Major Article



The Pampulha reservoir remains a potential urban focus of schistosomiasis mansoni in Brazil: changes in the occurrence patterns of Biomphalaria species and a new record of the parasite

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ABSTRACT

Introduction: The Pampulha reservoir has long been a focus of schistosomiasis transmission in Belo Horizonte, State of Minas Gerais, Brazil. The last malacological study conducted in this urban reservoir was more than two decades ago, and thus, an update on the distribution of the species of *Biomphalaria* as well as new data on the presence of *Schistosoma mansoni* in this water body are required. **Methods:** The current distribution of *Biomphalaria* spp. in the Pampulha reservoir and their infection with S. mansoni was evaluated during 55 malacological surveys conducted between 2009 and 2012. Results: Biomphalaria straminea displayed a high population density and distribution, and 13.7% (2,233/16,235) of the specimens collected were infected with larval trematodes other than Schistosoma mansoni. Biomphalaria tenagophila and Biomphalaria glabrata, species currently presenting a restricted distribution and small populations, displayed trematode infection rates of 15.2% (98/644) and 13% (83/640), respectively. Thirteen (2%) specimens of B. glabrata were found to be infected with S. mansoni. In addition, a historical review based on previous and new data on the occurrence patterns of Biomphalaria species in this reservoir is presented. Conclusions: The results indicate that the Pampulha reservoir remains a potential focus of urban schistosomiasis in Brazil, and significant changes in the occurrence patterns of *Biomphalaria* species were verified.

Keywords: Schistosomiasis, Snails, Cercaria, Urban lake, Tourism, Minas Gerais.

INTRODUCTION

Schistosomiasis mansoni is a helminth disease that affects approximately 6 million people in Brazil and was previously considered a disease typically endemic in rural areas. However, in recent decades, mainly due to human migration, a change has occurred in the transmission pattern of this parasite, as observed in different metropolitan areas of the country²⁻⁵. With the goals of eliminating active transmission foci and preventing outbreaks of schistosomiasis in urban areas from Brazil, the monitoring of molluscan transmitters of Schistosoma mansoni Sambon, 1907, is an important aspect of epidemiological surveillance of the disease⁶.

The Pampulha reservoir is one of the first urban foci of schistosomiasis in Brazil. The reservoir was built in the late 1930s, and its architectural complex is still one of the main tourist attractions in Belo Horizonte, which is the capital of the State of Minas Gerais in southeastern Brazil. This water body

was initially intended to serve as a water supply and to capture runoff from rainfall, but it has become an important area for recreational activities, which has contributed to the emergence of a schistosomiasis focus in Belo Horizonte. The first record of snails that were naturally infected with S. mansoni in the Pampulha reservoir was reported by Martins and Versiani7, who observed that Biomphalaria glabrata (Say, 1818) was infected with this trematode. These same authors conducted a coproparasitological survey of individuals who frequented the reservoir and uncovered an outbreak of schistosomiasis. In fact, this reservoir contributed to the increase of the prevalence of the disease in the capital of Minas Gerais from 0.5% in 19208 to 11.7% in 19389. Approximately three decades later, Paulini et al.10 obtained positive parasitological results from 18.7% of local residents in the same region. Given the presence of a transmission focus of schistosomiasis in the reservoir, several malacological studies that emphasize planorbids were also conducted at the Pampulha reservoir¹¹⁻¹⁷. However, the latest published information dates to more than two decades ago; thus, an update is required.

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METHODS

Malacological surveys were conducted at the margins of the Pampulha reservoir (19°51' S, 43°58' W), Belo Horizonte, State of Minas Gerais, Brazil, between January 2009 and November 2012. This reservoir has an amoeboid shape and is situated at an altitude of 810m. The reservoir is formed by the impounding of eight streams (AABB, Água Funda, Baraúnas, Mergulhão, Olhos D'água, Ressaca, Sarandi and Tijuco) that drain within the metropolitan region of Belo Horizonte. Although the Pampulha reservoir had a water volume of 18 million/m³ and a 21km perimeter at the time of its construction, the current volume is approximately 10 million/m³, with a maximum depth of 13.3m and a perimeter of 14km. This reduction has primarily resulted from silt accumulation, mainly due to the discharge of untreated sewage into the reservoir, which since the 1970s has contributed to the current state of eutrophication, and phenomena such as algal blooms and the proliferation of aquatic macrophytes are commonly observed 18-19, factors that contribute to the establishment of high densities of snails in this water body.

Six convenience sampling points (**Figure 1**) were selected without the use of any theoretical approach but, rather, based on criteria such as facility access to margins of the reservoir and previous reports of the occurrence of populations of snails. However, the sampling points where the largest numbers of samples (sampling points 1 and 4) were collected are relatively distant from each other (approximately 3.5km) and are at opposite ends of the reservoir. Sampling was conducted without defined periodicity; however, considering all sampling points, the average interval between sampling was one month. In total, 25, 3, 5, 14, 4 and 4 malacological samples were collected at sampling points 1 to 6, respectively. Each field excursion at each sampling point was counted once, for a total of 55 samples.

Snails were collected with the aid of a D-shaped nylon hand net (50cm wide and 40cm high with a 30cm opening and 1mm² mesh) for 60min of sampling effort, spanning approximately 100m at each sampling point. The collected material was transferred to plastic bags, labeled and transported to the laboratory, where the snails were separated and individually placed in microtiter plates containing 3ml of chlorine-free water. The snails were then exposed to artificial photostimulation for two hours and examined under a stereomicroscope for the presence of larval trematodes. A new examination of snails was performed on the day after collection before and after photostimulation. The cercariae obtained were studied under a light microscope with the aid of vital stain (0.05% neutral red), and those trematodes with morphology consistent with S. mansoni were used to experimentally infect groups of five mice via the subcutaneous route (100 cercariae/animal). Stool examinations were conducted using the spontaneous sedimentation technique²⁰ 40 days after inoculation with cercariae, and perfusion of the hepatic portal system was performed 60 days after infection²¹. Specimens of the planorbids were killed in water at 70°C and processed according to Deslandes²² for taxonomic identification, which was based on morphological criteria according to Paraense²³. In addition, a historical review of the occurrence patterns and distribution of Biomphalaria species at the sampling points was performed to emphasize the main changes in these parameters over the history of the Pampulha reservoir.



FIGURE 1 - Current map of the Pampulha reservoir, Belo Horizonte, State of Minas Gerais, Brazil, and the six sampling points where malacological surveys were performed between January 2009 and November 2012. Scale bar: 500m. Source: Google Earth.

RESULTS

During the malacological samplings performed in the Pampulha reservoir, 17,519 specimens of Biomphalaria spp. were collected. Of these, 2,514 (14.4%) were infected with 14 non-S. mansoni larval trematodes species belonging to different cercarian types, including brevifurcate, clinostomatoid, echinostome, gymnocephalous, magnacauda, monostome and strigea cercariae, which were subjected to taxonomic and life cycle studies. The three species of snails with epidemiological importance in the transmission of S. mansoni in Brazil, namely, B. glabrata, Biomphalaria tenagophila (d' Orbigny, 1835) and Biomphalaria straminea (Dunker, 1848), are currently present in the Pampulha reservoir. However, the distribution of these planorbid snails in the areas evaluated is markedly different from that observed during malacological surveys previously performed by other authors. A representation of changes in the species distribution of the intermediate hosts of S. mansoni at the Pampulha reservoir over more than seven decades is shown in Figure 2.

Currently, *B. straminea* is the species with the highest population density and distribution in the Pampulha reservoir. It was the only species of planorbid verified at all sampling points. From 16,235 specimens (92.8% of the total of the snails examined), 2,233 (13.7%) were observed to harbor larval trematodes other than *S. mansoni. Biomphalaria tenagophila* had a restricted distribution and was found primarily in small populations at sampling points 3 and 4. Infection rates of 15.2% (98/644) were observed for larval trematodes other than *S. mansoni. Biomphalaria glabrata* also displayed a restricted distribution primarily in a low population density at sampling point 4. A total of 13% (83/640) of *B. glabrata* evaluated were infected with larval trematodes, and 13 (2%) specimens of this

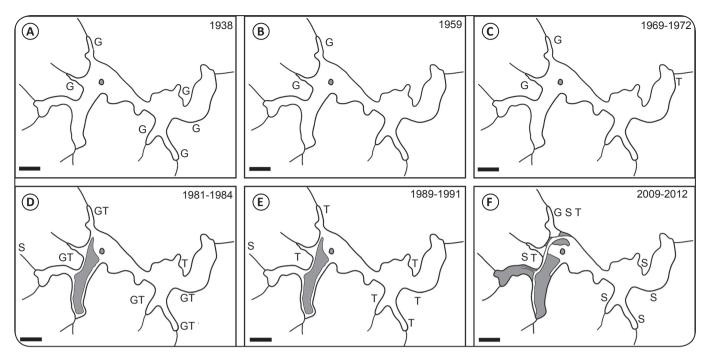


FIGURE 2 - Sketch maps of the Pampulha reservoir, Belo Horizonte, State of Minas Gerais, Brazil, showing the distribution of the *Biomphalaria* species in the six sampling points evaluated during the present and previous malacological surveys by other authors at different times. The sites of introduction of *Biomphalaria tenagophila* and *Biomphalaria straminea* are also indicated with the corresponding dates. A: 1938 (Martins & Versiani 1939; B: 1959 (Milward-de-Andrade 1959); C: 1969-1972 (Milward-de-Andrade 1969, 1972); D: 1981-1984 (Carvalho et al. 1985); E: 1989-1991 (Freitas et al. 1992, Freitas & Santos 1995); F: 2009-2012 (present study). Abbreviations: G: *Biomphalaria glabrata*, S: *Biomphalaria straminea*, T: *Biomphalaria tenagophila*. Scale bars: 500m. The gray areas represent islands or areas of silt accumulation.

species harbored *S. mansoni* larvae. This parasite was present in samples collected over the three years evaluated, with an average infection percentage of 2.5% (from 1.5 to 11.1%). At sampling point 4, where *B. glabrata* were observed in the Pampulha reservoir, the cercariae of *S. mansoni* were verified in 35.7% (5/14) of the sampling dates. Adult parasites of both sexes were recovered from mice that were experimentally infected with cercariae that emerged from naturally infected *B. glabrata*, and the eggs of the parasite were observed in the feces of the rodents 40 days after infection. The morphology of cercariae and adult trematodes were similar to those reported for other strains of *S. mansoni*.

DISCUSSION

Although methodological differences (e.g., malacological field methods, number of samples and duration of the studies) may make it difficult to compare the previous malacological studies performed at the Pampulha reservoir, the data shown in the present study, including those relating to the historical review of occurrence patterns and distribution of *Biomphalaria* species at the sampling points, suggest that changes most likely occurred in the distribution of the species in recent decades, as discussed below.

Biomphalaria straminea is a widely distributed species in Brazil, and among the intermediate hosts of *S. mansoni*, it is the least susceptible but is responsible for maintaining schistosomiasis in some endemic areas in the northeast region of

the country. Although their current relevance in the transmission of the parasite in southeastern and west-central Brazil appears to be minimal, the presence and growth of B. straminea in localities from these regions around Brazil are associated with environmental and social changes related to the epidemiology of schistosomiasis and should serve as a warning. It is possible that this species may become epidemiologically important in the transmission of parasites in these regions in the future²⁴⁻²⁷. This species was first reported in Belo Horizonte at the Pampulha basin (in the Baraunas stream) in the early 1980s²⁸, and until the early 1990s, it remained restricted to the introduction site and was not verified within the reservoir²⁹. However, since the mid-1990s, the species has been found inside the Pampulha reservoir near sampling point 2 (Dr. Alan Lane de Melo, unpublished results). The data obtained in the present study demonstrate that B. straminea is currently found at all sites evaluated, often with a high population density. Furthermore, B. straminea was the only species found in places previously colonized by B. tenagophila and/or B. glabrata, as reported by Carvalho et al. 15 and Freitas et al.¹⁷. Determining the factors related to the replacement and current distribution of B. straminea will require additional studies. Regarding the experimental susceptibility of B. straminea, which originated from the Baraunas stream, to S. mansoni, Souza et al.³⁰ found infection rates ranging between 5.7% and 19%. However, thus far, B. straminea that are naturally infected with S. mansoni have not been observed in the Pampulha reservoir. In contrast, the cercariae of avian schistosomes, which may be involved in cases of cercarial dermatitis in humans, were recently reported in B. straminea collected from this reservoir³¹.

Biomphalaria tenagophila displays intermediate susceptibility to S. mansoni and is responsible for maintaining the disease in some regions as well as isolated foci in southeastern Brazil. This species was originally reported at the Pampulha reservoir by Milward-de-Andrade¹⁴ and soon became the predominant planorbid species in the locality, occupying the entire edge of the reservoir between the early 1980s and the 1990s^{15,17,29}. This species of *Biomphalaria* was observed to be infected with S. mansoni at a very low rate, 0.03% (1/3,695), in the Pampulha reservoir by Carvalho et al.15. Later, Souza et al.32 reported two specimens that were naturally infected by the parasite but did not provide an infection rate. Data from experimental infection of B. tenagophila originating from the Pampulha reservoir with different strains of S. mansoni revealed infection rates ranging between 4 and 68%³²⁻³⁵; however, the sampling conducted during the present study revealed that this planorbid species was not verified to be infected with this parasite.

Biomphalaria glabrata is the snail species acting as an *S. mansoni* transmitter with the greatest epidemiological importance in Brazil given its wide distribution and high susceptibility to the parasite. After the finding of *S. mansoni* in *B. glabrata* in the Pampulha reservoir in the 1930s, the reservoir was temporarily emptied, which resulted in the elimination of approximately 15 million specimens of *B. glabrata*. Since then, the species has been found in the reservoir with low population densities but continues to harbor larvae of *S. mansoni*^{11-13,15,28}. In the early 1990s, *B. glabrata* was considered absent from the reservoir, although the species was present in tributary streams, and it was suggested that the occasional findings of this species in the reservoir resulted from snails carried by rainfall runoff^{17,29}.

However, the results obtained in the present study suggest that a population of B. glabrata was established at sampling point 4 of the reservoir, considering that specimens of this planorbid species of different sizes were collected and that specimens infected with S. mansoni were common in the dry seasons of the three years evaluated. Thus, B. glabrata not only remains present in the Pampulha reservoir but is also still infected with S. mansoni. The infection rates of B. glabrata with S. mansoni obtained in this study are consistent with those found in other endemic areas and are generally higher than those reported for this same species in the Pampulha reservoir, including 0.6% (1/176) by Martins and Falcão¹¹ and 0.5% (2/467) by Carvalho et al. 15. Only in the pioneering study of Martins and Versiani (2.4%, 1/42)10 and the results reported by Souza et al. (3.5%, 2/57)²⁸ were the rates of infection of B. glabrata by S. mansoni similar to that observed in the present study. Regarding the experimental susceptibility to S. mansoni, infection rates of 30% and 59% were obtained in B. glabrata from the Pampulha reservoir³⁶.

The factors related to changes in the distribution of *Biomphalaria* species in association with the natural history of the Pampulha reservoir require additional studies. Several authors have reported the possible natural competition or coexistence between *Biomphalaria* species in Brazil^{29,37-41}. It is interesting to note the unusual serial replacement of predominant species of snail intermediate hosts of *S. mansoni*.

Initially, B. glabrata predominated in this reservoir. However, this species was replaced by B. tenagophila, and subsequently, B. straminea became the predominant species. The replacement of B. tenagophila by B. straminea is reported here for the first time. These observations reflect the possible occurrence of interspecific competition between these species. In fact, the competition and elimination of B. glabrata by B. straminea has been reported in experimental and natural conditions^{38,40-42}. and we cannot rule out the possibility that the competitive advantages of B. straminea also exist with respect to B. tenagophila. However, the factors related to the overlap of one species of snail over another are complex, and changes in the environment appear to have great importance¹⁷. Indeed, the continuous increase in the eutrophication status of the reservoir over the past decades, as indicated by the deterioration of water quality indicators^{17,19}, may have made the water of the Pampulha reservoir unfavorable for the maintenance of B. tenagophila at most sampling points where this species is no longer observed.

The occurrence of two species of Biomphalaria with epidemiological importance in the transmission of schistosomiasis in the same body of water is rare and has been verified mainly in urban environments²⁹. However, unlike previous reports, all three species of snails that transmit S. mansoni were observed in the Pampulha reservoir during this study. In fact, the coexistence of these three species of *Biomphalaria*, although only at sampling point 4, has not been previously reported. This specific sampling point presents distinct features compared to the other points, primarily because it is located near the top of one of the tributary streams and therefore has a greater flow of water. In fact, the occurrence of B. glabrata at this sampling point has been reported since the late 1950s¹². In addition, some factors related to water quality, such as conductivity and oxygen, phosphorus, ammonia, nitrite, nitrate and chlorophyll levels, have different values when compared to the other sampling points¹⁹, but the true influence of these variables on the results obtained remains unknown. In addition, the influence of recent anthropogenic changes on the reservoir, which have partially isolated some areas of the water, have contributed, at least in part, to the reservoir becoming an environmentally unstable habitat.

Regarding the possible biological competition between the vectors of *S. mansoni* and other species of snails (*Pomacea* sp. and *Melanoides tuberculata*), several studies have been conducted on the Pampulha reservoir^{12,16,17,29}. However, over more than three decades of coexistence between these species in the Pampulha reservoir, the presence of these potential competitors has not eliminated the populations of *Biomphalaria* spp. or, consequently, the focus of transmission. In fact, high populations of these prosobranchs remain at the locality (data not shown), which appears to have no noticeable effect on the current population of *Biomphalaria* species or on the epidemiology of schistosomiasis in the locality, which has also been recorded in other eutrophic and unstable environments where food for the snails is widely available^{43,44}.

A high percentage of snails infected with the larvae of other trematode species was found during the malacological surveys in the present study. These findings are mainly the result of factors such as the large densities of second intermediate hosts (fish) and definitive hosts (mostly birds) currently observed in the reservoir due its high eutrophication state. Although some species of these trematodes may have antagonistic relationships with the *Biomphalaria* snails and direct relationships with *S. mansoni* ^{45,46}, the effects of their presence on the transmission of *S. mansoni* are, as of yet, poorly studied in Brazil.

The source of infection of *S. mansoni* for the snails in the Pampulha reservoir is most likely related to the direct discharge of untreated sewage into its interior, which can contain feces of humans infected with the parasite. Human cases of schistosomiasis have been registered in the metropolitan region of Belo Horizonte, including recent outbreaks related to rural and ecological tourism^{3,47-50}. Although the Pampulha reservoir remains a place for recreational activities, its contribution to maintaining the prevalence of schistosomiasis in Belo Horizonte is low given that its poor water quality discourages people from coming into contact with it. Thus, the risk of the Pampulha reservoir becoming an active focus of schistosomiasis transmission under the current conditions is attenuated but still persists because the margins of this reservoir are frequented by fishermen, who often come into contact with the water.

The implementation of measures to revitalize the Pampulha reservoir has been discussed over the last decade^{19,51,52}. In this context, any action taken to improve the conditions currently found in this urban reservoir should consider the presence of *S. mansoni*-transmitting mollusks. It is necessary to be alert to these dangers so that the Pampulha reservoir does not return to being an active urban focus of schistosomiasis in Brazil. Furthermore, studies must be performed to elucidate the phenomena involved in the substitution of one species of snail host of *S. mansoni* for another in a given aquatic collection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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