that allowed its detection in infected *B. glabrata*, with absence of non-specific (spurious) bands. This result points to the selectivity and specificity of this marker in the previous identification of infected snail hosts[4,7]. Since then, other studies aimed at performing the molecular diagnosis of infection in intermediate hosts have been developed and employed the loop-mediated isothermal amplification[4,5,8].

Currently, the Brazilian surveillance control program recommends a conventional method to diagnose *S. mansoni* infections, in which snails suspected of being infected are exposed to light to stimulate cercariae release[9]. Cercariae are usually observed approximately 40 days after miracidia infection, at the end of this parasitic asexual cycle[3].

The implementation of sensitive diagnostic tools to monitor the parasitic infection status in humans, mammalian reservoirs and intermediate hosts is part of the strategies discussed and recommended by WHO[10].

Thus, the combination between traditional and molecular methods will help to improve the malacological surveillance of *Schistosomiasis mansoni*, especially in areas of low endemicity.

This study corroborates this point of view by improving diagnosis in the prepatent stage of infection in snails.

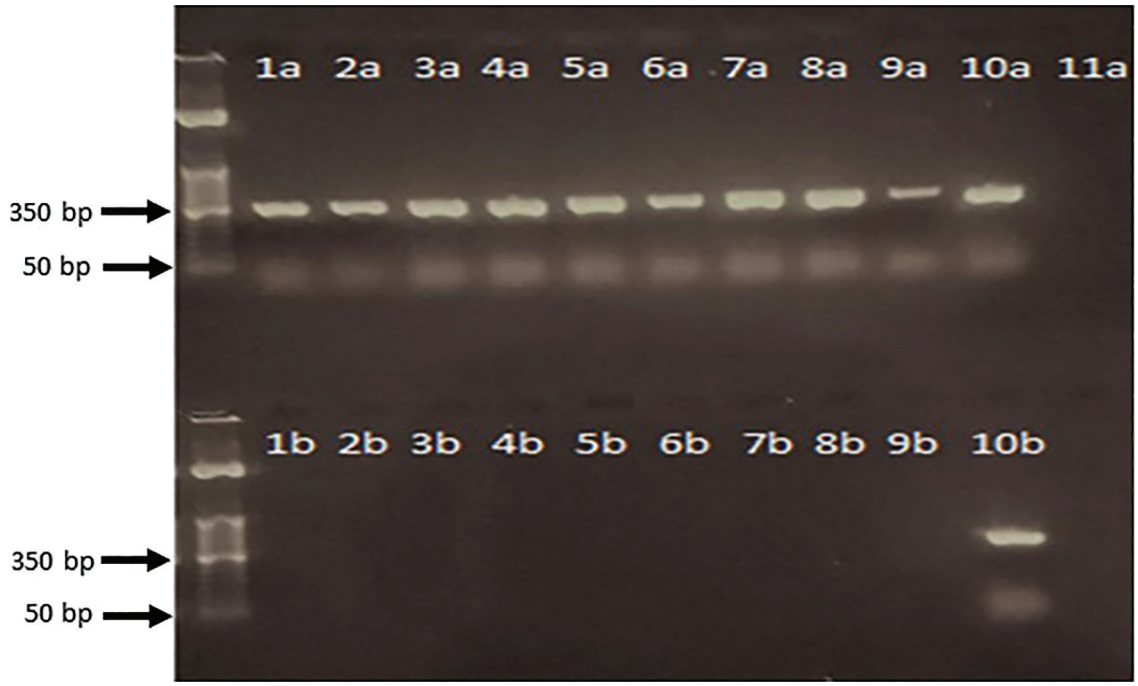

Figure 1 - The 350 bp amplification product from *S. mansoni* 28S rDNA gene using as template, DNA extracted from *B. glabrata* snails 14 days after their exposure to ~ 30 miracidia (1a to 10a) and *B. glabrata* snails not exposed to experimental infection (1b to 9b). 11a: negative control (ultrapure Milli-Q sterile water); 10b: positive control (DNA from adult *S. mansoni* worms). Molecular weight marker: 50 bp DNA ladder (Invitrogen TM Life Technologies Carlsbad, CA, USA).

Schistosomiasis is a chronic infection that may not progress to a severe form but can trigger debilitating sequelae that are secondary to parasitism[11]. Therefore, surveillance is critical in endemic areas. Nevertheless, considering that most infected snails can die during the vector surveillance process and that conventional techniques are efficient only for live snails after the completion of the period necessary for cercariae emergence, the prevalence of infection can be underestimated. These factors emphasize the need of a correct diagnosis prior to this stage, especially in areas of low endemicity, in which infections generally present low parasite loads[5,12,13].

It is known that *B. glabrata* snails are the most susceptible intermediate host of this trematode compared to other natural snail hosts, and the implementation of sensitive diagnostics is a crucial and valuable tool to monitor parasitic infection status in humans, mammalian reservoirs, and intermediate snail hosts.

CONCLUSION

The results presented here reinforce that conventional PCR can be used to detect the presence of *S. mansoni* infection in the prepatent period, suggesting that this methodology is a sensitive and specific tool that is able to detect the parasite infection in intermediate hosts approximately 15 days before the traditional techniques.

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AUTHORS' CONTRIBUTIONS

Conceived and designed the study: MCCES, MOC, EJAL, RT, MGG, RCBG; contributed with analyses/tools: FMP, JRRP; wrote the paper: MCCES, MOC. All authors have read and approved the manuscript.

CONFLICT OF INTERESTS

The authors have declared that there is no conflict of interests.

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REFERENCES

1. Colley DG, Bustinduy AL, Secor WE, King CH. Human schistosomiasis. Lancet. 2014;383:2253-64.
2. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Plano integrado de ações estratégicas de eliminação da hanseníase, filariose, esquistossomose e oncocercose como problema de saúde pública, tracoma como causa de cegueira e controle das geohelmintíases: plano de ação 2011-2015. Brasília: Ministério da Saúde; 2012. [cited 2020 Feb 10]. Available from: http://bvsms.saude.gov.br/bvs/publicacoes/plano_integrado_acoes_estrategicas_2011_2015.pdf
3. World Health Organization. Sixty-Sixth World Health Assembly: agenda item 16.2: neglected tropical diseases. Geneva: WHO; 2013. [cited 2020 Feb 10]. Available from: https://www.who.int/neglected_diseases/mediacentre/WHA_66.12_Eng.pdf
4. Gandasegui J, Fernández-Soto P, Hernández-Goenaga J, López-Abán J, Vicente B, Muro A. Biompha-LAMP: a new rapid loop-mediated isothermal amplification assay for detecting Schistosoma mansoni in Biomphalaria glabrata snail host. PLoS Negl Trop Dis. 2016;10:e0005225.
5. Jannotti-Passos LK, Vidigal TH, Dias-Neto E, Pena SD, Simpson AJ, Dutra WO, et al. PCR amplification of the mitochondrial DNA minisatellite region to detect Schistosoma mansoni infection in Biomphalaria glabrata snails. J Parasitol. 1997;83:395-9.
6. Sandoval N, Siles-Lucas M, Lopez Aban J, Pérez-Arellano JL, Gárate T, Muro A. Schistosoma mansoni: a diagnostic approach to detect acute schistosomiasis infection in a murine model by PCR. Exp Parasitol. 2006;114:84-8.
7. Allienne JF, Théron A, Gourbal B. Recovery of primary sporocysts in vivo in the Schistosoma mansoni/Biomphalaria glabrata model using a simple fixation method suitable for extraction of genomic DNA and RNA. Exp Parasitol. 2011;129:11-6.
8. Hamburger J, He-Na, Xin XY, Ramzy RM, Jourdane J, Ruppel A. A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (Schistosoma mansoni) from very early prepatency. Am J Trop Med Hyg. 1998;59:872-6.
9. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Vigilância e controle de moluscos de importância epidemiológica. 2ª ed. Brasília: Ministério da Saúde; 2008. [cited 2020 Feb 10]. Available from: https://portalarquivos2.saude.gov.br/images/pdf/2015/agosto/14/vigilancia-controle-moluscos-import-epidemio-2ed.pdf
10. World Health Organization. Sixty-Fifth World Health Assembly: Geneva, 21-26 May 2012: resolutions and decisions, annexes. Geneva: WHO; 2012. [cited 2020 Feb 10. Available from: http://apps.who.int/gb/DGNP/pdf_files/A65_REC1-en.pdf
11. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop. 2000;77:41-51.
12. Guaraldo AM, Magalhães LA, Rangel HA, Pareja G. Evolução dos esporocistos de Schistosoma mansoni Sambon, 1907 em Biomphalaria glabrata (Say, 1818) e Biomphalaria tenagophila (D'Orbigny, 1835). Rev Saude Publica. 1981;15:436-48.
13. Martins-Melo FR, Pinheiro MC, Ramos Jr AN, Alencar CH, Bezerra FS, Heukelbach J. Trends in schistosomiasis-related mortality in Brazil, 2000-2011. Int J Parasitol. 2014;44:1055-62.