ORIGINAL ARTICLE

A PRELIMINARY STUDY ON THE DISTRIBUTION OF

BREEDING SITES OF Biomphalaria glabrata

IN THE MUNICIPALITY OF PERI MIRIM,

A LOW ENDEMICITY AREA FOR

SCHISTOSOMIASIS IN NORTHEAST BRAZIL

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ABSTRACT

Information on areas colonized by snails that transmit *Schistosoma mansoni* is essential for planning schistosomiasis control measures. Therefore, the purpose of this study was to map the natural breeding sites of *Biomphalaria* spp. in the municipality of Peri Mirim, Maranhão, Brazil. The snails were manually collected and the breeding sites were geo-referenced (seven in the urban area and five in flooded fields), from November 2017 to March 2018. In the laboratory, the snails were examined for the presence of larval stages of *S. mansoni* identified by morphology and internal anatomy analysis. While no snails were found in the urban area, sixteen *B. glabrata* were collected in the flooded fields. No *S. mansoni* or other trematode infections were detected. Wild rodents, most likely *Holochilus sciureus* that could act as *S. mansoni* reservoirs, were also found in the flooded fields. These data indicate possible risk areas for further outbreaks of schistosomiasis transmission in the municipality of Peri Mirim.

KEY WORDS: Baixada Maranhense; malacological survey; planorbidae; spatial analysis.

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INTRODUCTION

Schistosomiasis is one of the most important parasitic diseases in the world, as it affects approximately 240 million people in more than 78 countries, mainly in tropical and subtropical regions, and is associated with severe morbidity in human populations in endemic areas (WHO, 2018). The etiologic agent of this disease in Brazil is the parasite of the species *Schistosoma mansoni* Sambon, 1907, a heteroxenous trematode that needs a vertebrate host, mostly humans and wild mammals (Rey, 1993; Miranda et al., 2017), and a susceptible invertebrate host, some snail species of the genus *Biomphalaria* (Coelho et al., 2008; Scholte et al., 2012). Three main *Biomphalaria* species are responsible for the transmission of schistosomiasis in Brazil: *B. glabrata* (Say, 1818), *B. tenagophila* (d'Orbigny, 1835) and *B. straminea* (Dunker, 1848; Brazil, 2008).

In malacological surveys carried out in the Brazilian State of Maranhão, *B. glabrata* and *B. straminea* were found, but only *B. glabrata* was naturally infected with *S. mansoni* (Santos et al., 2011; Oliveira et al., 2013; Cantanhede et al., 2014; Nogueira et al., 2016; Miranda et al., 2016; Rodrigues et al., 2017). The infected mollusks were found mainly in the northern region of the state, including São Luís (the state capital), and in the Baixada Maranhense Microregion (western Maranhão). Indeed, the Baixada Maranhense remains one of the main areas of active schistosomiasis transmission in Maranhão (Mendes et al., 2017). This region is characterized by extensive flooded fields, favoring activities such as fishing, hunting and agriculture, all of which enhance the risk of human infections by *S. mansoni* (Lira et al., 2017). Moreover, the maintenance of the *S. mansoni* life cycle in the Baixada Maranhense is possibly associated with the presence of the semi-aquatic rodents of the species *Holochilus sciureus*, naturally infected with *S. mansoni* (Veiga-Borgeaud et al., 1986; Do Carmo-Silva et al., 2019).

Peri Mirim is one of the 21 municipalities comprising the Baixada Maranhense and presented a *S. mansoni* prevalence of 2.4% in 2017 (Brazil, 2018). Despite being included in a recent qualitative malacological survey (Cantanhede et al., 2014), the detailed distribution of host snails in the surroundings of this municipality remains unknown. Thus, we aimed to evaluate the spatial distribution of natural *Biomphalaria* spp. breeding sites in the municipality of Peri Mirim, Maranhão, Brazil, which may improve the strategies for the local control of schistosomiasis.

MATERIAL AND METHODS

Study area

Peri Mirim (2°34'41.6"S 44°51'32.8"W) has a population of 14,345 inhabitants, with an area of 398,721 km² (Brazil, 2020), divided into two main

zones: the urban area and the flooded fields (Figure 1). It has a humid tropical climate, with two well-defined seasons: the rainy season from January to July, and the dry season from August to December (Costa-Neto et al., 2001). It is a region with low endemicity for schistosomiasis, with a prevalence of less than 5% (Brazil, 2005).

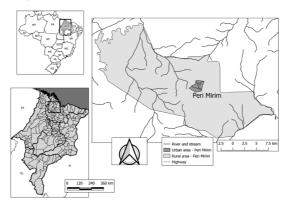


Figure 1. Geographic location of the municipality of Peri Mirim, State of Maranhão, Brazil.

Malacological survey

Freshwater mollusks were collected between November 2017 and March 2018 using an active search sampling technique in all water accumulation sites located in the urban area and the flooded fields in the study zone. The study was approved by the Chico Mendes Institute for Biodiversity Conservation (license number 40025/1) and registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (registration number A97213B). Manual snail collection was carried out by three trained persons using metal tweezers and metal dip nets in a radius of approximately 30 m (10 m/trained person) for 30 min in each collection point (adapted from Barboza et al., 2012). The collected mollusks were stored in plastic containers, which were identified with the date, location, and collection point and taken to the laboratory.

All collection points were geo-referenced (even those with no water accumulation and mollusks) with a GPS (Global Positioning System) receiver (Garmin Etrex®) and the spatial distribution of *Biomphalaria* spp. was built using the Qgis program, version 2.18. Rainfall data were obtained from a publically available database by PROCLIMA/CPTEC (http://proclima.cptec.inpe.br), and the average rainfall in each collection month was calculated.

In the laboratory, the mollusks were kept in glass aquariums with dechlorinated water and fed lettuce leaves. To verify the shedding of trematode larvae, the snails were individually placed in glass containers with 5 mL of dechlorinated water, exposed to light and heat from three incandescent light bulbs (60W) for 4 h and analyzed. Subsequently, the same snails were left in a dark room for 12 h before further analysis, as the cercariae from some species are shed at night. These procedures were performed once a week for sixty days. Subsequently, the negative mollusks were crushed to confirm the absence of other trematode life stages in soft tissues (Brazil, 2008). All live specimens underwent parasitological analysis and were then processed according to Fernandez et al. (2008) and identified using conchological and internal morphological parameters (Paraense, 1975).

RESULTS

Sixteen *Biomphalaria* spp. specimens were collected, belonging to the species *B. glabrata* found in the flooded fields (Paciência, n = 7; Agostinho, n = 4; Murriré, n = 3; Ilha da Pedra, n = 2) (Table). Despite the apparent absence of snails in the urban area, street names were recorded to enable further location of the collection points (Figure 2). None of the collected snails was found to be infected with either *S. mansoni* or other digenetic trematodes. The rainfall average of each month was quite irregular, varying from 0.06 mm in November/2017 to 6.8 mm in March/2018 (Table).

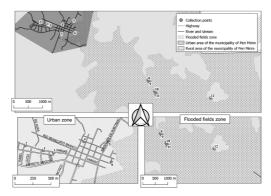


Figure 2. Distribution of *Biomphalaria glabrata* collection points in urban and flooded field zones in the municipality of Peri Mirim, State of Maranhão, Brazil. Points 1 and 2= Campo de Pouso; 3 and 4= Centro; 5 and 6 = Portinho; 7 = São Francisco; 8 = Canarã; 9 = Murriré; 10 = Paciência; 11 = Ilha da Pedra; 12 = Agostinho.

Regarding the characteristics of the collection points, Campo de Pouso and Centro had paved streets with no evident water collection. On the other hand, the Portinho and São Francisco points had small, shallow streams at the doors of the houses, but with no vegetation in such potential breeding sites. The flooded fields (Canarã, Agostinho, Ilha da Pedra, Murriré and Paciência) were characterized by the presence of both shallow and deep freshwater collection, with the presence of abundant aquatic vegetation (Figure 3, A-E). It is also important to highlight the presence of *Holochilus* spp. rodents, most likely *H. sciureus*, in the flooded fields, an animal regarded as a wild infection reservoir of *S. mansoni* in the region (Figure 3, F).

Table. Number of *Biomphalaria glabrata* collected in the flooded fields and neighborhoods of the municipality of Peri Mirim, State of Maranhão, Brazil.

Zone	Neighborhood	Point	Geographic coordinates	Number of snails	Collection data	Average Rainfall
Urban	Campo de Pouso	1	2°34' 40.7" S 44°51' 37.3" W	0	Nov./2017	
		2	2°34'38.4"S 44°51'45.4"W	0	Nov./2017	0.06mm
	Centro	3	2°34'41.6"S 44°51'32.8"W	0	Nov./2017	
		4	2°34'36.6"S 44°51'19.3"W	0	Nov./2017	
Flooded fields	Portinho	5	2°34'37.0"S 44°51'08.1"W	0	Dec./2017	
		6	2°34'46.1"S 44°51'07.0"W	0	Dec./2017	1.7mm
	São Francisco	7	2°34'47.4"S 44°51'22"W	0	Dec./2017	
	Canarã	8	2°35'41.2"S 44°49'44.1"W	0	Jan./2018	
	Murriré	9	2°35'43.0"S 44°49'42.9"W	3	Jan./2018	6.36mm
	Paciência	10	2°35'53.6"S 44°49'36.7"W	7	Jan./2018	
	Ilha da Pedra	11	2°35'56.8"S 44°49'35.2"W	2	Mar./2018	6.8mm
	Agostinho	12	2°36'00.9"S 44°48'34.2"W	4	Mar./2018	

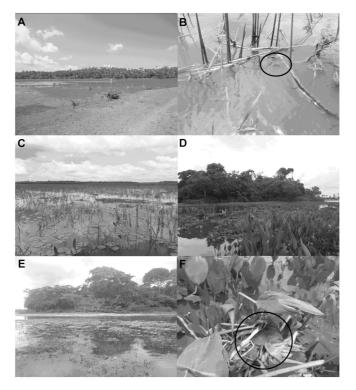


Figure 3. Environmental characteristics of collection points in the flooded field zone of the municipality of Peri Mirim, State of Maranhão, Brazil. A. Canarã. B. Agostinho (the circle indicates the presence of *Biomphalaria glabrata*). C. Ilha da Pedra. D. Murriré. E. Paciência. F. Presence of the rodent *Holochilus* spp. in the flooded fields (circle).

DISCUSSION

This is the first study to carry out a detailed spatial distribution of natural breeding sites for *B. glabrata* in the municipality of Peri Mirim, a low endemicity area for schistosomiasis in Northeastern Brazil, therefore a valuable record that can guide further interventions run by the epidemiological surveillance authorities to fight schistosomiasis in the region. The mapping of the geographic distribution of *S. mansoni*-transmitting snails has been used on a large scale in ecological and epidemiological studies (Guimarães et al., 2009; Barboza et al., 2012; Nogueira et al., 2016; Bezerra et al., 2018; David et al., 2018). This method allows the construction of maps that are fundamental in the planning of schistosomiasis control actions, as they enable visualization of snail breeding sites and increase knowledge regarding their distribution areas.

Some *Biomphalaria* species were reported in Peri Mirim in a previous qualitative malacological survey, such as *B. glabrata* and *B. schrammi* (Crosse, 1864) (Cantanhede et al., 2014). However, this work is the first attempt to map out in detail the occurrence of *B. glabrata* in the municipality with greater precision, considering it is the most important species in the transmission of schistosomiasis in Brazil (Souza et al., 1995; Carvalho et al., 2008).

Although *S. mansoni* infection is considered active at low levels in the municipality, only a few *B. glabrata* snails were found and no infection was detected. The absence of snails at the collection points located in the urban area may be related to street paving in the Peri Mirim neighborhoods. Even though they may also inhabit artificial breeding sites, *B. glabrata* prefer habitats that are rich in organic matter, and urbanization may affect optimal conditions for a potential breeding site, reducing food availability and essential minerals (Bini et al., 1999; Abílio et al., 2006; de Assis Janasi et al., 2007). Moreover, rainfall in the studied period was irregular, and some previous studies correlate a higher density of snails with the rainy season (Giovanelli et al., 2001; Favre et al., 2002). Additionally, Miranda et al. (2016) and David et at. (2018) showed a positive relationship between high precipitation and increased *Biomphalaria* spp. occurrence. However, we did not investigate the collection points in all seasons, which did not allow us to establish a relationship between rainfall and the number of snails collected in the area.

The absence of trematode-infected snails may be due to the low number of specimens collected, as previous malacological surveys in the Baixada Maranhense have already shown that this area has a high trematode biodiversity, including *S. mansoni* (Cantanhede et al., 2014; Rodrigues et. al, 2017). Additionally, the low occurrence of *S. mansoni* larval stages in snails can be associated with the majority of the infected human population presenting low levels of parasitism, eliminating only a few eggs in feces (low parasite burden), which could make environmental contamination by eggs less likely and consequentially lead to lower infection rates in *B. glabrata* (Lira et al., 2017). However, the absence of infected *B. glabrata* does not rule out the possibility of there being active transmission of *S. mansoni* in the collection points or these potential breeding sites becoming future sources of *S. mansoni* human infections.

Among the investigated areas, the flooded fields may be potential sources of transmission, since only this area presented *B. glabrata* snails. Lira et al. (2017) demonstrated a higher prevalence of infection in individuals who constantly visited these fields for fishing, suggesting that the infection occurs more actively in the flooded fields than in the urban area. Furthermore, in these same flooded fields, Do Carmo-Silva et al. (2019) first reported the presence of the rodent *H. sciureus* naturally infected with *S. mansoni* and that their infection rate did not vary significantly in relation to distance from the urban zone. Therefore, the rodent *H. sciureus* might be the cause of a constant active focus of schistosomiasis in the location; however, this hypothesis requires further work.

Although the malacological survey was only performed for five months, some main sites used for housing and fishing by the local population were preliminarily investigated. However, further work is necessary to better assess the influence of seasonality on the occurrence of *B. glabrata* in the municipality of Peri Mirim.

In summary, this study mapped in detail the natural breeding sites of *B. glabrata*, indicating areas at risk of possible outbreaks of *S. mansoni* transmission in the municipality of Peri Mirim, State of Maranhão. Our results also contribute to a better understanding of the *B. glabrata* area of occurrence in Northeastern Brazil and can help Peri Mirim health services in the control of schistosomiasis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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