

Case Report

Resistance of *Biomphalaria tenagophila* (Mollusca: Gastropoda) From The State of Espírito Santo, Brazil to *Schistosoma mansoni* (Platyhelminthes: Trematoda) Infection

Roberta Lima Caldeira^{1*}, Liana Konovaloff Jannotti-Passos², Tatiana Maria Teodoro³, and Omar dos Santos Carvalho¹

¹Grupo de Pesquisa em Helminthologia e Malacologia Médica, Instituto René Rachou/Fiocruz Belo Horizonte, MG, Brazil

²Moluscário “Lobato Paraense”, Instituto René Rachou/Fiocruz Belo Horizonte, MG, Brazil

³Departamento de Entomologia do Instituto Aggeu Magalhães/Fiocruz Recife, Pernambuco, Brazil

*Corresponding author

Roberta Lima Caldeira, Instituto René Rachou/Fiocruz Belo Horizonte, MG, Brazil, Av. Augusto de Lima, 1715 – Barro Preto, 30190-009 Belo Horizonte/MG, Brazil; Tel: +55-31-3349-7746; Fax: +55-31-3295-3115; Email: caldeira@minas.fiocruz.br

Submitted: 28 April 2018

Accepted: 15 May 2018

Published: 17 May 2018

ISSN: 2373-9282

Copyright

© 2018 Caldeira et al.

OPEN ACCESS

Keywords

- *Biomphalaria tenagophila*
- Resistance
- *Schistosoma mansoni*
- LS-PCR

Abstract

The susceptibility of *Biomphalaria tenagophila* from Espírito Santo (ES), Brazil was evaluated. Specimens F1 of *B. tenagophila* (ES) and *B. glabrata* (MG) control, were individually exposed to miracidia of the LE, SJ and AL strains. The snails were examined 30 days after the exposure and weekly thereafter for a period of 80 days. The snails that died during the experiment were subjected to the low-stringency polymerase chain reaction (LS-PCR) technique to detect *Schistosoma mansoni* DNA. The infection rate for *B. tenagophila* (ES) was 0% to all strains used, whereas the rates for *B. glabrata* were 81, 90 and 94.3% for the SJ, AL and LE strains, respectively. *B. tenagophila* snails that died during the experiments were not positive for *S. mansoni*, while all of the *B. glabrata* were positive for *S. mansoni*. In addition, to verify if the miracidia had penetrated in the molluscs and how long it was eliminated, *B. tenagophila* specimens were exposed to miracidia and ten snails were sacrificed at different times and subjected to LS-PCR. Forty-eight hours after the exposure, *S. mansoni* was not detected in *B. tenagophila*. We concluded that this *B. tenagophila* population is resistant to infections of *S. mansoni* (100 miracidia/snail).

ABBREVIATIONS

ES: Espírito Santos; LE: Luis Evangelista; SJ: São José dos Campos; AL: Alagoas; CMM-Fiocruz: Medical Malacology collection from Fundação Oswaldo Cruz; LS-PCR: low stringency polymerase chain reaction; PCR-RFLP: polymerase chain reaction and analyses of the restriction fragment-length polymorphism; ITS: internal transcribed spacer; DNA: deoxyribonucleic acid; rDNA: ribosomal deoxyribonucleic acid.

INTRODUCTION

Biomphalaria glabrata (Say, 1818), *B. tenagophila* (Orbigny, 1835), *B. straminea* (Dunker, 1848) are found naturally infected by the trematode *Schistosoma mansoni* (Sambon, 1907), the agent of schistosomiasis. *Biomphalaria peregrina*, *B. amazonica* and *B. cousini* were reported as potential hosts of the parasite since they had been infected experimentally [1-4].

Schistosoma - has a complex life cycle comprising sexual reproductive stage in the definitive mammalian host and asexual reproductive stage in the snail intermediate host.

Studies of the host-parasite interaction using the relationship between *B. glabrata* and *S. mansoni* as a model have demonstrated this species shows a varied degree of susceptibility to this trematode infection [5-6]. This susceptibility of the planorbid snails to *S. mansoni* infection is a trait genetically controlled and inherited over generations [7], being controlled both by parasite and snail genes [8].

The snail *B. tenagophila* exhibits populations that are susceptible to *S. mansoni* infection [5] and a population that is resistant from the Taim Ecological Reserve, Rio Grande do Sul - RS. In the laboratory this population has been exposed by different strains of *S. mansoni* with a variable number of miracidia and was always resistant to infection [9-13].

A range of studies has already been conducted to improve our understanding of the resistance of snails to trematode infections [14-17]. The snail internal defense system comprises hemocytes and soluble factors in the hemolymph. These two components act together in the snail defense, thus defining those molluscs susceptible and/or resistant to pathogens [18]. Few studies have

defined whether uninfected snails are resistant to *S. mansoni* infection. Souza & Jannotti-Passos [19] showed that the parasite was destroyed at the first week after its penetration in the *B. occidentalis* by low stringency polymerase chain reaction (LS-PCR). Pimenta-Nacif et al. [20], focusing on the initial phase (from 1h to 10h after exposure) of the interaction process between the snails and the *S. mansoni* sporocysts, compared by histology, susceptible versus resistant *B. tenagophila* populations. They showed that even at the earliest time point (1h a.e), fibrous host cells of both snail populations were arranged as a thin layer around the sporocysts.

The diagnosis of infection in snails is also an important aspect since it is not viable to detect infection in the prepatent period and in dead snails using traditional methods, and also it is not possible to differentiate *S. mansoni* from other trematodes. Molecular techniques have been used to aid in the diagnosis regarding the infection of snails [21-23].

The aim of this study is to analyze *B. tenagophila* population from the state of Espírito Santo (Brazil) exposed to different *S. mansoni* strains and elucidate whether the population is naturally resistant to this trematode.

MATERIALS AND METHODS

Snails

Specimens from the F1 generation of *B. tenagophila* collected in the Mãe Bá Lagoon (geographical coordinates: 20°45'19"W, 46°34'29"S) were used. As an experimental control of infection and mortality, *B. glabrata* originating from Belo Horizonte, Minas Gerais (MG), Brazil, was used. For species identification, one of the tentacles of the snails (the snails were not sacrificed at this stage) was removed for DNA extraction using the Wizard Genomic DNA Purification kit (Promega) and subjected to polymerase chain reaction and analyses of the restriction fragment-length polymorphism (PCR-RFLP) using the rDNA internal transcribed spacer (ITS) with the enzyme DdeI [24,4]. The profiles were compared to the standard profiles of the DNA extracted from snails tissue from the Medical Malacology Collection (CMM-Fiocruz). These snails were maintained and raised in the "Lobato Paraense" Mollusc Rearing of René Rachou Institute - IRR/FIOCRUZ, in Belo Horizonte, MG, Brazil according to Jannotti-Passos et al. [25].

Experimental research on vertebrates and invertebrates have been approved by an appropriate ethics committee CEUA/Fiocruz LW30/13.

Parasites

The LE, SJ and AL strains of *S. mansoni* were used. The LE strain was isolated from a patient residing in Belo Horizonte, MG, and maintained in the "Lobato Paraense" Mollusc Rearing since 1968. The SJ strain was isolated from naturally infected snails from the region of São José dos Campos, São Paulo state (Brazil), maintained in the "Lobato Paraense" Mollusc Rearing since 1975. The AL strain was isolated in 1980 from *B. glabrata* from Alagoas state (Brazil). The maintenance of the cycles of *S. mansoni* strains was performed through successive passages in hamsters (*Mesocricetus auratus*) and *B. glabrata*, according to

the technique described by Pellegrino and Katz [26] and modified by Jannotti-Passos et al. [25].

Susceptibility experiments

A. To verify the susceptibility status of the snails, 100 *B. tenagophila* and 100 *B. glabrata* (infection control) were individually exposed to 100 and 10 miracidia/snail, respectively, of the LE/SJ/AL strains according to Jannotti-Passos et al. [25]. The snail diameters were 4-6mm for *B. tenagophila* and 6-8mm for *B. glabrata*, the same diameter standardized in the Mollusc Rearing to *S. mansoni* routine infections. A total of 25 snails from each species and with the same diameters, were used as a mortality control and not exposed to miracidia.

C. To verify if the miracidia had penetrated in the molluscs and how long it was eliminated 60 *B. tenagophila* and 60 *B. glabrata* (control) were individually exposed to 100 and 10 miracidia SJ (strains), respectively. At intervals of 1, 5, 10, 24, 36 and 48 hr after exposure, 10 snails from each species were sacrificed and subjected for molecular studies.

Examination of the snails

The snails were individually introduced into recipient dishes with 5mL of unchlorinated water, exposed to artificial light for 30 min and then taken to the stereoscope for observation of possible *S. mansoni* cercariae (experiment "A"). The first examination was performed 30 days after the exposure of the snails to the miracidia, and the other examinations were performed weekly for a total period of 80 days after exposure [25]. At the end of this period, the snails that survived were examined by crushing them between glass plates. The snails that died during the experiment and experiment "B" snails were subjected to DNA extraction using the Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's instructions, and subjected to the LS-PCR technique to verify the presence of *S. mansoni* DNA [22]. The pair of primers used in these reactions was designed to amplify across adjacent in tandem minisatellite units from *S. mansoni* mtDNA [22]. This procedure was repeated for all strains used and the infection and mortality rates were calculated for each experiment.

RESULTS AND DISCUSSION

The snail *B. tenagophila* is an important intermediate host of *S. mansoni* in southern Brazil [27], and a population of this snail from Taim reserve (RS) has been shown to be resistant to *S. mansoni* infection [9-13]. In this study, descendants of *B. tenagophila* from Espírito Santo state, were exposed to miracidia of the three *S. mansoni* strains. Infection was not observed in any of the exposed specimens. On the other hand, the infection rates for *B. glabrata* (control) were 81, 90 and 94% for the SJ, AL and LE strains, respectively. The mortality rates of *B. glabrata* were 8, 16 and 11% for the AL, SJ and LE strains, respectively, and for *B. tenagophila*, were 25, 21 and 20% for the AL, SJ and LE strains, respectively. The specimens of *B. tenagophila* that died during the experiments were examined by LS-PCR and showed no profile of *S. mansoni* (Figure 1, lane 3-11). In figure 1, the pair of primers seemed to be highly specific for this parasite, as the DNA derived from uninfected snails did not show the typical pattern obtained with mtDNA. While, the *B. glabrata* specimens

that died during the experiments exhibited the presence of a ladder-type arrangement of the bands, corresponding to the amplification of the tandem repeated region of the 62-bp mtDNA fragment, the characteristic profile of *S. mansoni* (Figure 3 lane 9). To date, the study of snails susceptible to *S. mansoni* infection has been performed through the exposure of snails to miracidia and examination by light exposure or crushing. However, these techniques do not detect infection in the prepatent period or died snails, making it impossible to know if the miracidia penetrated the snails. LS-PCR is an important technique in susceptibility studies because it is capable of detecting the DNA of this trematode in the prepatent period [22]. Souza et al. [19], used the LS-PCR technique and showed that there was no *S. mansoni* DNA in *B. occidentalis* after 7 days of exposure. That is, the miracidia penetrated and were destroyed by the defense system of the snail, which was therefore considered resistant to *S. mansoni* infection. In our study, using the LS-PCR technique, it was possible to observe the presence of the a ladder-type arrangement of the bands in *B. tenagophila* exposed to *S. mansoni* (SJ strain) after 1, 5, 10, 24 and 36 hr only (Figure 2, lanes 3-7) as well as in adult *S. mansoni* worm (Figure 2, lane 2). Moreover, it was possible to observe the presence of this profile in all *B. glabrata*, in all intervals of sacrifice after exposure tested (Figure 3 lanes 3-8). It not was possible to observe the presence of *S. mansoni* in negative *B. glabrata* (Figure 3, lane 10), in the *B. tenagophila* exposed to *S. mansoni* after 48 hr (Figure 2, lane 8) and negative *B. tenagophila* (Figure 2, lane 9).

The host-parasite relationship is complex and there are still gaps in the knowledge about the susceptibility and resistance of snails to trematode infection and the capacity of development of this trematode in the intra-snail stage. It is known that in *B. glabrata*, 30% of the miracidia are capable of penetrating and transforming to sporocysts, 30% penetrate but do not transform,

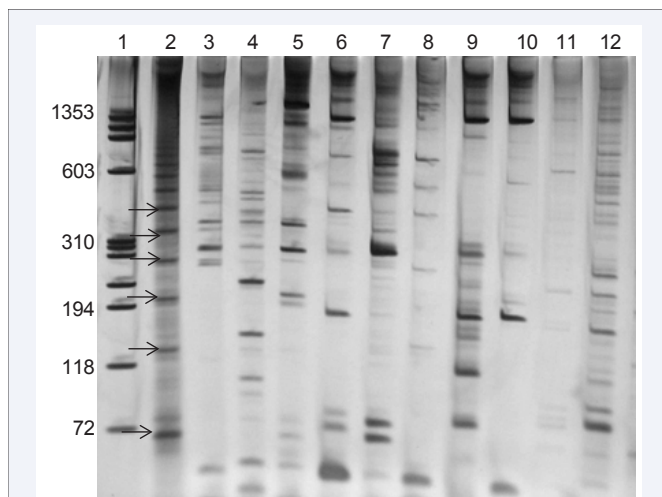


Figure 1 Silver stained polyacrilamide gel (6%) showing the profiles obtained by LS-PCR to detect the presence of *Schistosoma mansoni* in snails died during the experiment "A". Lane 1: Molecular-size marker phiX 174 digested with *HaeIII*; Lane 2: adult *Schistosoma mansoni* worm; Lanes 3-11: *Biomphalaria tenagophila* from Anchieta, Espirito Santo state (Brazil), exposed to miracidia that died during the experiment; Lane 12: negative *B. tenagophila*. Molecular-size markers are shown on the left of the gel.

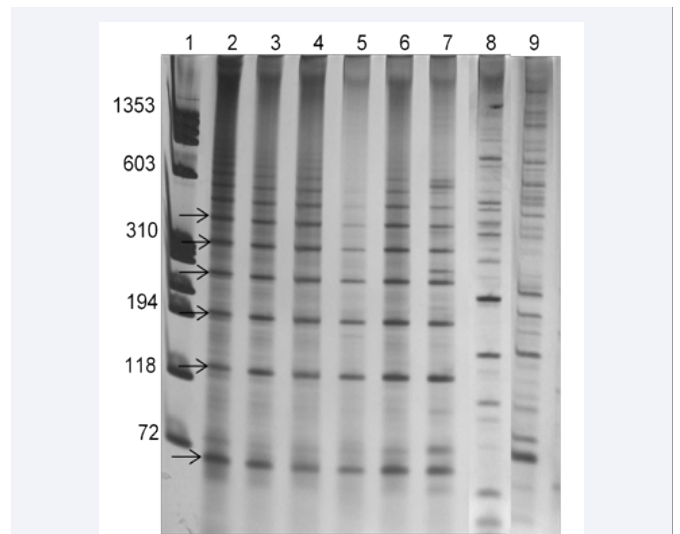


Figure 2 Silver stained polyacrilamide gel (6%) showing the profiles obtained by LS-PCR to detect the presence of *Schistosoma mansoni* in snails (experiment "B"). Lane 1: Molecular-size marker phiX 174 digested with *HaeIII*; Lane 2: adult worm *Schistosoma mansoni*; Lane 3: *Biomphalaria tenagophila* killed at intervals of 1 hr; Lane 4: *B. tenagophila* killed at intervals of 5 hours; Lane 5: *B. tenagophila* killed at intervals of 10 hr; Lane 6: *B. tenagophila* killed at intervals of 24 hr; Lane 7: *B. tenagophila* killed at intervals of 36 hr; Lane 8: *B. tenagophila* killed at intervals of 48 hr; Lane 9: negative *B. tenagophila*. Molecular-size markers are shown on the left of the gel.

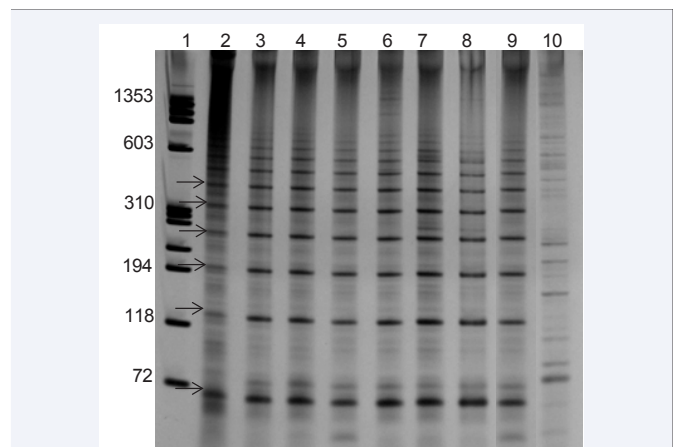


Figure 3 Silver stained polyacrilamide gel (6%) showing the profiles obtained by LS-PCR to detect the presence of *Schistosoma mansoni* in snails. Lane 1: Molecular-size marker phiX 174 digested with *HaeIII*; Lane 2: adult *Schistosoma mansoni* worm; Lane 3: *Biomphalaria glabrata* killed at intervals of 1 hr; Lane 4: *B. glabrata* killed at intervals of 5 hr; Lane 5: *B. glabrata* killed at intervals of 10 hr; Lane 6: *B. glabrata* killed at intervals of 24 hr; Lane 7: *B. glabrata* killed at intervals of 36 hr; Lane 8: *B. glabrata* killed at intervals of 48 hr; Lane 9: *B. glabrata* died during the experiment "A"; Lane 10: negative *B. glabrata*. Molecular-size markers are shown on the left of the gel.

and 40% are incapable of even penetrating the snail [28]. In resistant *B. glabrata* and *B. tenagophila*, the miracidia penetrate and are recognized as foreign bodies and destroyed by the immune defense system, composed of hemocytes and soluble factors present in the hemolymph, in the first hours following

penetration [16-17,20,28-30]. In refractory snails, the miracidia do not penetrate. In susceptible snails, the miracidia penetrate and develop within the snails, producing cercariae that are released to the external environment. According to Lewis et al. [8], parasite-host interactions are influenced by the snail genes that control susceptibility and the parasite genes that determine infectivity.

CONCLUSION

Biomphalaria tenagophila (ES) snails used in this study did not release cercariae after the exposure with *S. mansoni* miracidia and examination by light stimulation. Furthermore, the LS-PCR technique used detects the presence of *S. mansoni* DNA up to 36 hr after the exposure, demonstrating that the studied *B. tenagophila* population is resistant to strains used.

ACKNOWLEDGEMENTS

We would like to thank Fiocruz-MG, Fapemig (APQ-01766-15) and CNPq (308869/2017-6) for financial support for this study.

REFERENCES

- Corrêa LR, Paraense WL. Susceptibility of *Biomphalaria amazonica* to infection with two strains of *Schistosoma mansoni*. Rev Inst Med Trop Sao Paulo. 1971; 13: 387-390.
- Paraense WL, Corrêa LR. Susceptibility of *Biomphalaria peregrina* from Brazil and Ecuador to two strains of *Schistosoma mansoni*. Rev Inst Med Trop Sao Paulo. 1973; 15: 127-130.
- Teodoro TM, Jannotti-Passos LK, Carvalho Odos S, Caldeira RL. Occurrence of *Biomphalaria cousini* (Mollusca: Gastropoda) in Brazil and its susceptibility to *Schistosoma mansoni* (Platyhelminthes: Trematoda). Mol Phylogenet Evol. 2010; 57: 144-151.
- Teodoro TM, Jannotti-Passos LK, Carvalho OS, Grijalva MJ, Baus EG, Caldeira RL. Hybridism between *Biomphalaria cousini* and *Biomphalaria amazonica* and its susceptibility to *Schistosoma mansoni*. Mem Inst Oswaldo Cruz. 2011; 106: 851-855.
- Paraense WL, Corrêa LR. Differential susceptibility of *Biomphalaria tenagophila* populations to infection with a strain of *Schistosoma mansoni*. J Parasitol. 1978; 64: 822-826.
- Fernandez MA, Thiengo SC. Susceptibility of *Biomphalaria amazonica* and *Biomphalaria occidentalis* from Manso Dam, Mato Grosso, Brazil to infection with three strains of *Schistosoma mansoni*. Mem Inst Oswaldo Cruz. 2006; 101: 235-237.
- Richards CS. Genetics of a molluscan vector of Schistosomiasis. Nature. 1970; 227: 806-810.
- Lewis FA, Richards CS, Knight M, Coope LA, Clark B. *Schistosoma mansoni*: Analysis of an unusual infection phenotype in the intermediate host snail *Biomphalaria glabrata*. Exp Parasitol. 1993; 77: 349-361.
- Santos MBL, Freitas JR, Correa MCR, Coelho PMZ. Suscetibilidade ao *Schistosoma mansoni* de híbridos de *Biomphalaria tenagophila* do Taim, RGS, Cabo Frio, RJ, e Belo Horizonte. Rev Inst Med Trop Sao Paulo. 1979; 21: 281-286.
- Bezerra FS, Machado JÁ, Martins RL, Chaves MM, Correa RF, Coelho PMZ. Effect of gamma radiation on the activity of hemocytes and on the course of *Schistosoma mansoni* infection resistant in *Biomphalaria tenagophila* snails. Mem Inst Oswaldo Cruz. 2003; 98: 73-75.
- Martins-Souza RL, Pereira CA, Coelho PMZ, Negrão-Correa D. Silica treatment increases the susceptibility of the Cabo Frio strain of *Biomphalaria tenagophila* to *Schistosoma mansoni* infection but does not alter the natural resistance of the Taim strain. Parasitol Res. 2003; 91: 500-507.
- Rosa FM, Caldeira RL, Carvalho Odos S, Godard AL, Coelho PM. Dominant character of the marker of *Biomphalaria tenagophila* (Mollusca: Planorbidae) strain, resistant to *Schistosoma mansoni*. Mem Inst Oswaldo Cruz. 2004; 99: 85-87.
- Rosa FM, Godard AL, Negrão-Correa D, Rodrigues HA, Carvalho OS, Caldeira RL, et al. *Biomphalaria tenagophila*: dynamics of populations of resistant and susceptible strains to *Schistosoma mansoni*, with or without pressure of the parasite. Mem Inst Oswaldo Cruz. 2006; 101: 247-251.
- Granath WO JR, Yoshino TP. Lysosomal enzyme activities in susceptible and refractory strains of *Biomphalaria glabrata* during the course of infection with *Schistosoma mansoni*. J Parasitol. 1983; 69: 1018-1026.
- Bayne CJ, Yoshino TP. Determination of compatibility in mollusc-trematode parasitism. Integrative and Comparative Biology. 1989; 29: 399-407.
- Martins-Souza RL, Pereira CA, Rodrigues L, Araújo ES, Coelho PMZ, Corrêa Jr A, et al. Participation of N-acetyl-D-glucosamine carbohydrate moieties in the recognition of *Schistosoma mansoni* sporocysts by haemocytes of *Biomphalaria tenagophila*. Mem Inst Oswaldo Cruz. 2011; 106: 884-891.
- Mitta GC, Adema CM, Gourbal B, Loker ES, Théron A. Compatibility polymorphism in snail/schistosome interactions: from field to theory to molecular mechanisms. Dev Comp Immunol. 2012; 37: 1-8.
- Van Der Knaap WP, Loker ES. Immune mechanisms in trematode snail interactions. Parasitol Today. 1990; 6: 175-182.
- Souza CP, Passos LK. Resistance of *Biomphalaria occidentalis* from Varzea das Flores Dam, Minas Gerais, to *Schistosoma mansoni* infection detected by low stringency polymerase chain reaction. Mem Inst Oswaldo Cruz. 2001; 96: 381-383.
- Nacif-Pimenta R, de Mattos AC, Orfanó Ada S, Barbosa L, Pimenta PF, Coelho PM. *Schistosoma mansoni* in susceptible and resistant snail strains *Biomphalaria tenagophila*: in vivo tissue response and in vitro hemocyte interactions. Plos one. 2012; 7: 1-12.
- Hanelt B, Adema CM, Mansour MH, Loker ES. Detection of *Schistosoma mansoni* in *Biomphalaria* using nested PCR. J Parasitol. 1997; 83: 387-394.
- 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. Journal of Parasitology. 1997; 83: 395-399.
- Caldeira RL, Jannotti-Passos LK, Carvalho OS. Use of Molecular Methods for the Rapid Mass Detection of *Schistosoma mansoni* (Platyhelminthes: Trematoda) in *Biomphalaria* spp. (Gastropoda: Planorbidae). Jo Trop Med. 2017; 1-6.
- Vidigal TH, Caldeira RL, Simpson AJG, Carvalho OS. Further studies on the molecular systematic of *Biomphalaria* snails from Brazil. Mem Inst Oswaldo Cruz. 2000; 95: 57-66.
- Jannotti-Passos LK, Caldeira RL, Carvalho OS. Técnicas utilizadas no estudo dos moluscos do gênero *Biomphalaria* e na manutenção do ciclo de *Schistosoma mansoni*. In: Carvalho OS, Coelho PMZ, Lenzi HL, editors. *Schistosoma mansoni* e Esquistossomose, Uma visão multidisciplinar. Rio de Janeiro, Brazil. 2008; 1-124.
- Pellegrino J, Katz N. Experimental chemotherapy of schistosomiasis mansoni. In: Ben Dawes editors. Advances in Parasitology. London and New York: Academic Press. 1968; 233-290.

27. Paraense WL, Corrêa LR. Probable extension of schistosomiasis mansoni to southern most Brazil. Mem Inst Oswaldo Cruz. 1987; 82: 577.
28. Chernin E, Antolics VM. Penetrative capacity of *Schistosoma mansoni* miracidia. Journal of Parasitology. 1975; 63: 560-561.
29. Bayne CJ, Buckley PM, Dewan PC. Macrophagelike hemocytes of resistant *Biomphalaria glabrata* are cytotoxic for sporocysts of *Schistosoma mansoni* in vitro. J Parasitol. 1980; 66: 413-419.
30. Granath WO Jr, Yoshino TP. *Schistosoma mansoni* passive transfer of resistance by serum in the vector snail *Biomphalaria glabrata*. Exp Parasitol. 1984; 58: 188-193.

Cite this article

Caldeira RL, Jannotti-Passos LK, Teodoro TM, dos Santos Carvalho O (2018) Resistance of *Biomphalaria Tenagophila* (Mollusca: Gastropoda) From The State of Espírito Santo, Brazil to *Schistosoma Mansoni* (Platyhelminthes: Trematoda) Infection. Ann Clin Pathol 6(2): 1136.