Evidence for local transmission and maintenance of schistosomiasis in an urban neighborhood in Northeast Brazil

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Abstract

Schistosomiasis is a tropical neglected disease commonly associated with rural areas; however, urban schistosomiasis has been reported world-wide, and increasing urbanization is one of the most important demographic shifts of the 20 th and now 21 st centuries. The pattern of urbanization is not uniform so that within the same city the rates and sources of population increase vary. Here we report on the parasite composition in one neighborhood in the metropolitan area of Salvador, Bahia, Brazil. Using epidemiological data and population genetics we find evidence for local transmission and maintenance of *Schistosoma mansoni* infection within an urban population and little contribution from rural-urban migration. Our findings provide direction for local mitigation strategies and to assist the public living in this neighborhood to interrupt the local transmission cycle.

Introduction

Since the 2000s, Brazil has seen a decrease in the rate of rural-urban migration, however, the urban population continues to grow and the rural population is decreasing (Alves and Marra, 2009; United Nations, 2018). The city of Salvador went through an urbanization process even more intense than the country as a whole (Souza et al., 2012). This rapid process produce areas with precarious housing and limited public utilities that put some communities at risk for transmission of parasites such as *Schistosoma mansoni* the cause of schistosomiasis (Santana and Batista, 2012).

S. mansoni is the second most important parasitic infection after malaria for its prevalence and morbidity. It infects hundreds of millions in the Americas, Africa, Middle East, and East Asia. Infection is commonly considered a disease of rural populations related to agriculture, fishing, recreation, and other activities associated with contact with freshwater (McManus et al., 2018). While urban disease is considered unusual; it has been well documented historically in Africa, China, and Brazil (Blanton et al., 2015; Klohe et al., 2021). In Brazil, cities like Salvador, Bahia, in the Northeast have historically seen transmission of *S. mansoni* . Pirajá da Silva first distinguished*S. mansoni* from the species *S. haematobium* based on human cases in the city in 1911 (Katz, 2008). The main conditions for active transmission of *S. mansoni* are prevalent in some of the poorer sections of cities i.e., infected humans, contact with surface waters (agricultural work, recreation), presence of susceptible snails and poor sanitation (Zanardi et al., 2019; Klohe et al., 2021). Measures to eliminate schistosomiasis as a public health threat are aided by understanding the presence and persistence of the infection in each location. Rural to urban immigration is likely to contribute, but it is important for public health planning to understand how much of a factor this is. The pattern of distribution is also key to the management of the infection. Transmission in urban centers is thought to be highly focal

(McManus et al., 2018; Montgomery, 2019), but without knowledge of demography and parasite populations themselves this is difficult to verify.

Here using population genetics, we addressed for one urban community whether the presence of a population of *S . mansoni*resulted primarily from migration or local acquisition. We assessed place of birth and percentage of time living in a local neighborhood designated Pirajá, in the city Salvador, Bahia in Northeastern Brazil. We also evaluated genetic differences by gender, age, and water contact points. Using epidemiologic and population genetic evidence we show infection is present primarily due to local transmission in this section of a major Brazilian metropolis.

Materials and Methods

Ethical approval

The study was approved by the Ethical Committee (CEP) of the Oswaldo Cruz Foundation, Bahia, the Brazilian Commission for Research Ethics (CONEP) under no. 33779414.7.0000.0040, and the Institutional Review Board (IRB) of Tulane University administrative review 2019-1799. All participants signed or marked informed consent forms. Participants under 18 years of age filled an assent form and their parents or guardians provided informed consent on their behalf.

Study area and population survey

The Pirajá neighborhood (12º 53'45.00" S 38º 27'53.27" W) (Figure 1) is within a densely populated area of the major city of Salvador, Bahia, Brazil whose population is nearly 3 million (CONDER, 2016). The community borders a large city park, São Bartolomeu, that formerly protected a river and reservoir that served as a source of drinking water for the city. Population pressure surrounding the park has meant the reservoir no longer serves this purpose but is an area for recreation and some small-scale agriculture (CONDER, 2016).

According to the 2010 census, Pirajá had a population of 33,341 inhabitants of which >50% are age 20 - 49. Piped water is available to more than 95% of households and sewage is accessible to 91% (CONDER, 2016), however, many households are not connected to the system. Thirty percent earn less than one minimum monthly salary (US$213) and 40% between one and three minimum salaries (CONDER, 2016). Proximity to bodies of water and especially water development activities have been identified as a risk factor for infection with schistosomes (Clennon et al., 2004; Kabuyaya et al., 2017; Mogeni et al., 2020). Therefore, for this study, a band of homes approximately two city blocks wide that boarders the park, river, and reservoir was selected for sampling.

The study area comprised 650 households where 2,011 residents were interviewed and 1,134 provided at least one stool sample for Kato-Katz assay. Interviews and stool sampling were performed as previously described with stools collected on three different days (Blanton et al., 2015) (FigureS1). Interview data were directly entered into a REDCap database (version 9.3.1-2021 Vanderbilt University) using Android-based tablets (Android version 9.1, Samsung Galaxy Tab A 8.0).

Stool survey and egg isolation

All stools samples collected from participants were weighed, and single slides prepared as previously described (Katz et al., 1972). On the following day, the slides were read, and the number of schistosome eggs per gram and the presence of other helminths were recorded. Stools positive for *S . mansoni* were homogenized in a blender with 200 mL of 2% saline solution and then processed through a series of metal sieves and nylon filter bags (300 μm - 55 μm mesh pore size, FSI, Michigan City, Indiana, USA) and gravity sedimentation to concentrate the eggs (Dresden and Payne, 1981). The bottom 5 mL of sediment was collected and then kept frozen at -20 °C for DNA extraction. In accordance with Brazilian Ministry of Health guidelines (Ministério da Saúde do Brasil, 2014), participants with one or more egg-positive stools samples were given a one-time dose of praziquantel (60 mg/kg for children 4 to 15 years old and 50 mg/kg for adults), and 4–6 weeks later

three follow-up stool examinations were performed on those treated. For other helminthiases a single dose of albendazole was provided (FigureS1).

DNA extraction and genotyping

DNA was isolated from the sediment by a standard phenol/chloroform protocol, and then treated with cetyltrimethylammonium bromide (CTAB) to remove PCR inhibitors as previously described (Ausubel, 1987; Blanton et al., 2011). For genotyping, duplicate 2 µL of DNA samples per primer pair was used to PCR amplify 15 polymorphic microsatellite loci with a SeqStudio-3200 Genetic Analyzer (Thermo Fisher, Carlsbad, CA, USA). Allele peaks were identified and measured using Peak Scanner software version 2.0 online workstation (Thermo Fisher, Carlsbad, CA, USA), and the data were transferred to an Excel template for processing. Data trimming and organization were automated by custom designed macros. Allele peaks not conforming to the stepwise mutation model and peaks <100 pixels in height were excluded from analysis. Allele frequencies were calculated based on the ratio between each allele peak´s height and the sum of allele peak heights for each microsatellite locus (Barbosa et al., 2016). Microsatellite loci and samples with less than 50% genotyping success were excluded from analyses (Table S1). Final analyses were performed for 51 infrapopulations (parasites aggregated within one host) and 15 loci (smms2, smms13, smms16, smms3, smms17, smms18, smms21, smda23, sm13-478, 1f8a, 29e6a, smu31768, lg3_sc36b, sc23b, smd28 (Kovach et al., 2021).

Genetic differentiation

The differentiation index, Jost's D (Jost, 2008), was calculated from the allele frequencies using SpadeR (Chao and Jost, 2015). Where Jost's D between replicates was >0.01, the replicates were re-examined or eliminated (Silva et al., 2020). Differentiation of <0.05 was considered little differentiation, 0.05-0.25 moderate or great differentiation, and >0.25 as very great differentiation (Cormack et al., 1990).

The average differentiation among pairwise infrapopulations (parasite aggregated within one host) is designated the Di, between component populations the Dc (parasites aggregated within a group of hosts), and between an infrapopulation and a component population is the Dic. Genetic differences were analyzed the Dic with component populations based on place of birth (native-born vs immigrants); percentage of lifetime spent in Salvador (< 50 %, [?] 50% to < 90%, [?] 90%); age; sex; history of traveling outside of Salvador; contact with freshwater during traveling and contact with freshwater at five risk points within Piraja (P1: Represa do Cobre; P2: Barragem Sete Quedas; P3: Corrego do Campo; P4: Vala da Baixa da Fonte; P5: Cachoeira de Nana). Principal component analysis of the Dic was used to identify clustering based on the selected traits (Freeman and Jackson, 1992). To assess genetic relationship based on the Dic, we also conducted a network analysis (Kivela et al., 2015). Principal component and network analyses were performed with an open-source statistical package PAST version 4.03 (Hammer et al., 2001).

Data analysis

Univariate comparisons between *S. mansoni* infection and categorical variables were performed by a chi-square test with Yates' correction or Fisher's Exact test when appropriate, and continuous variables were performed using Kruskal-Wallis's test. The crude odds ratio (OR) and 95% confidence interval (CI) was obtained as a measure of the strength of association with and schistosomiasis. Adjusted OR was obtained from multivariate logistic regression analysis. All descriptive, univariate, and multivariate analyses were performed in Epi Info 7.2.2.6 (https://www.cdc.gov/epiinfo/index.html). We also performed Student's t test to evaluate differences between Dics from immigrants and natives and Mann-Whitney to assess differences between Dics for percent of lifetime spent in Salvador.

Results and Discussion

Of 2,011 residents interviewed, 1,134 (56%) provided at least one stool sample of which 62 were positive for *S. mansoni* (5.5%, 95% CI 4.2% - 7.0%, Table S2) and 75 for geohelminths (6.6%). Thus, schistosomiasis in the evaluated group living in Piraja is five times higher than the national average of 1% and more than twice as high as the Bahia state average of 2.1% (Ministerio da Saude do Brasil, 2014; Katz, 2018). This is typical

of many rural areas of the state. There was an association between *S. mansoni* infection and male sex (OR 3.0, 95% CI 1.7 - 5.1) and age >20 years old (OR 2.4, 95% CI 1.2 - 4.7, Table 1, FigS2). This is consistent with what we have observed in other urban areas of Salvador (Blanton et al., 2015; Barbosa et al., 2016; Silva et al., 2020) and in urban infection in Pernambuco State (Gomes et al., 2022). However, it contrasts with the younger age-specific risk of infection in rural areas (Blanton, 2019; Klohe et al., 2021) and with an urban area of Sergipe (Calasans et al., 2018). The mean intensity of *S. mansoni* infection was 89 +- SD 172 eggs per gram of feces (epg) was low by WHO criteria (Committee, 2002; World Health Organization, 2019) (Table S2). This was similar to two recent studies of urban schistosomiasis in Brazil (Calasans et al., 2018; Gomes et al., 2022).

Specific local risk was assessed by reported contact with surface water and association with *S. mansoni* infection. We observed an overall high risk to water contact in any of the evaluated points (OR 3.4, 95% CI 1.9 - 6.0) and specific risk points P1 (OR 2.8, 95% CI 1.7 - 4.8), P2 (OR 3.5, 95% CI 2.0 - 6.0), P3 (OR 2.2, 95% CI 1.2 - 4.2) and P5 (OR 8.6, 95% CI 3.6 - 20.8) (Table 1).

Migration has been indicated as a risk factor for urban schistosomiasis (Blanton, 2019; Klohe et al., 2021), but being born in Salvador was neither protective or a risk for infection. Immigrants (those not born in Salvador) were 17% of the sample (Table S2) and were no more likely to be infected than the native born (OR 1.98, 95% CI 0.84 - 4.67) (Table 1). Distribution of schistosomiasis by age revealed higher risk among male young adults and higher parasitic load among those above 60 years old (FigS2). Mean percentage of lifetime in Salvador was associated with *S. mansoni* or infection intensity (Table 1). While intensity of infection was numerically higher in immigrants (141 vs 83 eggs per gram, respectively), this was not statistically significant ($p = 0.65$). The higher intensity of infection in the 60+ age group is likely due to the small numbers of samples. A history of traveling was protective (OR 0.4, 95% CI 0.1 - 0.9) (Table 1). These data suggest that immigration was not associated with risk for schistosomiasis, and local acquisition was more important. Indeed, the more time spent in the city, the greater the risk of infection.

Analysis of genetic differentiation of parasite populations further supports primarily a local transmission pattern. Of the 62 stool samples collected, 51 were successfully genotyped (80%). The average differentiation between infra-populations (Di) was 0.22 (Table S1) and the differentiation between infra-populations from immigrants compared to the native-born (Dc) was 0.06. While the average Di indicates individuals were acquiring genetically different groups of parasites, this is typical of areas with low infection intensities. The Dc between parasites of immigrants and parasites of native-born was low relative to the Di, indicating that the two groups were drawn from the same source.

The genetic distance of individuals from the group is another approach to evaluating whether parasites of immigrants largely originated outside of the Piraja community. We have shown that communities separated by 6 km on the same river can be distinguished by Dc (Blanton et al., 2011) and principal coordinate with k-means analysis of the Dic or Nei's genetic distance (Long et al., 2022). Using parasites of those born in Salvador as the component population, no clustering was observed by principal component (PCA) or network analyses (FigureS3). We also stratified by percentage of lifetime in Salvador and no clustering was observed as well (Figure 2). No clustering was observed by PCA analyses when comparing differences by age, sex, history of contact with freshwater bodies when traveling and at the distinct points in Piraja neighborhood (FigureS4).

It is often unclear if the problem of schistosomiasis is rising or falling with increasing urbanization or if rural-urban migration is fueling the presence and persistence of the infection in cities (Klohe et al., 2021). In Piraja, immigrants may be more sinned against than sinning (King Lear, 3.2 49-60, Shakespeare) as far as schistosomiasis is concerned. Although some importation of *S. mansoni* is possible due to immigration, even necessary to stablish local transmission, the evidence in this urban neighborhood suggests that immigrants primarily become infected in the city. The conditions of crowding and sanitation are what perpetuate transmission and focusing on these conditions will powerfully resolve the issue of urban schistosomiasis.

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Authors' Contributions

CFC, LMB, LKS, MGR and REB conceived and designed the study. CFC, GSS, FMC, PSM, VSZ, VTM, APCS, FS and LMB involved in acquisition of data. CFC, GSS, FMC, LMB, LKS, MGR, and REB analyzed and interpreted of data. CFC, GSS and REB drafted the original version of the manuscript. CFC, GSS, FMC, LMB, LKS and REB were involved in reviewing and editing. MGR and REB contributed with reagents/material and analyzed tools. All authors contributed for the final version of the manuscript.

Competing Interests

The authors declare no conflict of interest.

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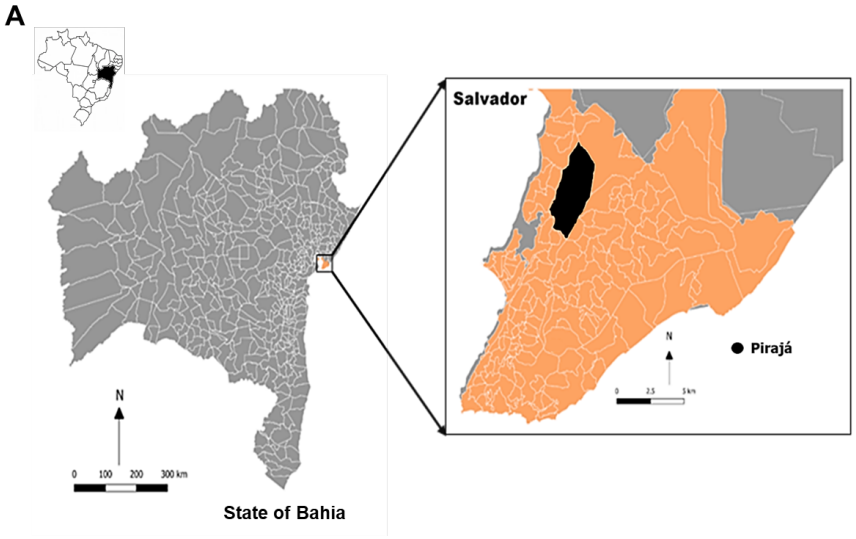


Figures

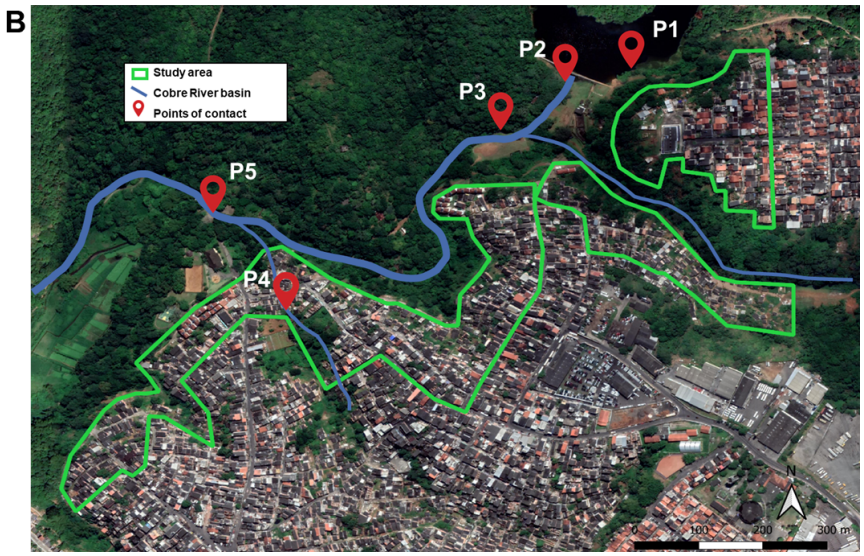
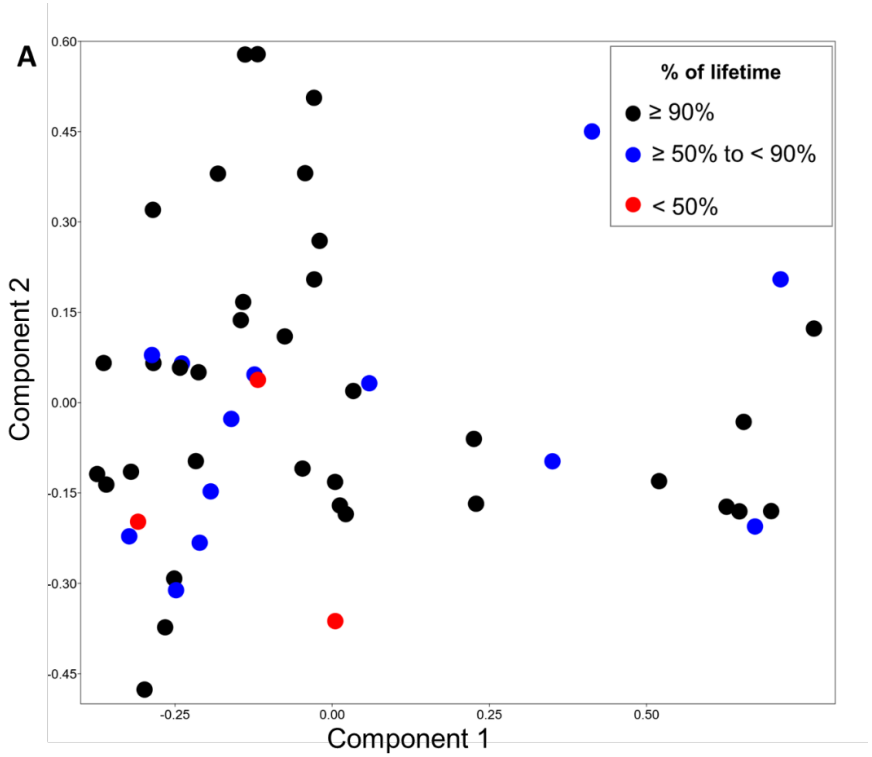


Figure 1. Study area highlighting Pirajá neighborhood in Salvador city, State of Bahia, north-eastern Brazil. (**A**) The map highlights Pirajá neighborhood craved in the urban area of Salvador city, Bahia, Brazil. (**B**) Study area and distribution of points along the Cobre River basin where the community in Pirajá often gets in contact with water: (**P1**) Represa do Cobre, (**P2**) Barragem Sete Quedas, (**P3**) Córrego do Campo, (**P4**) Vala da Baixa da Fonte, (**P5**) Cachoeira de Nanã. This map was generated using QGIS version 3.18.3 (Zürich-CH).



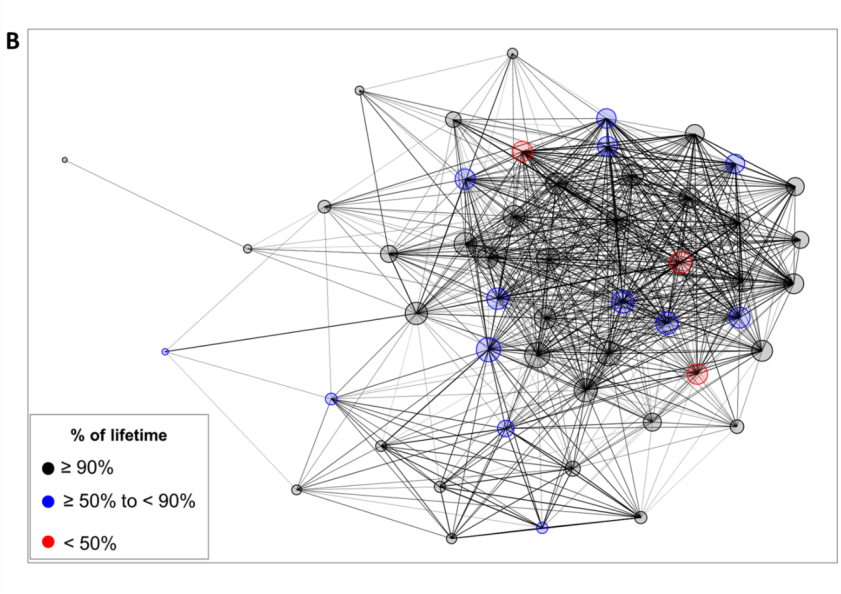


Figure 2. Genetic differentiation for *Schistosoma mansoni* : percentage (%) of lifetime between 51 infra-populations and the component population of parasites of the native-borne (Dic) in Pirajá neighborhood, Salvador-BA, Brazil, 2019, using 15 microsatellite loci (smms2, smms13, smms16, smms3, smms17, smms18, smms21, smda23, sm13-478, 1f8a, 29e6a, smu31768, lg3_sc36b, sc23b, smd28). **(A)** Principal component analysis comparing genetic distances for the % of lifetime in Salvador-BA, Brazil. Support values were calculated by bootstraps with 1000 iterations. The first two principal components were graphed. Component 1: eigenvalue= 0.112, 39% of variance; Component 2: eigenvalue= 0.059, 21% of variance.**(B)** Network array comparing genetic distances for % of lifetime in Salvador-BA, Brazil. Network nodes represent individuals from the infra-population. Links represent genetic distances. Larger nodes mean more interaction and thicker lines more similarities. For all analyses genetic distances using Dic were applied as variance-covariance matrix.

Tables

Traits	Crude OR*	95%CI	Adjusted OR**	95% CI
Male sex	3.0	1.7 - 5.1	3.0	1.7 – 5.4
> 20 years old	2.4	1.2 - 4.7	3.5	1.7 – 7.3
Born in Salvador	2.0	0.8 - 4.7	NS	NS
Immigrants	0.7	0.3 - 2.1	NS	NS
% Lifetime in Salvador	-	-	1.6	1.2 - 2.0
History of travelling	0.4	0.1 - 0.9	0.3	0.1 – 0.7
Water contact - Traveling	1.9	0.3 - 11.4	NS	NS
SSE D/E vs B/C	1.4	0.9 – 2.4	NS	NS
Sewage	0.6	0.1 – 4.9	NS	NS
Water contact – Pirajá	3.4	1.9 – 6.0	2.7	1.0 – 6.8
P1- Represa do Cobre	2.8	1.7 – 4.8	2.2	1.2 – 3.9
P2- Barragem Sete Quedas	3.5	2.0 – 6.0	2.2	1.2 – 4.0

Traits	Crude OR*	95%CI	Adjusted OR**	95% CI
P3- Córrego do Campo	2.2	1.2 – 4.2	1.3	0.6 – 2.7
P4- Vala da Baixa da Fonte	1.7	0.4 – 7.3	NS	NS
P5- Cachoeira de Nanã	8.6	3.6 – 20.8	6.9	2.7 – 17.8
Types of contact				
Leisure	2.6	1.5 – 4.3	0.8	0.4 – 2.0
While walking	3.2	1.8 – 5.5	1.3	0.6 – 2.8
Doing laundry	4.5	1.2 – 16.4	2.9	0.7 – 12.6
Fishing	3.2	1.8 – 6.0	0.9	0.4 – 1.9
Work	2.1	0.5 – 9.2	NS	NS
Frequency >7 times a week	3.3	1.9 – 5.8	1.3	0.2 – 2.6
Duration >1 hour	3.3	1.9 – 5.9	1.5	0.7 – 3.1

Table 1. Characteristics associated with *Schistosoma mansoni*infection among the population who participated in the epidemiological and parasitological surveys (n= 1,134) at Pirajá neighborhood in Salvador, Bahia, Brazil.

P = Point of contact with water with risk of infection.

* OR calculated by cross product. ** Logistic Regression including all variables significant in univariate analysis. The effect is in logistic-scale and OR was calculated as $e^{coefficient}$for each variable in the logistic model. "-" hyphen = comparison not made.

Supporting Information

FigureS1. Working flow chart for Pirajá neighborhood.

FigureS2. *Schistosoma mansoni* parasitic load based on eggs per gram and prevalence by age classes and sex (male and female).

FigureS3. Genetic differentiation for Schistosoma mansoni: place of birth between 51 infra-populations and the component population in Pirajá neighborhood, Salvador-BA, Brazil, 2019.

FigureS4. PCA analysis of genetic differentiation for Schistosoma mansoni between 51 infra-populations and the component population for distinct traits and points of contact with water in Pirajá neighborhood, Salvador-BA, Brazil, 2019.

Table S1. Genotyping success of microsatellites (markers) for*Schistosoma mansoni* .

Table S2. General features from the participants in the survey.