Genetic Population Structure of Cercariae from an Urban Foci of *Schistosoma mansoni*, Brazil

Samaly S. Souza, Lúcio M. Barbosa, Isabel C. Guimarães, Walter A. Blank, Renato Barbosa Reis, Mitermayer G. Reis, Ronald E. Blanton,* and Zilton A. Andrade

Gonçalo Moniz Research Centre, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil; Center for Control of Zoonoses, Municipal Secretariat of Health, Salvador, Bahia, Brazil; Center for Global Health and Diseases, Case Western Reserve University, Cleveland, Ohio

Abstract. Rapid urbanization in Brazil has meant that many persons from rural areas where *Schistosoma mansoni* is endemic have migrated to cities. Discovery of a focus of active transmission in the city of Salvador prompted a citywide survey for active and potential transmission sites. Cercariae shed from infected snails collected from four locations were used to determine how these samples were related and if they were representative of the parasite population infecting humans. Each cercarial collection was greatly differentiated from the others, and diversity was significantly lower when compared with eggs from natural human infections in one site. Egg samples collected 7 years apart in one neighborhood showed little differentiation (Jost’s D = 0.01–0.03). Given the clonal nature of parasite reproduction in the snail host and the short-term acquisition of parasites, cercariae from collections at one time point are unlikely to be representative of the diversity in the human population.

INTRODUCTION

Infection with *Schistosoma mansoni* remains one of the most important public health problems in Brazil. In this country, the parasite infects 2–6 million persons in 9 states despite > 30 years of control programs. Since 2008, it has been responsible for 400–500 hospitalizations per year and a highly prevalent but poorly measured chronic burden of disease. In addition, it contributes to 500 deaths each year (http://portal.saude.gov.br/portal/arquivos/pdf/obitos_1990_2008_06_04_11.pdf). Across Brazil, the northeastern states are the most affected by schistosomiasis. In the northeastern state of Bahia, 146 of 417 municipalities are endemic and show widespread transmission, 144 have focal transmission, and 127 are unaffected. However, even those unaffected have a high potential for transmission, given the presence of the snail host and human and/or snail infections in neighboring areas and migration.5

Although all of Brazil has experienced a demographic shift from a rural to an urban concentration of the population, the state of Bahia and its capital city of Salvador have seen much faster growth than the country as a whole (Figure 1). During 1980–2010, the greater metropolitan area of Salvador grew by more than 200% and now has more than 3 million persons. Although often considered primarily a rural problem, schistosomiasis today is found even in peri-urban and central regions of Salvador. Recently, autochthonous cases were identified around São Bartolomeu Park in the Ferroviária Suburban region of Salvador where in one neighborhood 30% of children surveyed were found to be infected, although most had never left the city.6

In surveys conducted by the municipal Center for Control of Zoonosis (CCZ), Biomphalaria glabrata, the principal snail vector of schistosomiasis in Bahia, has been identified in water bodies throughout the city. Natural foci of *S. mansoni* infection in the city were already known in the 1950s and 1960s,7 and the urbanization of schistosomiasis in Brazil has been observed in other studies.6,8–13 Although the presence of urban schistosomiasis is not new, its increasing recognition indicates deterioration or inadequacy of the sanitation infrastructure combined with human migration as relevant factors in the transmission and maintenance of the disease in urban spaces.

The fundamental basis for population genetic analysis is that organisms that tend to breed together have characteristic allele frequency patterns when stable or show perturbations in the patterns when the population experiences forces that change its composition. We wished to compare the relatedness of cercariae released from several geographically separated populations of snails within the city of Salvador to each other and to eggs from the infected human population in one neighborhood of the city.

The CCZ, in partnership with the Experimental Pathology Laboratory Research Center of the Oswaldo Cruz Institute Bahia conducted a malacologic survey of all major bodies of water in the city of Salvador for the presence of *B. glabrata*. The survey was designed to map and to determine the distribution and prevalence of schistosome infections with *S. mansoni* across the city. We used samples collected in this ongoing mapping effort to determine the relationship between cercariae in isolated urban water systems and those populations of parasites infecting humans.

Understanding the genetic distribution and parasite population dynamics has direct relevance for public health because it provides insights into how parasite populations recover, the nature of parasite persistence,14 geographic clustering, and movement among communities. It might contribute to public health by measuring the true impact of control efforts on parasite population reduction. This study concerns cercarial populations collected in newly identified areas of transmission in the state capital, Salvador, a modern metropolis of more than 3 million persons.

MATERIALS AND METHODS

Study areas and sample collection. Salvador is the most populous city in northeastern Brazil and has 3.6 million inhabitants in the greater metropolitan area.15 It is located on 706.8 km² of hilly terrain in a humid tropical climate and is mostly composed of densely packed neighborhoods. In some areas, such as parks, there is dense vegetation typical of the coastal

---

*Correspondence to Ronald E. Blanton, Center for Global Health and Diseases, Case Western Reserve University, Wolstein Research Building, 2301 Cornell Road, Cleveland, OH 44106. E-mail: reb6@case.edu
Atlantic forest. For public health purposes, the city is divided into 12 sanitary districts. A survey of snail populations was conducted district by district that included 158 major, permanent surface water sites consisting of dam and reservoir combinations, rivers, streams, drainage ditches/open sewers, streams, wetlands, ponds, springs, channels, wells and dikes. Agents of the CCZ responsible for the snail collections were given a one-week basic course on parasite and snail biology, snail identification, biosafety, use of personal protective equipment, and transport of biological material. For collection, each agent was equipped with scoops, forceps, rubber boots, and gloves.

The CCZ agents collected up to 50 snails within defined 10-meter segments along the targeted waterway or basin. Collections occurred at various times of the year. The coordinates of each snail collection were recorded with a handheld differential global positioning system unit, and the program ArcGis 9.3 (ESRI, Redlands, CA) was use for analysis of geographic positioning. These coordinates were then mapped to an outline of metropolitan Salvador at an initial scale of 1:2,000. The collected snails were speciated and placed in a covered, darkened tank with dechlorinated water for 48 hours. Pools of snails from each location were exposed to light from a 40-watt incandescent bulb in dechlorinated water to stimulate cercarial release for 20 minutes. After thorough washing, individual snails from positive pools were placed in 10 mL of dechlorinated water, exposed to light, and the water was examined for cercariae. The number of shedding snails was recorded. For each location, cercariae were pooled and fixed in 70% ethanol. Final cercarial numbers were estimated by microscopic examination of two 1-mL samples, except for the Lago de Urubu collection, for which numbers were estimated by extrapolation from results of a quantitative real-time polymerase chain reaction (PCR) and cercarial counts from collections from other sites (Dique do Cabrito = 6.5 \times 10^{-5} \text{ pg of DNA/cercaria}; Rio do Cobre = 5.6 \times 10^{-5} \text{ pg of DNA/cercaria}; and Pituaçu = 7.5 \times 10^{-5} \text{ pg of DNA/cercaria}).

To compare cercarial diversity with parasite egg diversity, we genotyped *S. mansoni* eggs collected in 2004 from 8 infected persons living in the São Bartolomeu neighborhood of Salvador and who had never visited rural schistosomiasis-endemic sites. Fecal samples were pooled in this instance before egg isolation. Egg isolation was performed as described \(^{17}\) by using selective sieving and sedimentation. In addition, in 2011, we genotyped individual infrapopulations collected from eggs found in fecal samples of 36 infected persons living in 3 of 6 defined microareas of São Bartolomeu. The microareas are administrative units used by the Family Health Program and eachcontains approximately 1800 persons. The microareas selected were not immediately adjacent to one another. These samples were not pooled, but genotyped as separate infrapopulations and analyzed together as component populations for each microarea. Cercariae from a laboratory strain maintained at the Oswaldo Cruz Foundation, Bahia (Feira de Santana strain) were used for comparison. DNA from 200 adult worms from a laboratory strain maintained at Case Western Reserve University (CWRU strain) was used for the PCR-positive control. Written consent was obtained from all human subjects. The Committee on Ethics in Research of the Oswaldo Cruz Foundation of Salvador, Bahia, the Brazilian National Committee on Ethics in Research and the Institutional Review Board for Human Investigation of University Hospitals Case Medical Center, Cleveland, Ohio approved the study design.

**DNA extraction and S. mansoni DNA quantification.** Before DNA extraction, tubes with cercariae in 70% ethanol were centrifuged at 14,000 \times g, the ethanol was drained, and the pellet was dried briefly. DNA was then extracted using the DNeasy Blood and Tissue DNA Isolation Kit (QIAGEN, Valencia, CA) according to the manufacturer’s protocol. To quantify the *S. mansoni* DNA, a PCR was performed using primers specific to the *S. mansoni* small ribosomal RNA subunit \(^{18}\) as described. \(^{14}\)

**Genotyping and analysis.** All samples were genotyped in duplicate by PCR amplification of microsatellite loci using fluorescent-labeled primers to 14 microsatellite markers, \(^{14}\) followed by capillary electrophoresis for peak detection as described. \(^{17}\)

For each site, the total number of alleles and the average effective allele number over all markers were calculated. The population effective allele number \(^{19}\) was calculated according to the equation \(1 \sum_{r=1}^{S} \rho_i^2\) where \(\rho_i\) is the frequency of the \(i\)th allele for each marker. Note that this is a simple transformation of expected heterozygosity. \(^{20}\) Differences in effective allele number were compared by using the Wilcoxon signed rank test.

To measure genetic differentiation, Jost’s \(D^2\) \(^{21}\) was calculated by using SPADE software. \(^{22}\) We used Jost’s \(D\) as a measure of the degree of relatedness between groups. This index has been shown to perform best when the markers are highly polymorphic and in populations with high diversity. \(^{21,23,24}\) In previous studies, we found that Jost’s \(D\) is proportionally similar to the \(F_{ST}\).

---

humans was determined by using the allele frequency from the six infections in each microarea weighted by the intensity of infection. Pairwise comparisons of allele frequencies between samples were performed with a bootstrap of 1,000 replicates. The allele counts used in SPADE were obtained by multiplying the allele frequencies for each locus by the number of cercariae that made up each sample.

RESULTS

Of 158 sites investigated, 120 were positive for *B. glabrata*, which was the only *S. mansoni* intermediate host found. Snails produced cercariae at seven sites (Figure 2 and Table 1), and cercariae were available from five of these sites for DNA extraction. Infected snails were obtained from a variety of water body sizes and types (Table 2), but all were found to drain areas with dense human populations and a mixture of housing qualities. The prevalence of infection in snails ranged from 14.5% to 56%. The smallest number of cercariae shed from snails collected in the field (Table 1) was from the Itacaranhas sample (76) and the largest from the Rio do Cobre sample (14,800). Fourteen microsatellite markers were used to genotype the samples. DNA of cercariae shed from snails collected at the Lagoa do Urubú amplified poorly for all markers. Therefore, this sample was excluded from analysis of differentiation.

The total number of alleles observed in cercariae from all sites was 124, but the number of alleles found in cercariae sampled from any one site ranged from 44 in the district of Itacaranhas to 91 in the district of Pituacu. The average effective allele number was similar across all cercarial samples. Eggs from infected persons in São Bartolomeu had a larger number of alleles compared with samples of cercariae, and a significantly greater effective allele number (3.48–3.96 versus 1.86–2.75) and therefore greater diversity (Table 2). DNA from cercariae of a laboratory strain in Brazil and more than 200 worms from the CWRU strain demonstrated 87 and 101 discrete alleles, respectively.

The genetic differentiation index Jost’s $D$ was calculated for the cercaria, egg, and worm populations (Table 3). Cercarial samples from the Cachoeira (São Bartolomeu), Avenida Vasco da Gama, and Lagoa do Urubú were excluded from analysis because of poor amplification. A pairwise comparison of the available cercarial collections around the city showed that each was highly differentiated from the other to a much greater extent than was observed for stool eggs between persons and communities in rural Bahia. We also observed that

**Table 1**

*Schistosoma mansoni* sample characteristics, Brazil

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Name</th>
<th>Source description</th>
<th>Parasite count (stage)</th>
<th>No. infected persons (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dique do Cabrito</td>
<td>Snails from small neighborhood lake</td>
<td>1,178* (cercariae)</td>
<td>10 (22.0)</td>
</tr>
<tr>
<td>2</td>
<td>Itacaranhas</td>
<td>Snails from drainage ditch/sewer</td>
<td>76 (cercariae)</td>
<td>17 (23.0)</td>
</tr>
<tr>
<td>3</td>
<td>Rio do Cobre</td>
<td>Snails from shore of river outside São</td>
<td>14,800 (cercariae)</td>
<td>20 (30.0)</td>
</tr>
<tr>
<td>4</td>
<td>Guachiria</td>
<td>Snails from base of São Bartolomeu waterfall</td>
<td>Cercariae*</td>
<td>12 (14.5)</td>
</tr>
<tr>
<td>5</td>
<td>Pituacu</td>
<td>Snails from temporary water collections from a municipal park</td>
<td>2,000 (cercariae)</td>
<td>34 (56.6)</td>
</tr>
<tr>
<td>6</td>
<td>Av. Vasco da Gama</td>
<td>Snails from median strip drainage ditch/sewer</td>
<td>(cercariae)†</td>
<td>4 (27.3)</td>
</tr>
<tr>
<td>7</td>
<td>Lagoa do Urubú</td>
<td>Snails from small neighborhood lake</td>
<td>2,800 (cercariae)</td>
<td>4 (40.0)</td>
</tr>
<tr>
<td>8</td>
<td>São Bartolomeu</td>
<td>Infected children from São Bartolomeu neighborhood (2004)</td>
<td>(eggs)†</td>
<td>8 (30.2)</td>
</tr>
<tr>
<td>9</td>
<td>MA1</td>
<td>Infected persons from São Bartolomeu neighborhood microarea 1 (2011)</td>
<td>127,728 (eggs)</td>
<td>12 (22.8)</td>
</tr>
<tr>
<td>10</td>
<td>MA3</td>
<td>Infected persons from São Bartolomeu neighborhood microarea 3 (2011)</td>
<td>30,616 (eggs)</td>
<td>12 (23.1)</td>
</tr>
<tr>
<td>11</td>
<td>MA6</td>
<td>Infected persons from São Bartolomeu neighborhood microarea 6 (2011)</td>
<td>153,273 (eggs)</td>
<td>12 (55.6)</td>
</tr>
<tr>
<td>12</td>
<td>Feira de Santana</td>
<td>Oswaldo Cruz Bahia laboratory strain</td>
<td>29,600 (cercariae)</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>CWRU</td>
<td>Case Western Reserve University laboratory strain</td>
<td>=200 (adult worms)</td>
<td>–</td>
</tr>
</tbody>
</table>

*Estimated cercarial count by a quantitative polymerase chain reaction.
†Samples no longer available for counting.
samples from humans in the same community (Table 3). Sáo Bartolomeu; MA1, MA3, and MA6) showed the least differentiation despite sample collections separated by seven years. Cercariae in field collections were less diverse (total number of alleles = 102, weighted effective allele number = 2.72) than eggs from human infections (118 and 3.61). The difference between most field collections was as great as that between a field collection and laboratory specimens. The only two collections that suggested potential gene flow between them were the laboratory strain of Feira de Santana and Dique do Cabrito (mean Jost’s $D = 0.017$). However, this result was likely spurious because these collections were reproductively isolated from each other for many years and no other samples from Brazil were similarly close. Therefore, there was no correlation geographic location and differentiation indices for cercariae from around the city.

**DISCUSSION**

Schistosomiasis mansoni is an endemic infectious disease with worldwide repercussions on the health of populations. The urbanization of schistosomiasis has come about through a process of human migration and settlement patterns that have left many cities of the developing world with areas as characterized of the countryside as of the metropolis. Accordingly, the city of Salvador, Bahia, Brazil, has grown nearly 200% in the past 20 years and continues to show S. mansoni infections because of immigration from rural schistosomiasis-endemic areas. Identification of infected snails leaves little doubt that one component of urban schistosomiasis is local transmission, and this finding requires a different response from public health institutions than for imported cases alone. More than 75% of sites sampled in the city were positive for *B. glabrata*, and there was active shedding of cercariae detected at 4.4% of these sites. At sites in which infected snails were present, the prevalence among snails was high (> 20%). At almost all sites studied, children and adults used the water or nearby areas for leisure activities, making these sources risks for continued transmission. Under these circumstances, the identification and mapping of areas harboring snails infected by *S. mansoni* are aids to surveillance and intervention against this infection.25

Cercariae within the city had a high degree of genetic differentiation between sites. This finding was as great as between samples geographically isolated from Salvador and elsewhere in Brazil. This could be consistent with reproductive isolation for these samples either because of geographic isolation of infected persons or more precisely, isolation of their wastes. It may also represent immigration of infected humans from different schistosomiasis-endemic zones in which parasite populations would be reproductively isolated from one another. However, an additional consideration should also be the nature of how snails sample parasites from the human population. Reproduction in the snail is asexual, and individual snail infections represent only 1–4 parasites and thus a small number of genotypes.26 In addition, because the maximum life span of snails is estimated to be 18 months,27 there is a limited time for acquisition of new genotypes. This lower diversity in cercariae in field collections is reflected in the total allele number and weighted average effective allele number (102 and 2.72, respectively) compared with the higher number for eggs from human infections (118 and 3.61, respectively). The effective number of alleles represents the number of alleles of equal frequency necessary to reproduce the observed genetic diversity. However, comparisons of effective allele numbers need to be interpreted with caution because population size and reproductive mechanisms across developmental stages differ between samples. Cercariae from snail collections at a single point in time are not likely to represent the full genetic diversity of worms present in humans.

In contrast, in a study in Kenya, Steinhauer and others found little differentiation between cercariae shed from infected snails around Lake Victoria, but a higher diversity than in Salvador (calculated mean ± SD effective allele number = 5.3 ± 0.3).28 The intensity of transmission, geography of infection, genotyping techniques, numbers of infected snails, and underlying parasite population structure in humans may all contribute to these differences. Lake Victoria represents a single, unobstructed ecosystem in relation to snails and parasites, as shown by low differentiation indices. However, the landscape for snails in Salvador is fragmented and likely inhabited by parasite populations that were founded independently and with little exchange between them. Intensity of transmission is also a likely factor. For example, in a study in
Table 3
Pairwise comparison of Jost’s D differentiation index for *Schistosoma mansoni*, Brazil

<table>
<thead>
<tr>
<th>Location</th>
<th>Cerariae from field collections</th>
<th>Eggs in human infections</th>
<th>Laboratory life cycle of worms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dique do Cabrito</td>
<td>Itacaranhas</td>
<td>Rio do Cobre</td>
</tr>
<tr>
<td>Dique do Cabrito</td>
<td>–</td>
<td>0.50 (0.50–0.51)</td>
<td>0.37 (0.36–0.37)</td>
</tr>
<tr>
<td>Itacaranhas</td>
<td>–</td>
<td>–</td>
<td>0.31 (0.28–0.33)</td>
</tr>
<tr>
<td>Rio do Cobre</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pituacuí</td>
<td>0.16 (0.15–0.17)</td>
<td>0.18 (0.17–0.18)</td>
<td>0.17 (0.17–0.18)</td>
</tr>
<tr>
<td>São Bartolomeu†</td>
<td>–</td>
<td>0.02 (0.02–0.02)</td>
<td>0.03 (0.02–0.03)</td>
</tr>
<tr>
<td>MA1</td>
<td>0.02 (0.02–0.02)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MA3</td>
<td>0.02 (0.02–0.02)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MA6</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Feira de Santana</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CWRU</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Values in parentheses are 95% confidence intervals. Values in bold are statistically significant. CWRU = Case Western Reserve University.

†Stools aggregated from 8 children in São Bartolomeu in 2004. MA1, 2, and 3 were component populations composed of genotyped infrapopulations from 12 persons. Markers SMMS 2, 3, and 17 were excluded from comparison for Itacaranhas because of poor amplification.
that these are urban infections only makes the similarity between these infrapopulations all the more striking. This finding suggests that most of these infections were locally acquired. Further sampling of these microareas will be important for confirming this observation.

The epidemiologic profile of schistosomiasis in Salvador is that of a chronic and potentially serious endemic disease because of the high prevalence of snails at water contact sites combined with the pollution of the environment with human waste where there is disorganized development. Assays of snail infections will have an important role in control measures and confirming interruption of transmission. The parasite population in snails also represents the subpopulation actively being transmitted. However, they might be less useful in cities such as Salvador for assessing parasite population structure and dynamics in the human host.

Received October 13, 2011. Accepted for publication July 29, 2012.

Financial support: This study was supported by the Brazilian Federal Agency for the Support and Evaluation of Graduate Education and the Council for Scientific and Technological Development.

Authors’ addresses: Samaly S. Souza, Lúcio M. Barbosa, Renato Barbosa Reis, Mitremayer G. Reis, and Zilton A. Andrade, Gonçalo Moniz Gonçalo Moniz Research Centre, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil; E-mails: samalyssouza@hotmail.com, lmacedo@aluno.bahia.fiocruz.br, georeis@gmail.com, miter@bahia.fiocruz.br, and zilton@cpqpm.fiocruz.br. Isabel C. Guimaraes, Center for Control of Zoonoses, Municipal Secretariat of Health, Alto do Trobogy, Salvador, Bahia, Brazil; E-mail: belcgumaraes@gmail.com. Walter A. Blank and Ronald E. Blanton, Center for Global Health and Diseases, Case Western Reserve University, Biomedical Research Building, Cleveland, OH; E-mails: wab25@case.edu and reb6@case.edu.

REFERENCES