

Potential for schistosomiasis in a municipality of Rondônia, Brazilian Amazon

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ABSTRACT

Schistosomiasis is a parasitic disease, caused by helminths of the genus *Schistosoma* and transmitted in Brazil by snails of the genus *Biomphalaria*. The municipality of Ouro Preto do Oeste, Rondônia, in the Brazilian Amazon Region, has unusually registered more than 900 cases of schistosomiasis in the last 10 years. The aim of this study was to investigate the potential of transmission of schistosomiasis in Ouro Preto do Oeste. A total of 1,196 people in a risk area for the disease transmission were requested to answer a clinical-epidemiological survey and to collect feces samples for examination. All the samples that underwent examination resulted negative for *S. mansoni*. Two hundred and sixty-eight snails were collected in the locality of Ouro Preto do Oeste in 32 different locations. Among these, 44% were classified as belonging to the genus *Biomphalaria*. Another sample of snails (146 specimens), collected at the same sites, were submitted to an *in vitro* challenge with *Schistosoma mansoni*, and none of them were able to transmit the parasite. Finally, we discuss the epidemiological importance of these findings and the lack of attention to a patient with the disease in a non-endemic area. We failed to detect any association between schistosomiasis and the snails from the genus *Biomphalaria*, that exists in the local, as the planorbids were unable to transmit *Schistosoma mansoni*. Perhaps the small sample and/or the stool examination technique can have contributed to the results. Further studies, in other localities of Rondônia and with a greater sample could put some light in this question.

KEYWORDS: Schistosomiasis; Amazon; Transmission.

Potencial de transmissão de esquistossomose em município de Rondônia, Amazônia brasileira

RESUMO

A esquistossomose é uma doença parasitária, causada por helmintos do gênero *Schistosoma* e transmitida no Brasil por caramujos do gênero *Biomphalaria*. O município de Ouro Preto do Oeste, Rondônia, Amazônia brasileira, notificou, de forma não usual, mais de 900 casos de esquistossomose mansônica nos últimos 10 anos. O objetivo deste estudo foi investigar o potencial de transmissão da esquistossomose em Ouro Preto do Oeste. Um total de 1.196 pessoas residindo em áreas de risco para a transmissão da doença foram abordadas para responderem um questionário clínico-epidemiológico e realização de exame parasitológico de fezes. Todas as amostras foram negativas para *S. mansoni*. Duzentos e sessenta e oito caramujos foram coletados em Ouro Preto do Oeste, de 32 localidades diferentes. Entre estes, 44% foram classificados como pertencendo ao gênero *Biomphalaria*. Outra amostra de caramujos (146 exemplares), coletada nos mesmos locais, foi submetida *in vitro* a cepas de *Schistosoma mansoni*, sendo todos incapazes de transmitir o parasito. Por fim, discute-se a importância epidemiológica desses achados e a falta de atenção ao paciente portador da parasitose em áreas endêmicas. O estudo não conseguiu demonstrar a ocorrência de transmissão na localidade e nem a habilidade dos planorbídeos locais transmitirem *Schistosoma mansoni*. Talvez o pequeno tamanho da amostra e/ou a técnica de exame de fezes (pouco sensível) possam ter contribuído para este resultado. Estudos futuros, em outras localidades e com uma amostra maior podem colocar um pouco de luz nesta questão.

PALAVRAS-CHAVE: Esquistossomose; Amazônia; Transmissão.

INTRODUCTION

Schistosomiasis is one of the most prevalent parasitic diseases in the world (Katz and Almeida 2003). It is caused by a trematode belonging to the genus *Schistosoma* that uses humans as its main definitive host and freshwater snails as intermediate hosts. Its prevalence is closely related to poor public sanitation and the presence of host snails.

Schistosomiasis is a disease known since antiquity, and its arrival in Brazil was related to slave trade. However, only *S. mansoni* became established because the existence of intermediate hosts and environmental conditions similar to those found in Africa (Silva 1984). The main intermediate host of this parasite is *Biomphalaria glabrata*, but other species such as *B. tenagophila* and *B. straminea* also act as hosts (Fernandez and Thiengo 2006).

Schistosoma mansoni has low mortality, and the major causes of death are related to severe clinical forms of the disease, which are generally characterized by liver cirrhosis, portal hypertension, colitis, pulmonary and neurological forms, which occur decades after the initial infection (Maroja 1953).

Approximately 779 million people are estimated to be at risk of contracting the disease, and approximately 207 million people are infected worldwide (Ibikounlé *et al.* 2009). In Brazil, the disease is considered endemic, reaching 19 federal units, with 2,297,352 confirmed cases from 1995 to 2011, and 524 deaths from the disease from 1996 to 2011 (Ministério da Saúde 2008).

In Brazil, the disease expanded into previously uninfected areas and occurred mainly through the migration of infected people from endemic areas (Coimbra Jr *et al.* 1984; Silva 1984). In the Amazon, Pará was the unique state who recorded the transmission of schistosomiasis (Maroja 1953) but it is currently already eradicated. Rondônia, although it is a non-endemic area (Coimbra Jr *et al.* 1984), is at particularly high risk because it has high migration rates (IBGE 2010). Most individuals immigrating to Rondônia are from states from the southeast and northeast regions of Brazil (IBGE 2010), where schistosomiasis is highly endemic.

Data from the Brazilian Ministry of Health about Ouro Preto do Oeste, Rondônia, shows that 963 cases of schistosomiasis were reported between 2002 and 2012, mainly in individuals 20–49 years old; all were considered to be allochthonous cases. Of these, 12 cases occurred in children under 10 years of age, suggesting possible autochthonous transmission of the disease (FUNASA 2012). The aim of this study was to investigate the potential transmission of schistosomiasis in Ouro Preto do Oeste.

MATERIALS AND METHODS

The study was conducted in the city of Ouro Preto do Oeste, Rondônia, (10°44'53"S and 62°12'57"W), 332 km from Porto Velho. The city has a population of 37,928 inhabitants, of which 28,208 (74.37%) live in the urban areas (IBGE 2010). This work was approved by the Research Ethics Committee of the Institute of Biomedical Sciences, University of São Paulo - ICB / USP (CAAE: 03687512.0.0000.5467).

Population Survey

A population of 5,233 inhabitants lives in an urban area with no sewage and close to water streams ("palafitas"), considered risk areas for schistosomiasis transmission. One thousand one hundred and ninety six (22.8% of the population), living in this urban area were asked to answer the clinical and epidemiological questionnaire and to participate in the parasitological examination. Participants were chosen by convenience and sampled from 11 of these areas. The research team consisted of a medical doctor, medical and biology students and the staff of the epidemiological surveillance system team from Ouro Preto do Oeste. Field activities were conducted from December 7th to 13th, 2013.

In larger sites, 50% of households were approached; all inhabitants who had lived on the site for at least one year were asked to participate. In smaller sites, all residents within 500 m of the main water collection site were approached.

Data from the clinical and epidemiological surveys were analyzed using the program OpenEpi, version 2.2.1 (http://openepi.com/Menu/OE_Menu.htm). A p-value < 0.05 was considered significant.

Coprological Survey

After participants had signed the Informed Consent and Clear Explanation Form, which gave details about the disease, and after they completed the questionnaire, they were given containers with the preservative MIF (Merthiolate-Iodine-Formaldehyde) for collection and storage of stool samples. These samples were analyzed using the Hoffman-Pons-Janer method; positive samples were further subjected to the Kato-Katz method (Katz and Almeida 2003).

Collection and identification of snails and mapping of the area

Malacological collection was performed simultaneously with the population census, and 268 snails were collected from 32 points distributed along the main water collection route. The sample was preserved in 70% alcohol, and georeferenced. The capture technique followed the procedures described by Tibiriçá (2006): thirteen pairs of tweezers and thirteen dip nets, with a 1-m long metal rod and a 35 cm × 35 cm central frame surrounded by a 30-cm deep nylon net were

used to capture snails. The dip nets were used for shallow and deep collecting on the banks of water collection locations in peridomestic sites.

Snails were identified using traditional methods, by dissection of specimens removed from their shells under a conventional stereomicroscope. The methodology and taxonomy described by Paraense (1975), Paraense (1981) and Paraense (1983) were applied. A sample (n = 20) of snails were deposited in the collection of the Zoology Museum of the University of São Paulo (USP).

Snail Infection

The snails used in the *in vitro* infection experiments were captured in aquatic areas in urbanized areas (ditches for drainage of rainwater and domestic sewage), in two different sites. Snails from each site were kept in separate tanks of dechlorinated water and were fed with a standard diet supplemented and with lettuce leaves. All snails used in the experiment were identified as *B. occidentalis* and *B. amazonica*. From the total of 146 captured snails, 60,1% died during transportation (100% of the *Biomphalaria amazonica* specimens) to São Paulo, where the *in vitro* challenge against *S. mansoni* took place.

Snails were selected by shell size, and 30 snails with an average diameter of 10.5 mm (SD ± 1.09 mm) were individually placed in plastic cups containing dechlorinated water for verification of natural infection with larval trematodes, according to the methodology described in Ministério da Saúde (2008). Thus, the snails were exposed weekly to artificial light (four table lamps with 60-W halogen lamp) for two hours. After this period the contents of each container were examined using a stereoscopic microscope at 20× magnification for detection of cercariae. Snails that did not excrete cercariae in the previous phase were divided into two groups for evaluation of the experimental infection rate with miracidia of the Belo Horizonte strain of *S. mansoni*.

Miracidia were obtained from *S. mansoni* eggs deposited in hamster livers and incubated for seven weeks. The miracidia were concentrated by phototropism and their numbers were estimated by counting under a microscope, following the procedures described in Ministério da Saúde (2008).

Thus, group I, containing 14 samples of *B. occidentalis*, was subjected to a single exposure to 10 miracidia, and group II, containing 15 samples, was subjected to four periods of seven days of exposure intervals to the same ratio (10: 1). A specimen positive for the elimination of furcocercariae was exposed in the same manner as group I and monitored separately.

As a control, 20 *B. glabrata* samples (albino variety, Belo Horizonte strain) were used. These were kept in the Enteroparasites Center of the Adolfo Lutz Institute, São Paulo, for experimental maintenance of the biological cycle. The

conditions of exposure to *S. mansoni* miracidia were similar to those of group I.

After 30 days of contact with the miracidia, the snails were exposed to artificial light to ensure the elimination of cercariae, following the steps already described. Snails in group I were exposed weekly to artificial light for four weeks. In contrast, parasite research on group II was performed 30 days after the last exposure to miracidia, for up to four weeks. In the control group, the cercariae investigation was conducted once, 30 days after exposure to the miracidia. At the end of the experimental period, snails that tested negative were crushed, and the ovotestis and digestive gland were disrupted using metallic tweezers to verify a possible infection with cercariae of *S. mansoni*.

RESULTS

Population Survey

No significant differences were observed between genders. Participants aged 0–20 years composed the majority of the sample. Among the surveyed individuals, 13% reported a family history of helminthiasis and 28% reported regularly visiting to urban water streams. A total of 43% of the population reported being born in other regions of the country, especially the southeast (22%) and northeast (7%), where schistosomiasis is endemic. In Table 1 there is more specific information about de population.

Table 1. Socio-epidemiological profile of the sample of the population of Ouro Preto do Oeste, Rondônia, 2013.

VARIABLE	N (%)
AGE (years)	
0—15	389 (32.5)
16—30	326 (27.3)
31—60	381 (31.8)
>60	100 (8.4)
Total	1,196 (100)
Gender	
Male	564 (47)
Female	632 (53)
Stool Parasite Tests	883 (73.8)
Average Family Income	
< 1 minimum wage (MW)	442 (37)
1 to 2 MW	383 (32)
2 to 3 MW	228 (19)
> / MW 4	143 (12)
Place of birth	
Rondônia	663 (55)
Northern Region (excluding RO)	25 (2)
Northeastern Region	84 (7)
Midwestern Region	59 (5)
Southeastern Region	267 (22)
Southern Region	98 (9)
Individuals who visit urban water streams	329 (28)

Coprological Survey

Of the 1,196 people surveyed, 883 (73.8%) sent material for coproscopy. All were negative for *S. mansoni*, and only 3 of the individuals (total= 39) who reported a personal history of schistosomiasis did not send material for parasitological examination of stools.

Sixty-eight percent of the samples were positive for one or more intestinal parasites, especially *Entamoeba histolytica* (31%), *Giardia intestinalis* (19%) and/or *Ascaris lumbricoides* (4%). Other parasites were also observed to be highly prevalent, such as *Entamoeba coli* (40%) and *Endolimax nana* (13%).

Snail identification and infection

Of the 268 snails identified, 44% belonged to the genus *Biomphalaria*, and of these, 46.7% were identified as *B. amazonica* and 53.3% as *B. occidentalis*.

Of the snails exposed to artificial light before experimental infection, a single specimen of *B. occidentalis* released furcocercariae.

As can be seen in Table 2, *in vitro* infection of *B. occidentalis* from Ouro Preto do Oeste with the Belo Horizonte strain of *S. mansoni* was unsuccessful; in group I, nine snails were negative (9/14) and five died (5/14). Similarly, in group II, six snails were negative for infection (6/15) and nine died (9/15). However, after exposure to miracidia, the 30-day control group (*B. glabrata*) showed an 85% rate of infection (17/20), 10% (2/20) negativity, and 5% (1/20) mortality. The *B. occidentalis* specimen with a natural furcocercariae infection, that was subjected to contact with *S. mansoni*, miracidia died two days after the procedure.

Table 2. Rate of infection and mortality of *Biomphalaria occidentalis* from Ouro Preto do Oeste (Rondônia) by the Belo Horizonte strain of *Schistosoma mansoni*.

Snail Species and Origin	Snails exposed to infection	Mortality N (%)	Number of exposures	Positivity N (%)
<i>B. occidentalis</i> (RO) Group I	14	5 (35.7)	1	0
<i>B. occidentalis</i> (RO) Group II	15	9 (60)	4	0
<i>B. glabrata</i> (MG) Control	20	1 (5)	1	17 (85)

DISCUSSION

In the Amazon region, mansonic schistosomiasis has previously been restricted to isolated outbreaks in Fordlândia and other municipalities in the state of Pará (Maroja, 1953), where it has already been eradicated (Valadão and Milward-De-Andrade 1991).

In the city of Ouro Preto do Oeste, although more than 900 cases of schistosomiasis were reported between 2002 and 2012, the coprological survey for *S. mansoni* resulted negative. However, the methods used for detection have low sensitivity in cases of mild infections or in chronic cases, which may have led to false negative results.

It should be noted that, 68% of the individuals were positive for one or more intestinal parasites, including *Entamoeba histolytica* (31%) and *Giardia intestinalis* (19%). Parasites of lesser medical importance, such as *Entamoeba coli* (40%) and *Endolimax nana* (13%), were also found to be highly prevalent, indicating that the water in these districts may be contaminated, probably because of a lack of adequate sanitation.

Intestinal parasites are highly prevalent in the Brazilian Amazon (Muniz *et al.* 2007; Souza *et al.* 2007; Borges *et al.* 2009) and have also been reported in other regions of Brazil, such as the cities of Parnaíba, Piauí (Furtado and Melo 2011; Oliveira *et al.* 2001), and São José da Bela Vista, as well as São Paulo, where the infection rate was 44.4% (Tavares-Dias and Grandini 1999), all these locations had inadequate sanitation.

The transmission of *S. mansoni* depends strictly on human contact with standing contaminated water that contains competent planorbids for transmission. In places where there is no supply of running water or other suitable source of drinking water, the population is dependent on these water sources for their daily activities. These urban water streams have been reported to be frequented by residents of surrounding areas for bathing, household, and occupational tasks, as well as leisure activities, facilitating transmission of intestinal parasites. In addition, 43.5% of the population of Rondônia state consists of immigrants from other Brazilian states surveyed in this study, especially from endemic areas of schistosomiasis (IBGE 2010). In this study, approximately 1/3 of individuals originated from areas with schistosomiasis transmission foci.

The malacological survey did not show the presence of *B. glabrata* in the municipality, concordant with the survey performed by Coimbra Jr. and Santos (1986). However, *B. amazonica* and *B. occidentalis*, which had been identified in the State of Rondônia by Paraense and Corrêa (1982), Coimbra Jr *et al.* (1984), Coimbra Jr. and Santos (1986) were identified. The sites where these species were found possessed ecological conditions suitable for their development, including a favorable pH (6–7), sufficient aquatic vegetation, and a muddy substrate and with little or no water current (Coimbra Jr. and Santos 1986).

The rate of infection of *B. occidentalis* from Ouro Preto do Oeste by the *S. mansoni* strain from Belo Horizonte was zero, both for a single exposure to miracidia (group I) and for multiple infections (group II), which mimics natural conditions. The high mortality rates observed (group I: 35.7%,

5/14; group II, 40%, 6/15) may be related to the different parasite loads that the specimens were subjected (ten miracidia for the experimental groups and five for the control group). Mortality can also be influenced by excessive handling of specimens during exposure to artificial light, or in the case of group II, by multiple infections. However, the natural infection of one of the specimens with furcocercariae from other trematodes, identified here as *Cercaria amplicoeata*, Ruiz, 1953 (Naruto 1984), should be emphasized, as it warns of the possible future adaptation of different strains of *S. mansoni* to other species of snails.

Although *B. amazonica* and *B. occidentalis* have not yet been found to be naturally infected with *S. mansoni* (Coimbra Jr. *et al.* 1984; Valadão and Milward-De-Andrade 1991), *in vitro* studies performed by Paraense and Correa (1982), and Coimbra Jr. and Engel (1982), using strains of the pathogen and snails from Rondônia, showed that *B. amazonica* is susceptible to infection by the trematode, at very low rates. However, *B. occidentalis* was not found to be a good host, either in the above-mentioned studies or in others performed by Fernandez and Thiengo (2006) using *B. occidentalis* from the state of Mato Grosso. In addition, the observed failure of *S. mansoni* strains from Belo Horizonte to infect *B. occidentalis* from Ouro Preto do Oeste does not allow us to conclude that this species cannot transmit the disease in this area, because strains of *S. mansoni* may adapt to the local strains of *B. occidentalis*, as Paraense and Correa (1963) observed with *B. tenagophila* in the Paraíba River valley in the state of São Paulo (Coimbra Jr and Engel 1982).

Similar to Brazil, some areas of China are endemic for schistosomiasis (*S. japonicum*), whereas other areas are non-endemic. According to Lu *et al.* (2013), with the improved economic development in China in the recent decades, the Schistosomiasis Control Program was redefined in 2004, resulting in greater assistance to the affected population. However, the authors emphasized the need to improve the program, since it operates only in endemic areas, and chronic carriers in non-endemic areas are underserved.

In Brazil, Rondônia is an non-endemic region, and for this reason, it has no specific programs to carry out active surveys, nor there are professionals trained in the early diagnosis and treatment of schistosomiasis.

Given the results of the coprological survey and the lack of transmission observed in the *in vitro* experiment, the municipality of Ouro Preto do Oeste, appears to have presently no current potential for establishment of active foci for disease transmission. However, given the occurrence of allochthonous cases of schistosomiasis in the municipality, the social and environmental conditions, and the possibility of adaptation of the parasite to local snails, epidemiological surveillance measures should be implemented for immigrants

from endemic areas, to treat illness and prevent progression to severe forms, and to reduce the risk of transmission in the region, by treating immigrants infected with the parasite..

CONCLUSIONS

We failed to detect any association between schistosomiasis and the snails from the genus *Biomphalaria*, that exists in the local, as the planorbids were unable to transmit *Schistosoma mansoni*. Perhaps the small sample and/or the stool examination technique (low sensitivity) can have contributed to the results. Further studies, in other localities of Rondônia and with a greater sample could put some light in this question.

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