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RAPID COMMUNICATION

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Evidence for local transmission and maintenance of schistosomiasis in an urban neighbourhood 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 worldwide, and increasing urbanization is one of the most important demographic shifts of the 20th and now 21st 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 neighbourhood 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 neighbourhood to interrupt the local transmission cycle.

KEYWORDS
migration, population genetics, *Schistosoma mansoni*, urbanization

1 | 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 & 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 produces 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 & Batista, 2012).

Schistosoma 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

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*In memoriam: to whom this work is dedicated.

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, that is infected humans, contact with surface waters (agricultural work, recreation), presence of susceptible snails and poor sanitation (Klohe et al., 2021; Zanardi et al., 2019). 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 centres is thought to be highly focal (McManus et al., 2018; Montgomery, 2020), 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 neighbourhood designated Pirajá, in the city Salvador, Bahia in Northeast 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.

2 | MATERIALS AND METHODS

2.1 | 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.

2.2 | Study area and population survey

The Pirajá neighbourhood (12°53′45.00″ S 38°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 aged 20–49 years. 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 per cent 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 borders the park, river and reservoir was selected for sampling.

The study area comprised 650 households where 2011 residents were interviewed and 1134 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) (Figure S1). 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).

2.3 | Stool survey and egg isolation

All stool samples collected from participants were weighed, and single slides were prepared as previously described (Katz & Pellegrino, 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 (55–300 μm mesh pore size; FSI, Michigan City, Indiana, USA) and gravity sedimentation to concentrate the eggs (Dresden & 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 stool samples were given a one-time dose of praziquantel (60 mg/kg for children 4–15 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 (Figure S1).

2.4 | DNA extraction and genotyping

DNA was isolated from the sediment by a standard phenol/chloroform protocol, and then treated with cetyltrimethylammonium bromide to remove PCR inhibitors as previously described (Ausubel, 1987;

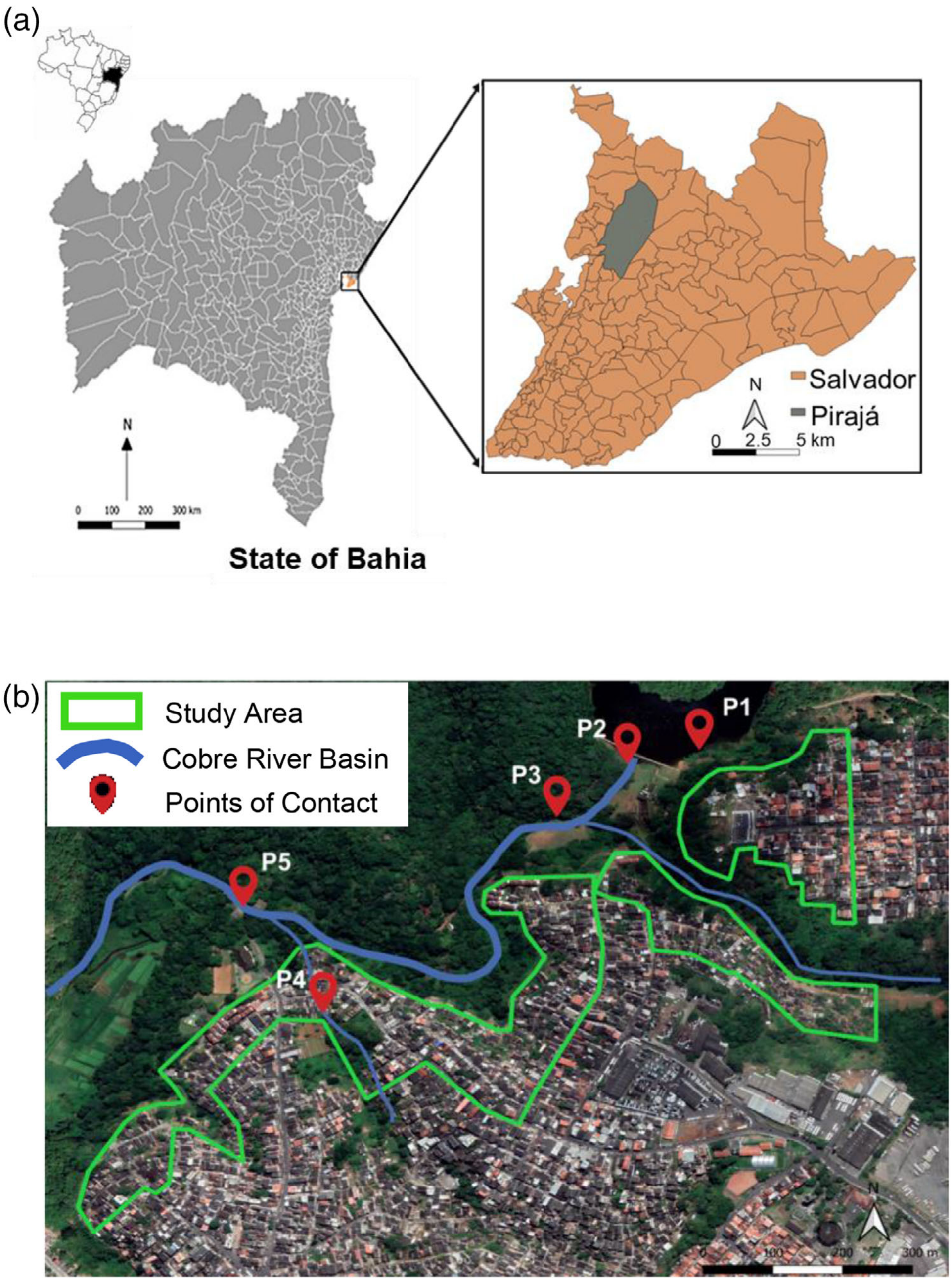

FIGURE 1 Study area: Pirajá neighbourhood in Salvador city, State of Bahia, north-eastern Brazil. (a) Pirajá (black) is shown within the municipal area of Salvador, Bahia, Brazil. (b) Study area and distribution of points along the Cobre River basin where the community in Pirajá often gets in contact with water: (P1) Represa do Cobre, (P2) Barragem Sete Quedas, (P3) Córrego do Campo, (P4) Vala da Baixa da Fonte, and (P5) Cachoeira de Nanã. This map was generated using QGIS version 3.18.3 (Zürich-CH)

Blanton et al., 2011). For genotyping, duplicate 2 µl of DNA samples per primer pair was used to PCR amplify 15 polymorphic microsatellite loci (Table S1) 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, and smd28) (Kovach et al., 2021).

TABLE 1 *Schistosoma mansoni* infection associations for the participating population in the Pirajá neighbourhood in Salvador, Bahia, Brazil ($n = 1134$)

Traits	Crude OR[a]	95% CI	Adjusted OR[b]	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**
Immigrants	0.7	0.3–2.1	–	–
Immigrant percent 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	–	–
SES D/E vs. B/C	1.4	0.9–2.4	–	–
Adequate Sewage	0.6	0.1–4.9	–	–
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**
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	–	–
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	–	–
Frequency >7 times a week	3.3	1.9–5.8	1.3	0.2–2.6
Duration >1 h	3.3	1.9–5.9	1.5	0.7–3.1

Note: En dash '–' indicates value was undefined or there were cells equal to zero. The effect is in logistic scale and OR was calculated as $e^{\text{coefficient}}$ for each variable in the model. Statistically significant results based on the adjusted OR are presented in bold print. The crude OR is based on univariable analysis of bivariable categories against the dependent variables: infection or no infection with *Schistosoma mansoni*. Percent lifetime spent in Salvador, however, is a continuous independent variable assessed for those not born in Salvador. History of traveling was positive if the respondent had left the city of Salvador in the last year. Water contact while traveling was also asked. P = Water contact point. SES D/E vs. B/C: socio-economic status in Brazil is categorized by letters. The most disadvantaged (D and E) grouped here in one category and those with better conditions (B and C) forming another group. There were no participants in the A category. Adequate sewage was a connection to the municipal system or septic tank.

[a] OR calculated by cross product.

[b] Logistic regression including all variables significant in univariate analysis.

2.5 | Genetic differentiation

The differentiation index, Jost's *D* (Jost, 2008), was calculated from the allele frequencies using SpadeR (Chao & 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 infra-populations (parasite aggregated within one host) is designated Di, between component populations Dc (parasites aggregated within a group of hosts) and between an infra-population and a component population is Dic. Genetic differences were analysed among component populations of *S. mansoni* based on the following traits: age, sex, place of birth, percentage of time living in Piraja, history of traveling outside of Piraja and contact with freshwater during traveling and contact with freshwater at five points within Piraja (P1: Represa do Cobre; P2: Barrage Sete Quedas; P3: córrego do campo; P4: vala da baixa da fonte; P5: Cachoeira de Nanã). Principal component analysis of Dic was used to identify clustering based on the selected traits (Freeman & Jackson, 1992). To assess genetic relationship based on Dic, we also conducted a network analysis (Kivelä et al., 2015). Principal component and network analyses were performed with an open-source statistical package PAST version 4.03 (Hammer et al., 2001).

2.6 | 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 examined using Kruskal–Wallis test. The crude odds ratio (OR)

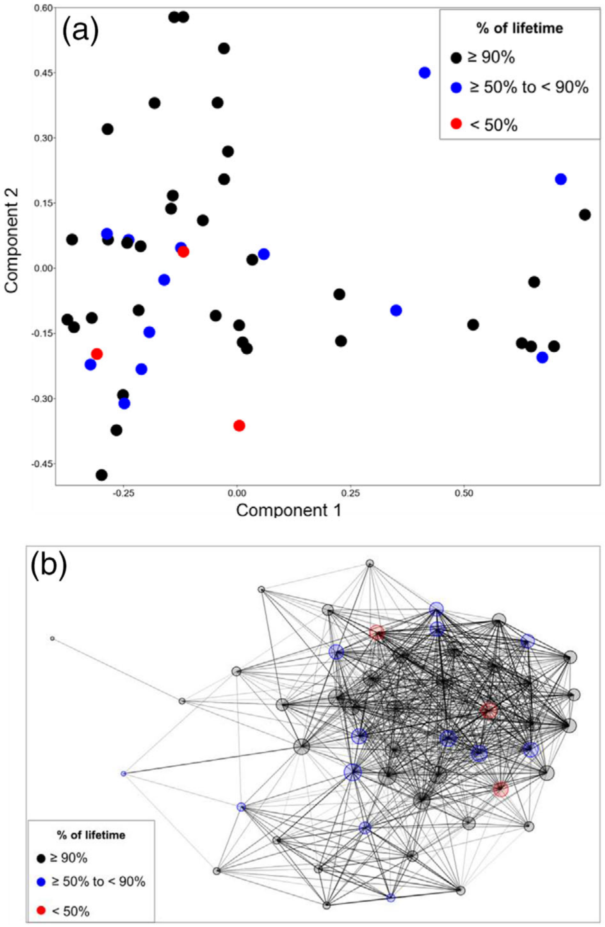

FIGURE 2 Genetic differentiation for *Schistosoma mansoni*: percentage (%) of lifetime between 51 infra-populations and the component population of parasites of the native-borne (Dic) in Pirajá neighbourhood, Salvador-BA, Brazil, 2019, using 15 microsatellite loci (smms2, smms13, smms16, smms3, smms17, smms18, smms21, smda23, sm13-478, 1f8a, 29e6a, smu31768, lg3_sc36b, sc23b, and smd28). (a) Principal component analysis comparing genetic distances for the percentage 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 percentage 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

and 95% confidence interval (CI) were obtained as a measure of the strength of association with the corresponding predictor and schistosomiasis. Adjusted OR was obtained from multivariate logistic regression analysis with the corresponding predictor and *S. mansoni* infection. 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.

3 | RESULTS AND DISCUSSION

Of 2011 residents interviewed, 1134 (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 Pirajá is five times higher than the national average of 1% and more than twice as high as the Bahia state average of 2.1% (Katz, 2018; Ministério da Saúde do Brasil, 2014). This is typical of many rural areas of the state. There was an association between *S. mansoni* infection and male sex (adjusted OR: 3.0, 95% CI: 1.7–5.4) and age >20 years old (adjusted OR: 3.5, 95% CI: 1.7–7.3; Table 1; Figure S2). This is consistent with what we have observed in other urban areas of Salvador (Barbosa et al., 2016; Blanton et al., 2015; 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). For those infected, the mean intensity was 89 ± SD 172 eggs per gram of faeces (epg), low by WHO criteria (WHO Expert 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 risk with water contact (adjusted OR: 2.7, 95% CI: 1.0–6.8), but the risk was specifically associated with points P1 (adjusted OR: 2.2, 95% CI: 1.2–3.9), P2 (adjusted OR: 2.2, 95% CI: 1.2–4.0) and P5 (adjusted OR: 6.9, 95% CI: 2.7–17.8) (Table 1). At all of the associated sites, residents tended to have full body contact or emersion. The adjusted OR was not significant for P3 (Corrego do Campo) and P4 (Vala da Baixa da Fonte). The Córrego is a small stream that courses along a soccer field, and residents only have occasional contact. The Vala is the site where an underground spring surfaces and has little faecal contamination or frequent contact by the population.

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 nor 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). Spending a greater percent of lifetime in Salvador for immigrants was associated with infection (adjusted OR: 1.6, 95% CI: 1.2–2.0). Distribution of schistosomiasis by age revealed higher risk among male young adults and higher parasitic load among those above 60 years old (Figure S2). 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* = .65). The higher intensity of infection in the 61–70 and 70+ age groups (represented by two and one individual, respectively) is likely due to the small numbers. A history of traveling away from Salvador was protective (adjusted OR: 0.3, 95% CI: 0.1–0.7) (Table 1). These data suggest that immigration was not associated with risk for schistosomiasis, and local acquisition was more important. Indeed, the more the 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 that individuals were acquiring genetically different groups of parasites, this is typical of areas with low infection intensities. Dc between parasites of immigrants and parasites of native-born was low relative to 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 Pirajá 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 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 or network analyses (Figure S3). We also stratified by percentage of lifetime spent in Salvador and no clustering was observed as well (Figure 2). No clustering was observed by principal component analyses when comparing differences by age, sex and history of contact with freshwater bodies when traveling and at the distinct points in Pirajá neighbourhood (Figure S4).

It is often unclear if the problem of schistosomiasis is rising or falling with increasing urbanization or if rural–urban migration is fuelling the presence and persistence of the infection in cities (Klohe et al., 2021). In Pirajá, 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 establish local transmission, the evidence in this urban neighbourhood suggests that immigrants primarily become infected in the city. The conditions of crowding and sanitation perpetuate transmission and focusing on these conditions will powerfully resolve the issue of urban schistosomiasis.

AUTHOR CONTRIBUTIONS

Camila F. Chaves, Lucio M. Barbosa, Luciano K. Silva, Mitermayer G. Reis and Ronald E. Blanton conceived and designed the study. Camila F. Chaves, Gilberto Sabino-Santos, Fernanda Mac-Allister Cedraz, Pedro Santos-Muccillo, Vanessa S. Zanardi, Vanessa T. Moretto, Adriano P. C. Santos, Fabiano Simões and Lucio M. Barbosa were involved in acquisition of data. Camila F. Chaves, Gilberto Sabino-Santos, Fernanda Mac-Allister Cedraz, Lucio M. Barbosa, Luciano K. Silva, Mitermayer G. Reis, and Ronald E. Blanton analysed and interpreted of data. Camila F. Chaves, Gilberto Sabino-Santos and Ronald E. Blanton drafted the original version of the manuscript. Camila F. Chaves, Gilberto Sabino-Santos, Fernanda Mac-Allister Cedraz, Lucio M. Barbosa, Luciano K. Silva and Ronald E. Blanton were involved in reviewing and editing. Mitermayer G. Reis and REB contributed with reagents/material and analysed tools. All authors contributed for the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request

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