

## MALACOLOGICAL SURVEY OF POTENTIALLY CONTAMINATED WATERS AT OUTBREAKS OF TRANSMISSION OF SCHISTOSOMIASIS MANSONI IN THE CITY OF BETIM, MINAS GERAIS, BRAZIL

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### ABSTRACT

*Schistosoma mansoni*, which causes schistosomiasis mansoni, has humans as the primary definitive host and the mollusks *Biomphalaria glabrata*, *B. straminea* and *B. tenagophila* as intermediate hosts. The goal of this research was to undertake a malacological survey on watercourses in the city of Betim, using the data provided by the City Health Department. The mollusks were collected from pre-selected sites, examined for the presence of cercariae and taxonomically-identified, by morphological and molecular techniques for species. 168 mollusks were collected in eight collection sites. The species found were *B. glabrata*, *B. straminea* and *B. tenagophila*. Four *B. glabrata* from one of the sites were positive for *S. mansoni*. These results and the presence of intermediate hosts throughout the region studied, even if not found to be infected, is of epidemiological importance and suggests that the health authorities from the city of Betim should implement disease control measures.

**KEY WORDS:** public health; bioindicators; malacology; malacofauna; distribution ecology; vector expansion.

### INTRODUCTION

In Brazil, schistosomiasis mansoni is a disease caused by the digenetic trematode *Schistosoma mansoni* whose primary definitive host are humans and its intermediate hosts snails of the genus *Biomphalaria*. In this country, there are three species of *Biomphalaria* that are found naturally infected: *B. glabrata*

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(Say, 1818), *B. straminea* (Dunker, 1848) and *B. tenagophila* (Orbigny, 1835). These snails are widely distributed throughout Brazil (Brasil, 2013; Brasil, 2014; Carvalho et al., 2018;). In the last Brazilian National Inquiry of the Prevalence of Schistosomiasis Mansoni and Geo-Helminths 14 States and the Federal District were considered endemic for *S. mansoni* (Katz, 2018). The percentage of municipalities endemic for schistosomiasis by State varied from 2.4% to 68.2%. According to Katz (2018) in the State of Minas Gerais the positivity rate for schistosomiasis was 5.8% of infected individuals, clearly indicating the need for more effective control measures, since the State is among the regions that had the highest prevalence of infection in municipalities with a population of up to 500,000 inhabitants, with fewer cases only than the State of Sergipe (10.7% of infected individuals).

Schistosomiasis occurs in locations either without sanitation or with inadequate basic sanitation, and is acquired through the skin and mucous membranes as a consequence of human contact with water containing cercariae, the infective forms of *S. mansoni* (Paraense, 1975; WHO, 2023). The transmission of the disease depends on the presence of humans passing helminth eggs in their feces, and on the intermediate snail hosts releasing infective larval forms of the trematode into the watercourses used by human beings (Paraense, 1975; Neves, 2016). Other important factors also contribute to the occurrence of schistosomiasis in a given locality, which are: socioeconomic status, occupation, behavior (i.e., leisure activities) and level of information of the population exposed to disease (Paraense, 1975; Neves, 2016; WHO, 2023).

Currently, epidemiological surveillance of schistosomiasis aims to identify early the conditions that favor the occurrence of cases and the establishment of foci of transmission of the disease (Farias et al., 2011; Brasil, 2013; Brasil, 2014; Katz, 2018). These important conditions include: the wide geographical distribution of the intermediate snail hosts; migratory movement, whether transitory or permanent, of people within endemic areas; lack of, or inadequate, domestic and/ or environmental sanitation; poor awareness and health education of the populations at risk of transmission of schistosomiasis (Farias et al., 2011; Brasil, 2013; Katz, 2018). In areas without disease circulation these conditions must be permanently monitored. Along with imported cases in endemic areas must be detected and treated early to avoid the introduction of the disease. Therefore, in these areas, the notification of schistosomiasis is compulsory, and the regulations of municipalities and the States must be observed (Brasil, 2013; Vitorino et al., 2012; Brasil, 2014).

The general objective of this research was to identify foci of transmission of schistosomiasis mansoni, through the collection, examination and specific identification of the intermediate mollusk hosts of *S. mansoni* in regions where the occurrence of the disease has been previously reported based on the active search for cases undertaken by the Municipal Health Department in Betim, in the State of Minas Gerais, Brazil.

These data were collected from information provided by the Zoonosis and Endemic Control Center of the municipality of Betim (CCZE/ Betim), which in 2015 had carried out a coprological survey in the municipality through the active search for positive cases for schistosomiasis.

## MATERIAL AND METHODS

### *Epidemiological survey*

In order to identify sites for the strategic collection of mollusks of the genus *Biomphalaria*, an epidemiological analysis was performed on data provided by the Zoonosis and Endemic Control Center (CCZE) from the Betim Health Surveillance, a metropolitan region in the State of Minas Gerais. The epidemiological data consulted for the identification and selection of the regions to be studied were taken from the Betim's Schistosomiasis Control Program (PCE/ Betim). This program undertakes prospective coproscopic surveys in order to notify, prevent and provide information about the disease to residents of the municipality of Betim.

The regions selected for carrying out the malacological survey were defined in partnership with the CCZE/ Betim based on data obtained from the coprological survey carried between 2011 and 2015 in the municipality. CCZE/Betim carried out an active search for positive human cases for schistosomiasis as one of the goals defined in the Schistosomiasis Control Program (PCE) (Table 1). Thus, the regions with the highest number of individuals infected with schistosomiasis and living close to water bodies were selected.

The following regions were selected for the research and collection of mollusks of the genus *Biomphalaria*: Icaivera, Várzea das Flores, Homero Gil and Tupã from Regional Icaivera; Estrada Serra Negra/ Barra Liberatus, Cachoeira and Marimbá from Regional Vianópolis (Figure A and B). Bodies of water in which the population used as a source of leisure and recreation were selected. The predominant vegetation in the regions visited is riparian forest. The climate is warm throughout the year and, in general, the temperature varies from 55 °F to 86 °F and is rarely below 50 °F or above 92 °F. The rainy season is humid and cloudy and the dry season is almost cloudless.

### *Collection, examination and identification of the mollusks*

The methodology utilized for the collection of the mollusks of the genus *Biomphalaria* was previously described by Jannotti-Passos et al., 2008. The snails were individually introduced into recipient dishes with 5 mL of unchlorinated water, exposed to artificial light for 30 min, and then examined under a stereoscope for the observation of possible cercariae of *S. mansoni*, when negative snails were submitted to squeezing (Jannotti-Passos et al., 2008). The identification of the cercaria species was done by anatomy, type of swimming and resting movement (Jannotti-Passos et al., 2008).

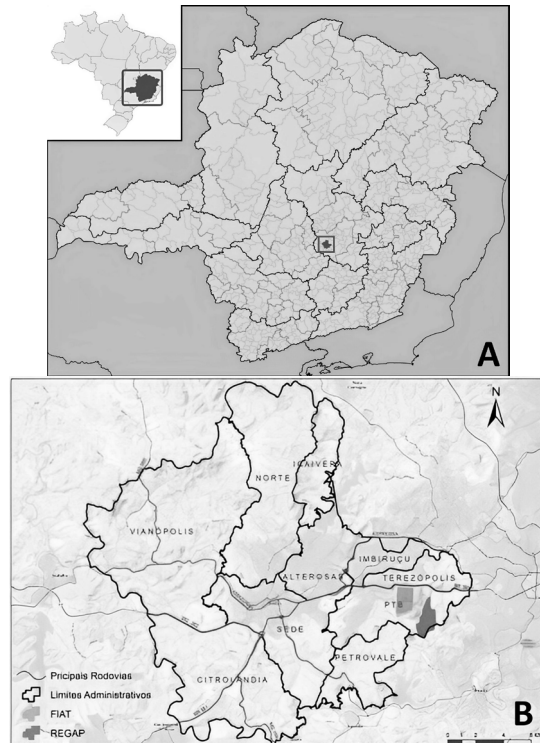
One mollusk from each collection site was identified using both morphological (Deslandes, 1951; Paraense, 1975) and molecular techniques (Caldeira et al., 1998, 2009). For the DNA extraction the foot of each snail was lysed mechanically with 200 mL of 50 mM Tris-HCl at pH 8.0, 100mM NaCl, 50 mM EDTA and 0.5% SDS and incubated overnight at 37 °C with 50mg/mL proteinase K. Posteriorly, 200 mL of a buffer solution containing 10 mM Tris-HCl and 2.5 mM EDTA at pH 8.0 and 1% polyvinyl-polypyrrolidone (PVPP, insoluble in water) was added and incubated for 20 min at 95 °C. This solution was placed on ice for 5 min, then centrifuged at 14,000 rpm for 10 min. After centrifugation the DNA was transferred to tubes of 1.5 mL and precipitated by the addition of 0.1 vol. Na acetate (3 M, pH 7) and 3 vol. absolute ethanol. The precipitated DNA was washed in 70% ethanol, resuspended in 20 mL water and cooled to -20 °C. The entire ITS (which includes the 5.8S rDNA gene together with the flanking ITS1 and ITS2 spacers) was amplified using the primers ETTS2 (5/-TAACAAGGTTTCCGTAGGTGAA-3/) and ETTS1 (5/-TGCTTAAGTTCAGCGGGT-3/) anchored respectively in the conserved extremities of the 18S and 28S ribosomal genes. The PCR amplification was undertaken in a volume of 10 mL consisting of 1-10 ng of template DNA, 10mM TrisHCl, pH 8.5, 200 mM each dNTP, 1.5 mM MgCl<sub>2</sub>, 0.8U of Taq DNA polymerase (Cenbiot<sup>®</sup>, RS, Brazil), 50 mM KCl, together with 1.0 pmol of each primer. The reactions were covered with a drop of mineral oil and subjected to initial denaturation step for 5 min at 95 °C, and then 32 cycles with annealing of 54 °C for 1 min, extension at 72 °C for 2 min, denaturation at 95 °C for 45 sec and a final extension step at 72 °C for 5 min. A negative control (no template DNA) was included in all experiments. Three mL of the amplification products were visualized on 0.8% ethidium bromide stained agarose gels to check the quality of amplification.

## RESULTS

After analyzing the epidemiological data provided by the CCEZ from the Health Department of the city of Betim, the sites were selected to carry out the collection of mollusks for the malacological study (Table 1). After completion of the morphological and molecular analysis of the mollusks collected in these regions, the results generated were organized, analyzed and presented in Table 2. The data presented describes the locality of the collection site, the number of mollusks collected, whether the mollusks collected were positive or negative for the presence of cercariae, and the morphological and molecular identification of the mollusks encountered. In some places, there was no identification of the names of the streets where the mollusks were collected.

*Table 1.* Number of schistosomiasis positive individuals per region assessed by the Schistosomiasis Control Program (PCE) between 2011 and 2015 in Betim, Minas Gerais, Brazil.

Location	Evaluated individuals/ positive individuals 2011/2012	Evaluated individuals/ positive individuals 2013	Evaluated individuals/ positive individuals 2014	Evaluated individuals/ positive individuals 2015
Region Icaivera	358/340	NE	196/163	164/149
Region Vianópolis	NE	138/110	NE	NE



*Figure.* Map. (A) Location of the city of Betim, in the state of Minas Gerais, Brazil. (B) Map of the city of Betim with the identification of regional of the city. [wikimedia.org/wikipedia/commons/4/42/MinasGerais\\_Municip\\_Betim;](https://wikimedia.org/wikipedia/commons/4/42/MinasGerais_Municip_Betim;) [vitruvius.com.br/revistas/read/arquitextos/17.197/6260.](http://vitruvius.com.br/revistas/read/arquitextos/17.197/6260)

Table 2. The mollusks collected and their *Schistosoma* infection status in Betim, Minas Gerais, Brazil in 2016.

Location	Collection date	Mollusks collected	Cercariae	Morphological identification	Molecular identification
S: 19.92402° W: 044.19307° E: 846 meters	03/01/2016	15	Positive	<i>B. glabrata</i>	<i>B. glabrata</i>
S: 19.91235° W: 044.15042° E: 841 meters	03/01/2016	58	Negative	<i>B. straminea</i>	<i>B. straminea</i>
S: 19.91190° W: 044.16238° E: 837 meters	03/01/2016	03	Negative	<i>B. straminea</i>	<i>B. straminea</i>
S: 19.92974° W: 044.26876° E: 735 meters	05/23/2016	0	Not applicable	Not applicable	Not applicable
S: 19.92534° W: 044.27166° E: 758 meters	05/23/2016	48	Negative	<i>B. tenagophila</i>	<i>B. tenagophila</i>
S: 19.51228° W: 044.9286° E: 853 meters	05/23/2016	0	Not applicable	Not applicable	Not applicable
S: 19.51588° W: 044.9035° E: 852 meters	06/07/2016	13	Negative	<i>B. straminea</i>	<i>B. straminea</i>
S: 19.51588° W: 044.843° E: 820 meters	06/07/2016	0	Not applicable	Not applicable	Not applicable
S: 19.54.689° W: 044.14721° E: 835 meters	08/24/2016	31	Negative	<i>B. tenagophila</i>	<i>B. tenagophila</i>
S: 19.5453° W: 044.14639° E: 809 meters	08/24/2016	02	Negative	<i>B. glabrata</i>	<i>B. glabrata</i>
S: 19.5407° W: 044.14528° E: 844 meters	09/31/2016	0	Not applicable	Not applicable	Not applicable
S: 19.50.841° W: 044.9636° E: 870 meters	09/31/2016	0	Not applicable	Not applicable	Not applicable
S: 19.5105° W: 044.9342° E: 870 meters	09/31/2016	0	Not applicable	Not applicable	Not applicable

Cercariae = whether the mollusks collected were shown to release (positive) or not release (negative) cercariae in the laboratory. S: south, W: west, E: elevation.

## DISCUSSION

The data show that in only one of the collection sites were mollusks infected with cercariae of *S. mansoni*. This result and the presence of the intermediate hosts throughout the studied region, even if not infected, is of great epidemiological importance and it makes action by the Health Department of Betim essential for the resolution of this public health problem. Betim is a Brazilian municipality in the state of Minas Gerais, in the southeast region of the country. It belongs to the metropolitan region of the city Belo Horizonte and it is the fifth most populous municipality in the state, bringing together 450,024 inhabitants according to an estimate for 2021, an area of 345.99 square kilometers and 122 kilometers of perimeter according to Betim's City Hall(PMB) (IBGE, 2023).

According to the DATASUS (2023), epidemiological surveillance of schistosomiasis disease has been included in the compulsory notification in the municipality since August, 2011. In 2016, when this study was carried out, the municipality of Betim was considered an endemic area for this infection, since contamination with *S. mansoni*-infected feces may occur in lakes, rivers, springs, streams and wells of the municipality, where already the snails that transmit the schistosomes to humans were found (DATASUS, 2023). The PCE/ Betim actions to combat the spreading of this disease, by preventively carrying out the referrals necessary for the early treatment of the confirmed cases in humans.

The complexity of the mechanisms of transmission of schistosomiasis, and the diversity of contributing factors, means that the control of the disease depends on various preventive actions, which include: (i) early diagnosis and treatment; (ii) surveillance and control of the mollusks of the genus *Biomphalaria*, which are the intermediate hosts of *S. mansoni*; (iii) improvement of health education, and increasing public awareness of the disease and its transmission; (iv) interventions that promote sanitation through modification of the domestic and environmental factors favorable to transmission (Brasil, 2013; Brasil, 2014; Katz, 2018).

Therefore, it is essential that the Health Department of the city of Betim, given the current results, takes measures to ensure the eradication of foci of transmission, especially in the regions where mollusks of the genus *Biomphalaria* were found. It is important to highlight that the locations selected in this current study for the collection of mollusks were those in which there were the highest number of human cases of schistosomiasis. As such, *S. mansoni* is already known to be well established and the disease transmission on-going in these environments, as confirmed by our findings of the presence of the susceptible intermediate host in this region.

The host mollusks were collected from January 3<sup>rd</sup>, 2016 to September 31<sup>st</sup>, 2016, and it is important to note that after six years it is possible that the environmental conditions of the water collections in which the collections were carried out may have changed. Thus, we suggest that further studies be carried out to verify the current distribution of schistosomiasis intermediate hosts in these regions.

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## CONFLICT OF INTERESTS.

The authors declare that there is no conflict of interests to disclose.

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