FIOCRUZ

FUNDAÇÃO OSWALDO CRUZ

CENTRO DE PESQUISAS GONÇALO MONIZ

Curso de Pós-Graduação em Biotecnologia e Medicina Investigativa

TESE DE DOUTORADO

AVALIAÇÃO DA ESTRUTURA POPULACIONAL DO *Schistosoma mansoni* EM DUAS COMUNIDADES RURAIS E EM UMA LOCALIDADE URBANA

LÚCIO MACEDO BARBOSA

Salvador - Bahia
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Tese submetida à coordenação do Curso de Pós-Graduação em Biotecnologia em Saúde e Medicina Investigativa, Fundação Oswaldo Cruz, para a obtenção do Título de doutor.

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Aos meus pais, pelo afeto, carinho, dedicação e conselhos;

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À minha família e amigos, pela alegria e confiança;

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“You are never fully dressed without a smile -

Você nunca está devidamente vestido sem um sorriso”

Harry Connick Jr.

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RESUMO

Os esforços para o controle da esquistossomose no Brasil foram iniciados na década de 70, no entanto, a doença permanece como um problema de saúde pública no país, com prevalência aproximada de 5%. As atuais estratégias de controle são focalizadas no tratamento dos indivíduos infectados e obtiveram redução considerável na prevalência, morbidade e mortalidade da esquistossomose. O praziquantel é o medicamento utilizado atualmente no Brasil e apresenta uma eficácia de 80 a 90%. Apesar da redução da prevalência e das formas graves da doença, a sucessiva repetição do tratamento quimioterápico levanta questionamentos sobre a eficácia do mesmo ao longo do tempo e a respeito do surgimento de resistência ao medicamento. Com o objetivo de estudar o efeito do tratamento quimioterápico repetido nas populações de *Schistosoma mansoni* foram selecionadas duas localidades rurais e uma urbana do estado da Bahia. Foi utilizada uma estratégia de amostragem robusta e um número de marcadores polimórficos neutros suficientes para demonstrar diferenças entre microrregiões em momentos de equilíbrio entre deriva genética e migração, nas comunidades da zona rural e urbana, e em momentos de estresse evolutivo pós-tratamento, apenas nas comunidades rurais. Em termos epidemiológicos as localidades estudadas se mostraram com uma alta prevalência de esquistossomose. As características epidemiológicas das populações rurais e urbanas foram muito similares em diversos aspectos, principalmente nos fatores de risco para aquisição da doença. Repetidos tratamentos com o praziquantel reduziram significativamente as taxas de prevalência, reinfecção, incidência e intensidade de infecção da esquistossomose. Os parâmetros genéticos das populações de *Schistosoma mansoni* fornecem evidências de focos de transmissão local na zona rural e urbana. Foi observada uma estabilidade no ponto de vista genético, com baixa influência migratória, indicando que os indivíduos tendem a se infectar na região e permanecer no ambiente ao longo da vida. As infecções persistentes, pós tratamento, demonstraram fazer parte das populações susceptíveis, pré tratamento. Por esta razão é improvável que a razão da persistência da infecção seja devido a resistência ao praziquantel. Por fim, indivíduos não tratados ou que apresentam infecção persistente são capazes de recuperar qualitativamente a população parasitária de forma a representar a diversidade inicial, pré-tratamento, após sucessivos tratamentos. Isso indica que para ser possível obter alguma mudança na estrutura genética do *S. mansoni*, o tratamento deve ser distribuído de uma maneira mais eficáz para as populações infectadas. Desta forma, ações integradas incluindo saneamento básico, fornecimento de água potável, educação em saúde e tratamentos sucessivos são essenciais para o controle e eliminação da esquistossomose.

Palavras-chave: *Schistosoma mansoni,* Genética de Populações, Epidemiologia molecular, Praziquantel.

BARBOSA, Lúcio Macedo. Evaluation of *Schistosoma mansoni* population structure in two rural comunities and one urban locality. 115 f. il. Tese (Doutorado) – Fundação Oswaldo Cruz, Centro de Pesquisas Gonçalo Moniz, Salvador, 2013.

ABSTRACT

Efforts to control schistosomiasis in Brazil started in the 70s, however, the disease remains a public health problem in the country, with a prevalence of approximately 5%. The current control strategies are focused on the treatment of infected individuals and obtained significant reduction in the prevalence, morbidity and mortality from schistosomiasis. Praziquantel is the drug currently used in Brazil, and has an efficiency of 80 to 90%. Despite the reduction in the prevalence and severe forms of the disease, the successive rounds of chemotherapy raise questions about the effectiveness over time and in regard of the emergence of drug resistance. In order to study the effect of repeated chemotherapy in populations of *Schistosoma mansoni* two rural locations and urban area in the state of Bahia were selected. We used a robust sampling strategy and a number of neutral polymorphic markers sufficient to demonstrate differences between microregions in migration-drift equilibrium, in rural communities and urban areas, and in post-treatment evolutionary stress, only in rural communities. Epidemiological characteristics of rural and urban populations were very similar in many aspects, especially those related to the risk factors for acquiring the disease. Repeated treatments with praziquantel significantly reduced prevalence, reinfection and incidence rates and intensity of infection of schistosomiasis. Genetic parameters of *Schistosoma mansoni* populations indicated foci of local transmission in rural and urban areas. A high degree of genetic stability was observed in the studied areas with little influence of migration, indicating that individuals tend to get infected in the region and remain in the environment throughout life. Persistent infections, post-treatment, were demonstrated to be drawn from susceptible parasite populations, pre-treatment. Therefore, it is unlikely that persistence of infection is due to resistance to praziquantel. Finally, untreated or persistent infections are able to recover qualitatively the parasite population to represent the initial diversity, pretreatment, after repeated rounds of treatment. This indicates that in order to obtain some change in the *S. mansoni* genetic structure treatment must be distributed more efficiently for the infected populations. Thus, integrated actions including sanitation, potable water supply, health education and successive treatments are essential for the the control and elimination of schistosomiasis.

Keywords: *Schisosoma mansoni,* Population Genetics, Molecular Epidemiology, Praziquantel.

SUMÁRIO

1 INTRODUÇÃO

A esquistossomose é uma doença tropical causada por parasitas do gênero *Schistosoma.* Esta doença afeta mais de 243 milhões de pessoas no mundo em 74 países diferentes (WHO, 2013). No Brasil, onde apenas o *Schistosoma mansoni* é encontrado, estima-se que aproximadamente 9 milhões de pessoas estão infectadas sendo que mais de 30 milhões encontram-se em risco de infecção (COURA e AMARAL, 2004; WHO, 2013). No entanto, estes números podem ser ainda maiores, tendo em vista que a esquistossomose é assintomática em aproximadamente 90% dos casos, quando o indivíduo infectado apresenta uma baixa carga parasitária.

As formas sintomáticas da esquistossomose são classificadas em agudas ou crônicas. O perfil agudo é descrito principalmente em indivíduos de áreas não endêmicas, enquanto o crônico é encontrado predominantemente em regiões endêmicas. O tipo crônico da doença é sub-dividido em intestinal, hepato-intestinal e hepato-esplênica. A manifestação intestinal se caracteriza pela presença de diarréias, na maioria das vezes, ovos nas fezes e eosinofilia. A forma hepato-intestinal apresenta as características das formas intestinais com o aumento do lobo esquerdo do fígado e na forma hepato-esplênica é possível observar também o aumento do baço (GRYSEELS, 1989). Desta maneira, em fases mais avançadas é possível observar um quadro de hipertensão portal intensa com circulação colateral e varizes esofágicas que é a principal causa de óbito dos indivíduos infectados (GRYSEELS *et al.*, 2006). Vale ressaltar ainda que em qualquer das formas clínicas o indivíduo infectado pode apresentar comprometimento do sistema nervoso central com mielites decorrentes da presença de vermes e principalmente ovos na medula espinhal (BOTTIEAU *et al.*, 2006).

O tratamento para *S. mansoni* preconizado pela Organização Mundial de Saúde (OMS) acontece com o uso de Praziquantel (50 mg/kg em adultos e 60 mg/kg em crianças) em dose única, o que o torna relativamente simples, pouco oneroso e com

uma boa efetividade com eliminação do parasita em 80 a 90% dos casos (SAVIOLI *et al.*, 2002).

O ciclo de vida do *S. mansoni* é complexo envolvendo diferentes hospedeiros. Nos seres humanos, hospedeiro definitivo, o parasita apresenta-se na sua forma adulta que se reproduzem sexuadamente. Contudo, para a continuação do ciclo, os ovos produzidos devem migrar do hospedeiro definitivo para o meio ambiente através das fezes. Quando os mesmos entram em contato com água, eles eclodem liberando miracídios. Estes penetram no seu hospedeiro intermediário, caramujos do gênero *Biomphalaria*. Nestes, os miracídios se reproduzem de forma assexuada dando origem a cercárias. O ciclo se completa quando o hospedeiro definitivo entra em contato com água infestada por caramujos infectados. A cercária é o estágio evolutivo do *S. mansoni* que possui a capacidade de infectar o hospedeiro definitivo, sendo capaz de penetrar a pele humana. Após percorrer diferentes regiões do hospedeiro e passar por transformações evolutivas até se tornar o verme adulto, o parasita alcança o espaço porta, no fígado, onde encontram um verme do sexo oposto e reiniciam o ciclo (NEVES, 2005).

Com base nestes conhecimentos, torna-se fácil identificar que esta doença está associada a locais com baixo saneamento básico onde as fezes humanas entram em contato com coleções hídricas contendo caramujos. Desta forma, as estratégias de controle ao parasita devem se basear no saneamanto básico e ambiental, educação em saúde, tratamento dos indivíduos infectados e controle dos moluscos (VIEIRA, 1993; CARMO e BARRETO, 1994; COURA e AMARAL, 2004). Apesar algumas variações desde o seu início na década de 70, estas estratégias conseguiram reduzir a morbidade, mortalidade e prevalência da doença, mas muito precisa ser alcançado para a erradicação da mesma (COURA e AMARAL, 2004).

Para ser possível desenvolver estratégias de controle mais eficázes para a esquistossomose é necessário entender melhor características do parasita, sua distribuição e como ela se comporta em determinadas situações, como em estase

evolutiva e de estresse. A melhor maneira desses parâmetros serem avaliados é através da avaliação da genética do parasita por meio de métodos moleculares. Por se tratar de um ser sexuado, clones não vão ser gerados e os indivíduos serão diferentes entre si. Desta forma, as caraterísticas e variabilidade genética existentes podem ser definidas através da distribuição do material genético entre a população como um todo. Então, para ser possível compreender as características citadas, é necessário observar os parasitas como uma população. Indivíduos que são da mesma família ou próximos irão demonstrar traços genéticos e estruturas populacionais semelhantes. Uma população no sentido genético não se trata apenas de um grupo de indivíduos, mas sim um grupo de indivíduos da mesma espécie que intercruzam, ou seja, interessa a composição genética da população avaliada e como deve se comportar no futuro (FALCONER e MACKAY, 1996; HARTL e CLARK, 2007).

A compreensão da estrutura genética das populações de *S. mansoni* pode fornecer informações úteis para as estratégias de controle, como por exemplo, a avaliação da eficiência no intervalo entre tratamentos, o uso de estratégias de distribuição da droga, características da população infectada, fontes de infecção, preferência de hospedeiro. É possível também avaliar o efeito de repetidas doses de tratamento com o praziquantel a fim de, por exemplo, prever uma possível resistência ao tratamento de escolha. Contudo, para o desenvolvimento deste tipo de pesquisa três elementos são de vital importância: a) um grande números de marcadores polimórficos neutros, b) uma estratégia apropriada de amostragem, e c) utilização de bons índices para avaliação da estrutura populacional.

a) Marcadores polimórficos para caracterização genética do *S. mansoni*

Diferentes tipos de marcadores genéticos podem ser utilizados para diferenciar subpopulações do *S. mansoni*, porém trabalhos descrevem os marcadores microsatélites como ideais para este tipo de pesquisa (ROSS *et al.*, 1999). Variabilidade, facilidade de determinar um resultado e alta carga de informação são características fundamentais para a utilização dos microsatélites. Para serem

informativos a respeito de indivíduos ou populações, os marcadores devem apresentar uma significativa variabilidade, ou seja, quanto maior a variação, melhor a capacidade discriminatória. É também de fundamental importância, a formulação de escores com o mínimo de ambiguidade, e que esses testes possam ser repetidos em outros laboratórios. Em um material em que pode haver DNA de diferentes origens, como as amostras de fezes, o marcador deve ser específico para a espécie de interesse. Os microsatélites englobam todas essas características de forma mais eficáz que todos os outros marcadores disponíveis no momento. Outros tipos de marcadores que podem oferecer o mesmo valor discriminatório seriam os polimorfismos pontuais (SNPs – *Single Nucleotide Polymorphisms*), contudo, é necessário uma grande quantidade de ensaios diferentes para obter a representatividade dos microsatélites. Ross *et al.,* em 1999 utilizaram quatro diferentes tipos de marcadores genéticos - microsatélites, aloenzimas, RAPDs codominantes e RAPDs dominantes - para avaliar qual apresentava melhor poder de diferenciação em colônias de formigas da espécie *Solenopsis invicta*. Eles demonstraram que os microsatélites forneciam a maior proporção do total da variância das populações em cada nível de amostragem e uma maior diferenciação entre os grupos (ROSS *et al.*, 1999). Em relação aos marcadores mitocondriais na esquistossomose, Curtis *et al.*, em 2001 descrevem que apesar da detecção de subpopulações, a análise utilizando sete marcadores microssatélites fornecem mais informações revelando moderada diferenciação quando vilas ou comunidades são utilizadas para definir subpopulação parasitária e uma diferenciação maior ainda quando os hospedeiros humanos são utilizados como subpopulações (CURTIS *et al.*, 2001).

Por razões que serão descritas no subtópico posterior, as amostras utilizadas consistiram de parasitas naturalmente agregados, ou seja, foram utilizados todos os ovos eliminados nas fezes de cada indivíduo. Para a obtenção de resultados consistentes utilizando amostras agregadas é importante a utilização de marcadores polimórficos que possuam um número limitado de alelos e que possam ser amplificados com o mínimo de DNA molde. Repetições de 3 ou 4 nucleotídeos apresentam melhor amplificação que os de duas repetições, e desta forma, são melhores escolhas para

este tipo de trabalho. Elas também tendem ser menos polimórficas que as repetições de dois nucleotídeos (3-5 alelos, ao invés de 10-20), característica que torna a interpretação dos resultados mais simples em amostras agregadas. Foram escolhidos 15 marcadores microsatélite para este trabalho, sendo 11 descritos por nossa equipe (SILVA *et al.*, 2006; BLANK *et al.*, 2010; BLANTON *et al.*, 2011) e os outro quatro por Durand *et al.* 2000, Curtis *et al.* em 2001 e Rodrigues *et al.* em 2002 (DURAND *et al.*, 2000; CURTIS *et al.*, 2001; RODRIGUES *et al.*, 2002).

b) Estratégia de amostragem das populações de *S. mansoni*

Um dos maiores problemas encontrados nos estudos que visam avaliar as populações do *S. mansoni* em humanos é a amostragem, que geralmente se apresenta limitada e com diversos vieses. Para quase todas as análises estatísticas, um aumento no tamanho amostral leva a um aumento no poder do estudo. A amostra deve também ser representativa da população total, ou de uma subpopulação de interesse, um fator auxiliado pelo tamanho da amostra. Além de ser representativa, a melhor amostragem é aquela que acontece com a menor quantidade de viés de seleção, ou que estes possam ser corrigidos. Quando detalhes relacionados com o número e distribuição das populações são conhecidos, ajustes podem ser feitos para compensar essas falhas. A biologia e distribuição dos humanos em uma comunidade pequena é muito melhor compreendida que a distribuição dos hospedeiros intermediários. Como existem poucos hospedeiros definitivos não-humanos para o *S. mansoni* e estes não devem ser de importância para a manutenção desta doença no Brasil, a amostragem de humanos pode ser mais facilmente compreendida e inclusive as falhas mais facilmente reconhecidas para que as correções possam ser aplicadas. Em áreas urbanas, onde as taxas de migrações podem ser altas, os caramujos locais podem não representar de forma fidedigna as populações humanas. E, finalmente, a população parasitária mais importante para a morbidade e a transmissão para os hospedeiros intermediários são aquelas que residem nos hospedeiros humanos.

Para estudos de genética de populações do *S. mansoni*, a abordagem mais utilizada se baseia na passagem de determinados estágios do parasita por animais, o que acarreta na diminuição do número de amostras utilizadas e a alguns viéses de seleção. Esse esquema de amostragem subestima as características da população parasitária selecionando uma pequena porcentagem de ovos a partir de um pequeno número de indivíduos e utiliza estes para infectar caramujos provenientes de laboratórios. É conhecido que linhagens locais de parasita vão ser específicas a cepas de caramujos locais (WEBSTER e DAVIES, 2001). A infecção com uma linhagem não adaptada de caramujo com uma determinada cepa de parasita produz seleção e viés. Se este parasita for utilizado para infectar caramujos de laboratório, o mesmo princípio pode ser aplicado, e apenas uma fração dos genótipos de *S. mansoni* será encontrada nos hospedeiros intermediários (LOVERDE *et al.*, 1985). Desta forma, o processo de amostragem mais fidedigno acontece através da obtenção completa dos ovos nas fezes de humanos infectados, como realizado neste estudo. Esse tipo de abordagem é o que produz a menor quantidade de erros e fornece um grande número amostral.

Outra vantagem apresentada para o uso da realização da genotipagem de amostras agregadas naturalmente deve-se ao fato de que a infecção humana por este trematódeo não ser associada com reprodução assexual. Desta forma, parasitas diferentes se acumulam apenas por re-infecção e, assim, a população se desenvolve. Por este motivo a análise das populações de *S. mansoni* se torna de maior relevância que o estudo de indivíduos do parasita. Além disso, em infecções humanas, apenas as amostras agregadas nas fezes são disponíveis diretamente e são mais representativas da população total no indivíduo. Vale a pena ressaltar que as frequências alélicas de uma população de amostras agregadas se correlacionam bem com as frequências avaliadas de forma individual. Shaw e colaboradores (SHAW *et al.*, 1998) descreveram que o mapeamento do gene de susceptibilidade para a doença de Hirschsprung poderia ser obtida de forma eficaz utilizando amostras agregadas de 27, 54 e 76 indivíduos. Esses achados estão de acordo com outros trabalhos que analism frequências em amostras agregadas (PACEK *et al.*, 1993; COLLINS *et al.*, 2000; KIROV *et al.*, 2000; SILVA *et al.*, 2006; HANELT *et al.*, 2009).

Por fim, determinados parasitas, como o *S. mansoni*, apresentam diferentes estágios de vida em diferentes hospedeiros. Desta forma, é suposto que devido às diferentes características dos hospedeiros, os parasitas exibem diferentes estruturações genéticas a depender do seu hospedeiro. Para facilitar a compreensão deste estudo, foram utilizadas as nomeclaturas e conceitos descritos por Bush e colaboradores em 1997: *infrapopulações*, que é a população parasitária que está presente em um dos hospedeiros; *população componente*, todas as infrapopulações de um dos hospedeiros em um determinado local geográfico; *suprapopulação*, que consiste em todas as populações componentes juntas (BUSH *et al.*, 1997).

c) Utilização de bons índices para avaliação da estrutura populacional

Nas pesquisas envolvendo genética de populações busca-se a compreensão de determinados parâmetros para que a estrutura populacional possa ser avaliada e certos aspectos possam ser inferidos. Os principais pontos pesquisados são a diversidade e a diferenciação genética entre subpopulações. A diversidade genética é obtida geralmente através da heterozigosidade (HET) enquanto que a diferenciação genética é medida pelo o uso dos chamados índices de fixação, sendo o mais utilizado o Fst, que é calculado com base na HET (HARTL e CLARK, 2007). A interpretação dos resultados de similaridade genética segue o proposto por Wright onde: valores abaixo de 0.05 indicam baixa diferenciação genética; entre 0.05 e 0.25 demonstram uma diferenciação moderada; e acima de 0.25 mostram que as subpopulações avaliadas são muito diferentes (HARTL e CLARK, 2007).

Estes índices vêm sido utilizados há muito tempo em inúmeros trabalhos diferentes, contudo, alguns pesquisadores descreveram algumas falhas conceituais e metodológicas para a sua obtenção. Em determinados cenários, os resultados encontrados fornecem dados errôneos que, consequentemente, levarão a interpretações falsas. O grande problema ao utilizar HET para estimar diversidade genética é que este índice é uma probabilidade, que sempre apresentará limite superior

igual a 1. Desta forma, torna-se difícil a interpretação de qual subpopulação terá uma maior variabilidade caso as mesmas apresentem-se com uma diversidade alta (JOST, 2008). Para suplantar esse possível viés, foi desenvolvido o cálculo do número de alelos efetivos (Ne) utilizando os valores de HET (WAPLES, 1989). Os valores obtidos, através da fórmula 1/[1-HET], não apresentam limite superior, fornecendo a real diversidade ao se comparer diferentes populações.

Os principais problemas com o uso do Fst encontram-se relacionados a utilização da HET. O Fst é calculado através da fórmula [HET total – HET subpopulação]/ HET total. Desta maneira, caso a diversidade da população seja alta, partindo do pressuposto que a HET indica diversidade, o resultado de Fst tenderá para o zero, indicando que as populações avaliadas são mais próximas geneticamente mesmo que não compartilhem alelos (JOST, 2008). Assim, para suprir as falhas metodológicas descritas foi desenvolvido um verdadeiro índice de diferenciação, o D de Jost. Este é calculado a partir das frequências alélicas avaliadas e demonstrou uma constância nos valores obtidos independente da diversidade da população (JOST, 2008).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a estrutura genética do *S. mansoni* em duas populações humanas rurais e uma população humana urbana do estado da Bahia

2.2 OBJETIVOS ESPECÍFICOS

- Descrever as características epidemiológicas das populações humanas em duas comunidades rurais no município de Ubaíra no estado da Bahia e no bairro do subúrbio ferroviário da cidade de Salvador no estado da Bahia.
- Determinar as características genéticas das populações de *S. mansoni* em duas comunidades rurais no município de Ubaíra no estado da Bahia e no bairro do subúrbio ferroviário da cidade de Salvador no estado da Bahia.
- Descrever a estrutura genética das populações do *S. mansoni* antes e após o tratamento com praziquantel.
- Verificar a existência de associação entre padrões de estrutura genética do *S. mansoni* e dados demográficos dos hospedeiros.
- Descrever a origem da recuperação das infecções após repetidas doses de tratamento.

3 RESULTADOS

Os resultados desta tese encontram-se apresentados em 4 capítulos representando em ordem cronológica os manuscritos produzidos no período do curso de pós-graduação, tendo como títulos: (1) *Schistosoma mansoni* population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil; (2) Host characteristics have little influence on which local *Schistosoma mansoni* populations are acquired; (3) Sources of Schistosomiasis Urbanization in Salvador, Bahia, Brazil; (4) *Schistosoma mansoni:* population dynamics after repeated treatments.

Capítulo 1

Manuscrito 1 - *Schistosoma mansoni* population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil.

Este artigo apresenta pela primeira vez, de forma resumida, uma descrição das áreas rurais estudadas. Os dois vilarejos, Jenipapo e Volta do Rio, apresentaram prevalência de esquistossomose muito superior aos números relatados pelo Ministério da Saúde no Brasil. Contudo, o principal objetivo deste trabalho foi a descrição da estrutura genética do *Schistosoma mansoni* antes e 4-6 semanas após o tratamento, nos indivíduos que permaneceram eliminando ovos do parasita nas fezes, apesar da administração do praziquantel nas doses preconizadas pela OMS. Este estudo também mostra a relação entre: a) amostras do mesmo indivíduo coletada em dias diferentes; b) entre infrapopulações; c) entre os vilarejos estudados.

Principais resultados encontrados:

1- *S. mansoni* demonstra produção constante de frequências alélicas, devido a baixa variação genotípica dia a dia encontrada;
2- Cada hospedeiro abriga uma amostragem incompleta da população geral, devido a diferenciação moderada entre infrapopulações reportada;
3- Há diferenciação moderada entre as comunidades vizinhas indicando algum obstáculo no fluxo gênico;
4- Os parasitas persistentes não são diferentes genotipicamente dos susceptíveis ao tratamento, logo, não há indicação de seleção ao tratamento com praziquantel.

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Schistosoma mansoni population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil

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ABSTRACT

Praziquantel has been used to treat schistosome infections since 1979 and currently is the only chemotherapeutic agent in production for this purpose, raising concerns about the potential for the emergence of drug resistance. In practice, 10–20% of infected patients will continue to excrete eggs after treatment. It is not understood to what degree this represents selection of a resistant population or incomplete elimination due to the presence of immature worms at the time of treatment. We used a population genetics approach to test whether or not persistent *Schistosoma mansoni* parasites were drawn from the same population as susceptible parasites. In this study, stool samples were collected from 96% of individuals in two small Brazilian communities (populations 482 and 367) and examined for *S. mansoni* eggs. The combined prevalence of *S. mansoni* infections in the villages was 41%. Total egg DNA was extracted from each sample and was genotyped at 15 microsatellite markers. Day-to-day variation of the infrapopulation from an individual human host was low (median differentiation using Jost's $D = 0.010$), so that a single stool was representative of the genotypes present in stool eggs, at least in the short term. Average pairwise analysis of D among all pre-treatment infrapopulations suggested moderate differentiation (mean $D = 0.082$ and 0.122 for the two villages), whereas the pre-treatment component population differentiation between the two communities was 0.047. The differentiation of the component population remaining after treatment from the fully susceptible component population was low (mean $D = 0.007$ and 0.020 for the two villages), suggesting that the persistent parasites were not selected by praziquantel treatment. We will continue to follow these communities for evidence of selection or changes in population structure.

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1. Introduction

Schistosomiasis is a global health problem. It is an important cause of morbidity wherever it occurs (King et al., 2005). In Brazil, however, it is second only to Chagas disease as a cause of death from parasitic infections and leads important viral and bacterial diseases such as dengue, yellow fever and leptospirosis (Ministry of Health Brazil, DataSUS: Health Information – Vital Statistics: General Mortality, http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sim/cnv/obtuf.def, accession date 12-4-2010). Significant progress has been made in controlling the disease by means of the repeated mass administration of drugs, first oxamniquine and now praziquantel (PZQ). Treatment results in an immediate reduction in the prevalence and intensity of infection but because immunity to infection is weak, reinfection often brings the prevalence back to pre-treatment levels within 2 years (McManus and Loukas, 2008). Currently only PZQ is in use against all species of schistosomes, generating concern about the emergence of drug resistance. In the laboratory, resistance to PZQ can be induced relatively easily using six to seven passages with ~400 worms in each passage (Fallon and Doenhoff, 1994). Several studies have also indicated that resistance to PZQ can develop in practice (Danso-Appiah and De Vlas, 2002; Melman et al., 2009), although other studies suggest that this occurs quite slowly if at all or that PZQ resistance is associated with reduced parasite fitness (King et al., 2000), also seen for drug resistance in malaria (Babiker et al., 2009). A consistent finding following treatment of populations with PZQ is that 10–20% of individuals will continue to excrete eggs, usually at a reduced intensity (Wegner, 1984; Cioli and Pica-Mattoccia, 2003). Because immature worms are relatively insensitive to PZQ, the persistence of worms after treatment has commonly been attributed to immature schistosomes from recent infections which subsequently mature (Cioli and Pica-Mattoccia, 2003). It is also possible, however, that these persistent parasites or a portion of them represent a population of adult worms resistant to the drug.

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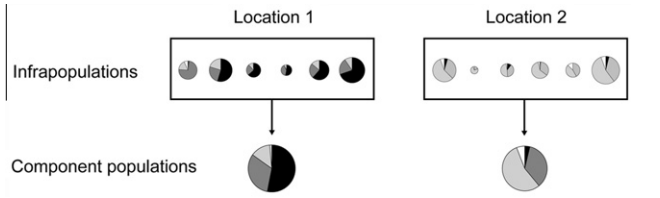

Fig. 1. Diagrammatic representation of allele frequency distribution between two hypothetical locations. In each location, six infrapopulations of varying sizes are combined to represent the component population.

The biology of schistosomes leads to an irregular distribution of genotypes that does not conform to many standard population models and affects how they should be sampled. In the cycle of human schistosome infection, paired male and female worms residing in the intestinal or urinary vasculature produce progeny (eggs) that are excreted. Hatched miracidia undergo asexual reproduction in the snail intermediate host followed by shedding of cercariae and subsequent infection of humans. The population of schistosomes within an individual host, the "infrapopulation" (Bush et al., 1997), can represent infections acquired from multiple sites over years and possibly decades. New individuals join the infrapopulation only by infection (in-migration) and all progeny are exported. Infrapopulations, therefore, need not conform to Hardy-Weinberg expectations. Within a specified geographical area at a given time, the aggregate of infrapopulations in humans – the "component population" – is the pool from which future rounds of infection in that location are drawn (Fig. 1). As a consequence of this uneven distribution, the infrapopulations of a small number or biased sample of individuals may poorly represent the component population.

In addition to the parasite's distribution, population genetic studies on schistosomes present several other difficulties with respect to sampling: worm populations are generally inaccessible in the human host and the cost and effort involved in genotyping individual eggs or miracidia is prohibitive when analysing many eggs from many infrapopulations. Our approach to these problems is to use allele frequencies obtained from the aggregate of excreted eggs. Data from the individual infrapopulations can then be combined to obtain a portrait of the component population. We and others have previously demonstrated that allele frequencies measured from aggregates of offspring from *Schistosoma mansoni* (Silva et al., 2006; Blank et al., 2009; Hanelt et al., 2009) and other parasites (Redman et al., 2008) reflect the aggregate of allele frequencies obtained by discrete genotyping. Mean error rates for allele frequency estimates of pooled samples range from 2% to 11% in these studies. Furthermore, we have shown in laboratory infections of mice that the allele frequencies obtained from eggs also reflect the allele frequencies of the infecting worms (Blank et al., 2011). Genetic differentiation indices using these allele frequencies also follow the known population dynamics of *S. mansoni* laboratory strains (Blank et al., 2010). By combining a large number of infrapopulations stratified by geographic origin or response to PZQ treatment, this sampling approach allows for population analyses at multiple scales and over time, while avoiding errors due to under sampling.

As part of a comprehensive *S. mansoni* epidemiological survey and treatment program covering nearly all residents of two communities in the Brazilian state of Bahia, we analysed the microsatellite allele frequencies of aggregated schistosome eggs recovered from the stool of each infected host. This study investigates the consistency of *S. mansoni* allele frequencies excreted over multiple days and examines the changes in allele frequencies observed in those schistosome infections that persist after PZQ treatment. Finally, we explore the potential for this approach to detect the hypothetical resistance to PZQ in persistent *S. mansoni* populations.

2. Materials and methods

2.1. Population

The villages of Jenipapo and Volta do Rio in the East Central region of the state of Bahia, Brazil were selected for their reported high prevalence of *S. mansoni* infection and their well-defined geographic limits. The two communities are 12 km apart and are connected by the Jiquiriçá River and a two-lane highway. The region consists of steep river valleys with rolling hills and is primarily devoted to raising cattle and other livestock. A census was performed for all households at the start of the study and all residents >1 year of age were invited to participate. Participants or guardians responded to a brief questionnaire on demographic characteristics and prior treatment for parasites. They were also asked to provide whole stool samples for examination on three different days within the same week. Both communities are administratively governed from the district's largest city, Ubaíra. Jenipapo (population 482 in 2009) has a Federal Family Health Program post with a permanent staff of a nurse, dentist and part-time physician. A laboratory adjacent to the post was used for all stool examinations. The primary and secondary schools for the nearby small communities, including Volta do Rio, are located in Jenipapo. Volta do Rio (population 367 in 2009) has a simple health post staffed by a group of nurses. 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 Centre, Cleveland, Ohio approved the study design.

2.2. Stool survey and egg isolation

Morning stools collected from participants were weighed, single slides were prepared by the Kato-Katz method (Katz et al., 1972), and the slides were read on the same day to determine the number of eggs per gram in each sample. The following day, whole stools that were positive for *S. mansoni* were processed to obtain eggs. Stools were homogenised in a blender with 200 ml of 2% saline followed by selective sieving (Dresden and Payne, 1981) through 300 and 55 μm mesh nylon filter bags (FSI, Michigan City, Indiana, USA) and sedimentation in 2% saline. The bottom 5 ml of sediment was collected and then kept frozen at −20 °C until used for DNA isolation. In accordance with Brazilian Ministry of Health guidelines (Ministry of Health Brazil, 2005), participants with one or more egg-positive stools were given PZQ (50 mg/kg) once, and three follow-up stool examinations were performed on these individuals 4–6 weeks later, together with egg isolations as before.

2.3. DNA isolation

The 5 ml frozen stool sediment was mixed with 5 ml 2× extraction buffer (50 mM NaCl, 100 mM Tris–HCl, pH 7.5, 10 mM EDTA, 1.0% SDS) and 10 ml H_2O-saturated and Tris-buffered phenol, pH 7.5. This was mixed by rocking for 5 min, then 10 ml of chloroform/isoamyl alcohol (23:1) was added and rocking continued for another 5 min. Following centrifugation, the aqueous portion was removed and extracted twice with 10 ml chloroform/isoamyl alcohol. The DNA was then ethanol precipitated and suspended in 10 mM Tris, pH 7.5, 1 mM EDTA. Further cleanup using

Table 1
Summary of microsatellite markers used in this study.

Genotyping group	Locus	References	Size range (nt[a])	Alleles observed
I	SMMS2	Silva et al. (2006)	212–235	4
	SMMS13	Silva et al. (2006)	183–192	4
	SMMS16	Silva et al. (2006)	211–229	7
II	SMMS3	Silva et al. (2006)	176–209	12
	SMMS17	Silva et al. (2006)	286–304	7
	SMMS18	Silva et al. (2006)	195–228	12
	SMMS21	Silva et al. (2006)	174–186	5
IV	13TAGA	Rodrigues et al. (2002)	103–143	11
	SMDA23	Curtis et al. (2001)	193–237	12
	1F8A	Blank et al. (2010)	151–172	8
	SM13-410	This study	192–207	6
V	29E6A	This study	160–178	7
	SM13-478	This study	225–258	12
	15J15A	This study	208–232	9
	SMU31768	Durand et al. (2000)	190–226	13

[a] nt, Nucleotide.

hexadecyltrimethyl ammonium bromide (CTAB) was performed to remove PCR inhibitors (Ausubel et al., 1987).

2.4. Quantitative PCR (qPCR) and PCR amplification

Since the faecal sediment was not exclusively composed of *S. mansoni* eggs, the amount of specific *S. mansoni* DNA was quantified by qPCR by the method of Gomes et al. (2006) which amplifies a *S. mansoni* ssrRNA fragment. Real-time PCR was performed with the AB 7300 Real-Time PCR system (Applied Biosystems) using SYBR-Green qPCR master mix (Roche). Serial dilutions of adult worm DNA were used to generate a standard curve based on concentrations determined with a NanoDrop 1000 spectrophotometer (Thermo Scientific).

2.5. Microsatellite genotyping

Fifteen microsatellite markers (Table 1) were used in this study. Eleven were previously published (Durand et al., 2000; Curtis et al., 2001; Rodrigues et al., 2002; Silva et al., 2006; Blank et al., 2010), and primers for markers not previously described are as follows: SM13-410 (F: TGACTTTGAATCCAACAGAGACC, R: GTTTGCTCAGAG ACCTGAACCTAC), 29E6A (F: ACATCCAGCTGACGAGTCC, R: ACTGCC CTATTCCTAACTGGC), SM13-478 (F: CAGGAATTTGTATTGTTCTGC TGTC, R: ACAGTGGCTAACTGACTACG), and 15J15A (F: TGTGGTTA ATCGCTGCTACC, R: GTTTCATGCCAACTGCGTCTC). Primer design and PCR genotyping were performed as described previously (Blank et al., 2010). Briefly, duplicate PCR reactions using 2 µl of extracted DNA were performed for each of the 15 marker loci, totalling 30 reactions per sample. PCR products from each sample were combined into groups of three or four markers and processed on an Applied Biosystems 3730xl DNA Analyzer. PeakScanner software (Applied Biosystems) was used to determine peak heights from which allele frequencies were calculated. Successful PCR reactions were defined as those in which measurable peaks in the size range expected for a given marker were observed. Subsequent population analyses were limited to those samples that had a minimum of 25 successful PCRs out of the 30 performed on each sample.

2.6. Data analysis

Categorical comparisons of survey data between villages (sex ratios, birthplace, treatment history) were made using Yates' corrected chi-square analysis. Age, years of residence and *S. mansoni* infection intensity were analysed by Student's *t*-test. For all statistical tests, a P value of <0.05 was deemed significant. Unless otherwise indicated, averages are reported as means ± S.D. when values approximated a normal distribution and as medians with the first and third quartile values for skewed distributions. Statistical analyses were performed using R version 2.12.1 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org).

Allele counts for each sample were calculated by multiplying the allele frequencies at a microsatellite locus by the total egg counts. Infrapopulations were stratified according to the host's village of residence and whether or not eggs were observed in follow-up samples after treatment with PZQ (persistent and susceptible, respectively). Persistent infrapopulations were also stratified by whether they were collected pre- or post-treatment. Component population allele frequencies were calculated as the sum of the allele counts from individual infrapopulations. Genetic differentiation between populations was expressed as the index D (Jost, 2008) calculated using the program SPADE (Chao and Shen, 2010, SPADE: Species Prediction and Diversity Estimation, URL: http://chao.stat.nthu.edu.tw). D is a true differentiation index and does not rely on assumptions of Hardy-Weinberg equilibrium that may not hold for infrapopulations. The D value reported for any given comparison in this study was the mean D across all the markers used. Following the convention used for interpreting F_{ST} values (Hartl and Clark, 2007), D values up to 0.05 indicate little differentiation; from 0.05 to 0.15, moderate differentiation; and above 0.15, great differentiation.

3. Results

3.1. Infection prevalence and intensity

The populations of the two communities were comparable in terms of sex ratios, age, place of birth, portion of life spent in the community, previous treatment and infection intensity (Table 2). Approximately 30% of the total population reported prior treatment for schistosomiasis, and 62% of these could identify the medication used. Only 24% of those who reported prior treatment said they had been treated with PZQ. The proportion that reported taking PZQ in Jenipapo was twice that of Volta do Rio. These responses appear to be consistent with the history of PZQ use in the region. One individual, however, reported treatment with PZQ in 1994, prior to this drug's common use by public health authorities, and another reported treatment with pyrantel pamoate, which is not used for schistosomiasis. Otherwise the reporting was credible.

Of the 482 residents registered in Jenipapo, 458 (95%) participated in the stool survey, while 357 of the 367 (97%) residents of Volta do Rio participated. Two of the 34 residents not examined were lost from the study due to migration; the remainder refused to provide a stool sample. At least one stool sample was provided by 96% of the total population of both communities, 94% provided a second sample and 93% provided a third. Stool examination by the Kato-Katz method showed that 41% of the overall population was positive for *S. mansoni* on at least one stool exam. The prevalence of schistosomiasis was significantly higher in Jenipapo (43.6 versus 33.8%, $P = 0.005$), although the mean intensity of infection was not significantly different between the communities. In Jenipapo, 92.3% of those infected became egg negative following treatment and 84.7% in Volta do Rio.

Table 2
Demographics, treatment history and *Schistosoma mansoni* infection status of communities examined in this study.

Characteristic	Community		
	Jenipapo n = 482	Volta do Rio n = 367	
Sex			
Male (%)	234 (48.5)	166 (47.8)	
Female (%)	248 (51.5)	201 (52.2)	
Age (years)			
Mean (S.D.)	30.5 (21.6)	32.5 (25.3)	
Minimum–maximum	1–101	1–91	
Birthplace			
Within district (%)	407 (84.4)	300 (81.7)	
Other (%)	75 (15.6)	67 (18.3)	
% of lifespan in district	92.8	90.2	
Previous treatment[a]			
Treated (%)	166 (34.4)	121 (33.0)	
Not treated (%)	14 (2.9)	6 (1.6)	
Do not know (%)	302 (62.7)	240 (66.4)	
Medication used[a]			
Oxamniquine (%)	112 (70.4[b])	66 (85.7)	P = 0.02[c]
Praziquantel (%)	47 (29.6)	11 (14.3)	
S. mansoni infection			
Pretreatment			
Prevalence (%)	210 (43.6)	124 (33.8)	P < 0.01[c]
Intensity[e] (S.D.)	278 (4.1)	435 (4.4)	P = 0.26[d]
Post-treatment			
Prevalence (%)	16 (7.6)	17 (15.3)	P = 0.07
Intensity[e] (S.D.)	25 (3.9)	71 (3.8)	P = 0.03

[a] Self-reported.
[b] Percentage of those identifying a medication.
[c] Yates' corrected chi-square test.
[d] Two-sided t-test with unequal variances comparing Jenipapo and Volta do Rio.
[e] Log transformed mean eggs per gram of faeces.

3.2. Sample selection and genotyping success

Samples chosen for genotyping in this study were from two overlapping groups. The first group, selected to evaluate the day-to-day variation in allele frequencies of excreted egg populations, consisted of samples from individuals with three egg-positive stools collected prior to PZQ treatment (348 samples from 116 individuals). The second group was selected to determine the effect of PZQ treatment on persistent infections and therefore consisted of pre- and post-treatment samples from individuals whose post-treatment stool was found to contain eggs (120 samples from 33 individuals). Thirteen individuals with persistent infections were also among the individuals with three positive pre-treatment samples. In total, 429 samples from 136 individuals were subjected to duplicate PCRs at 15 microsatellite markers.

Three hundred and fifty-eight samples (83% of the total) were genotyped successfully as defined by a minimum of 25 of the 30 PCRs producing scorable results. Of these, 269 (75%) were typed at all 15 markers, 65 (18%) at 14 markers, and the remaining 24 (7%) at 13 markers. Sample genotyping success correlated with the amount of *S. mansoni* DNA present in the sample (Spearman's rank correlation coefficient = 0.58; Fig. 2). Greater than 90% of samples that contained at least 25 pg/μl of *S. mansoni* DNA were genotyped successfully.

Of the 33 individuals with persistent infections, 23 had samples of sufficient quality for analysis both before and after PZQ treatment. In an additional three samples, only post-treatment samples were successfully typed. With respect to microsatellite success, 11 of the 15 markers were scorable in over 90% of reactions, 14 markers in over 80% of reactions. In those samples with at least 25 successful reactions, 14 markers had >90% success rate and the success of the poorest marker (SMMS13) was 74% (Table 3).

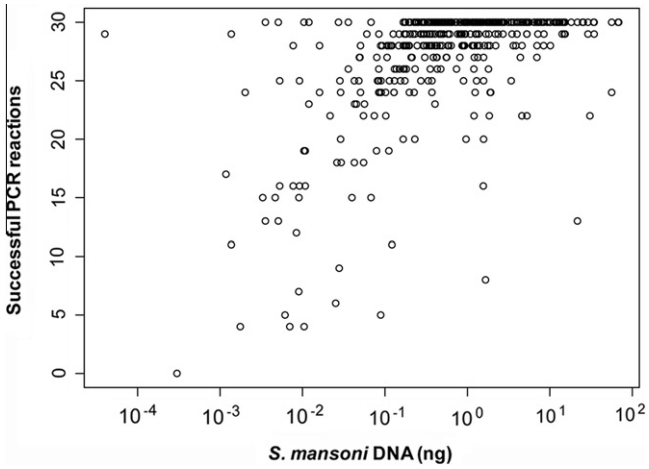

Fig. 2. PCR success versus quantity of *Schistosoma mansoni* DNA. DNA quantity was determined by quantitative PCR. Duplicate reactions were performed for 15 markers and the number of amplifications obtained for each sample was plotted. n = 429 samples.

Table 3
Genotyping success for samples in this study by microsatellite marker.

Marker	All samples (n = 449)		Samples ⩾25 reactions (n = 358)	
	Reaction success[a] (%)	Sample success[b] (%)	Reaction success (%)	Sample success (%)
SMMS2	91	94	98	100
SMMS13	63	71	74	82
SMMS16	94	95	99	100
SMMS3	86	88	96	98
SMMS17	80	83	91	94
SMMS18	97	98	100	100
SMMS21	92	94	99	100
13TAGA	96	97	99	100
SM13-410	91	94	97	99
1F8A	88	91	96	98
SMDA23	95	97	98	100
SM13-478	94	96	99	100
29E6A	94	95	98	99
15J15A	94	96	99	100
SMU31768	99	100	100	100

[a] Percentage of all reactions which were successful.
[b] Percentage of samples with at least one successful duplicate at that marker.

3.3. Infrapopulation differentiation

To determine the day-to-day consistency of genotype excretion, we determined D between stool samples collected from the same individual on different days. A total of 282 samples from 103 individuals were examined, giving 255 within-individual, day-to-day pairwise comparisons. The median day-to-day differentiation within an individual host was 0.010 (first quartile 0.004, third quartile 0.019). To establish a baseline for consistency, the median D between duplicate assays for the same sample (282 comparisons) was 0.007 (first quartile 0.004, third quartile 0.015), which may be considered near the limit of detection for differentiation between infrapopulations using this approach.

Pairwise D values between individual infrapopulations in Jenipapo and Volta do Rio were determined based on mean infrapopulation allele frequencies and egg counts. The pre-treatment infrapopulations of Volta do Rio were significantly more differentiated from each other than were those of Jenipapo (Table 4), although similar differentiation was observed among

Table 4
Average pairwise genetic differentiation of *Schistosomiasis mansoni* egg infrapopulations within and between Jenipapo and Volta do Rio, Brazil.

	Pre-treatment						Post-treatment	
	All		Susceptible		Persistent[a]		Persistent	
	n[b]	Mean D (S.D.)	n	Mean D (S.D.)	n	Mean D (S.D.)	n	Mean D (S.D.)
Within Jenipapo	84 (3486)	0.082 (0.045)	70 (2415)	0.084 (0.046)	14 (91)	0.070 (0.033)	9 (36)	0.113 (0.057)
Within Volta do Rio	45 (990)	0.122 (0.057)	29 (406)	0.130 (0.060)	16 (120)	0.099 (0.043)	17 (136)	0.116 (0.080)
P value[c]		<0.001		<0.001		<0.001		0.780
Between villages[d]	129 (3780)	0.134 (0.046)	99 (2030)	0.136 (0.048)	30 (224)	0.125 (0.037)	26 (153)	0.161 (0.066)

[a] Pre-treatment infrapopulations of those individuals who continued to excrete eggs after treatment.
[b] Number of infrapopulations (number of pairwise comparisons).
[c] Two-tailed t-test with unequal variances, between villages.
[d] Inter-village pairwise comparisons.

Table 5
Genetic differentiation (Jost's D) between pre- and post-treatment susceptible (S) and persistent (P) component populations of *Schistosoma mansoni* in Jenipapo and Volta do Rio, Brazil.

		Jenipapo				Volta do Rio			
		Pre		Post		Pre		Post	
		S + P	S	P	P	S + P	S	P	P
Jenipapo	Pre S + P	–	0.000	0.004	0.006	0.047	0.046	0.050	0.059
	Pre S		–	0.005	0.007	0.046	0.046	0.050	0.060
	Pre P			–	0.006	0.054	0.053	0.058	0.063
	Post P				–	0.052	0.052	0.056	0.063
Volta do Rio	Pre S + P					–	0.004	0.001	0.010
	Pre S						–	0.009	0.020
	Pre P							–	0.007
	Pre P								–

post-treatment infrapopulations in both villages. Mean pairwise differentiation among all pre- and post-treatment infrapopulations was significantly higher between villages than within either village (with the exception of susceptible infrapopulations from Volta do Rio, $P = 0.06$, not shown), indicating a degree of reproductive isolation between the villages.

3.4. Component population differentiation

Egg allele frequencies from the pre- and post-treatment infrapopulation groups were combined by village to give allele frequencies of the component populations. Pairwise D analysis of these component populations (Table 5) showed that there likely were some barriers to gene flow between village populations ($D = 0.047$ for pre-treatment populations; $D = 0.063$ post-treatment).

Prior to treatment, the susceptible component populations from the two villages were little differentiated from their respective persistent populations ($D \leqslant 0.010$; Table 5). When stratified by village, differentiation was likewise low between PZQ susceptible and post-treatment persistent component populations in Jenipapo ($D = 0.007$) and slightly higher for Volta do Rio ($D = 0.020$). The pre- and post-treatment persistent component populations showed little differentiation within each village ($D = 0.006$, Jenipapo; $D = 0.007$, Volta do Rio).

A reduction in intensity of infection is expected after PZQ treatment, even if the individual is not ultimately egg-free. The mean intensity reduction in persistent infections was 63% in Jenipapo and 83% in Volta do Rio. For some of the persistent infections, however, there was no reduction or there was even an increase in the intensity of infection. Some of these likely represented individuals who did not take PZQ. To assess whether there were population differences in those with or without reduction in egg burden, we grouped the persistent infections by whether or not they exhibited a reduction in infection intensity and compared the infrapopulation pre- versus post-treatment D values for the two groups (Fig. 3). The distribution of Ds between the reduced and non-reduced populations was not found to differ significantly (medians 0.024 and 0.029, respectively; Wilcoxon rank-sum test $P = 0.841$). Analysed from several perspectives, the persistent populations were not differentiated from the susceptible populations.

4. Discussion

Much of the focus on selection in population genetics is on events that occur over an evolutionary time frame, but when selection pressure is exerted by use of antimicrobial agents, the effect of

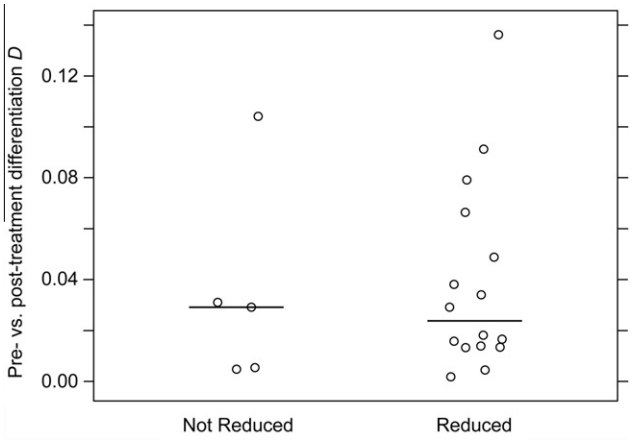

Fig. 3. Distribution of D values for infrapopulations from the same individual pre- and post-treatment. Infrapopulations for which there was no reduction in egg counts ($n = 5$) and those with a reduction ($n = 16$) were grouped together. The horizontal bar represents median D for each group.

a selection event can be observed after a very short period of time. In prokaryotes, antimicrobial selection often results in overgrowth of a clone (Soares et al., 1993; Davis et al., 1999; Manges et al., 2001; Martin et al., 2002) and is thus easy to identify using common molecular tools. Sexual reproduction never produces clones, but rather families in which the genotypes among the members are more closely correlated than unrelated or more distantly related members of a population. Since useful mutations often develop on one or a few genotypes, in the short-term, the average degree of relationship among resistant organisms is likely to be higher than the average degree of relationship among susceptible organisms. In the absence of isolation and over many generations, polymorphisms associated with resistance may be distributed to other genotypes, and familial relationships may be less apparent. At the moment of a selective sweep and prior to reproduction, however, the distribution of surviving genotypes is likely not to be random. They are likely to represent an extended family of individuals more closely related to each other at many loci than the susceptible population.

Measurement of accuracy and error are complex for the population genetics of schistosomes since there are two scales to be considered. At one scale there is the error of individual genotyping compared with genotyping pools of individuals. This error ranges from 2% to 11% based on our own studies (Silva et al., 2006; Blank et al., 2009; Hanelt et al., 2009) and those of others (Redman et al., 2008). However, when we compared the F_{ST} based on genotyping individuals or pools, we found that the differences had little effect on the F_{ST} and is unlikely to significantly affect other measures of differentiation. The second source of error exemplified in Fig. 1 results from the compartmentalisation of genotypes in infrapopulations. The almost complete sampling of all infrapopulations here avoids error due to undersampling, which we show could potentially be substantial given the degree of differentiation between infrapopulations.

Given a model where resistance arises in a limited number of organisms, we would predict that if schistosomes persist following treatment with PZQ due to a shared selective advantage, the persistent infrapopulations would be more related to each other than to the susceptible populations. What we observed was that the schistosome populations that persist are essentially randomly drawn from the overall population and not a selected population. This is consistent with the recent introduction of PZQ for widespread treatment of *S. mansoni* infections in Brazil and its limited use to date in these communities. The lack of differentiation from the overall population and lack of similarity within the persistent populations supports the common presumption that persistent populations represent recent transmission (Cioli, 2000; Gryseels et al., 2001; Cioli and Pica-Mattoccia, 2003). Since for the first 4 weeks following infection *S. mansoni* is relatively insensitive to PZQ, any recently acquired infections will not be affected by the administration of these drugs. These will appear as persistent infections, although the parasites will be drawn from the overall susceptible population. It is also possible worms may be in a location protected from the drug or that host factors governing drug metabolism randomly affect worm survival.

There are other models for the development of resistance under which these assumptions would not hold. Resistance to an antimicrobial agent may develop on multiple genotypes or by means of mobile elements in which case the genomes of resistant organisms will not show an overall correlation. Another caveat for any interpretation is that the marker panel used is not large and does not provide dense coverage of the genome. We are, however, able to show infrapopulation variation within these small communities, genetic drift within a laboratory strain (Blank et al., 2010), and demonstrate variation between subsamples of an *S. mansoni* laboratory population using a marker panel of similar size (Blank et al., 2011). Finally, there is a precedent for selection producing detectable differentiation across neutral unlinked markers (Merilä and Crnokrak, 2001; Freeland et al., 2010; Johansson et al., 2010). Since in this study we see little pre- and post-treatment differentiation for the component populations of the communities studied, this is suggestive evidence of a lack of selection.

One other study has examined *S. mansoni* population structure in the context of a treatment program. Norton et al. (2010) conducted a study in Tanzania analysing individual miracidia from 80 children prior to PZQ treatment and from 47 children at follow-up 1 year post-treatment. The individuals included in the pre- and post-treatment groups did not completely overlap. The study found a marked reduction in parasite allelic richness, which was attributed primarily to a population bottleneck resulting from mass community-wide administration of PZQ. Pre- and post-treatment populations a year later were not similar, however this study primarily examined re-infection rather than persistent infections. The potential for drug resistance was considered as an explanation for some observations, but thought unlikely given the modest prior exposure of the population to PZQ in these communities. It is difficult to make comparisons with the present study since the location and sampling strategies were so different and Norton et al. (2010) did not analyse infrapopulation differentiation. Two-year follow-up studies in Jenipapo and Volta do Rio are planned to determine the longer-term impact on the parasite populations.

In addition to investigating selection, the approach taken in this study has allowed us to sample whole communities widely and begin to understand important aspects of baseline genetic diversity within and among subpopulations. We find that at least over the period of 1 week, the excretion of parasite genotypes is consistent day-to-day. In theory, a single stool would suffice to represent the individual host's infrapopulation. We also found that in these communities, there is a moderate degree of differentiation of the infrapopulations among hosts. Parasites are probably acquired a few at a time by each host at different locations and under different circumstances. When the component population is very heterogeneous, any individual host will carry a very incomplete sample of the component population. For this reason the component population differentiation between villages is lower than the infrapopulation differentiation within each of the communities. This is consistent with each infrapopulation representing a limited draw from the overall pool. Sampling of infrapopulations needs to be extensive to capture the variability in the component population and under sampling is likely to overestimate differentiation at this level. We will continue to monitor the response to therapy and the population structure in these communities following repeated rounds of PZQ treatment using the techniques we have developed.

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References

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., 1987. Current Protocols in Molecular Biology. Green Publishing Associates and Wiley Inter-science, New York.

Babiker, H.A., Hastings, I.M., Swedberg, G., 2009. Impaired fitness of drug-resistant malaria parasites: evidence and implication on drug-deployment policies. Expert Rev. Anti. Infect. Ther. 7, 581–593.

Blank, W., Liu, S., Prasad, J., Blanton, R., 2011. Mouse strain type is not selective for *Schistosoma mansoni*. J. Parasitol. 97, 518–521.

Blank, W.A., Reis, E.A., Thiong'o, F.W., Braghiroli, J.F., Santos, J.M., Melo, P.R., Guimaraes, I.C., Silva, L.K., Carmo, T.M., Reis, M.G., Blanton, R.E., 2009. Analysis of *Schistosoma mansoni* population structure using total fecal egg sampling. J. Parasitol. 95, 881–889.

Blank, W.A., Test, M.R., Liu, S.F., Lewis, F.A., Blanton, R.E., 2010. Long-term genetic stability and population dynamics of laboratory strains of *Schistosoma mansoni*. J. Parasitol. 96, 900–907.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.

Chen, A., Shen, T-J., 2010. SPADE (Species Prediction And Diversity Estimation). Available from: <http://chao.stat.nthu.edu.tw/softwareCE.html> (Last updated 03 April 2010).

Cioli, D., 2000. Praziquantel: is there real resistance and are there alternatives? Curr. Opin. Infect. Dis. 13, 659.

Cioli, D., Pica-Mattoccia, L., 2003. Praziquantel. Parasitol. Res. 90 (Suppl. 1), S3–s9.

Curtis, J., Fraga, L.A., de Souza, C.P., Correa-Oliveira, R., Minchella, D.J., 2001. Widespread heteroplasmy in schistosomes makes an mtVNTR marker "nearsighted". J. Hered. 92, 248–253.

Danso-Appiah, A., De Vlas, S.J., 2002. Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. Trends Parasitol. 18, 125–129.

Davis, M.A., Hancock, D.D., Besser, T.E., Rice, D.H., Gay, J.M., Gay, C., Gearhart, L., DiGiacomo, R., 1999. Changes in antimicrobial resistance among *Salmonella enterica* Serovar *typhimurium* isolates from humans and cattle in the Northwestern United States, 1982–1997. Emerg. Infect. Dis. 5, 802–806.

Dresden, M.H., Payne, D.C., 1981. A sieving method for the collection of schistosome eggs from mouse intestines. J. Parasitol. 67, 450–452.

Durand, P., Sire, C., Theron, A., 2000. Isolation of microsatellite markers in the digenetic trematode *Schistosoma mansoni* from Guadeloupe Island. Mol. Ecol. 9, 997–998.

Fallon, P.G., Doenhoff, M.J., 1994. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. Am. J. Trop. Med. Hyg. 51, 83–88.

Freeland, J.R., Biss, P., Conrad, K.F., Silvertown, J., 2010. Selection pressures have caused genome-wide population differentiation of *Anthoxanthum odoratum* despite the potential for high gene flow. J. Evol. Biol. 23, 776–782.

Gomes, A.L., Melo, F.L., Werkhauser, R.P., Abath, F.G., 2006. Development of a real time polymerase chain reaction for quantitation of *Schistosoma mansoni* DNA. Mem. Inst. Oswaldo Cruz 101 (Suppl. 1), 133–136.

Gryseels, B., Mbaye, A., De Vlas, S.J., Stelma, F.F., Guisse, F., Van Lieshout, L., Faye, D., Diop, M., Ly, A., Tchuem Tchuenté, L.A., 2001. Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. Trop. Med. Int. Health 6, 864–873.

Hanelt, B., Steinauer, M.L., Mwangi, I.N., Maina, G.M., Agola, L.E., Mkoji, G.M., Loker, E.S., 2009. A new approach to characterize populations of *Schistosoma mansoni* from humans: development and assessment of microsatellite analysis of pooled miracidia. Trop. Med. Int. Health 14, 322–331.

Hartl, D.L., Clark, A.G., 2007. Principles of Population Genetics. Sinauer Associates, Sunderland, Massachusetts.

Johansson, A.M., Pettersson, M.E., Siegel, P.B., Carlborg, O., 2010. Genome-wide effects of long-term divergent selection. PLoS Genet. 6, e1001188.

Jost, L., 2008. G_{ST} and its relatives do not measure differentiation. Mol. Ecol. 17, 4015–4026.

Katz, N., Chaves, A., Pellegrino, J., 1972. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev. Inst. Med. Trop. São Paulo 14, 397–400.

King, C.H., Muchiri, E.M., Ouma, J.H., 2000. Evidence against rapid emergence of praziquantel resistance in *Schistosoma haematobium*, Kenya. Emerg. Infect. Dis. 6, 585–594.

King, C.H., Dickman, K., Tisch, D.J., 2005. Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. Lancet 365, 1561–1569.

Manges, A.R., Johnson, J.R., Foxman, B., O'Bryan, T.T., Fullerton, K.E., Riley, L.W., 2001. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. N. Engl. J. Med. 345, 1007–1013.

Martin, J.M., Green, M., Barbadora, K.A., Wald, E.R., 2002. Erythromycin-resistant group A streptococci in schoolchildren in Pittsburgh. N. Engl. J. Med. 346, 1200–1206.

McManus, D.P., Loukas, A., 2008. Current status of vaccines for schistosomiasis. Clin. Microbiol. Rev. 21, 225–242.

Melman, S.D., Steinauer, M.L., Cunningham, C., Kubatko, L.S., Mwangi, I.N., Wynn, N.B., Mutuku, M.W., Karanja, D.M., Colley, D.G., Black, C.L., Secor, W.E., Mkoji, G.M., Loker, E.S., 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. PLoS Negl. Trop. Dis. 3, e504.

Merilä, J., Crnokrak, P., 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evol. Biol. 14, 892–903.

Ministry of Health, Brazil, 2005. Guide to Epidemiological Surveillance [Guia de Vigilância Epidemiológica]. Ministry of Health, Brazil, Brasilia.

Norton, A.J., Gower, C.M., Lamberton, P.H.L., Webster, B.L., Lwambo, N.J.S., Blair, L., Fenwick, A., Webster, J.P., 2010. Genetic consequences of mass human chemotherapy for *Schistosoma mansoni*: population structure pre-and post-praziquantel treatment in Tanzania. Am. J. Trop. Med. Hyg. 83, 951.

Redman, E., Packard, E., Grillo, V., Smith, J., Jackson, F., Gilleard, J.S., 2008. Microsatellite analysis reveals marked genetic differentiation between *Haemonchus contortus* laboratory isolates and provides a rapid system of genetic fingerprinting. Int. J. Parasitol. 38, 111–122.

Rodrigues, N.B., Loverde, P.T., Romanha, A.J., Oliveira, G., 2002. Characterization of new *Schistosoma mansoni* microsatellite loci in sequences obtained from public DNA databases and microsatellite enriched genomic libraries. Mem. Inst. Oswaldo Cruz 97 (Suppl. 1), 71–75.

Silva, L.K., Liu, S., Blanton, R.E., 2006. Microsatellite analysis of pooled *Schistosoma mansoni* DNA: an approach for studies of parasite populations. Parasitology 132, 331–338.

Soares, S., Kristinsson, K.G., Musser, J.M., Tomasz, A., 1993. Evidence for the introduction of a multiresistant clone of serotype 6B *Streptococcus pneumoniae* from Spain to Iceland in the late 1980s. J. Infect. Dis. 168, 158–163.

Wegner, D.H., 1984. The profile of the trematodicidal compound praziquantel. Arzneimittelforschung 34, 1132–1136.

Capítulo 2

Manuscrito 2 - Characteristics of the human host have little influence on which local *Schistosoma mansoni* populations are acquired

Neste manuscrito foi realizada uma descrição epidemiológica das populações rurais estudadas, em Ubaíra-Bahia. Utilizando características demográficas e comportamentais, os participantes foram separados em grupos (populações componentes) para que pudesse ser avaliado se diferentes populações de *Schistosoma mansoni* eram selecionadas pelos diferentes atributos pesquisados. Além disso, foram descritas pela primeira vez diferentes aplicações para o índice de diferenciação genética D de Jost.

Principais resultados encontrados:

1- Características epidemiológicas similares a outras localidades do mundo;
2- Dados genéticos indicando transmissão local com baixa influência migratória;
3- Não foi encontrada seleção genética de parasitas baseada em características do hospedeiro.

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Characteristics of the Human Host Have Little Influence on Which Local *Schistosoma mansoni* Populations Are Acquired

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Abstract

Background: Brazil remains the country in the Americas with the highest prevalence of schistosomiasis. A combination of control efforts and development, however, has sharply reduced its intensity and distribution. The acquisition of specific schistosome populations may be dependent on host characteristics such as sex, age, geography, work, habits and culture. How these and other host characteristics align with parasite subpopulations may guide approaches to improve control.

Methodology: A cohort of more than 90% of the residents in two rural communities in Brazil participated in an epidemiologic survey of demographic, socio-economic and behavioral characteristics. The variables sex, age, intensity of infection, socio-economic index, % lifetime spent on site, previous infection, and trips outside the district were used to group parasites infecting individuals. *Schistosoma mansoni* infection status was determined by examination of stools submitted on 3 different days. The aggregate of eggs collected from the whole stool was used to determine degree of population differentiation from allele frequencies for 15 microsatellites.

Conclusions/Significance: Infection prevalence was 41% for these communities, and the epidemiologic characteristics were similar to many of the endemic areas of Brazil and the world. Parasite population structuring was observed between the two communities (Jost's D 0.046, CI95% 0.042–0.051), although separated by only 8 km and connected by a highway. No structuring was observed when infected individuals were stratified by host's biologic, demographic or epidemiologic characteristics. Those most heavily infected best reflected the communities' overall parasite diversity. The lack of differentiation within villages suggests that individuals are likely to get infected at the same sites or that the same parasite multilocus genotypes can be found at most sites. The geographic structuring between villages and the lack of structuring by age of the host further supports the impression of a population little affected by migration or drift.

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Introduction

The transmission of schistosomiasis is influenced by human culture, occupations and demographics among other factors. Also, our group and others have demonstrated that each individual host carries only a portion of the total available parasite genetic variability [1,2,3,4,5,6,7], and thus host-to-host structuring may exist due to each individual's personal characteristics, such as age, sex, social status or residence. These are factors that may bring them into contact with genetically distinct populations of parasites or even influence their susceptibility. While these epidemiologic relationships are usually explored by associating human demographics with infection prevalence or intensity, by using genetic markers we can also determine if these host characteristics are associated with acquiring different parasite subpopulations.

An immediate problem for any such analysis is how to sample the parasite population. Due to the biology and local distribution of the parasite *Schistosoma mansoni*, sampling for genetic analysis is not straightforward. The snail host, where asexual reproduction takes place, lives an average of 3 months, and cercariae collected at one point in time do not represent the whole genetic diversity found in humans [7]. In addition to differences in behavioral factors and biological susceptibility of the human host, the intermittent presence in the snail host increases the potential for differential acquisition of parasite genotypes.

Sexual reproduction takes place in the human host where the adult worms are inaccessibly located in mesenteric veins. A portion of the hundreds of eggs produced daily by worm pairs remains trapped in tissues and will not contribute to the succeeding generation, whereas the majority of these progeny is shed in stool.

Author Summary

Schistosomiasis is one of the world's most important parasitic infections. Its elimination has proved difficult even in countries such as Brazil where access to treatment is readily available. Infection is the result of human contact with surface water where there are infected snails, so that human biology and habits may bring different individuals in contact with different groups of parasites. Identification of schistosome subpopulations may assist understanding transmission patterns and guide control efforts. We compared microsatellite allele frequencies from all of the infections in 2 small villages and determined that the movement of parasites between them was limited. Individual infections were distinct composites of parasites, but if infected humans were grouped by demographic and epidemiologic characteristics, there was no evidence that specific parasite subpopulations were being selected in these types of hosts. Infections were also not differentiated when stratified by host's age indicating that the populations were stable over time. Since the infection cycle requires human fecal contamination of water, local human behavior can to some degree be inferred from the patterns of schistosome subpopulation distribution.

New individuals enter the host only by infection, a form of migration. Since the adult parasites are long-lived, humans can accumulate a variety of individuals over time. Our approach to the population genetics of *S. mansoni* has been to analyze allele frequencies obtained by extracting DNA from the aggregate of eggs isolated from single stools. In this way the reproducing population of schistosomes from many individuals (e.g., most of the residents of small communities) can be analyzed with a large sample size and a minimum of selection bias.

An important problem for all genetic studies is determining appropriate sample size and avoiding selection bias. The population structure of most organisms is studied by collecting a sample of discrete genotypes and then aggregating or pooling these into allele frequencies for the whole population. This approach is dependent on the quality of the sampling performed. Depending on the organism and the specific population, sample sizes of 30 [8] or hundreds [9,10] have been deemed necessary to provide an adequate sample. Parasite populations add unique challenges to the problem of sampling since they are not simply structured as discrete organisms scattered or clustered across a landscape. They exist as populations within individual hosts (infrapopulations) as well as the collection of parasites within one host species (component populations) [11]. The latter represents the full genetic potential of which the infrapopulations are each a small sample. For the individual human infection with *S. mansoni*, a typical 200 g stool with a light infection of 40 eggs/g will have a total of 8,000 eggs. The miracidial stage can be hatched from eggs and collected for study. Samples of 10, 20, 30 individual miracidia may be small when diversity is high, and there may be bias for which eggs will hatch into miracidia and which can be collected. Further, the process of hatching and collecting individual parasites limits the number of infected people that can be examined. How to sample, what to sample and how much to sample has never been defined for schistosomes. Our approach to the population genetics of *S. mansoni* has been to analyze allele frequencies obtained by extracting DNA from an aggregate of eggs isolated from the whole stool of infected individuals. In this way the transmitted population of schistosomes from many or even all individuals (in the case of a small community) can be analyzed with a large sample size from many infrapopulations and a minimum of selection bias. Sampling larger numbers of infrapopulations also allows for stratifying hosts for comparisons. Finally, using this approach we have shown that the stool egg population has a similar genetic composition to the adult worm population [12,13].

In this paper we assessed risk factors for infection and differentiation of parasite infrapopulations by genotyping the aggregate of eggs obtained from infected individuals in two small rural villages. We divided parasites into "component" populations based on host geography as well as host biology, demography and epidemiology. We then estimated differentiation between these groups from their infrapopulation or component population allele frequencies. Although we previously observed structure based on geographic distance between these two nearby communities [3], we found little population structuring within the villages or between hosts. Finally, we explore the implications of these findings for the nature of schistosome populations in rural communities.

Methods

Ethics Statement

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. All subjects provided written informed consent or in the case of minors, consent was obtained from their guardians. All aspects of the study have been conducted according to the principles expressed in the Declaration of Helsinki.

Study Area

Two rural Brazilian communities – Jenipapo (population 482) and Volta do Rio (population 367) – were studied because of their high prevalence of schistosomiasis, their size and their relative isolation. They are administered by the municipality of Ubaíra (roughly equivalent to a county in the USA) and are located in the Jiquiriçá River valley in the state of Bahia. By road they are 270 km SE of the State capitol and principal city, Salvador. Each was at least 12 km from a major town and 8 km distant from each other. Volta do Rio is also divided geographically into an upper and lower section with a 40 m difference in height above the river (Figure 1). The major sources of livelihood are planting cacao, bananas cassava, cattle raising and other animal production. There is a Federal Family Health Program clinic in Jenipapo with a permanent staff consisting of a nurse, dentist and part-time physician. Volta do Rio has a simpler health post that employs only a group of nurses. Jenipapo also has primary and secondary schools attended by all of the nearby small communities, including Volta do Rio.

Study Design and Protocol

As previously described [3], an epidemiologic and parasitologic survey was conducted for all inhabitants $\geq$1 year old who agreed to participate. Questions concerning housing, sanitary habits, socio-economic conditions and water contact were asked as part of the epidemiologic survey. For water contact, individuals or guardians for minors $<$10 years of age were asked if they frequently used any of the 8–9 previously identified major water contact sites and what activities they tended to perform there. The socio-economic evaluation was based on the Criteria for Economic Classification of Brazil (http://www.abep.org/novo/Content.

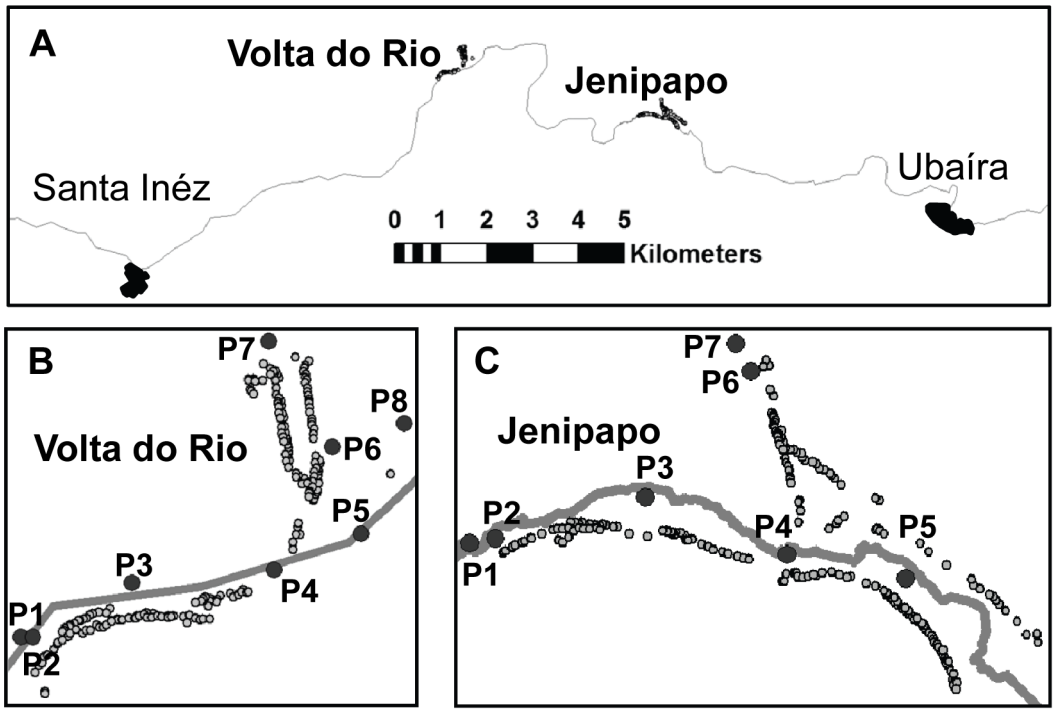

Figure 1. Study areas and water contact sites. A. Map of the Jiquiriçá River Valley between the towns of Santa Inês and Ubaíra. B. Volta do Rio. C. Jenipapo. The Jiquiriçá River is represented by the solid line dividing the communities. Open circles - homes, (P) numbered water contact sites.
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aspx?ContentID = 139). These criteria with revisions have been used nationally for more than a decade to characterize the purchasing power of the Brazilian population using possessions (color TV, radio, bathroom, car, washing machine, videocassette/DVD, refrigerator, freezer), services (maid/housekeeper) and degree of education of the head of household. The index places households within 8 categories ranging from minimum monthly wage to 13X minimum monthly wage. The interpretation of these categories is weighted for metropolitan regions of the country including Salvador, Bahia.

Three stool samples each on different days were requested from each resident over a period of 1 week for quantitative examination by the Kato-Katz method. Individuals who tested positive for *S. mansoni* infection were treated with a single oral dose of praziquantel according to Brazilian Ministry of Health guidelines [14]. Those found to have intestinal nematodes were treated with mebendazole.

Egg Purification

All stools were weighed to the nearest 0.01 g on a digital balance upon arrival in the laboratory. Whole stools from single individuals that were positive for *S. mansoni* were homogenized in a blender containing 200 ml of 2% saline followed by selective sieving [15] through two mesh nylon filter bags (FSI, Michigan City, Indiana, USA) with 300 and 55 μm pore sizes, respectively. The retained material was then sedimented in 2% saline. Since eggs were among the densest elements in the stool [16], the bottom 5 ml of sediment was collected and kept frozen at −20°C until used for DNA isolation.

DNA Isolation

The 5 ml frozen stool sediment was mixed with 5 ml 2X extraction buffer (50 mM NaCl, 100 mM Tris–HCl, pH 7.5, 10 mM EDTA, 1.0% SDS) and 10 ml H_2O-saturated and Tris-buffered phenol, pH 7.5. This was followed by two chloroform/Isoamyl extractions [3]. The DNA was then ethanol precipitated and suspended in 10 mM Tris, pH 7.5, 1 mM EDTA. Finally, the sample was treated with cetyl trimethylammonium bromide (CTAB) to remove PCR inhibitors [17].

Microsatellite Genotyping

To genotype *S. mansoni* eggs, 15 microsatellite markers were used as described previously [2,3]. For each marker a duplicate PCR reaction using 2 μL of extracted DNA from stool was performed, totaling 30 reactions per sample. PCR products from each sample were combined into groups of three or four markers and processed on an Applied Biosystems 3730xl DNA Analyzer. PeakScanner software (Applied Biosystems, Carlsbad, CA) was used to determine peak heights from which allele frequencies were calculated. Successful PCR reactions were defined as those in which there was at least one peak >500 pixels in the size range expected for a given marker. All peaks less than 100 pixels were excluded. We attempted to genotype all samples, and if multiple samples from the same individual amplified, their mean allele frequency was used. Subsequent population analyses were limited to those samples where a minimum of 12 out of 15 markers genotyped successfully.

Statistical Analyses

Information collected during the study was double-entered into the program Epi Info version 3.5.3 [18]. Pearson's chi-square and Student's t-test were used to compare categorical and continuous data, respectively, and a p-value of 0.05 was used as the criterion for statistical significance. Multivariable analyses were carried out using logistic or linear regression in SPSS (Version 17). Individuals with missing data were dropped for the analysis of that variable.

For population genetic analyses, allele counts for each sample were calculated by multiplying the allele frequencies at a microsatellite locus by the total egg counts found on the Kato-Katz assay. Infrapopulations were stratified by the host's residence, sex, age, intensity of infection, household, travel history, number and location of water contacts and socio-economic condition. Genetic differentiation between populations was expressed as the index Jost's D [19] calculated using the program SPADE (http://chao.stat.nthu.edu.tw). D is a true differentiation index and does not rely on assumptions of Hardy-Weinberg equilibrium, which do not apply to infrapopulations. After grouping, each pair of infrapopulations can be compared within a group or the combined allele numbers and allele frequencies can be used to form a component population. We make the following differentiation and diversity comparisons:

Di - Jost's D values for pairs of **I**nfrapopulations in the group as defined by host characteristics. The Di indicates how differentiated the parasites infecting individual members of the group are on average from other infections in the group. For any host group a matrix of pairwise Di's is generated, and the mean of this matrix for the different groups was compared.

Dc - Jost's D for the combined allele numbers of two **C**omponent populations grouped based on shared geography or host characteristics. Differentiation here indicates whether certain host characteristics lead to a preference for selected populations of parasites or vice-versa.

Dic – Jost's D between individual **I**nfrapopulation compared to its geographic **C**omponent population. A single value (Dic) is produced for each individual host that indicates how differentiated his or her infrapopulation of worms/eggs is from the pool of available genotypes. The mean Dic was compared for infrapopulations from humans with and without the selected characteristic.

AE - The effective allele number [20] is a measure of diversity. It was calculated as $1/\sum_{j=1}^{n} p_j^2$ where p_j is the frequency of the j^h allele for each marker.

Egg counts were recorded as eggs per gram of stool (epg) and log-transformed to approximate a normal distribution for analyses. Arithmetic means were calculated for group Di, Dic and AE. For the Di and the Dic group means were compared by bootstrapped Student's t-test with 1000 resamples, since the distribution of these measures is unknown. There is no standard for effect size for these new types of comparison. For the Dc, we follow the convention used for interpreting F_{ST} values [21]. Dc values from 0–0.05 indicate little differentiation; from 0.05–0.15, moderate differentiation; and above 0.15, great differentiation [22]. Changes in D rather than the absolute value below the 0.05 range, however, may still indicate a significant obstacle to gene flow.

Results

Study Population Characteristics

The study group consisted of 814 of the 849 (96%) inhabitants residing in the 243 households of the two villages. The mean age was 31.5 years (±22.2), and slightly more women than men were enrolled (53.7%). Most subjects were born in their current municipality (83.7%), and the average percent of lifetime spent in the municipality of Ubaíra was 93.5%. Considering the history of travel outside of the district, 25.3% reported any travel, and a minority (19.5%) of those who traveled reported contact with surface water. There were some differences for the two geographically distinct areas of Volta do Rio (VdR). The percent of those traveling outside of the district was greater for individuals from lower VdR than upper VdR (34.2 vs. 22.2%, p = 0.02), but they remained outside of the area for similar lengths of time (61.92 vs. 57.82 days, p = 0.51). There were significantly more individuals in upper VdR who had at least one family member infected (36.9 vs. 25.2%, p = 0.02).

Most demographic and epidemiologic characteristics were similar for both villages (Table 1), with the exception of the socio-economic index and sanitation. Jenipapo had a somewhat greater purchasing power for (12.0 vs. 10.7, p = 0.017). The mean socio-economic index for the two localities was 11.4±4.3, which corresponds to the second lowest of the 8 income categories used nationwide. A socio-economic index of 11 points translated to a family income of approximately $330/month in 2009. Nearly all homes in both villages have piped water and indoor flush toilets. The 2 most common destinations for these toilets was either a septic tank or the river. Despite a lower socio-economic index, the disposal of human waste was more adequate in VdR than Jenipapo, and upper VdR had better waste disposal than lower VdR. In VdR the Jiquiriçá River is shallow, sluggish and seasonal, while at Jenipapo the Jiquiriçá is joined by a major stream that maintains flow in the river throughout the year. This may explain the different approaches to sanitation. Drinking water in both communities comes from sources several km away from the river.

Infection Risk Factors

The prevalence of *S. mansoni* infection was higher in Jenipapo (45.8%) than VdR (35.1%), but the mean intensity of infection was similar (Table 1). The lower limit of detection was 8 epg and the highest mean intensity observed was 3,792 epg. Some 31.3% of residents knew someone with current or past infection with *S. mansoni*, and 34.2% had one or more relatives with schistosomiasis. Two hundred and ninety seven individuals (37%) reported past infection with *S. mansoni*, and 93.6% of those reporting infection also reported being treated, most often with oxamniquine (64.0%). None had been treated with praziquantel, which was newly approved in Brazil for treatment of schistosomiasis at the time of the study. No variables or contact points were correlated with intensity of infection.

Characteristics that were associated with a higher risk for *S. mansoni* infection were living in Jenipapo, age (2nd, 3rd and 4th decades compared to 1st, Figure 2) and male sex (Table 2). Traveling outside of the municipality of Ubaíra in the past year was not associated with an increased risk for infection, but water contact while traveling was (OR of 2.3, p = 0.012) compared to those reporting no contact. A self-reported history of past infection overall had no correlation with risk, but reporting past treatment for *S. mansoni* did (OR 3.07, p = 0.02).

Eight water contact points in Jenipapo and nine in VdR were identified as those most commonly visited by villagers. The number of visits and nature of activities at each site were asked during the epidemiologic survey. The risk of being infected with *S. mansoni* increased substantially as the individual had contact with an increasing number of sites (Table 2). After adjusting for age and sex, people who reported contact with one point in Jenipapo and two in VdR were significantly more likely to be infected (Table 3). All of these points were common crossings to reach from one side of the river to the highway. A log was used as a temporary bridge at one point each in the two villages. However, at contact point 5 in Jenipapo (Figure 1C) the activity most associated with infection was fishing (OR = 2.96, p = 0.012), which is usually performed

Table 1. Demographic, epidemiologic and infection characteristics of Jenipapo and Volta do Rio.

		Combined	Jenipapo	VdR	*p*	Volta do Rio		*p*
						Upper	Lower	
Characteristic	Total	n = 814	n = 461	n = 353		n = 195	n = 158	
Male Sex		377 (46.3)	221 (47.9)	156 (44.2)	0.288	92 (47.2)	64 (40.5)	0.209
Mean age in years		31.5±22.2	30.6±21.7	32.6±22.7	0.211	32.5±21.8	32.7±23.8	0.922
Birth place (%)	Ubaíra	681 (83.7)	391 (84.8)	290 (82.2)	0.309	159 (81.5)	131 (82.9)	0.738
	Other	133 (16.3)	70 (15.2)	63 (17.8)		36 (18.5)	27 (17.1)	
% Lifetime in Ubaira		93.5±41.4	93.6±19.9	93.5±58.6	0.982	89.0±23.2	92.5±19.3	0.116
Travel last year		204 (25.3)	105 (22.8)	99 (28.7)	0.056	**45 (23.8)**	**54 (34.2)**	**0.033**
Travel water contact		39 (19.5)	17 (16.2)	22 (20.6)	0.264	8 (15.1)	14 (26.0)	0.319
Socio-Economic Index		**11.4±4.3**	**12.0±4.7**	**10.7±3.8**	**0.017**	10.5±4.2	11.1±3.2	0.390
Piped Water (%)		810 (99.4)	-	-	-	-	-	-
Indoor Toilet	Yes (%)	791 (97.4)	-	-	-	-	-	-
Sanitation	Septic tank	**515 (63.4)**	**200 (43.4)**	**316 (89.3)**	**<0.001**	**187 (95.9)**	**129 (81.6)**	**<0.001**
	River/Open air	**268 (33.0)**	**250 (54.2)**	**20 (7.4)**		**3 (1.5)**	**16 (10.1)**	
Infection Prevalence (%)		**335 (41.2)**	**211 (45.8)**	**124 (35.1)**	**0.002**	60 (30.8)	64 (40.5)	0.057
Infection Intensity (epg*)		60.8	56.6	68.6	0.248	69.2	68.0	0.949
Past *S. mansoni* infection	Yes (%)	297 (36.5)	175 (38.0)	122 (34.6)	0.318	68 (34.9)	54 (34.28)	0.891
Know someone with *S. mansoni*	Yes (%)	252 (31.3)	146 (32.0)	106 (30.0)	0.830	68 (35.4)	38 (24.2)	0.057
Family member with *S. mansoni*	Yes (%)	252 (34.1)	150 (36.1)	102(31.6)	0.194	**65 (36.9)**	**37 (25.2)**	**0.024**

Categorical variables were compared using Pearson's Chi squared. Means for continuous variables were compared by Students t-test. Significant values are in bold type. Values ± S.D and (%).

*epg - genometric mean of *S. mansoni* eggs per gram of stool. Statistically significant comparisons are in bold type.

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while wading in the river. In VdR, at the point not used for crossing the river (P3, Figure 1B) formed a pool, and bathing here was most associated with infection (OR = 3.55, $p<0.001$). Working and walking at this site were protective (OR = 0.1, p = 0.012 and OR = 0.043, p = 0.036, respectively), while fishing and playing in the water were also associated with a risk for infection (OR = 4.18, p = 0.048 and OR = 4.93, p = 0.026, respectively). The only significant activity associated with those who used the site and were uninfected was collecting water (OR = 5.1, $p<0.048$).

While individuals younger than 15 years old did not report more water contact than those older (p = 0.540), the type of contact may have involved more or longer exposure. Water contact for children tended to involved leisure activities such as walking (p = 0.02), swimming ($p<0.001$) and playing (p = 0.002) compared to older individuals who contacted water primarily through activities associated with labor, such as working (p = 0.02) and obtaining water (p = 0.05). Fishing was equally frequent between both age groups. Males did report visiting 1.5 times as many water contact points as females (p = 0.001).

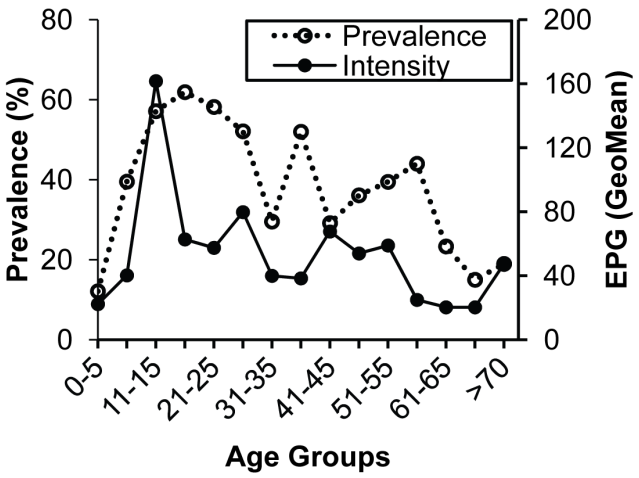

Figure 2. Age-related prevalence and intensity of infection. Prevalence (%) and intensity of infection (geometric mean epg) by age-group. epg - count of *S. mansoni* eggs per gram of stool.

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Genetic Differentiation

Our previous study [3] used only samples that were positive by Kato-Katz in all 3 stools (n = 116). For the analysis here we included all samples regardless of the number of stools positive for *S. mansoni*, thus, genotypes from 226 of the 335 infected individuals (67.5%) were included for analysis. Of those genotyped, 51.8% were genotyped for 3 samples, 14.6% for 2 samples and 33.6% for only 1 sample. The differentiation between the two geographic component populations of the two villages (D = 0.046, CI95% 0.042–0.051) was similar to that previously reported [3]. To determine whether related parasites clustered with host characteristics, component populations were formed by grouping infrapopulations based on host epidemiologic characteristics of sex, age, household, economic status, place of birth, frequency of travel, previous infection and number and location of water contacts. Differentiation between these component populations was analyzed for the Di, Dc, Dic and effective allele number.

Table 2. Risk for *S. mansoni* egg positive stools.

Variable		Total	Positive n (%)	Or (95% CI)	p value
Age	1–10	152	42 (27.6)	-	-
	11–20	**175**	**104 (59.4)**	**3.84 (2.41–6.12)**	**<0.001**
	21–30	**138**	**76 (55.1)**	**3.21 (1.97–5.23)**	**<0.001**
	31–40	**94**	**39 (41.5)**	**1.86 (1.08–3.20)**	**0.03**
	41–50	84	27 (32.1)	1.24 (0.70–2.22)	0.470
	51–60	63	26 (41.3)	1.84 (0.99–3.40)	0.052
	>60	108	21 (19.4)	0.63 (0.35–1.15)	0.130
Sex	Male	**378**	**192 (50.8)**	**2.12 (1.60–2.82)**	**<0.001**
	Female	**437**	**143 (32.7)**		
Outside Trips Last Year	0	602	247 (41.0)	-	
	1–3	148	64 (43.2)	1.10 (0.76–1.58)	0.624
	>4	56	22 (39.3)	0.93 (0.53–1.63)	0.800
Water contact during trip	Yes	**39**	**24 (61.5)**	**2.38 (1.18–4.81)**	**0.015**
	No	**176**	**68 (38.6)**		
Prior *S. mansoni* infection	Yes	297	128 (43.1)	1.13 (0.85–1.52)	0.393
	No	518	207 (40.0)		
Treatment for *S. mansoni*	Yes	**278**	**115 (41.4)**	**3.07 (1.13–8.32)**	**0.021**
	No	**19**	**13 (68.4)**		
Water Contacts	0	100	18 (17.8)	-	-
	1	**276**	**85 (30.8)**	**1.89 (1.06–3.37)**	**0.032**
	2	**236**	**99 (41.9)**	**2.84 (1.59–5.08)**	**<0.001**
	3	**90**	**56 (62.2)**	**6.07 (3.08–11.94)**	**<0.001**
	4	**56**	**38 (67.9)**	**7.41 (3.43–16.03)**	**<0.001**
	≥5	**56**	**39 (69.6)**	**7.77 (3.56–16.97)**	**<0.001**

Risk for *S. mansoni* infection compared to youngest category for age or to no surface water contact by chi-squared test. CI = confidence interval; OR = odds ratio. Statistically significant comparisons are in bold type.

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Di was significantly different for the individual villages and both villages combined when infections were grouped by sex, age, infection intensity and certain water contact sites (Table 4). We also tested similarity of infrapopulations within households. Only 11% and 5% of households in Jenipapo and VdR, respectively, had more than one member infected. The mean Di for household

Table 3. Risk at water contact points for *S. mansoni* infection.

Jenipapo (n = 461)					**Volta do Rio (n = 352)**			
	Infected (%)	**Not-Infected**	**OR (95% CI)**	**p-value**	**Infected (%)**	**Not-Infected**	**OR (95% CI)**	**p-value**
Contact point	n = 211	n = 250			n = 124	n = 230		
1	45 (21.3)	27 (10.8)	1.42 (0.77–2.62)	0.292	**52 (41.9)**	**21 (9.1)**	**2.97 (1.44–6.11)**	**0.001**
2	69 (32.7)	50 (20.0)	1.39 (0.83–2.30)	0.182	48 (38.7)	33 (14.3)	1.84 (0.97–3.49)	0.075
3	31 (14.7)	14 (5.6)	*		**41 (33.1)**	**12 (5.2)**	**3.60 (1.49–8.72)**	**0.004**
4	184 (87.2)	215 (86.0)	*		76 (61.3)	126 (54.8)	*	
5	**110 (52.1)**	**74 (29.6)**	**2.27 (1.52–3.39)**	**<0.001**	15 (12.1)	10 (4.3)	*	
6	49 (23.2)	37 (14.8)	*		17 (13.7)	9 (3.9)	*	
7	11 (5.2)	9 (3.6)	*		37 (29.8)	55 (23.9)	*	
8	-	-	-		8 (6.5)	9 (3.9)	*	
Other	16 (7.6)	13 (5.2)	*		18 (14.5)	17 (7.4)	2.05 (0.90–4.66)	0.091

Reported use of a water contact point (see Figure 1) was entered into a logistic regression model where the dependent variable was *S. mansoni* infection status. The model was controlled for age and sex.

*Indicates contact points that did not enter the model. Statistically significant comparisons are in bold type.

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Table 4. Subpopulation differentiation and diversity.

	D_i (p-value)	D_c	D_{ic} (p-value)	AE
Combined				
Sex (male vs female)	0.125 vs 0.127 (0.105)	0.002	0.061 vs 0.060 (0.961)	3.36 vs 3.31 (0.683)
Age (≤15 vs >15 y/o)	**0.108 vs 0.132 (<0.001)**	0.005	0.053 vs 0.064 (0.069)	3.40 vs 3.31 (0.072)
Intensity of infection (<400 vs >400 epg)	**0.134 vs 0.082 (<0.001)**	0.005	**0.066 vs 0.032 (<0.001)**	**3.40 vs 3.43 (<0.001)**
Socio-economic Index (<11 vs >11)	**0.118 vs 0.129 (<0.001)**	0.004	**0.054 vs 0.067 (0.036)**	3.40 vs 3.29 (0.050)
% lifetime in Ubaira (100% vs <100%)	0.124 vs 0.127 (0.183)	0.013	0.060 vs 0.064 (0.620)	3.35 vs 3.33 (0.555)
Previous Infection (Yes vs No)	**0.124 vs 0.126 (0.028)**	0.003	0.058 vs 0.062 (0.529)	3.36 vs 3.34 (0.661)
Trips outside of the district (Yes vs No)	**0.134 vs 0.122 (<0.001)**	0.007	0.065 vs 0.059 (0.287)	3.32 vs 3.36 (0.358)
Jenipapo				
Sex (male vs female)	**0.112 vs 0.101 (<0.001)**	0.003	0.062 vs 0.055 (0.392)	3.35 vs 3.32 (0.653)
Age (≤15 vs >15 y/o)	**0.097 vs 0.112 (<0.001)**	0.006	0.054 vs 0.062 (0.322)	3.28 vs 3.34 (0.228)
Intensity of infection (<400 vs >400 epg)	**0.116 vs 0.054 (<0.001)**	0.006	**0.065 vs 0.028 (<0.001)**	**3.32 vs 3.40 (<0.001)**
Socio-economic Index (<11 vs >11)	**0.098 vs 0.115 (<0.001)**	0.007	0.054 vs 0.065 (0.176)	**3.35 vs 3.31 (0.042)**
% lifetime in Ubaira (100% vs <100%)	0.107 vs 0.097 (0.111)	0.008	0.059 vs 0.061 (0.848)	3.36 vs 3.32 (0.520)
Previous Infection (Yes vs No)	**0.102 vs 0.110 (<0.001)**	0.002	0.056 vs 0.062 (0.435)	3.39 vs 3.33 (0.195)
Trips outside of the district (Yes vs No)	0.111 vs 0.105 (0.016)	0.011	0.062 vs 0.058 (0.610)	3.33 vs 3.37 (0.456)
Contact point 5 (Yes)	**0.092 vs 0.124 (<0.001)**	0.006	**0.050 vs 0.070 (0.014)**	3.39 vs 3.32 (0.188)
Volta do Rio				
Sex (male vs female)	**0.108 vs 0.128 (<0.001)**	0.007	0.059 vs 0.070 (0.191)	3.37 vs 3.19 (0.136)
Age (≤15 vs >15 y/o)	**0.091 vs 0.121 (<0.001)**	0.009	**0.049 vs 0.067 (0.035)**	3.42 vs 3.27 (0.216)
Intensity of infection (<400 vs >400 epg)	**0.124 vs 0.079 (<0.001)**	0.008	**0.068 vs 0.039 (<0.001)**	**3.26 vs 3.48 (0.016)**
Socio-economic Index (<11 vs >11)	**0.104 vs 0.128 (<0.001)**	0.005	0.055 vs 0.071 (0.074)	3.32 vs 3.43 (0.133)
% lifetime in Ubaira (100% vs <100%)	0.115 vs 0.116 (0.870)	0.020	0.063 vs 0.065 (0.814)	3.34 vs 3.34 (0.974)
Previous Infection (Yes vs No)	0.116 vs 0.116 (0.970)	0.018	0.063 vs 0.063 (0.968)	3.31 vs 3.37 (0.319)
Trips outside of the district (Yes vs No)	**0.129 vs 0.112 (<0.001)**	0.013	0.071 vs 0.060 (0.301)	3.32 vs 3.35 (0.619)
Contact point 1 (Yes)	**0.107 vs 0.126 (<0.001)**	0.006	0.057 vs 0.069 (0.176)	3.34 vs 3.26 (0.970)
Contact point 3 (Yes)	**0.102 vs 0.130 (<0.001)**	0.008	**0.049 vs 0.073 (0.005)**	3.37 vs 3.25 (0.320)

Di - pairwise D for all members of the group. Student's t-test was used to compare group means. Di and Dic were compared by ANOVA Other variables were trips outside of the region, co-infection with other helminths, all water contact points, number of water contacts visited, a history of past infections, socio-economic index. The Dc was estimated using the program Spade (http://chao.stat.nthu.edu.tw). Statistically significant comparisons for Dic are in bold type.

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members in Jenipapo was 0.065 ± 0.040 and 0.086 ± 0.047 in VdR compared to 0.095 ± 0.033 and 0.123 ± 0.066 for all those infected in Jenipapo and VdR, respectively. The bootstrapped t-tests for the mean Di of household clusters versus all individuals in the village were significantly smaller ($p = 0.004$, $p = 0.030$; Jenipapo and VdR, respectively).

The Dc indicates that the composition of the populations based on host characteristics differ little in their genetic composition. The Dic was significant for age overall, but this was mainly due to a difference in VdR where children ≤15 acquired parasites that were more genetically differentiated from the whole community of parasites than those infecting adults. Overall and in both communities, infrapopulations from heavy infections were less differentiated from the community's component population than lighter ones.

The AE was only significantly different for intensity of infection for the two villages combined as well as separately. This is a measure of diversity, and higher intensity infections averaged higher effective allele numbers. AE was also associated with the socio-economic index in Jenipapo.

Since different age groups may be exposed to different subpopulations of *S. mansoni*, we further stratified age into 4 groups: 0–7, 8–15, 16–40 and >40. We found that the youngest age group gave the highest Dc in pairwise comparisons and the highest mean Dic of any group, but this age group was also the smallest ($n = 12$), had the lowest prevalence and the lowest intensity of infection. When 12 individuals with similar intensities of infection (sample sizes) were compared from each age group, these differences resolved.

Discussion

The communities of Jenipapo and Volta do Rio are typical of the region in their level of development and access to sanitation. They also are similar to many other areas endemic for schistosomiasis in their age-specific prevalence and intensity of infection [23]. Differences in the prevalence of infection between these otherwise similar communities may be due to differences in how human waste is handled. In part these choices may be the result of the presence of constant flow in the river in Jenipapo and seasonal flow in VdR. Consistent with this, upper VdR which is much further from the river had higher use of septic tanks and fewer homes reporting using the river. In Brazil, economic development and control efforts using education and the drug

oxamniquine (used prior to praziquantel) have greatly reduced the amount of hepatosplenic disease, but the infection prevalence in many areas has not changed. In these two villages, the current prevalence when based on a single stool examination is no different from the 15–20% prevalence observed for the state of Bahia in the 1950's [24,25] and at the start of control programs in 1976 [24,26,27]. When multiple stool samples are examined, the true prevalence of infection is even two to threefold higher.

Some common risk factors found in other communities can be identified in this study. Age between 10 and 20 was associated with the highest prevalence and intensity. In Brazil, male sex is associated with increased risk [28], but in other parts of the world infection can be more prevalent in females [29]. This difference is likely due to differing sexual roles in work, play and ultimately water contact. Water contact is an essential step in transmission of schistosomiasis. While this variable would seem to be a strong risk factor with high correlation with infection, it has been difficult to measure and then associate with intensity [30]. Even when water contact is directly observed [29], the frequency of contacts is not always predictive. Questionnaires have been the simplest and least expensive way to assess risk factors. In Brazil, questionnaires have been shown to produce reliable responses that correlate with risk of infection [31,32], but even here there can be significant place-to-place variation [33] requiring questions tailored to the specific location. Water contact has been solicited in multiple ways in terms of location, type of activity, time of contact and percent body exposure. We asked only which sites were visited and what activities were commonly performed there. The questionnaire was administered prior to all stool examinations and thus not biased by knowledge of the infection status of respondents. We found that simply counting the number of sites visited was most associated with prevalence of infection. Further, while travel away from the area was not associated with infection, self-reported travel combined with surface water contact was associated. These associations tend to validate the responses given by the residents.

In addition to risk for prevalence and intensity of infection, we sought to identify risk factors for acquiring specific parasite populations. The moderate differentiation between infrapopulations indicates that each individual collects a limited portion of the total genetic variability from the component population. This non-homogeneous distribution together with differences in water contact, occupation, habits, sex, years of exposure, etc are all reasons for structuring of the parasite population within different demographic categories of the human host. No population structuring, however, was observed. In another human population with a different intensity of transmission or different economic, cultural or geographic organization the distribution of parasites might be different.

Geographic structuring showed that over a short distance schistosome gene flow is limited in this region (Dc Jenipapo/VdR = 0.046). By contrast, within the two villages, when we assessed differentiation based on individual water contact sites, we found no difference in Dc among the sites or for number of sites visited. In VdR in particular, where there is a significant geographic difference in the height of the two parts of the city relative to the Jiquiriçá River and a highway between them, we were unable to demonstrate geographic differentiation. This indicates that, within the resolution of our methodology, local gene flow is high within the villages, but not between them. Individuals tend to be infected at the same sites or the same parasite multilocus genotypes can be found at most sites. They do not tend to contaminate the waters in nearby villages, and the school sanitation system (serving children from both villages) is unlikely to contribute to the local parasite population.

The marked difference in age-specific prevalence, intensity and perhaps increased exposure during water contact suggest that children are likely to be more exposed than adults to the current component population present in the resident snails. However, the lack of differentiation by age suggests that current and past populations are largely undifferentiated, and that over at least the last 5 years (the 95% CI for parasite life-span is 5.7–10.5 years [34]) there has not been a large degree of migration or selection, also supported by the geographic structuring between the villages.

The amount of differentiation within the groups of infrapopulations (Di) defined by host characteristics was often significantly different for multiple host factors, but we have no basis for comparison to say if this is biologically meaningful. By contrast the index Dic was significantly different for only intensity of infection in both villages. The socio-economic index and age are variably associated, but these may be secondarily related to intensity. The Dic is a measure of how differentiated an individual infrapopulation is from the whole adult worm/egg parasite community. It serves as a useful measure of how effectively individual hosts within a group sample the component population. The higher the intensity of infection, the more samples are present, which results in a better representation of the component population. This will reduce D for the infrapopulation relative to the component population. For the mean effective allele number, a measure of diversity, only intensity of infection ($<$400 or $>$400 epg) showed significant difference for both villages. This is consistent with expectation. In this area, the sampling of the most heavily infected, who are usually between 7 and 15 years of age, might be the best way of estimating the composition of the component population without sampling everyone. This limited sample would still lack precision, and age alone was not significantly associated with the Dc or Dic for Jenipapo.

An important issue for these conclusions is the sensitivity of the methods employed for differentiating parasite subpopulations. We do know that our approach is sensitive enough to differentiate the component population for the two villages, and that in the laboratory, different laboratory-maintained *S. mansoni* populations from the same laboratory and different lots of parasites from the same life cycle can be distinguished [12]. We were able to genotype at least 1 sample from 67% of those infected. Since we have shown infrapopulation allele frequencies are stable over at least the span of a week, obtaining a single stool is unlikely to be a source of error. Most of those we were unable to genotype had low egg counts and low DNA concentrations [3]. Most of the cryptic infections we failed to detect are also likely to have been of low intensity. They were, therefore, less likely to contribute significantly to the genetic composition of their component populations. The relative relationship of the genetic composition of eggs to adult worms is unknown in natural infections, but in laboratory infections in mice, allele frequencies between these two stages were very similar [12].

There is no one approach that address all problems in population genetics, but the approach taken here is well suited to measure differentiation, since it allows for many large samples. Certain population genetic indices, such as the FIS, cannot be well estimated from aggregated data, however. In addition, we are unable to identify null alleles. This should not affect estimates of differentiation for populations in which the rate of null alleles is likely to be similar.

Attempts to control *S. mansoni* infection in Brazil were successful in decreasing intensity of infection, and therefore, morbidity and

mortality of the disease, but the infection has far from disappeared. An understanding of the dynamics of transmission and the distribution of the parasite at the population level can contribute to planning control measures. We show that there is little population sub-structure by host characteristics to influence how praziquantel therapy should be distributed. There are no special reservoirs of distinct parasite populations within the community, and much of transmission is local with good evidence for a barrier to gene flow with a nearby community. Future studies will examine how applicable the patterns seen in these communities are to others in Brazil and elsewhere. Until elimination has been achieved, surveillance and treatment will need to be continued and improvements in sanitation advanced.

Supporting Information

Checklist S1 STROBE checklist.
(PDF)

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Author Contributions

Conceived and designed the experiments: REB MGR. Performed the experiments: LKS JMC EAR TMA WAB. Analyzed the data: LMB REB WAB. Contributed reagents/materials/analysis tools: MGR REB. Wrote the paper: LMB REB.

References

1. Agola LE, Steinauer ML, Mburu DN, Mungai BN, Mwangi IN, et al. (2009) Genetic diversity and population structure of *Schistosoma mansoni* within human infrapopulations in Mwea, central Kenya assessed by microsatellite markers. Acta Trop 111: 219–225.
2. Blank WA, Reis EA, Thiong'o FW, Braghiroli JF, Santos JM, et al. (2009) Analysis of *Schistosoma mansoni* population structure using total fecal egg sampling. J Parasitol 95: 881–889.
3. Blanton RE, Blank WA, Costa JM, Carmo TM, Reis EA, et al. (2011) *Schistosoma mansoni* population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. Int J Parasitol 41: 1093–1099.
4. Curtis J, Sorensen RE, Minchella DJ (2002) Schistosome genetic diversity: the implications of population structure as detected with microsatellite markers. Parasitology 125 Suppl: S51–S59.
5. French MD, Churcher TS, Basanez MG, Norton AJ, Lwambo NJ, et al. (2012) Reductions in genetic diversity of *Schistosoma mansoni* populations under chemotherapeutic pressure: The effect of sampling approach and parasite population definition. Acta Trop 4: e897.
6. Hanelt B, Steinauer ML, Mwangi IN, Maina GM, Agola LE, et al. (2009) A new approach to characterize populations of *Schistosoma mansoni* from humans: development and assessment of microsatellite analysis of pooled miracidia. Trop Med Int Health 14: 322–331.
7. Souza SS, Barbosa LM, Guimaraes IC, Blank WA, Reis RB, et al. (2012) Genetic population structure of cercariae from an urban foci of *Schistosoma mansoni*, Brazil. Am J Trop Med Hyg 87: 843–849.
8. Hale ML, Burg TM, Steeves TE (2012) Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS One 7: e45170.
9. Bashalkhanov S, Pandey M, Rajora OP (2009) A simple method for estimating genetic diversity in large populations from finite sample sizes. BMC Genet 10: 84.
10. Gapare W, Yanchuk A, Aitken S (2008) Optimal sampling strategies for capture of genetic diversity differ between core and peripheral populations of Picea sitchensis (Bong.) Carr. Conserv Genet 9: 411–418.
11. Bush AO, Kennedy CR (1994) Host fragmentation and helminth parasites: hedging your bets against extinction. Int J Parasitol 24: 1333–1343.
12. Blank WA, Liu SF, Prasad J, Blanton RE (2011) Host mouse strain is not selective for a laboratory adapted strain of *Schistosoma mansoni*. J Parasitol 97: 518–521.
13. Blank WA, Test MR, Liu SF, Lewis FA, Blanton RE (2010) Long-term genetic stability and population dynamics of laboratory strains of *Schistosoma mansoni*. J Parasitol 96: 900–907.
14. Ministério da Saúde (2010) [National Therapeutic Formulary 2010] Formulário Terapêutico Nacional 2010. Brasília, DF: Ministry of Health.
15. Dresden MH, Payne DC (1981) A sieving method for the collection of schistosome eggs from mouse intestines. J Parasitol 67: 450–452.
16. Coelho PM, Jurberg AD, Oliveira AA, Katz N (2009) Use of a saline gradient for the diagnosis of schistosomiasis. Mem Inst Oswaldo Cruz 104: 720–723.
17. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, et al. (1987) Current protocols in molecular biology. New York, NY: Green Publishing Associates and Wiley Inter-science.
18. Dean AG, Dean JA, Burton AH, Dicker RC (1991) Epi Info: a general-purpose microcomputer program for public health information systems. Am J Prev Med 7: 178–182.
19. Jost L (2008) G_{ST} and its relatives do not measure differentiation. Molecular Ecology 17: 4015–4026.
20. Kimura M, Crow JF (1964) The Number of Alleles That Can Be Maintained in a Finite Population. Genetics 49: 725–738.
21. Hartl DL, Clark AG (2007) Principles of Population Genetics. Sunderland, MA: Sinauer Associates.
22. Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. Mol Ecol 11: 155–165.
23. Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. Lancet 368: 1106–1118.
24. Carmo EH, Barreto ML (1994) [*Schistosomiasis mansoni* in Bahia, Brazil: historical trends and control measures]. Cad Saude Publica 10: 425–439.
25. Pellon AB, Teixeira I (1950) Distribuição Geográfica da Esquistossomose Mansônica no Brasil. Rio de Janeiro: Departamento Nacional de Saúde. 108 p.
26. Coura JR, Amaral RS (2004) Epidemiological and control aspects of schistosomiasis in Brazilian endemic areas. Mem Inst Oswaldo Cruz 99: 13–19.
27. Vieira JBF (1993) O programa brasileiro de controle de esquistossomose. IV Simpósio Internacional de Esquistossomose. Rio de Janeiro - Fundação Oswaldo Cruz.
28. Conceição MJ, Coura JR (2012) Epidemiology of Schistosomiasis Mansoni in Brazil. In: Mohammad Bagher Rokni, editor. Schistosomiasis. Rijeka, Croatia: InTech. pp. 183–192.
29. Satayathum SA, Muchiri EM, Ouma JH, Whalen CC, King CH (2006) Factors affecting infection or reinfection with *Schistosoma haematobium* in coastal Kenya: survival analysis during a nine-year, school-based treatment program. Am J Trop Med Hyg 75: 83–92.
30. Payne G, Carabin H, Tallo V, Alday P, Gonzalez R, et al. (2006) Concurrent comparison of three water contact measurement tools in four endemic villages of the Philippines. The schistosomiasis transmission ecology in the Philippines project (STEP). Trop Med Int Health 11: 834–842.
31. Barreto ML (1993) Use of risk factors obtained by questionnaires in the screening for *Schistosoma mansoni* infection. Am J Trop Med Hyg 48: 742–747.
32. Friedman JF, Kurtis JD, McGarvey ST, Fraga AL, Silveira A, et al. (2001) Comparison of self-reported and observed water contact in an *S. mansoni* endemic village in Brazil. Acta Trop 78: 251–259.
33. Lima e Costa MF, Rocha RS, Firmo JO, Guerra HL, Passos VA, et al. (1998) Questionnaires in the screening for *Schistosoma mansoni* infection: a study of socio demographic and water contact variables in four communities in Brazil. Rev Inst Med Trop Sao Paulo 40: 93–99.
34. Fulford AJ, Butterworth AE, Ouma JH, Sturrock RF (1995) A statistical approach to schistosome population dynamics and estimation of the life-span of *Schistosoma mansoni* in man. Parasitology 110 (Pt 3): 307–316.

Capítulo 3

Manuscrito 3 - Sources of Schistosomiasis Urbanization in Salvador, Bahia, Brazil

Partindo do pressuposto que a esquistossomose é uma doença rural, uma região da maior cidade do estado da Bahia, Salvador, foi estudada utilizando os mesmos critérios das comunidades rurais (Manuscrito 2). Foram descritas as características epidemiológicas da população estudada. Informações relativas a origem das infecções de esquistossomose foram relatadas a partir do perfil genético das populações de *Schistosoma mansoni* nos diferentes grupos estudados. Neste trabalho, as diferentes aplicações do índice de diferenciação genética D de Jost também foram utilizadas.

Principais resultados encontrados:

1- Perfil de infecção urbana similar com área rural;
2- Características genéticas das infrapopulações foram similares as encontradas na zona rural. Este resultado indica que a população parasitária local não sofreu influência migratória ou de deriva genética. Ao comparar amostras ovos do *S. mansoni* coletados em 2002 com as amostras atuais não foi encontrada diferenciação;
3- Não foi encontrada associação entre características do hospedeiro e genótipos do *S. mansoni;*
4- A cobertura de saneamento básico de 70% não foi suficiente para interromper a transmissão local.

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4 Sources of Schistosomiasis Urbanization in Salvador, Bahia, Brazil

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Abstract

Urbanization is an increasing trend across the globe, and diseases once considered rural can now be found in urban areas either due to the migration of population from rural endemic areas, the inability of city services to cope with rapid population growth or a combination of both factors. We investigated a focus of transmission of *Schistosoma mansoni* in Salvador, the third largest urban area of Brazil, and examined all inhabitants in 3 demarcate geographic areas to identify risk factors and understand the dynamics of urban transmission. We determined microsatellite allele frequencies for parasite infrapopulations and for component populations composed of individuals with the same epidemiologic characteristics. Many features of the urban infection were similar to those of rural areas. The peak age for intensity and prevalence was 15, and males were more affected than females. No differences were noted for parasite populations infecting individuals with differing epidemiologic characteristics, such as age or sex. There was little day-to-day differentiation in the allelic composition of excreted eggs. The pairwise infrapopulation differentiation (Jost's D 0.063) and the effective allele number (3.36) were also similar to values observed in a rural district >200 km from Salvador. Eggs from stool samples taken 7 years earlier showed little differentiation from the current population. This all indicates that the parasites being transmitted here represent a single stable population. This urban focus is primarily the result of local transmission rather than migration indicating municipal improvements in sanitation have been insufficient to interrupt transmission.

Key words: urbanization, schistosomiasis, migration, sanitation, population genetics

1. Introduction

Schistosomiasis is a widely distributed disease of the tropics and a major cause of overt and subclinical morbidities leading to organ fibrosis, malnutrition, anemia and impaired intellectual development (King and Dangerfield-Cha, 2008). Its transmission depends on human contact with water where the appropriate snail host has been infected by parasites originating in human feces or urine. Schistosomiasis is ultimately a disease of inadequate sanitation. The disease is usually thought of as a rural problem, but it is increasingly recognized in large urban areas of Brazil (Firmo et al., 1996; Amorim et al., 1997; Rocha et al., 2000; Ximenes et al., 2000; Barbosa et al., 2004; Barbosa et al., 2011). In addition to being the world's 7th largest economy, Brazil is the 5th most populous country. Some aspects of the social and environmental context of urban schistosomiasis may be specific to Brazil, but much is common to the general process of the urbanization of parasitic diseases. Urbanization has been the great global demographic trend of the last 100 years. In Brazil urbanization has been rapid, and today 86% of Brazilians live in cities (Victora et al., 2011). Salvador, the capital of the state of Bahia, has grown by 300% in just 20 years. Most of this growth has been due to migration from rural areas to regions of the city with the highest proportion of informal housing (Copque et al., 2011; Pereira, 2011). Directly or indirectly this has lead to the presence or persistence and even extension of schistosomiasis in the urban environment.

The city of Salvador has a historic role in the study of schistosomiasis. *Schistosoma mansoni* was definitively set apart from infection with *S. haematobium* in 1908 by the Brazilian scientist, Pirajá da Silva based on patients resident in Salvador (Pirajá da Silva, 1909). Some risk of urban infection has been present

since at least the early 20th century, and public health authorities have surveyed for it. Between 2000 and 2006, schistosomiasis was a reportable infection. All regions of the city reported cases from clinics **(Figure 1A)**, and there were some hospitalizations. Still, overall prevalence in the last 10 years has been between 2 and 5% (http://tabnet.datasus.gov.br/, accessed 07-07-2013). Cases were concentrated in the central and northern regions of the city consistent with the Municipal Health Department reports of positive stool exams and predictably overlying areas with the greatest combination of low income, high population density and new migration since 1970 (Rocha, 1995; Herold, 2004). What percentage of infections in the city is due to migration and what percentage represents local transmission is not known, but it is an important question when considering public health measures. There clearly is some degree of local transmission. A 2011 cross-sectional survey of major water bodies in Salvador (Souza et al., 2013) found *S. mansoni* infected snails in 7 of 158 locations. In 2004, 30% of school-aged children in the São Bartolomeu neighborhood of Salvador was found to have schistosomiasis (Guimarães and Tavares-Neto, 2006). Some of these had stool egg counts in the thousands, where 400 eggs/gram of feces (epg) is considered heavy. Three of the 298 children examined had splenomegaly.

We studied the distribution of infections within the human population and the genetic epidemiology of *S. mansoni* in São Bartolomeu to better understand the risks, sources and persistence of the infection within a large metropolitan area.

2. Methods

2.1 Study site and population.

The neighborhood of São Bartolomeu is located in the northwestern part of the city between the Cobre Reservoir with its nature park and the Bay of Todos os Santos. The Cobre River drains from the Reservoir into a small mangrove swamp and out to sea. The neighborhood of São Bartolomeu surrounds this outlet of the Cobre River (**Figure 1**) and is home to nearly 5,000 inhabitants. Socially, São Bartolomeu is considered a low-income and high-crime area with housing that dates from the 1970's, but informal new housing is continually being added. A recently established unit of the Federal Family Health Program has divided the region into 6 microareas, each with approximately 800 residents and each with an assigned health agent who is a liason between the community and the clinic. The 3 microareas with the highest prevalence of *S. mansoni* infection in school-aged children in 2004 (Guimarães and Tavares-Neto, 2006) were selected for study. All households were visited, numbered and their location registered with a hand-held Trimble/Nomad GPS unit (Model 65220-11). Responses to a one-page questionnaire were recorded for each resident. Parents and guardians responded to some questions for minors <12 years of age. Water contact information was obtained directly from each resident. Age, sex, race, years of residence in Salvador, previous residence, travel outside of the city of Salvador, education, occupation, household goods, city services, frequency of flooding, history of *S. mansoni* infection, treatment for schistosomiasis and surface water contact sites commonly visited. Each resident >4 years of age was asked to provide 3 stools for parasitologic examination. We also analyzed stool from 9 infected residents collected in 2004 for comparison. All subjects provided written informed consent. In the case of minors, consent was obtained from their guardians. 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.

2.2 Stool examination and egg collection.

For fecal examination, the stools were weighed to the nearest 0.1 g with a digital balance and then examined by the Kato-Katz method to identify ova of *S. mansoni* and other helminths. The number of *S. mansoni* ova were quantified, recorded and finally expressed as eggs per gram of feces (epg). Total egg counts were determined based on stool weight. Each stool positive for *S. mansoni* ova was homogenized, filtered and sedimented as described (Blanton et al., 2011) to obtain a sample enriched for these ova. A standard phenol-chloroform extraction was followed by treatment with hexadecyltrimethylammonium bromide (CTAB) to removed PCR inhibitors. Primers for 15 microsatellite markers (Blank et al., 2010) were used to amplify *S. mansoni* DNA. These markers have previously been shown to have specificity for this parasite in stool samples.

2.3 Microsatellite genotyping.

The intensity of resultant amplicons were measured by automated sequencer and analyzed using the Peak Scanner program (Applied Biosystems). Peak heights for alleles within the expected size ranges for the marker were summed and the allele frequency calculated for each peak by dividing its height by the total intensity in all peaks in the sample. The number of alleles for each marker was calculated by multiplying the allele frequency by sample total egg counts. Allele numbers for each

infrapopulation were used for calculation of genetic differentiation (Jost, 2008). Jost's D and it confidence interval were calculated with the program SPADE (http://chao.stat.nthu.edu.tw, Chao, A. and Shen, T.-J., last accessed 6-9-2013).

2.4. Data analysis.

Genetic differentiation based on Jost's D was used to compare the pairwise infrapopulation infection in one infected person to another (Di). The mean Di was calculated for stratified subsets of infected individuals based on demographic, geographic or parasitologic characteristics (**Figure 2**). When the combined allele counts for infrapopulations from one group of hosts were compared to a different group, this comprised the component population differentiation (Dc). Finally, each individual's parasite infrapopulation was compared to the component population in São Bartolomeu to yield the Dic. The effective allele number (AE), a measure of diversity, was calculated as described (Kimura and Crow, 1964; Souza et al., 2013). Means for continuous variables were compared by a bootstrapped Student's t-test using SPSS (Version 19). Egg counts were normalized by log-transformation. Logistic regression was performed using a forward stepwise conditional method. A p-value of 0.05 was used as the criterion for statistical significance.

3. Results

3.1 Study Population

A total of 1,335 residents were identified in the 3 selected microareas (MA1, MA3, and MA6) and 1,223 (91.6%) provided at least one stool sample. Although geographically within less than 1 km, the sample characteristics of MA3 were significantly different than the other MAs. A higher proportion of residents of MA3 were born outside of Salvador, had shorter times of residence and were more likely to be white. The area itself had lower coverage with septic tanks and flooding was more common (**Table 1**).

Univariate analyses showed that male sex, age group, prior infection or treatment for schistosomiasis and number of water sites visited were risk factors. Trips outside of Salvador, an adequate sewer system and lack of household crowding were protective. Age groups were divided into 5-year intervals with the 4-5 year olds used as the reference group. The following intervals each produced odds ratios of 4-8. The prevalence and intensity of infection with *S. mansoni* was highest in the 11-15 age group, and prevalence remained significantly higher in older age groups up to the age of 45 (**Table 2** and **Figure 3**). These factors have also been associated with risk of disease in our studies of infection in a rural area (manuscript submitted). The protective effects of travel, low levels of household crowding and use of septic tank or municipal sewers may well reflect socio-economic development, although crude household income was not associated with infection status.

Three water contact sites were significantly associated with infection (Table 3). The OR's were similar (~2 fold) for these sites. Contact site 1 is at the outlet for the Cobre Reservoir Dam. The site is relatively shallow and is used for netting fish. Site 2 was used for crossing small streams within an area used for small-scale commercial vegetable production along the flood plain of the river. Contact site 5 is

located at the bridge that spans the river on a road that joins MA's 1 and 6. The bridge is a gathering point for young people as well as used for fishing.

3.2 Genetic Analysis

Component populations represent all of the parasites in one species and typically in a given geographic area. We have extended the concept to include all the parasites infecting hosts with similar epidemiologic characteristics. There was no appreciable genetic differentiation (Dc) between the parasites of infected males and females, young and old, heavy and light infections or between microareas. In a 2004 pilot study, stools from 9 infected children living in São Bartolomeu were collected and pooled. *S. mansoni* eggs isolated from this sample were genotyped and compared to the 2011 sample from the community. The Dc for these samples was 0.007. This indicates that in the São Bartolomeu region there is a single population with no internal obstacles to gene flow and few new introductions of genetically diverse parasites.

The mean pairwise Di for São Bartolomeu was 0.063 (**Table 4**). All comparisons were significant, but we currently have no measure for effect size. The large number of eggs analyzed generally means that even the confidence intervals will not overlap. While the Dic was significantly lower for those individuals under 15 years old overall, this was not the case when the analysis was stratified by microarea. This suggests that this association is not very strong and requires larger numbers to be observed. The Dic is also lower for those with heavy infections, and this may explain the difference between age groups, since those <15 were more heavily infected than those >15 (**Figure 3**). The effective allele number (Ae) was

also significantly higher for those more heavily infected as well as for infected individuals reporting use of contact site 5.

4. Discussion

Multiple Brazilian cities have seen outbreaks of schistosomiasis (Firmo et al., 1996; Coura-Filho, 1997; Ximenes et al., 2000; Enk et al., 2003; Barbosa et al., 2010; Kloos et al., 2010). In some cities like Salvador, this is not so much a new introduction as it is a low level continuation of a pattern of infection present for some time. Although Bahia is a state where schistosomiasis is endemic, Salvador, its capital, is considered a non-endemic area. This would be a valid designation if the infection were not transmitted and only found in immigrants to the city. This is clearly not the case.

Molecular techniques were not required to show that *S. mansoni* is transmitted in Salvador. Guimarães et al. (Guimarães and Tavares-Neto, 2006) demonstrated this in the São Bartolomeu neighborhood by virtue of finding infected children who had never left the area even for visits or vacations and the presence of infected snails. São Bartolomeu, however, like other poor parts of the city is likely to be an area most subject to recent immigration and introduction of parasites from rural areas. On average >90% of lifetime had been spent in the capital, though older residents had spent a lower percentage of their time in the city than children (not shown). The prevalence and intensity of infection, however, was typical of an area with endemic transmission where the most affected range from preteens to 20 year olds. The population genetic characteristics of the parasite population in São Bartolomeu are quite similar to those of rural areas, where the scale of migration and

the area from which migrants are drawn are less. A large number of parasite migrants arriving with their host would be expected to produce a large degree of pairwise infrapopulation differentiation. Instead we see a value (Di = 0.063) that is the same or less than 2 distant rural communities we have studied in Bahia (0.095 ± 0.033 and 0.123 ± 0.066 (Blanton et al., 2011). There was little differentiation between parasite populations from host with varying epidemiologic risk factors. Hosts are not being selected on these characteristics, and all hosts are drawing from a single population. Finally, the Dc between the 2004 samples and the current population is nearly zero, although the 2004 sample was small. This suggests that the parasite population is closed and little influenced by migration. It is likely that many of today's adults as well as children became infected in São Bartolomeu rather than that transmission is being sustained by the arrival of newly infected people.

One of the factors that makes infections like schistosomiasis unexpected in cities is the presence of city services and sanitation. Worldwide, drinking water is a first priority in municipal development before sanitation, and essentially everyone in São Bartolomeu has municipal water piped to their homes. For Salvador, at the turn of the 20th century, a series of reservoirs were constructed outside what was then the city limits. As the city expanded rapidly after World War II, these became surrounded by new housing with poor sanitation such that many of these collections became polluted and the waterways used to occupy new areas became open sewers. São Bartolomeu is downstream from one of these early development projects. The Cobre Reservoir is still relatively protected, while it's outflow, the Cobre River, is not. It is a recipient of raw sewage not only from the São Bartolomeu community itself but all of the densely populated hills that surround it. The city of Salvador has grown so rapidly that attempts to keep up with the infrastructure

requirements around it can be described as heroic. In 2004, a citywide program for the introduction of sewer systems increased the coverage from 40% to 70% in São Bartolomeu. The Bahia Azul Project, as it was known, was demonstrated to have an enormous effect on the incidence of diarrheal diseases in the city (Barreto et al., 2007). The effect on schistosomiasis in this area, however, appears to have been negligible. The prevalence of infection appears to be the same in children today as in 2004, this despite a degree of coverage by the municipal sewer superior to many emerging countries of the world. Nevertheless the 70% coverage is not sufficient where raw sewage makes its way to waterways that large numbers of people use for recreation and commerce. The persistence of schistosomiasis represents a failure of city services. Fortunately, our analysis indicates that transmission in the city is focal and local. Elimination or even reduction of prevalence in this focal area is likely to be long lasting despite continued immigration at current rates.

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281 References

282 Amorim, M.N., Rabello, A., Contreras, R.L., Katz, N., 1997. Epidemiological
283 characteristics of Schistosoma mansoni infection in rural and urban endemic
284 areas of Minas Gerais, Brazil. Mem Inst Oswaldo Cruz 92, 577-580.
285 Barbosa, C.S., Araujo, K.C., Antunes, L., Favre, T., Pieri, O.S., 2004. Spatial
286 distribution of schistosomiasis foci on Itamaraca Island, Pernambuco, Brazil.
287 Mem Inst Oswaldo Cruz 99, 79-83.
288 Barbosa, C.S., Araujo, K.C., Sevilla, M.A., Melo, F., Gomes, E.C., Souza-Santos, R.,
289 2010. Current epidemiological status of schistosomiasis in the state of
290 Pernambuco, Brazil. Mem Inst Oswaldo Cruz 105, 549-554.
291 Barbosa, C.S., Leal-Neto, O.B., Gomes, E.C., Araujo, K.C., Domingues, A.L., 2011.
292 The endemisation of schistosomiasis in Porto de Galinhas, Pernambuco,
293 Brazil, 10 years after the first epidemic outbreak. Mem Inst Oswaldo Cruz 106,
294 878-883.
295 Barreto, M.L., Genser, B., Strina, A., Teixeira, M.G., Assis, A.M., Rego, R.F., Teles,
296 C.A., Prado, M.S., Matos, S.M., Santos, D.N., dos Santos, L.A., Cairncross,
297 S., 2007. Effect of city-wide sanitation programme on reduction in rate of
298 childhood diarrhoea in northeast Brazil: assessment by two cohort studies.
299 Lancet 370, 1622-1628.
300 Blank, W.A., Test, M.R., Liu, S.F., Lewis, F.A., Blanton, R.E., 2010. Long-term
301 genetic stability and population dynamics of laboratory strains of Schistosoma
302 mansoni. J Parasitol 96, 900-907.
303 Blanton, R.E., Blank, W.A., Costa, J.M., Carmo, T.M., Reis, E.A., Silva, L.K.,
304 Barbosa, L.M., Test, M.R., Reis, M.G., 2011. Schistosoma mansoni population

structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. Int J Parasitol 41, 1093-1099.

Copque, A.C.d.S.M., Souza, F.A., Santos, D.V.d.C., Paixão, R.C.d., 2011. Expansão urbana e redução de áreas verdes na localidade do Cabula VI Região do miolo da cidade do Salvador, Bahia. In, XV Simpósio Brasileiro de Sensoriamento Remoto, Curitiba, PR, Brazil, pp. 706 - 713.

Coura-Filho, P., 1997. [*Schistosomiasis mansoni* in urban territory. 2. A theoretical approach to the accumulation, concentration, and centralization of capital and the production of disease]. Cad Saude Publica 13, 415-424.

Enk, M.J., Amorim, A., Schall, V.T., 2003. Acute schistosomiasis outbreak in the metropolitan area of Belo Horizonte, Minas Gerais: alert about the risk of unnoticed transmission increased by growing rural tourism. Mem Inst Oswaldo Cruz 98, 745-750.

Firmo, J.O., Lima Costa, M.F., Guerra, H.L., Rocha, R.S., 1996. Urban schistosomiasis: morbidity, sociodemographic characteristics and water contact patterns predictive of infection. Int J Epidemiol 25, 1292-1300.

Guimarães, I.C., Tavares-Neto, J., 2006. [Urban transmission of schistosomiasis in children from a neighborhood of Salvador, Bahia]. Rev Soc Bras Med Trop 39, 451-455.

Herold, M.W., 2004. Between Sugar and Petroleum: Bahia and Salvador, 1920–1960. Revista Espaço Acadêmico 42, Online.

Jost, L., 2008. G_{ST} and its relatives do not measure differentiation. Molecular Ecology 17, 4015-4026.

Kimura, M., Crow, J.F., 1964. The Number of Alleles That Can Be Maintained in a Finite Population. Genetics 49, 725-738.

King, C.H., Dangerfield-Cha, M., 2008. The unacknowledged impact of chronic schistosomiasis. Chronic Illn 4, 65-79.

Kloos, H., Correa-Oliveira, R., dos Reis, D.C., Rodrigues, E.W., Monteiro, L.A., Gazzinelli, A., 2010. The role of population movement in the epidemiology and control of schistosomiasis in Brazil: a preliminary typology of population movement. Mem Inst Oswaldo Cruz 105, 578-586.

Pereira, J.M.P.D., 2011. Desconcentração, Migração E Diferenciais Por Estrato De Renda Na Bahia (Deconcentration, Migration and differentials by income strata in Bahia State). Informe Gepec (Grupo de Pesquisa em Agronegócio e Desenvolvimento Regional) 15, 546-563.

Pirajá da Silva, M.A., 1909. Contribution to the study of Schistosomiasis in Bahia, Brazil. Brazil. J. Trop. Med. Hyg. 12, 159-164.

Rocha, R.S., Silva, J.G., Peixoto, S.V., Caldeira, R.L., Firmo, J.O., Carvalho, O.d.S., Katz, N., 2000. [Assessment of schistosomiasis and other intestinal parasitoses in school children of the Bambui municipality, Minas Gerais, Brazil]. Rev Soc Bras Med Trop 33, 431-436.

Rocha, S., 1995. Metropolitan Poverty in Brazil: Economic Cycles, Labour Market and Demographic Trends. 19, 383–394.

Souza, S.S., Barbosa, L.M., Guimaraes, I.C., Blank, W.A., Reis, R.B., Reis, M.G., Blanton, R.E., Andrade, Z.A., 2013. Genetic population structure of cercariae from an urban foci of Schistosoma mansoni, Brazil. Am J Trop Med Hyg 87, 843-849.

Victora, C.G., Barreto, M.L., do Carmo Leal, M., Monteiro, C.A., Schmidt, M.I., Paim, J., Bastos, F.I., Almeida, C., Bahia, L., Travassos, C., Reichenheim, M.,

354 Barros, F.C., 2011. Health conditions and health-policy innovations in Brazil:
355 the way forward. Lancet 377, 2042-2053.
356 Ximenes, R.A., Southgate, B., Smith, P.G., Guimaraes Neto, L., 2000. Migration and
357 urban schistosomiasis. The case of Sao Lourenco da Mata, northeast of
358 Brazil. Rev Inst Med Trop Sao Paulo 42, 209-217.
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Figure Legends

Figure 1. A. Distribution of mean annual *S. mansoni*-positive fecal examinations in Salvador by neighborhood 2000-2006. No information was available for the blank areas. Source: Salvador Municipal Secretariate of Health (http://www.tabnet.saude.salvador.ba.gov.br). B. Map of São Bartolomeu showing homes in the 3 microareas (MA) and rivers running through the community as well as streets in surrounding neighborhoods. Water contact points are indicated in italics.

Figure 2. Small circles represent infrapopulations infecting a single host. The individuals in the infrapopulations are hetergeneous, but they are all represented by their average allele frequency a single color. Large circles represent component populations made up of all the infrapopulations in an area or a group of hosts with the same epidemiologic characteristic. D is the calculation of Jost's D.

Figure 3. Intensity and prevalence of *S. mansoni* infection in 5 year intervals.

Table 1 – General characteristics of the studied population in São Bartolomeu

		Total	MA1	MA3	MA6	[a]pMA_{1-3} (OR)	pMA_{1-6} (OR)	pMA_{3-6} (OR)
Characteristic	Total	n=1221	n=439	n=335	n=447	-	-	-
Male Sex (%)		554 (45.1)	187 (42.6)	157 (46.9)	206 (46.1)	0.236	0.296	0.829
Mean Age (SD)		29.2±17.8	28.6±18.0	29.0±17.5	29.8±17.7	0.784	0.309	0.483
Ethnicity (%)	White	93 (7.6)	29 (6.6)	33 (9.8)	31 (6.9)	0.004	0.052	0.003
	Brown	670 (54.9)	244 (55.6)	201 (60.0)	225 (50.3)	-	-	-
	Black	429 (35.1)	162 (36.9)	91 (27.2)	176 (39.4)	-	-	-
	Yellow/Red	29 (2.4)	4 (0.9)	10 (3.0)	15 (3.4)	-	-	-
Birth place (%)	Salvador	946 (77.6)	355 (81.2)	232 (69.3)	359 (80.3)	<0.001 (1.92)	0.728 (1.06)	<0.001 (0.55)
% Life in Salvador (SD)		90.7 (20.2)	92.1 (18.9)	87.7 (22.0)	91.6 (19.7)	0.006	0.721	0.011
Travel last year (%)		399 (32.8)	163 (37.1)	133 (39.8)	103 (23.1)	0.446 (1.12)	<0.001 (0.51)	<0.001 (0.46)
Water contact traveling (%)		202 (51.4)	80 (49.1)	70 (52.6)	52 (51.0)	0.644 (0.90)	0.916 (0.97)	0.756 (1.09)
Socio-economic index								
Piped water (%)		1080 (99.9)	-	-	-	-	-	-
Sanitation (%)	Indoor Toilet	1029 (95.9)	386 (98.7)	294 (96.4)	349 (92.6)	0.042 (2.89)	<0.001 (6.19)	0.033 (2.14)
	Septic tank/ Sewer	711 (67.2)	303 (78.9)	167 (56.2)	241 (63.9)	<0.001 (0.34)	<0.001 (0.47)	0.042 (1.38)
	River/Open air	347 (32.8)	81 (21.1)	130 (43.8)	136 (36.1)	-	-	-
Flooding		601 (49.4)	170 (39.0)	224 (67.1)	207 (46.4)	<0.001 (0.31)	0.026 (0.738)	<0.001 (2.35)
Infection	Prevalence (%)	300 (24.7)	101 (23.1)	81 (24.3)	118 (26.5)	0.712 (0.94)	0.250 (0.84)	0.484 (0.89)
	Intensity[b] (SD)	60.8 (4.6)	50.3 (3.0)	69.7 (5.0)	64.9 (4.9)	0.065	0.214	0.764
Past *S. mansoni* infection	Yes (%)	225 (18.5)	52 (12.0)	60 (18.0)	113 (24.4)	0.018 (0.62)	<0.001 (0.40)	0.014 (0.65)
Friend with *S. mansoni*	Yes (%)	358 (29.5)	97 (22.2)	95 (28.4)	167 (37.4)	0.047 (0.72)	<0.001 (0.48)	0.009 (0.66)
Family with *S. mansoni*	Yes (%)	342 (28.0)	93 (21.2)	85 (25.4)	164 (36.7)	0.170 (1.27)	<0.001 (2.16)	0.001 (1.70)

[a]pMA - the p-value for the comparison of the 2 subscripted microareas.

[b]Geometric mean epg

Table 2. Risk for *S. mansoni* egg positive stools.

Variable		Total	Positive n (%)	Or (95% CI)	p value
Age	≤5	64	4 (6.3)	-	-
	6-10	124	23 (18.6)	3.41 (1.13-10.35)	0.027
	11-15	147	55 (37.4)	8.97 3.09-26.04)	<0.001
	16-20	122	40 (32.8)	7.32 (2.48-21.56)	<0.001
	21-25	122	38 (31.1)	6.79 (2.30-20.03)	<0.001
	26-30	137	43 (31.4)	6.86 (2.34-20.10)	<0.001
	31-35	105	24 (22.9)	4.44 (1.46-13.48)	0.005
	36-40	74	19 (25.7)	5.18 (1.66-16.18)	0.003
	41-45	94	20 (21.3)	4.05 (1.3`-12.500	0.012
	46-50	75	14 (18.7)	3.44 (1.07-11.06	0.055
	51-55	55	9 (16.4)	2.93 (0.85-10.13)	0.138
	56-60	36	4 (11.1)	1.88 (0.44-8.00)	0.454
	>60	67	8 (11.9)	2.03 (0.58-7.12)	0.366
Sex	Male	549	199 (66.3)	3.33 (2.52-4.42)	<0.001
	Female	668	101 (33.7)	-	-
Outside Trips Last Year	Yes	400	76 (19.0)	0.62 (0.46-0.83)	0.001
	No	820	225 (27.4)	-	-
Water contact during trip	Yes	203	46 (22.7)	1.63 (0.982.74)	0.059
	No	191	29 (15.2)	-	-
Sewage Adequate	Yes	711	153 (21.5)	1.50 (1.10-2.00)	0.009
	No	343	99 (28.9)	-	-
Persons/Bedroom<3	Yes	378	89 (23.5)	0.57 (0.42-0.76)	<0.001
	No	385	154 (40.0)	-	-
Prior *S. mansoni* infection	Yes	226	90 (39.8)	2.80 (1.51-2.87)	<0.001
	No	991	211 (21.3)	-	-
Previous Treatment	Yes	195	72 (36.9)	2.70 (1.10-6.62)	0.016
	No	28	17 (60.7)	-	-
Water Contacts	0	237	26 (11.0)	0.29 (0.19-0.44)	<0.001
	1	358	61 (17.0)	1.66 (1.01-2.74)	0.046
	2	184	38 (20.7)	1.92 (1.10-3.33)	0.021
	3	146	42 (28.8)	2.91 (1.67-5.07)	<0.001
	4	83	34 (41.0)	5.18 (2.82-950)	<0.001
	≥ 5	126	100 (46.5)	5.78 (3.49-9.58)	<0.001

Age compared to youngest category for age, no surface water contact, no septic tank or municipal sewer connection, median density of 2/bedroom. Compared by two-tailed Pearson's chi-squared test. CI = confidence interval; OR = odds ratio

Table 3 - Risk at contact points for *S. mansoni* infection

Contact point	Contact (%)	Infected (%)	OR	p-value
1	210 (17.3)	103 (48.4)	1.98 (1.38 - 2.84)	<0.001
2	245 (20.1)	112 (45.5)	1.91 (1.34 - 2.72)	<0.001
3	270 (22.2)	107 (39.5)	-	-
4	308 (25.3)	123 (39.8)	-	-
5	354 (29.1)	153 (43.1)	2.15 (1.56 - 2.96)	<0.001
6	297 (24.4)	96 (32.1)	-	-
7	370 (30.4)	130 (34.8)	-	-
8	237 (19.5)	93 (39.1)	-	-
Other	571 (47.0)	165 (28.6)	-	-

1. Reservoir dam; 2. Gardens; 3. Gardens entrance; 4. Fountain Street; 5. Iron Bridge; 6. São Rafael Passageway; 7. Swamp beside soccer field; 8. Manguete and Snake Streets

Table 4. Subpopulation differentiation and diversity

		Dc	Di (p-value)	Dic (p-value)	Ae (p-value)
ALL	Sex (male vs female)	0.003	0.053 vs 0.071 (<0.001)	0.029 vs 0.039 (0.058)	3.360 vs 3.305 (0.051)
	Age (≤15 vs >15 y/o)	0.003	0.045 vs 0.063 (<0.001)	0.024 vs 0.034 (0.018)	3.329 vs 3.351 (0.341)
	Intensity of infection (<400 vs >400 epg*)	0.001	0.065 vs 0.023 (<0.001)	0.036 vs 0.012 (<0.001)	3.308 vs 3.506 (<0.001)
	Contact point 1 (Yes)	0.002	0.051 vs 0.062 (<0.001)	0.028 vs 0.034 (0.230)	3.341 vs 3.347 (0.255)
	Contact point 2 (Yes)	0.002	0.043 vs 0.068 (<0.001)	0.023 vs 0.038 (0.002)	3.339 vs 3.348 (0.197)
	Contact point 5 (Yes)	0.003	0.047 vs 0.070 (<0.001)	0.026 vs 0.039 (0.008)	3.362 vs 3.325 (0.038)
	MA1 vs MA3	0.005	0.069 vs 0.056 (<0.001)	0.038 vs 0.030 (0.170)	3.284 vs 3.400 (0.042)
	MA1 vs MA6	0.001	0.069 vs 0.042 (<0.001)	0.038 vs 0.023 (0.004)	3.284 vs 3.390 (0.048)
	MA3 vs MA6	0.006	0.056 vs 0.042 (<0.001)	0.030 vs 0.023 (0.140)	3.400 vs 3.390 (0.791)

Di - pairwise Jost's D for all members of the group. Dic - mean Jost's D for each infrapopulation in the group compared to the village component population. AE – mean effective allele number. Bootstrapped Student's t-test was used to compare group means for these indices. Dc - Jost's D for the component population formed by the total allele numbers for the group. Jost's D was estimated using the program Spade (http://chao.stat.nthu.edu.tw). Other variables tested but without significant differences were trips outside the region, co-infection with other helminths, all other water contact points, number of water contacts visited, a history of past infections.

* epg - mean count of *S. mansoni* eggs per gram of stool.

Dic MA1 - 0.0004, MA3 - 0.003 e MA6 - 0.0004

Figure 1

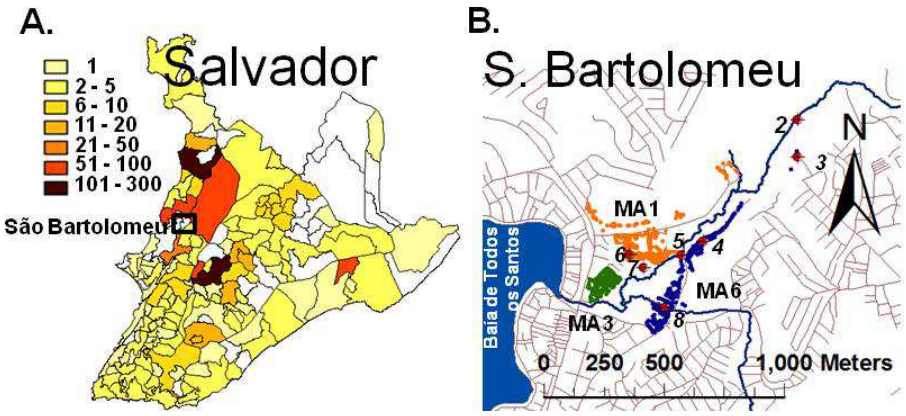


Figure 2

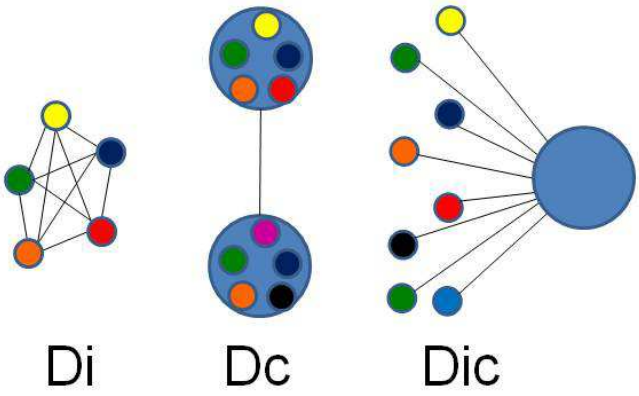
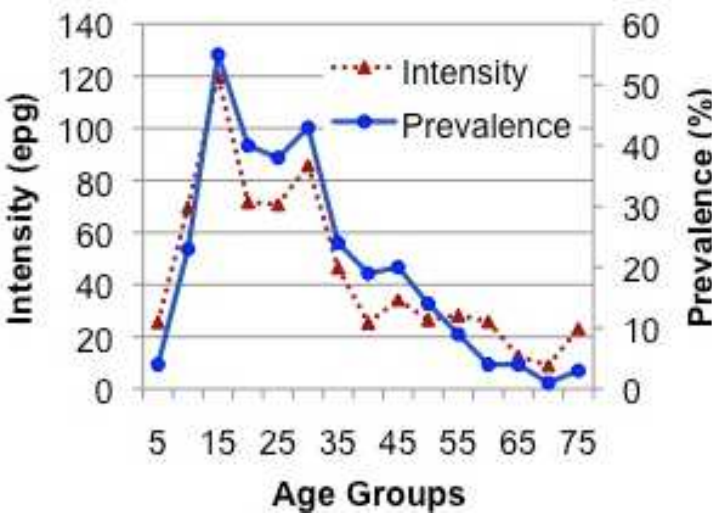


Figure 3



Capítulo 4

Manuscrito 4 – *Schistosoma mansoni:* population dynamics after repeated treatments

Este trabalho descreve Jenipapo e Volta do Rio, duas comunidades rurais do estado da Bahia, após a primeira rodada de tratamento com o Praziquantel. O principal objetivo deste manuscrito foi relatar o comportamento da população de *Schistosoma mansoni* após sucessivas rodadas de tratamento. É descrita a influência de imigrantes para estrutura populacional local do parasita e a origem da recuperação dos mesmos e como estes resultados podem interferir em políticas de saúde pública. Além disso, foram relatadas diferenças demográficas e epidemiológicas nos 5 anos em que o estudo aconteceu.

Prinicipais resultados encontrados:

1- O tratamento sucessivo reduziu significativamente as taxas de prevalência e intensidade de infecção. Taxas de incidência e reinfecção também diminuíram;
2- Efeito gargalo genético não foi alcançado, apesar de haver indícios que a redução de aproximadamente 10 vezes na intensidade parasitológica global seja o *cut-off* para o início de mudança na estrutura populacional;
3- Baixa influência dos migrantes para a estrutura populacional local;
4- Cada vez que o indivíduo se reinfecta ele coleta uma amostra da população parasitária diferente da anterior.
5- Para a eliminação do *S. mansoni,* uma melhor estratégia de distribuição do tratamento deve ser instituída, tendo em vista que os indivíduos não tratados são capazes de recompor a variabilidade genética parasitária inicial.

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Schistosoma mansoni: population dynamics after repeated treatments

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INTRODUCTION OUTLINE

- ➔ Importance of schistosomiasis in the world and Brazil.
- ➔ Treatment strategies
  - o Repeated rounds of treatment → Comment MDA
- ➔ Importance of monitoring the genetic structure of the parasites
  - o Resistance
  - o Source of reinfection
  - o Different distribution among hosts
- ➔ By the last paragraph make sure to indicate that this study is a follow-up of the first leg of the Project that happened in 2009.

METHODS

The study was developed in two rural communities in the northeast of Brazil in the state of Bahia. Jenipapo and Volta do Rio (VdR). They are administrated by the city of Ubaíra that is located 270 km from Salvador, the state's capitol. They are 12 km from Ubaíra and 8 km apart from each other. This relative isolation from each other and from larger cities, along with the historically high prevalence of schistosomiasis in the area, was the main reason for this region to be chosen. The first description of the two communities was made in our previous publications Blanton et. al. 2011 and Barbosa et. al. 2013. These papers were based on field work that took place in 2009, and very little has changed since then.

Study Design and Protocol

As previously described (Blanton et. al. 2011 and Barbosa et. al. 2013), we conducted an epidemiological and parasitological survey of all inhabitants ≥1 year old who agreed to participate in the study. For the epidemiological survey we asked questions regarding housing, sanitary habits, water contact. The parasitological survey was conducted using the Kato-Katz method for three stool samples collected in different days from each resident. Individuals who tested positive for *S. mansoni* infection had their stool sample processed through a selective sieving network, as previously described (Blank et al. 2009*;* Blanton et. al. 2011), to isolate the parasite's eggs and were treated with a single oral dose of praziquantel according to Brazilian Ministry of Health guidelines [9]. Those found to have other intestinal parasites were treated with mebendazole. After 4 weeks of treatment individuals who were *S.*

mansoni positive were re-tested to check treatment efficacy. Those participants who still had *S. mansoni* eggs in their stool were re-treated. It is noteworthy that only in year 3 and 4, was treatment directly observed.

First year of the project happened in 2009, and this year was the baseline for the analysis. This protocol was repeated in 2012 and 2013, however, in the last year data was restricted to Jenipapo (Figure 1).

Microsatellite genotyping

Genomic DNA from *S. mansoni* eggs isolated from participants' stools were extracted using phenol/chloroform technique and further treated with cetyl trimethylammonium bromide (CTAB) to remove PCR inhibitors, as previously described (Blank et al. 2009*;* Blanton et. al. 2011). Microsatellite genotyping was performed using 11 microsatellite markers previously described (Blank et al. 2009*;* Blanton et. al. 2011; Barbosa et. al. 2013). For each marker a duplicate PCR reaction using 2 µL of extracted DNA from stool was performed, totaling 22 reactions per sample. PCR products from each sample were combined into groups of three or four markers and processed on an Applied Biosystems 3730xl DNA Analyzer. PeakScanner software version 1.0 (Applied Biosystems, Carlsbad, CA) was used to determine peak heights from which allele frequencies were calculated. Successful PCR reactions were defined as those in which there was at least one peak >500 pixels in the size range expected for a given marker. No peak <100 pixels was identified as an allele. If both duplicate samples amplified, their mean allele frequency was used. Subsequent population analyses were limited to those samples where a minimum of 8 out of 11 markers genotyped successfully.

Data Analysis

Epidemiological data

Information collected during each year of the study was double-entered into the program Epi Info version 3.5. To compare categorical data we used Pearson's chi-square test and continuous data was evaluated by Student's t-test. A p-value of 0.05 was used as the criterion for statistical significance. Eggs per gram (epg) of feces found in Kato-Katz were log-transformed to approximate a normal distribution.

Reinfection rates were calculated by selecting individuals who were *S. mansoni* positive in consecutive years. Incidence rates were calculated selecting the individuals who were negative for schistosomiasis at the former data point or who were positive, received treatment and were found to be negative on reexamination. Those who still had parasite eggs in their stool after 4 weeks of treatment and received treatment again, were assumed to be clear of the infection.

Population genetic analysis

Allele counts were obtained to assess the contribution for each allele for that infrapopulation. They were calculated by multiplying total egg counts found on the Kato-Katz assay by the allele frequencies at a microsatellite locus. Total egg counts were calculated by multiplying EPG found in Kato-Katz by total stool weight obtained parasitological survey. Infrapopulations were used as the parasite population living within each definitive host. Component populations were all infected individuals within a shared geographic area or year of the analysis. To assess genetic

differentiation between populations we used Jost's D index calculated using the program SPADE (http://chao.stat.nthu.edu.tw). D was described in 2008 as a true differentiation index that does not rely on heterozygozity as the index most commonly used, Fst or its relatives. The use of Fst, can often give incorrect results regarding the differentiation between two populations. Also, D uses the actual population's allele frequencies, therefore does not rely on the assumptions of Hardy-Weinberg equilibrium, which are not applicable to infrapopulations. We have in previous papers (Barbosa et al 2013 and unpublished), described new ways to use Jost's D. After grouping, each pair of infrapopulations can be compared within a group or the combined allele numbers and allele frequencies can be used to form a component population. We make the following differentiation and diversity comparisons:

- Di - Jost's **D** values pairwise for each **I**nfrapopulations in the group as defined by locality or year of assessment. The Di is the mean differentiation between the groups of parasites collected by individuals. For any group a matrix of pairwise Di's is generated, and the average of this matrix for the different groups was compared and tested by a Welch's t-test.

- Dc - Jost's **D** for the combined allele numbers of 2 **C**omponent populations grouped based on shared geography or year of analysis. Dc indicates differentiation between the parasites collected from individuals from a defined group.

There is no standard for effect size for Di. The inferences that we are making are based using the absolute values comparing the assigned groups. For the Dc, we follow the convention, described by Wright, used for interpreting F_{ST} values. Dc values from 0 - 0.05 indicate little differentiation; from 0.05 - 0.15, moderate

differentiation; and above 0.15, great differentiation. However, changes in D rather than the absolute value below the 0.05 range may still indicate a significant result.

To evaluate genetic diversity we used the effective allele number (Ae). Ae is a measure of diversity, calculated as $1/\sum_{i=1}^{n} p_i^2$ where p_i is the frequency of the i^{th} allele for each marker.

Ethics Statement

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.

RESULTS

Study population characteristics

The study population at baseline was described in our previous publications (Blanton et al. 2011 and Barbosa et al. 2013). In 2009, of the 849 individuals residing in both villages, 814 (96%) provided at least one stool sample and were included in the study. In 2012, we identified 848 individuals and 810 participated in the study (95.5%). In 2013, only Jenipapo was evaluated, 422 out of 442 (95.5%) of the residents participated in the project providing at least one sample. We are able to see that demographic characteristics remained stable (Table 1). The mean age in year 1 was 31.5 ± 22.2, and showed a slight increase over the years (year 3 = 32.4 ± 21.9;

year 4 = 33.6 ± 20.9). Over the years, the number of females remained similar (year 1 = 46.3%; year 2 = 45.8%; year 3 = 46.3%).

Number of individuals migrating was the only significant difference we could see comparing the two villages. Immigration was more common in VdR compared to Jenipapo (19.0 vs 11.7%), however, the percentage of people moving out of the areas were similar (Jen = 13.9 vs VdR = 16.2%). In 2013, there were 5.9% of the population had moved to Jenipapo since 2009 and 12.2% left the region. This greater amount of individuals moving to VdR reflects a slightly lower, but not significant, number of individuals who were born in Ubaíra and a lower % of lifetime in the region compared to Jenipapo in 2012.

Infection Characteristics

S. mansoni prevalence was 41% at baseline and it decreased significantly in the year 3 (18.9%, $p < 0.001$). Intensity of infection also showed an important decrease with an initial geometric mean of eggs per gram of feces of 68.6 to 33.5 in year 3 ($p < 0.001$). Comparing the two villages, Jenipapo always showed a significant higher prevalence than VdR (year 1, 45.8 versus 35.1%, $p=0.002$; year 3, 23.7 versus 12.8%, $p < 0.001$) with a similar intensity of infection (Table 2). Evaluating only Jenipapo over the course of the study, the same trend is seen. In 2009, prevalence was 45.8% with a geometric mean intensity of 60.8 epg. In 2012, 23.7% of the participants were infected with a mean epg of 34.2, and in the last year studied, prevalence decreased to 16.1% with an intensity of 24.1 epg.

Treatment was available to every infected participant. In year 1, praziquantel was distributed to 88.1% (295/335) of the infected individuals, with a success rate of

90.4%. For year 3, drug was given to 96.8% (149/154) of the participants with schistosomiasis. Of those 98.7% cleared the infection. Since the persistently infected received praziquantel again, they were also included for the calculation of the incidence rate. The overall incidence rate between year 1 and 3 was 18.1%. Jenipapo presented a higher incidence than VdR (22 vs 12.9%). In 2013, incidence was also lower (14.9%). Intensity of infection was low among those who were newly infected. Reinfection rate was higher in Jenipapo (33.7 vs 18.4%). The reinfection rate in Jenipapo in 2012 fell to 28.4%.

Population Genetics

Samples collected from different years showed the same success rate in terms of PCR amplification. (2009 = 221/335 (66%); 2011 = 90/153 (58.8%); 2013 = 44/68 (64.7%)). Failure in genotyping has been associated with DNA concentration (Blanton et al 2011) which is associated with a lower intensity of infection, found after the community-wide treatment.

Di was used to determine mean differentiation between the group of parasites collected by individuals. In 2009, baseline of the study, Di indicated a moderate differentiation (0.103). This index similar in the next year evaluated (0.099). Assessing Di in all three years just for Jenipapo, the same result can be seen (2009 = 0.083; 2012 = 0.084). In year 2013, this index increased a little (0.103). Volta do Rio always presented higher levels of Di than Jenipapo (Table 4). Effective Allele Number did not show a significant decrease following treatment.

Dc was used to evaluate the mean differentiation between the parasites collected from individuals from a defined group. The component populations were defined by geography and between years of the study. Dc between villages decreased in year 2012 from 0.046 to 0.031. If we combine the 2 communities and compare their component populations from 2009 with 2011, we found a small Dc of 0.008. Assessing only Jenipapo for all three years (Table 5), a small Dc was found for consecutive years (2009 vs 2012 = 0.008; 2012 vs 2013 = 0.009), however, comparing the baseline in 2009 to the final survey in 2013 greater differentiation was found (D = 0.014).

The influence of the immigrants for the native population was assessed using the same tools. A similar trend with a moderate degree of differentiation found in the Di for the whole population over the years was observed in the immigrants (2012 = 0.071). Parasites infecting immigrants in Jenipapo, in 2012, were more homogenous when compared to the all infrapopulations of the same village in 2009 (0.056), however, in 2013, the same degree of differentiation was seen again (0.108). Volta do Rio presented a higher level of Di (0.148). Dc between immigrants and natives in the same year showed small differentiation (2012 = 0.012 and 2013 = 0.011). Assessing Dc for newly arrived individuals and natives from the posterior year, results were a little higher (immigrants 2012 vs natives 2009 = 0.019; immigrants 2013 vs natives 2012 = 0.019), however, analyzing immigrants from year 2013 with native from baseline, Dc was twice as large (0.031). Comparing natives from different years Dc was really small (2012 vs 2009 = 0.007; 2013 vs 2012 = 0.008).

Finally, we evaluated how differentiated are samples from the same individuals over time. Mean D in samples from the same individual showed moderate

differentiation over time (2009 vs 2012 = 0.055; 2013 vs 2012 = 0.077). A higher mean Dc was found comparing year 2013 and baseline (0.089).

228 Figure 1 – Study design over time

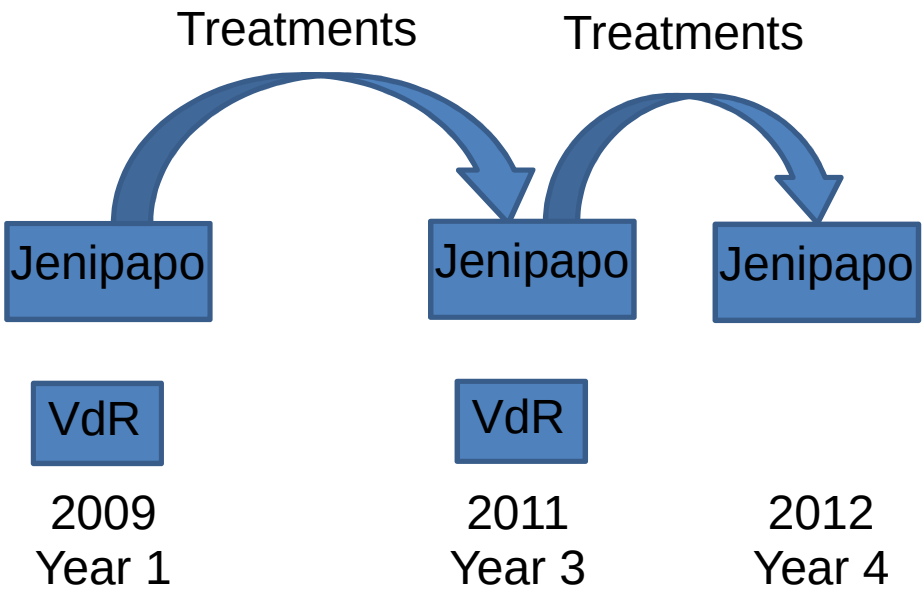

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Table 1 – Demographic characteristics in Jenipapo and Volta do Rio over the years.

	2009 (year 1)				2012 (year 3)				2013 (year 4)	
Characteristic	**Total**	**Jen**	**VdR**	***p***	**Total**	**Jen**	**VdR**	***p***	**Jen**	*p*
n (%) or mean ± SD	814	461	353		810	452	358		422	
Sex (Male)	377 (46.3)	221 (47.9)	156 (44.2)	ns	371 (45.8)	212 (46.9)	159 (44.4)	ns	196 (46.3)	ns
Age (y)	31.5 ± 22.2	30.6 ± 21.7	32.6 ± 22.7	ns	32.4 ± 21.9	30.9 ± 20.9	33.9 ± 23.0	ns	33.6 ± 20.9	ns
Birth place										
Ubaíra (%)	681 (83.7)	391 (84.8)	290 (82.2)	ns	668 (82.5)	378 (83.6)	290 (81.0)	ns	335 (84.2)	ns
Other	133 (16.3)	70 (15.2)	63 (17.8)		142 (17.5)	74 (16.4)	68 (19.0)		63 (15.8)	ns
% Lifetime in UB	93.5 ± 41.4	93.6 ± 19.9	93.5 ± 58.6	ns	90.9 ± 21.7	92.7 ± 20.1	89.4 ± 22.9	ns	93.7 ± 17.8	ns
Immigrants	-	-	-	-	121 (14.9)	53 (11.7)	68 (19.0)	*	25 (5.9)	*
Emigrants	-	-	-	-	122 (15.1)	64 (13.9)	58 (16.2)	ns	55 (12.2)	ns

* $p < 0.05$/ ns – Not significant

Table 2. Infection characteristic in Ubaíra, BA, in 2009, 2012 and 2013

	2009 year 1				**2012 year 3**				**2013 year 4**		
	Total (814)	**Jen (461)**	**VdR (353)**	***P between villages***	**Total (810)**	**Jen (452)**	**VdR (358)**	***P between villages***	**Jen (423)**	***p 2009 – 2012***	***p 2012 – 2013***
Schistosoma mansoni											
Prevalence (%)	41.2	45.8	35.1	*	18.9	23.7	12.8	**	**16.1**	**	**
Intensity ǂ	68.6	60.8	56.6	ns	33.5	34.2	32.0	ns	**24.1**	**	ns
Other Parasites											
Trichuris trichiura	21.3	18.4	25.0	*	18.4	16.2	21.2	ns	12.1	ns	ns
Ascaris lumbricoides	8.6	7.6	9.9	ns	10.4	8.0	14.3	*	6.2	ns	ns
Ancylostoma duodenale	7.6	11.1	3.1	**	14.2	12.0	17.1	*	**9.9**	**	ns
Enterobius vermicularis	7.4	10.0	4.0	**	26.2	28.1	23.7	ns	**14.0**	**	**
Taenia SP	2.7	3.9	1.1	*	15.1	16.6	13.1	ns	**3.5**	**	**

ǂ epg - *S. mansoni* eggs per gram of stool

* $p < 0.05$

** $p < 0.001$

Table 3 – Infection and Reinfection following treatment

	2012 (year 3)			2013 (year 4)
	Total	Jen	VdR	Jen
Incidence	(n=657)	(n=378)	(n=279)	(n=395)
S.mansoni - n (%)	119 (18.1)	83 (22.0)	36 (12.9)	59 (14.9)
Intensity *	27.7	29.2	24.6	19.5
Reinfection	(n=250)	(n=163)	(n=87)	(n=90)
S.mansoni - n (%)	71 (28.4)	55 (33.7)	16 (18.4)	16 (17.8)
Intensity *	35.0	32.6	44.7	23

*Geometric Mean eggs per gram of feces

Table 4 – Di and Ae following treatment

	2009 (year 1)			2012 (year 3)			2013* (year 4)
	Combined	**Jen**	**VdR**	**Combined**	**Jen**	**VdR**	**Jen**
Di							
All	0.103	0.083	0.096	0.099	0.084	0.116	0.102
Immigrants	-	-	-	0.071	0.056	0.148	0.108
Ae	6.8 (3.1)	6.8 (3.1)	6.7 (3.1)	5.4 (2.9)	5.3 (2.9)	5.4 (2.9)	5.5 (2.9)

Table 5 – Dc for Jenipapo: over time, Immigrants versus Natives and reinfected samples

	Jenipapo								
	All individuals			Immigrants vs Natives			Reinfected samples		
	2009	**2012**	**2013**	**2009**	**2012**	**2013**	**2009**	**2012**	**2013**
2009	-	0.008	0.014	-	0.019	0.031	-	0.055	0.089
2012		-	0.009		-	0.019		-	0.077
2013			-			-			-

4 DISCUSSÃO

Apesar do longo tempo de existência e de todos os conhecimentos a respeito da sua profilaxia e tratamento, a esquistossomose continua como um grande problema de saúde pública no mundo e no Brasil. A Organização Mundial de Saúde estima que mais de 200 milhões de pessoas estão infectadas com o parasita em mais de 74 países diferentes (WHO, 2010). No Brasil, o Ministério da Saúde estima que aproximadamente 9 milhões estão infectadas com o *Schistosoma mansoni* e que mais de 30 milhões estão em risco de infecção (COURA e AMARAL, 2004).

As estratégias de controle foram iniciadas na década de 70, obtendo uma redução na prevalência no país de aproximadamente 25% a cerca de 5%, de 1977 a 2011, com a morbidade e mortalidade acompanhando esta redução (PELLON e TEIXEIRA, 1950; VIEIRA, 1993; CARMO e BARRETO, 1994; MS/SVS, 1997; COURA e AMARAL, 2004). Estes dados, no entanto, por serem baseados em notificações hospitalares algumas vezes não refletem a realidade da totalidade do Brasil. Ao ser aplicado um protocolo de pesquisa direcionado em determinadas regiões, prevalências diferentes são descritas (BRITO *et al.*, 2006; GAZZINELLI *et al.*, 2006; GUIMARAES e TAVARES-NETO, 2006; BARBOSA *et al.*, 2010; ENK *et al.*, 2010; GALVAO *et al.*, 2010; PEREIRA, A. P. *et al.*, 2010; PEREIRA, W. R. *et al.*, 2010; REIS *et al.*, 2010). O mesmo pode ser observado em comunidades rurais pertencentes a cidade de Ubaíra, sudoeste da Bahia, mais precisamente nas comunidades de Jenipapo e Volta do Rio, e em uma região urbana na capital da Bahia, Salvador, chamada de Parque São Bartolomeu no bairro do Subúrbio Ferroviário. A prevalência da esquistossomose nas zonas rurais foi de 41%, sendo 45.8% em Jenipapo e 35.1% em Volta do Rio. No Parque São Bartolomeu 24.7% das pessoas encontravam-se infectadas com o parasita. Acreditamos que este número elevado na prevalência quando comparado aos dados do Ministério da Saúde (MS/SVS, 1997) deva-se à metodologia imposta no nosso protocolo que aumentou significativamente a sensibilidade do teste ao requisitar três amostras de fezes em dias diferentes. Ao avaliarmos apenas uma amostra, os números

observados assemelham-se aos descritos na literatura para a doença no estado da Bahia, que figuram entre 15-20%.

Apesar de estarem cerca de 270 km de distância, as comunidades rurais e o Parque São Bartolomeu apresentaram marcantes semelhanças ambientais, demográficas, comportamentais e de saneamento básico. Talvez por esta razão as características de infecção também se mostraram semelhantes. Um maior risco e uma maior intensidade de infecção foram associados com ser adolescente (faixa de idade entre 10 e 20 anos), ser do sexo masculino e relatar contato com um maior número de localidades de risco. Estas características podem ser vistas em outras regiões do país e do mundo (KABATEREINE *et al.*, 2004; BERHE *et al.*, 2007; IGREJA *et al.*, 2009; ENK *et al.*, 2010; PEREIRA, A. P. *et al.*, 2010), embora outros resultados também já tenham sido relatados (BARAKAT *et al.*, 2000). Ao avaliarmos as comunidades de Jenipapo e Volta do Rio nos anos posteriores a primeira rodada de tratamento em 2009, é possível observar a baixa migração dos residentes. Volta do Rio apresentou uma taxa de imigração maior que Jenipapo com números semelhantes de indivíduos que mudaram-se para fora da área. Contudo, as características demográficas se mantiveram semelhantes e na maior parte, os indíviduos migrantes residiam na proximidade dos vilarejos, no Vale do Jiquiriçá.

O projeto inicialmente previa a repetição dos inquéritos epidemiológicos e parasitológico seguido do tratamento nas zonas rurais e urbana. Contudo, no final de 2011 foram iniciadas obras de saneamento básico com a reconstrução de aproximadamente 70% das residências com os moradores das mesmas sendo realocados. Estas obras impossibilitaram o retorno ao Parque São Bartolomeu e a repetição da pesquisa ficou restrita às comunidades rurais. No ano de 2012, foi possível observar uma diminuição na prevalência e intensidade de infecção da esquistossomose. Em 2012, a porcentagem de indivíduos infectados reduziu em 55% (2009 = 41.2% vs 2012 = 18.9%) com uma redução de 45% na quantidade de ovos por grama de fezes (2009 = 60.8 vs 2012 = 33.5). Comparando as duas vilas entre si, houve uma diminuição muito mais significativa em Volta do Rio quando comparado com

Jenipapo, apesar de apresentarem intensidades de infecção semelhantes. As taxas de reinfecção e incidência também foram maiores em Jenipapo. As diferenças nos índices descritos entre as comunidades pode ser explicada em como o esgoto de cada residência é tratado nas duas localidades. Há uma diferença significante entre a quantidade de fossas sépticas em Volta do Rio (89.3% em VdR vs 43.4% em Jenipapo). Outra possível explicação se dá pela perenidade do rio Jiquiriçá em Jenipapo, enquanto o mesmo em Volta do Rio encontra-se sem fluxo em determinadas épocas do ano.

Devido a falta de recursos, não foi possível realizar a repetição dos inquéritos epidemiológicos e parasitológico seguido do tratamento pelo terceiro ano nos dois vilarejos. Desta forma, Jenipapo que apresentou os piores índices de infecção nos dois períodos avaliados foi selecionado. Em 2013, Jenipapo demonstrou os mesmos resultados que em 2012. Houve uma redução significativa na prevalência e uma diminuição, não significativa, na intensidade de infecção. As taxas de incidência e reinfecção também sofreram queda. Esses dados se assemelham com os demonstrados no país ao longo do tempo (PELLON e TEIXEIRA, 1950; VIEIRA, 1993; CARMO e BARRETO, 1994; MS/SVS, 1997; COURA e AMARAL, 2004) e, baseado no que é observado nesses e em outros trabalhos em outros locais do mundo (PICQUET *et al.*, 1998; UTZINGER *et al.*, 2000; KABATEREINE *et al.*, 2003; BLACK *et al.*, 2009; BARAKAT e EL MORSHEDY, 2010), podemos prever que se as mesmas intervenções forem mantidas, a porcentagem de indivíduos com *Schistosoma mansoni* diminuirá até atingir uma marca próxima aos 5% e se manterá nestes níveis ao longo dos anos.

De acordo com a OMS as estratégias de controle para a esquistossomose foram implementadas com sucesso nos últimos 20 anos em diversos países além do Brasil como Camboja, China, Egito e Árabia Saudita. Contudo, apenas em Marrocos que existe evidência de que a transmissão foi interrompida (WHO, 2010). As razões para que um *plateau* nas taxas de infecção de esquistossomose seja mantido e uma redução destes padrões não seja comumente visto não é bem elucidada. Um maior entendimento de características do parasita, da sua distribuição e de como o mesmo se

comporta em situações de estase evolutiva e em estresse, causado por exemplo pelo tratamento, pode fornecer informações valiosas a serem utilizadas no aperfeiçoamento de políticas de saúde pública. Para a compreensão destas características, a estrutura populacional genética dos parasitas foi avaliada no contexto rural e urbano.

Durante o inquérito parasitológico, um esforço foi realizado para que todos os ovos eliminados nas fezes fossem coletados. É de conhecimento que uma porcentagem dos ovos não são eliminados e ficam retidos nas veias mesentéricas causando as patologias associadas à esquistossomose. Contudo, estes ovos que não são liberados ao meio ambiente não influenciarão a geração seguinte por não dar prosseguimento ao ciclo de vida parasitário. Ao trabalhar com as amostras de ovos agregadas naturalmente, foi possível obter informações da estrutura genética dos parasitas que realmente contribuem para a infecção. Ao utilizar este tipo de amostra também foi possível obter uma quantidade de indivíduos infectados, infrapopulações, que não seria possível trabalhar caso fossem usadas amostras discretas, obtidas a partir de passagem por caramujos e/ou camundongos. Essa estratégia, foi validada através de artigos publicados por nosso grupo (SILVA *et al.*, 2006; BLANK *et al.*, 2010; BLANK *et al.*, 2011).

Para avaliação da similaridade genética entre as diferentes populações parasitárias avaliadas, foi utilizado o índice de diferenciação O coeficiente de endogamia (Fst) e outros índices similares utilizados comumente em estudos de diferenciação genética apresenta falhas metodológicas que, por vezes, torna o resultado incorreto. O Fst se aproxima de zero quando a diversidade na população avaliada é alta e por utilizar a heterozigosidade como base para o seu cálculo, diversas vezes os valores encontrados estarão incorretos (JOST, 2008). Outro fator importante para a utilização do D de Jost é que este baseia-se nas frequências alélicas e não nos critérios de Hardy-Weinberg, que não é aplicável para infrapopulações de *Schistosoma mansoni.* Durante o ciclo de vida do parasita, toda a progênie deve migrar do hospedeiro definitivo para que a espécie se perpetue, o que é uma violação grave do equilíbrio de Hardy-Weinberg (HARTL e CLARK, 2007). O índice de D de Jost se

mostrou consistente ao serem testadas a mesma amostra repetidas vezes. O valor encontrado foi muito próximo ao zero (D = 0.007) indicando que se tratava da mesma amostra e que a taxa de erro é muito baixa. Comparando as amostras provenientes dos locais de estudo com as amostras controle de uma cepa laboratorial foi encontrado uma grande diferenciação (D > 0.25) indicando a aplicabilidade do teste.

Com o intuito de aumentar a sensibilidade do exame parasitológico, realizado com o kit Kato-Katz, foram requisitadas dos participantes três amostras de fezes em dias diferentes. Contudo, ainda não era conhecido se o aumento da sensibilidade seria benéfico para a caracterização genética das infrapopulações e como este fato iria influenciar nas análises posteriores. O principal questionamento era se a frequência dos ovos liberados pelas fêmeas a cada dia era a mesma. Avaliando as amostras do mesmo indivíduo coletada em dias diferentes foi visto que a a freqüência alélica era constante, tendo em vista que o D de Jost entre as amostras diferentes indicou uma diferenciação baixa (D = 0.01). Este resultado serviu de base para as análises genéticas posteriores, principalmente quando a carga parasitária da amostra coletada era baixa dificultando a amplificação por PCR. Apenas uma amostra de fezes é suficiente para ter uma aproximação da real diversidade genética parasitária em cada infrapopulação.

Neste trabalho, foram descritos pela primeira vez na literatura diferentes aplicações do D de Jost com a finalidade de melhor caracterizar as populações parasitárias estudadas: a) D de Jost aplicado para as Infrapopulações par a par (Di) tem como objetivo observar em média quão diferenciados se encontram os parasitas que infectam indivíduos dentro de um grupo; b) D de Jost aplicado para as populações componentes (Dc) é utilizado para indicar a diferenciação entre grupos formados por localização geográfica, característica do hospedeiro ou ano avaliado; c) D de Jost entre Infrapopulações comparados com a População Componente geográfica (Dic). Este índice fornece um valor único para cada indivíduo que indica quanto a infrapopulação é diferenciada do *pool* de genótipos disponíveis.

Comparando cada infrapopulação agrupada por localidade geográfica, foi encontrado um Di moderada (0.082 para Jenipapo; 0.122 para Volta do Rio e 0.063 para São Bartolomeu), indicando que os parasitas provavelmente são adquiridos pouco a pouco em diferentes localidades e em diferentes circunstâncias. Quando a população componente é heterogênea, um hospedeiro irá carregar uma amostra incompleta da população componente. Por esta razão, a amostragem das infrapopulações deve ser feita de uma forma a captar a maior quantidade de indivíduos para que possa ser obtida a total variabilidade da população componente. Este fato valida mais uma vez a abordagem utilizando amostras agregadas naturalmente realizada neste trabalho. Ao avaliar o Di segregando as infrapopulações por características demográficas e epidemiológicas, como sexo, idade, carga parasitária, índice socio-econômico, infecções passadas com esquistossomose e viagens para fora da regiao, diferenças significativas foram encontradas em Ubaíra e no Parque São Bartolomeu. Apenas tempo de vida em Ubaíra não foi diferente entre os grupos. No entanto, o uso de infrapopulações faz com que sejam utilizados um número muito elevado de parasitas. Para qualquer teste estatístico, se o tamanho amostral for muito grande, qualquer diferença será estatisticamente significante independente do tamanho do efeito. No caso dos testes para Di, por se tratar de uma nova aplicação utilizada pela primeira vez na literatura, o tamanho do efeito que deve ser alcançado para a determinação da importância da diferença entre grupos ainda não é conhecida, ou seja, pouco pode ser inferido a partir dos resultados encontrados.

Na descrição original realizada por Bush e colaboradores em 1997 (BUSH *et al.*, 1997), as populações componentes são descritas como todos os hospedeiros, definitivo ou intermediário, de uma determinada região geográfica. No entanto, o conceito de População Componente foi extendido para incluir todos os indivíduos com características epidemiológicas semelhantes, desta forma, foi possível descrever como os parasitas encontram-se distribuídos nos hospedeiros definitivos. O Dc entre as vilas mostrou uma diferenciação baixa a moderada (0.046). Jenipapo e Volta do Rio encontram-se a uma distância de aproxidamente 8 km, portanto, as populações parasitárias apresentam uma pequena barreira no fluxo gênico entre si. Além disso,

esses resultados juntamente com as variações nas infrapopulações indicam que a quantidade de marcadores utilizados são suficientes para obter diferenciação entre uma microregião. Trabalhos da nossa equipe corroboram com este achado quando descreveram deriva genética em uma cepa laboratorial (BLANK *et al.*, 2011) e variação entre sub amostras de populações laboratoriais de *S. mansoni*, utilizando o mesmo painel de marcadores microsatelites (BLANK *et al.*, 2010).

Nenhuma estruturação genética foi encontrada quando as populações componentes foram estratificadas pelas características demográficas e epidemiológicas em nenhuma das populações estudadas. A interpretação evolutiva para a falta de diferenciação entre os grupos é que os indivíduos de Jenipapo, Volta do Rio e do Parque São Bartolomeu se infectam localmente, e além disso, tendem a permanecer nas proximidades de infecção ou o mesmo genótipo encontra-se nas diferentes coleções hídricas infectantes. Além disso, devido a baixa diferenciação nos parasitas que infectam diferentes gerações (estratificação por faixa etária), a população parasitária aparenta ser estável ao longo do tempo sem ter influência dos principais eventos evolutivos, como migração, deriva genética, seleção natural. Este fato é reforçado através da comparação realizada dos parasitas de residentes do Parque São Bartolomeu com amostras de indivíduos coletados em 2004, onde o valor de Dc foi baixo. É descrito que a distribuição deste parasita é altamente variada em populações naturais (CURTIS *et al.*, 2002). Contudo, é possível que sua variação seja distribuída igualmente por todos os humanos ou, como representado pelos dados de Di e Dc relatados, que haja uma considerável quantidade de heterogeneidade. Foi observado que cada infrapopulação contribui apenas com uma parte da variabilidade genética da população componente.

Falta de estruturação genética também foi vista ao avaliar os valores de Dic. Tendo em vista que se trata de um valor único para cada indivíduo os métodos de avaliação baseiam-se em uma simples comparação de médias entre os grupos avaliados. A única diferença entre os valores de Dic foi vista quando avaliado a intensidade de infecção. Indivíduos que apresentaram uma carga parasitária alta (acima

de 400 ovos por grama de fezes em média) compunham melhor as populaçoes componentes. O mesmo foi visto em relação aos números de alelos efetivos, quanto maior o número de ovos por grama de fezes, maior a diversidade genética. Este resultado é importante para mostrar que em determinadas situações, onde não é possível realizar uma boa amostragem da população, indivíduos com alta carga parasitária, geralmente na faixa etária entre os 10 e 20 anos, podem ser selecionados e eles podem ser uma amostra representativa da variabilidade genética total.

As evidências de transmissão local e a baixa influência de migrantes demonstradas através dos dados de Dc nas comunidades rurais foram, de certa forma, esperadas. A esquistossomose é historicamente descrita como uma doença rural e a falta de mobilidade nas populações levavam a crer que o Rio Jiquiriçá é realmente a fonte de contaminação das populações de Ubaíra. Já na comunidade urbana, a hipótese inicial era que a população parasitária circulante sofresse uma grande influência migratória, tendo em vista que, historicamente, comunidades similares ao Parque São Bartolomeu são formadas de indivíduos do interior do estado que procuram a capital em busca de melhores qualidade de vida. Para se estabilizarem localmente, os imigrantes procuram por vezes habitação em lugares de invasão que não apresentam um saneamento básico adequado. Desta forma, estes indivíduos tornam-se fator de risco para um fenômeno que vem acontecendo nas cidades grandes que é o de re-endemização de doenças consideradas controladas. Salvador é considerada não endêmica para a esquistossomose, contudo, uma vigilância malacológica que é realizada pelo Centro de Controle de Zoonoses (CCZ) identificou 7 localidades que apresentaram caramujos da espécie *Biomphalaria glabrata* liberando cercárias, sendo um deles o Parque São Bartolomeu (SOUZA *et al.*, 2012). O resultado encontrado pelo CCZ é reflexo dos problemas de saneamento básico vividos em Salvador. Em uma tentativa de melhoria desses serviços, em 2004 foi realizado na cidade um programa intitulado “Bahia Azul” que melhorou o sistema de esgotamento no Parque São Bartolomeu de 40 para 70% das residências locais. Esta melhoria foi responsável por uma diminuição significativa na incidência de diarréia na área (BARRETO *et al.*, 2007), embora, aparentemente os 70% de esgotamento na área não seja suficiente para

conter a transmissão de esquistossomose. Felizmente, os dados de genética populações indicam que os esforços para o controle e/ou eliminação do *Schistosoma mansoni* podem ser direcionados para as áreas estudadas e que dificilmente a influência dos imigrantes irá ser suficiente para manter ou reativar o ciclo de transmissão da doença.

As estratégias para o controle da esquistossomose são baseadas no tratamento dos infectados, melhorias de saneamento básico, educação em saúde e controle malacológico. Pela grande quantidade de benefícios diretos ao indivíduo infectado, o tratamento segue como principal mecanismos utilizado para o controle da doença. Em algumas situações, o tratamento em massa é descrito por ser capaz de contribuir para uma redução sustentada da transmissão (FRENCH *et al.*, 2010; NORTON *et al.*, 2010; STURROCK *et al.*, 2011; HODGES *et al.*, 2012). Por esta razão a OMS estabeleceu como meta que até 2020, a intensificação da administração de medicamentos em massa para atingir a cobertura geográfica de 100% em pelo menos 50% dos países endêmicos que requerem quimioterapia preventiva para esquistossomose em 2015 (WHO, 2010). Atualmente, o medicamento de escolha é o praziquantel (MCMANUS e LOUKAS, 2008). Trata-se de uma droga de dose única, barata, com poucos efeitos adversos e que apresenta eficácia de 80 - 90% (WEGNER, 1984; CIOLI, 2000; CIOLI e PICA-MATTOCCIA, 2003). No entanto, ao serem realizados repetidos tratamentos quimioterápicos, o questionamento acerca da possibilidade de desenvolvimento resistência ao medicamento é levantado. A tolerância ao praziquantel pelos parasitas já foi obtida em laboratório após 7 passagens por camundongos (FALLON e DOENHOFF, 1994). Por esta droga ser relativamente insensível aos vermes imaturos, a persistência à infecção é geralmente atribuída a contaminação recente (CIOLI e PICA-MATTOCCIA, 2003), mas é possível que os vermes persistentes, ou uma porção deles, estejam relacionados a resistência ao praziquantel. A avaliação da possibilidade de seleção de parasitas pelo tratamento foi realizada a partir de técnicas de genética de populações, onde as estruturas genéticas das populações pré e pós tratamento foram comparadas utilizando Dc, onde as populações componentes foram determinadas por resposta ao tratamento.

Em procariotos, a seleção a antibióticos pode ser observada em um curto período de tempo geralmente resultando no crescimento de um único clone (MANGES *et al.*, 2001; MARTIN, 2002) sendo, desta forma, de fácil identificação. Em seres sexuados, como o *S. mansoni*, clones não são formados, contudo, os genótipos entre membros da mesma família ou próximos apresentam maior correlação entre si que os mais distantes ou não relacionados. Como mutações acontecem geralmente em um ou poucos genótipos, em um período curto o grau de relação entre indivíduos que adquiram um genótipo de resistência, por exemplo, deverá ser maior que os que não apresentem esta característica. Com o passar do tempo, na ausência de um isolamento geográfico, as mutações causadoras dessa resistência seriam transmitidos para outros membros da população em questão e essa relação seria mais difícil de ser obtida. A partir deste modelo, as análises foram realizadas.

Os resultados encontrados indicam que os parasitas dos indivíduos que foram tratados e permaneceram infectados após o tratamento não apresentaram uma estrutura genética diferente do que os susceptíveis ao tratamento, indicando que é improvável que resistência ao praziquantel seja a explicação para a persistência de infeção. Esses dados são de certa forma, esperados já que o praziquantel foi recentemente introduzido para o tratamento no Brasil e a maior parte dos indivíduos não tiveram sido tratados com o mesmo (CIOLI, 2000; GRYSEELS *et al.*, 2001; CIOLI e PICA-MATTOCCIA, 2003). A baixa diferenciação na população geral e entre as infecções persistentes apoiam a idéia que estas representam uma transmissão recente e que a população persistente é uma coleção aleatória da população susceptível. A falha na cura dos indivíduos pode ser associada à recusa ao tratamento por parte dos mesmos, contudo, na maior parte daqueles persistentes foi possível observar uma diminuição na carga parasitária, o que demonstra que o praziquantel foi ingerido.

Até a publicação destes resultados (BLANTON *et al.*, 2011), apenas um trabalho havia tentado utilizar a genética de populações para verificar o efeito do tratamento com praziquantel na estrutura genética de *S. mansoni* (NORTON *et al.*, 2010). Contudo,

comparações com este trabalho são difíceis de ser realizadas tendo em vista que uma abordagem diferente foi utilizada. Na Tanzânia, analisando miracídios de crianças antes (n=80) e após um ano do uso do praziquantel (n=47), observou-se que a diversidade alélica diminuiu associando com o efeito gargalo resultante da quimioterapia. Eles encontraram também que as populações do parasita eram diferentes nos dois momentos avaliados. Contudo, devido ao longo período entre avaliações e por não terem sido observados o mesmo grupo de crianças nos dois momentos do trabalho, é mais provável que eles tenham avaliado re-infecções ao invés de infecções persistentes na sua maior parte. Estes resultados são consistentes com os encontrados neste trabalho onde foi encontrado que indivíduos se re-infectam com diferentes populações parasitárias.

Para verificar a natureza das re-infecções e como se comportam as populações de *Schistosoma mansoni* após repetidas doses de tratamento, os indivíduos de Ubaíra foram avaliados dois e três anos após o tratamento inicial, em 2009. A hipótese principal para este trabalho foi que o tratamento de 88.1 e 96.8% dos indivíduos infectados em 2009 e 2012, respectivamente, induzisse um efeito gargalo genético na população parasitária com a recuperação dos vermes sofrendo grande influência migratória e de deriva gênica. Caso a diminuição drástica no número de parasitas mudasse as frequências alélicas e genotípicas nas gerações futuras, caracterizando o efeito gargalo, o seguinte cenário deveria acontecer: 1) O valor de Di seria maior no ano posterior ao tratamento, indicando uma maior heterogeneidade na segunda população; 2) O valor de Dc entre vilas no mesmo ano seria mais alto no momento pós-tratamento; 3) O valor de Dc entre os anos seria alto.

Os resultados encontrados, entretanto, demonstraram que os tratamentos sucessivos foram eficazes na redução da carga parasitária global em Ubaíra, aproximadamente 4 vezes em cada ano sucessivo e cerca de 11 vezes em 2013 comparando com 2009, mas isso não foi suficiente para afetar a estrutura genética da população em anos sucessivos. O valor de Di entre sucessivos anos se manteve constante (Di em 2009 = 0.103; Di em 2012 = 0.099), indicando a mesma variabilidade

nas infrapopulações entre os anos, ou seja, a população parasitária não foi reduzida suficientemente para intereferir na heterogeneidade da mesma. O valor de Dc entre vilas no mesmo ano foi menor no ano de 2012 (Dc entre vilas em 2009 = 0.046; Dc entre vilas em 2012 = 0.031). Este resultado não só indica uma falha em alcançar o efeito gargalo genético como mostra um aumento de fluxo gênico entre as populações no segundo momento avaliado. Estes dados, no entanto, podem ser associados com os dados de prevalência e reinfecção que mostram que Jenipapo é o principal ponto de risco para a esquistossomose e indicam que os residentes de Volta do Rio tendem a se infectar no primeiro vilarejo.

Observando o valor de Dc entre o ano de 2009 e 2013, observa-se uma maior diferenciação (Dc em 2009 e 2012 = 0.008; Dc em 2012 e 2013 = 0.009; Dc em 2009 e 2013 = 0.014) o que talvez indique que uma redução de 11 vezes na carga parasitária seja o *cut-off* para a modificação da estrutura genética inicial. Os imigrantes demonstraram ter pouca influência na estruturação da população a cada ano, indicando que os novos indivíduos compartilham os mesmos parasitas dos indivíduos locais, o que parece ser improvável ao observarmos que entre 8 km de distância já é possível observar uma pequena barreira no fluxo gênico.

Outra hipótese é que a principal fonte de reinfecção sejam indivíduos que permaneceram infectados ao longo dos anos apesar da terapia, já que foi descrito por Souza e colaboradores (SOUZA *et al.*, 2012) que a população parasitária nos hospedeiros intermediários não representa as populações nos hospedeiros definitivos. As razões para que eles apresentem infecções persistentes passam por uma possível falha terapêutica, indivíduos que recusaram a quimioterapia ou recém infectados apresentando vermes imaturos, ou por apresentaram carga parasitária baixa, a ponto de não serem detectadas com o uso do Kato-Katz, mesmo com a melhora na sensibilidade do teste a partir da utilização de três amostras em dias diferentes.

Qualquer que seja a real razão para a persistência na infecção, a solução é identificar formas mais eficiente para a distribuição dos medicamentos. Uma diretriz

adotada principalmente nos países da África foi a administração de droga em massa. Essa estratégia obteve grandes reduções nos índices de prevalência, morbidade e mortalidade (FRENCH *et al.*, 2010; NORTON *et al.*, 2010; STURROCK *et al.*, 2011; HODGES *et al.*, 2012). No Brasil, este tipo de abordagem não vem sendo utilizado e o tratamento deve ser condicionado a um diagnóstico prévio. No entanto, neste trabalho foram observadas evidências dos potenciais benefícios do uso da terapia em massa. Além disso não foi encontrada evidência relativa ao desenvolvimento de resistência ao medicamento, principal preocupação com o uso repetido do praziquantel. Vale a pena ressaltar que o esforço para a cura de todos os indivíduos com esquistossomose deve ser aliado a políticas de saneamento básico com o objetivo de eliminar os pontos de risco para novas infecções. Esta é a única forma para que a doença seja eliminada de forma definitiva.

BARAKAT, R.; EL MORSHEDY, H. Efficacy of two praziquantel treatments among primary school children in an area of high Schistosoma mansoni endemicity, Nile Delta, Egypt. **Parasitology**, v. 138, n. 4, p. 440-446, apr. 2010.

BARAKAT, R., *et al.* The epidemiology of schistosomiasis in Egypt: patterns of Schistosoma mansoni infection and morbidity in Kafer El-Sheikh. **Am. J. Trop. Med. Hyg.**, v. 62, n. 2 Suppl, p. 21-27, feb. 2000.

BARBOSA, C. S., *et al.* Current epidemiological status of schistosomiasis in the state of Pernambuco, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 549-554, jul. 2010.

BARRETO, M. L., *et al.* Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies. **Lancet**, v. 370, n. 9599, p. 1622-1628, nov. 2007.

BERHE, N., *et al.* Intensity of Schistosoma mansoni, hepatitis B, age, and sex predict levels of hepatic periportal thickening/fibrosis (PPT/F): a large-scale community-based study in Ethiopia. **Am. J. Trop. Med. Hyg.**, v. 77, n. 6, p. 1079-1086, dec. 2007.

BLACK, C. L., *et al.* Impact of intense, longitudinal retreatment with praziquantel on cure rates of schistosomiasis mansoni in a cohort of occupationally exposed adults in western Kenya. **Trop. Med. Int. Health**, v. 14, n. 4, p. 450-457, apr. 2009.

BLANK, W. A., *et al.* Host mouse strain is not selective for a laboratory adapted strain of Schistosoma mansoni. **J. Parasitol.**, v. 97, n. 3, p. 518-521, jun. 2011.

BLANK, W. A., *et al.* Long-term genetic stability and population dynamics of laboratory strains of Schistosoma mansoni. **J. Parasitol.**, v. 96, n. 5, p. 900-907, oct. 2010.

BLANTON, R. E., *et al.* Schistosoma mansoni population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. **Int. J. Parasitol.**, v. 41, n. 10, p. 1093-1099, aug. 2011.

BRITO, L. L., *et al.* Moderate- and low-intensity co-infections by intestinal helminths and Schistosoma mansoni, dietary iron intake, and anemia in Brazilian children. **Am. J. Trop. Med. Hyg.**, v. 75, n. 5, p. 939-944, nov. 2006.

BUSH, A. O., *et al.* Parasitology meets ecology on its own terms: Margolis et al. revisited. **J Parasitol**, v. 83, n. 4, p. 575-583, aug. 1997.

CARMO, E. H.; BARRETO, M. L. [Schistosomiasis mansoni in Bahia, Brazil: historical trends and control measures]. **Cad. Saude. Publica**, v. 10, n. 4, p. 425-439, dec. 1994.

CIOLI, D. Praziquantel: is there real resistance and are there alternatives? **Curr. Opin. Infect. Dis.**, v. 13, n. 6, p. 659-663, dec. 2000.

CIOLI, D.; PICA-MATTOCCIA, L. Praziquantel. **Parasitol. Res.**, v. 90 Supp 1, n., p. S3-9, jun. 2003.

COURA, J. R.; AMARAL, R. S. Epidemiological and control aspects of schistosomiasis in Brazilian endemic areas. **Mem. Inst. Oswaldo Cruz**, v. 99, n. 5 Suppl 1, p. 13-19 2004.

CURTIS, J., *et al.* Schistosome genetic diversity: the implications of population structure as detected with microsatellite markers. **Parasitology**, v. 125 Suppl, n., p. S51-59 2002.

ENK, M. J., *et al.* Factors related to transmission of and infection with Schistosoma mansoni in a village in the South-eastern Region of Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 570-577, jul. 2010.

FALLON, P. G.; DOENHOFF, M. J. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in Schistosoma mansoni in mice is drug specific. **Am. J. Trop. Med. Hyg.**, v. 51, n. 1, p. 83-88, jul. 1994.

FRENCH, M. D., *et al.* Observed reductions in Schistosoma mansoni transmission from large-scale administration of praziquantel in Uganda: a mathematical modelling study. **PLoS Negl. Trop. Dis.**, v. 4, n. 11, p. e897 2010.

GALVAO, A. F., *et al.* Spatial distribution of Schistosoma mansoni infection before and after chemotherapy with two praziquantel doses in a community of Pernambuco, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 555-562, jul. 2010.

GAZZINELLI, A., *et al.* Socioeconomic determinants of schistosomiasis in a poor rural area in Brazil. **Acta Trop.**, v. 99, n. 2-3, p. 260-271, oct. 2006.

GRYSEELS, B., *et al.* Are poor responses to praziquantel for the treatment of Schistosoma mansoni infections in Senegal due to resistance? An overview of the evidence. **Trop. Med. Int. Health**, v. 6, n. 11, p. 864-873, nov. 2001.

GUIMARAES, I. C.; TAVARES-NETO, J. [Urban transmission of schistosomiasis in children from a neighborhood of Salvador, Bahia]. **Rev. Soc. Bras. Med. Trop.**, v. 39, n. 5, p. 451-455, oct. 2006.

HARTL, D. L.; CLARK, A. G. **Principles of Population Genetics**. Sunderland, MA: Sinauer Associates. 2007

HODGES, M. H., *et al.* Mass drug administration significantly reduces infection of Schistosoma mansoni and hookworm in school children in the national control program in Sierra Leone. **BMC Infect. Dis.**, v. 12, n., p. 16 2012.

IGREJA, R. P., *et al.* A 15-year follow-up study on schistosomiasis in a low-endemic area in Rio de Janeiro State, Brazil. **J. Helminthol.**, v. 84, n. 3, p. 229-233, sep. 2009.

JOST, L. G(ST) and its relatives do not measure differentiation. **Mol. Ecol.**, v. 17, n. 18, p. 4015-4026, sep. 2008.

KABATEREINE, N. B., *et al.* Epidemiology and morbidity of Schistosoma mansoni infection in a fishing community along Lake Albert in Uganda. **Trans. R. Soc. Trop. Med. Hyg.**, v. 98, n. 12, p. 711-718, dec. 2004.

KABATEREINE, N. B., *et al.* Efficacy and side effects of praziquantel treatment in a highly endemic Schistosoma mansoni focus at Lake Albert, Uganda. **Trans. R. Soc. Trop. Med. Hyg.**, v. 97, n. 5, p. 599-603, oct. 2003.

MANGES, A. R., *et al.* Widespread distribution of urinary tract infections caused by a multidrug-resistant Escherichia coli clonal group. **N. Engl. J. Med.**, v. 345, n. 14, p. 1007-1013, oct. 2001.

MARTIN, S. Fewer Canadians being prescribed antibiotics. **CMAJ**, v. 166, n. 6, p. 798, mar. 2002.

MCMANUS, D. P.; LOUKAS, A. Current status of vaccines for schistosomiasis. **Clin. Microbiol. Rev.**, v. 21, n. 1, p. 225-242, jan. 2008.

MS/SVS, M. D. S.-. **Programa de Controle da Esquistossomose:** Programa de Controle da Esquistossomose, 1997.Disponível em: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinan/pce/cnv/pce.def. 30/10/2013

NORTON, A. J., *et al.* Genetic consequences of mass human chemotherapy for Schistosoma mansoni: population structure pre- and post-praziquantel treatment in Tanzania. **Am. J. Trop. Med. Hyg.**, v. 83, n. 4, p. 951-957, oct. 2010.

PELLON, A. B.; TEIXEIRA, I. **Distribuição Geográfica da Esquistossomose Mansônica no Brasil**. Departamento Nacional de Saúde, Divisão Organização Sanitária. Rio de Janeiro. 1950

PEREIRA, A. P., *et al.* The prevalence of schistosomiasis in school-aged children as an appropriate indicator of its prevalence in the community. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 563-569, jul. 2010.

PEREIRA, W. R., *et al.* Schistosoma mansoni infection in a rural area of the Jequitinhonha Valley, Minas Gerais, Brazil: analysis of exposure risk. **Acta Trop.**, v. 113, n. 1, p. 34-41, jan. 2010.

PICQUET, M., *et al.* Efficacy of praziquantel against Schistosoma mansoni in northern Senegal. **Trans. R. Soc. Trop. Med. Hyg.**, v. 92, n. 1, p. 90-93, feb. 1998.

REIS, D. C., *et al.* Accessibility to and utilisation of schistosomiasis-related health services in a rural area of state of Minas Gerais, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 587-597, jul. 2010.

SILVA, L. K., *et al.* Microsatellite analysis of pooled Schistosoma mansoni DNA: an approach for studies of parasite populations. **Parasitology**, v. 132, n. Pt 3, p. 331-338, mar. 2006.

SOUZA, S. S., *et al.* Genetic population structure of cercariae from an urban foci of Schistosoma mansoni, Brazil. **Am. J. Trop. Med. Hyg.**, v. 87, n. 5, p. 843-849, nov. 2012.

STURROCK, H. J., *et al.* Planning schistosomiasis control: investigation of alternative sampling strategies for Schistosoma mansoni to target mass drug administration of praziquantel in East Africa. **Int. Health**, v. 3, n. 3, p. 165-175, sep. 2011.

UTZINGER, J., *et al.* Efficacy of praziquantel against Schistosoma mansoni with particular consideration for intensity of infection. **Trop. Med. Int. Health**, v. 5, n. 11, p. 771-778, nov. 2000.

VIEIRA, J. B. F. **O programa brasileiro de controle de esquistossomose**. In: IV Simpósio Internacional de Esquistossomose, 1993, Rio de Janeiro - Fundação Oswaldo Cruz.

WEGNER, D. H. The profile of the trematodicidal compound praziquantel. **Arzneimittelforschung**, v. 34, n. 9B, p. 1132-1136 1984.

WHO. **Schistosomiasis:** Schistosomiasis, 2010.Disponível em: http://www.who.int/mediacentre/facesheets/fs115/en/index.html. Oct 5

5 CONCLUSÕES

- A esquistossomose se mantem como problema de saúde pública no Brasil, onde ao serem avaliadas microrregiões é possível observar prevalências muito superiores aos dados do Ministério da Saúde.

- As populações rurais e urbanas compartilhavam marcantes semelhanças relativas as características epidemiológicas e demográficas.

- Repetidas rodadas de tratamento com o Praziquantel foi capaz de reduzir significativamente a prevalência da esquistossomose, a intensidade de infecção e os índices de incidência e reinfecção.

- As populações de *Schistosoma mansoni* avaliadas na zona rural e urbana demonstraram ser transmitidas localmente. Elas também demonstraram uma estabilidade no ponto de vista genético, com baixa influência migratória, indicando que os indivíduos tendem a se infectar na região e permanecer no ambiente ao longo da vida. Desta forma, eficientes intervenções pontuais nas regiões estudadas serão capazes de controlar a esquistossomose.

- As infecções persistentes, pós tratamento, fazem parte das populações susceptíveis, pré tratamento. Desta forma é improvável que a razão da persistência da infecção seja resistência ao praziquantel.

- Os parasitas persistentes ou não tratados de poucos indivíduos são capazes de recuperar qualitativamente a população parasitária de forma a representar a diversidade inicial pré-tratamento. Desta forma, medidas mais eficientes para a distribuição do tratamento, como administração de drogas em massa, aliadas a medidas de saneamento básico são as formas mais eficazes para o controle e eliminação o *Schistosoma mansoni.*

REFERÊNCIAS BIBLIOGRÁFICAS

BARAKAT, R.; EL MORSHEDY, H. Efficacy of two praziquantel treatments among primary school children in an area of high Schistosoma mansoni endemicity, Nile Delta, Egypt. **Parasitology**, v. 138, n. 4, p. 440-446, apr. 2010.

BARAKAT, R.*, et al.* The epidemiology of schistosomiasis in Egypt: patterns of Schistosoma mansoni infection and morbidity in Kafer El-Sheikh. **Am. J. Trop. Med. Hyg.**, v. 62, n. 2 Suppl, p. 21-27, feb. 2000.

BARBOSA, C. S.*, et al.* Current epidemiological status of schistosomiasis in the state of Pernambuco, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 549-554, jul. 2010.

BARRETO, M. L.*, et al.* Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies. **Lancet**, v. 370, n. 9599, p. 1622-1628, nov. 2007.

BERHE, N.*, et al.* Intensity of Schistosoma mansoni, hepatitis B, age, and sex predict levels of hepatic periportal thickening/fibrosis (PPT/F): a large-scale community-based study in Ethiopia. **Am. J. Trop. Med. Hyg.**, v. 77, n. 6, p. 1079-1086, dec. 2007.

BLACK, C. L.*, et al.* Impact of intense, longitudinal retreatment with praziquantel on cure rates of schistosomiasis mansoni in a cohort of occupationally exposed adults in western Kenya. **Trop. Med. Int. Health**, v. 14, n. 4, p. 450-457, apr. 2009.

BLANK, W. A.*, et al.* Host mouse strain is not selective for a laboratory adapted strain of Schistosoma mansoni. **J. Parasitol.**, v. 97, n. 3, p. 518-521, jun. 2011.

BLANK, W. A.*, et al.* Long-term genetic stability and population dynamics of laboratory strains of Schistosoma mansoni. **J. Parasitol.**, v. 96, n. 5, p. 900-907, oct. 2010.

BLANTON, R. E.*, et al.* Schistosoma mansoni population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. **Int. J. Parasitol.**, v. 41, n. 10, p. 1093-1099, aug. 2011.

BOTTIEAU, E.*, et al.* Imported Katayama fever: clinical and biological features at presentation and during treatment. **J. Infect.**, v. 52, n. 5, p. 339-345, may. 2006.

BRASIL. Ministério da Saúde. SVS. **Programa de Controle da Esquistossomose:** Programa de Controle da Esquistossomose, 1997. Disponível

em: <http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sinan/pce/cnv/pce.def>. Acesso em: 30 out. 2013.

BRITO, L. L.*, et al.* Moderate- and low-intensity co-infections by intestinal helminths and Schistosoma mansoni, dietary iron intake, and anemia in Brazilian children. **Am. J. Trop. Med. Hyg.**, v. 75, n. 5, p. 939-944, nov. 2006.

BUSH, A. O.*, et al.* Parasitology meets ecology on its own terms: Margolis et al. revisited. **J Parasitol**, v. 83, n. 4, p. 575-583, aug. 1997.

CARMO, E. H.; BARRETO, M. L. [Schistosomiasis mansoni in Bahia, Brazil: historical trends and control measures]. **Cad. Saude. Publica**, v. 10, n. 4, p. 425-439, dec. 1994.

CIOLI, D. Praziquantel: is there real resistance and are there alternatives? **Curr. Opin. Infect. Dis.**, v. 13, n. 6, p. 659-663, dec. 2000.

CIOLI, D.; PICA-MATTOCCIA, L. Praziquantel. **Parasitol. Res.**, v. 90 Supp 1, n., p. S3-9, jun. 2003.

COLLINS, H. E.*, et al.* A simple and accurate method for determination of microsatellite total allele content differences between DNA pools. **Hum. Genet.**, v. 106, n. 2, p. 218-226, feb. 2000.

COURA, J. R.; AMARAL, R. S. Epidemiological and control aspects of schistosomiasis in Brazilian endemic areas. **Mem. Inst. Oswaldo Cruz**, v. 99, n. 5 Suppl 1, p. 13-19 2004.

CURTIS, J.*, et al.* Widespread heteroplasmy in schistosomes makes an mtVNTR marker "nearsighted". **J. Hered.**, v. 92, n. 3, p. 248-253, jun. 2001.

CURTIS, J.*, et al.* Schistosome genetic diversity: the implications of population structure as detected with microsatellite markers. **Parasitology**, v. 125 Suppl, n., p. S51-59 2002.

DURAND, P.*, et al.* Isolation of microsatellite markers in the digenetic trematode Schistosoma mansoni from Guadeloupe island. **Mol. Ecol.**, v. 9, n. 7, p. 997-998, jul. 2000.

ENK, M. J.*, et al.* Factors related to transmission of and infection with Schistosoma mansoni in a village in the South-eastern Region of Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 570-577, jul. 2010.

FALCONER, D.; MACKAY, T. Genetic Constitution of a Population. In: P. E. Limited (Ed.). **Introduction to quantitative genetics**. England: Longman Pub Group, 1996. v. 4

FALLON, P. G.; DOENHOFF, M. J. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in Schistosoma mansoni in mice is drug specific. **Am. J. Trop. Med. Hyg.**, v. 51, n. 1, p. 83-88, jul. 1994.

FRENCH, M. D.*, et al.* Observed reductions in Schistosoma mansoni transmission from large-scale administration of praziquantel in Uganda: a mathematical modelling study. **PLoS Negl. Trop. Dis.**, v. 4, n. 11, p. e897 2010.

GALVAO, A. F.*, et al.* Spatial distribution of Schistosoma mansoni infection before and after chemotherapy with two praziquantel doses in a community of Pernambuco, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 555-562, jul. 2010.

GAZZINELLI, A.*, et al.* Socioeconomic determinants of schistosomiasis in a poor rural area in Brazil. **Acta Trop.**, v. 99, n. 2-3, p. 260-271, oct. 2006.

GRYSEELS, B. The relevance of schistosomiasis for public health. **Trop. Med. Parasitol.**, v. 40, n. 2, p. 134-142, jun. 1989.

GRYSEELS, B.*, et al.* Are poor responses to praziquantel for the treatment of Schistosoma mansoni infections in Senegal due to resistance? An overview of the evidence. **Trop. Med. Int. Health**, v. 6, n. 11, p. 864-873, nov. 2001.

GRYSEELS, B.*, et al.* Human schistosomiasis. **Lancet**, v. 368, n. 9541, p. 1106-1118, sep. 2006.

GUIMARAES, I. C.; TAVARES-NETO, J. [Urban transmission of schistosomiasis in children from a neighborhood of Salvador, Bahia]. **Rev. Soc. Bras. Med. Trop.**, v. 39, n. 5, p. 451-455, oct. 2006.

HANELT, B.*, et al.* A new approach to characterize populations of Schistosoma mansoni from humans: development and assessment of microsatellite analysis of pooled miracidia. **Trop. Med. Int. Health**, v. 14, n. 3, p. 322-331, mar. 2009.

HARTL, D. L.; CLARK, A. G. **Principles of Population Genetics**. Sunderland, MA: Sinauer Associates. 2007.

HODGES, M. H.*, et al.* Mass drug administration significantly reduces infection of Schistosoma mansoni and hookworm in school children in the national control program in Sierra Leone. **BMC Infect. Dis.**, v. 12, n., p. 16 2012.

IGREJA, R. P.*, et al.* A 15-year follow-up study on schistosomiasis in a low-endemic area in Rio de Janeiro State, Brazil. **J. Helminthol.**, v. 84, n. 3, p. 229-233, sep. 2009.

JOST, L. G(ST) and its relatives do not measure differentiation. **Mol. Ecol.**, v. 17, n. 18, p. 4015-4026, sep. 2008.

KABATEREINE, N. B.*, et al.* Epidemiology and morbidity of Schistosoma mansoni infection in a fishing community along Lake Albert in Uganda. **Trans. R. Soc. Trop. Med. Hyg.**, v. 98, n. 12, p. 711-718, dec. 2004.

KABATEREINE, N. B.*, et al.* Efficacy and side effects of praziquantel treatment in a highly endemic Schistosoma mansoni focus at Lake Albert, Uganda. **Trans. R. Soc. Trop. Med. Hyg.**, v. 97, n. 5, p. 599-603, oct. 2003.

KIROV, G.*, et al.* Pooled genotyping of microsatellite markers in parent-offspring trios. **Genome Res.**, v. 10, n. 1, p. 105-115, jan. 2000.

LOVERDE, P. T.*, et al.* Evidence for host-induced selection in Schistosoma mansoni. **J. Parasitol.**, v. 71, n. 3, p. 297-301, jun. 1985.

MANGES, A. R.*, et al.* Widespread distribution of urinary tract infections caused by a multidrug-resistant Escherichia coli clonal group. **N. Engl. J. Med.**, v. 345, n. 14, p. 1007-1013, oct. 2001.

MARTIN, S. Fewer Canadians being prescribed antibiotics. **CMAJ**, v. 166, n. 6, p. 798, mar. 2002.

MCMANUS, D. P.; LOUKAS, A. Current status of vaccines for schistosomiasis. **Clin. Microbiol. Rev.**, v. 21, n. 1, p. 225-242, jan. 2008.

NEVES, D. Schistosoma mansoni e a doença. In: (de MELO, A. L.; COELHO, P. M. Z.) **Parasitologia Médica.** São Paulo: Atheneu, 2005. v.12. p.192-211.

NORTON, A. J.*, et al.* Genetic consequences of mass human chemotherapy for Schistosoma mansoni: population structure pre- and post-praziquantel treatment in Tanzania. **Am. J. Trop. Med. Hyg.**, v. 83, n. 4, p. 951-957, oct. 2010.

ODONGO-AGINYA, E. I.*, et al.* Evidence of long term benefit of morbidity reduction due to praziquantel treatment against schistosoma mansoni in kigungu fishing village in entebbe, Uganda. **Afr. J. Infect. Dis.**, v. 5, n. 2, p. 33-39 2011.

PACEK, P.*, et al.* Determination of allele frequencies at loci with length polymorphism by quantitative analysis of DNA amplified from pooled samples. **PCR Methods Appl.**, v. 2, n. 4, p. 313-317, may. 1993.

PALMEIRA, D. C.*, et al.* [Prevalence of Schistosoma mansoni infection in two municipalities of the State of Alagoas, Brazil]. **Rev. Soc. Bras. Med. Trop.**, v. 43, n. 3, p. 313-317, jun. 2010.

PELLON, A. B.; TEIXEIRA, I. **Distribuição Geográfica da Esquistossomose Mansônica no Brasil**. Rio de Janeiro: Departamento Nacional de Saúde, Divisão Organização Sanitária. 1950.

PEREIRA, A. P.*, et al.* The prevalence of schistosomiasis in school-aged children as an appropriate indicator of its prevalence in the community. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 563-569, jul. 2010.

PEREIRA, W. R.*, et al.* Schistosoma mansoni infection in a rural area of the Jequitinhonha Valley, Minas Gerais, Brazil: analysis of exposure risk. **Acta Trop.**, v. 113, n. 1, p. 34-41, jan. 2010.

PICQUET, M.*, et al.* Efficacy of praziquantel against Schistosoma mansoni in northern Senegal. **Trans. R. Soc. Trop. Med. Hyg.**, v. 92, n. 1, p. 90-93, feb. 1998.

REIS, D. C.*, et al.* Accessibility to and utilisation of schistosomiasis-related health services in a rural area of state of Minas Gerais, Brazil. **Mem. Inst. Oswaldo Cruz**, v. 105, n. 4, p. 587-597, jul. 2010.

RODRIGUES, N. B.*, et al.* Characterization of new Schistosoma mansoni microsatellite loci in sequences obtained from public DNA databases and microsatellite enriched genomic libraries. **Mem. Inst. Oswaldo Cruz**, v. 97 Suppl 1, n., p. 71-75 2002.

ROLLEMBERG, C. V.*, et al.* [Epidemiological characteristics and geographical distribution of schistosomiasis and geohelminths, in the State of Sergipe, according to data from the Schistosomiasis Control Program in Sergipe]. **Rev. Soc. Bras. Med. Trop.**, v. 44, n. 1, p. 91-96, feb. 2011.

ROSS, K. G.*, et al.* Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant Solenopsis invicta. **Mol. Biol. Evol.**, v. 16, n. 4, p. 525-543, apr. 1999.

SAVIOLI, L.*, et al.* Schistosomiasis and soil-transmitted helminth infections: forging control efforts. **Trans. R. Soc. Trop. Med. Hyg.**, v. 96, n. 6, p. 577-579, dec. 2002.

SHAW, S. H.*, et al.* A genome-wide search for schizophrenia susceptibility genes. **Am. J. Med. Genet.**, v. 81, n. 5, p. 364-376, sep. 1998.

SILVA, L. K.*, et al.* Microsatellite analysis of pooled Schistosoma mansoni DNA: an approach for studies of parasite populations. **Parasitology**, v. 132, n. Pt 3, p. 331-338, mar. 2006.

SOUZA, S. S.*, et al.* Genetic population structure of cercariae from an urban foci of Schistosoma mansoni, Brazil. **Am. J. Trop. Med. Hyg.**, v. 87, n. 5, p. 843-849, nov. 2012.

STURROCK, H. J.*, et al.* Planning schistosomiasis control: investigation of alternative sampling strategies for Schistosoma mansoni to target mass drug

administration of praziquantel in East Africa. **Int. Health**, v. 3, n. 3, p. 165-175, sep. 2011.

UTZINGER, J.*, et al.* Efficacy of praziquantel against Schistosoma mansoni with particular consideration for intensity of infection. **Trop. Med. Int. Health**, v. 5, n. 11, p. 771-778, nov. 2000.

VIEIRA, J. B. F. O programa brasileiro de controle de esquistossomose. In: SIMPÓSIO INTERNACIONAL DE ESQUISTOSSOMOSE, 4., 1993, Rio de Janeiro: Fundação Oswaldo Cruz, 1993.

WAPLES, R. S. A generalized approach for estimating effective population size from temporal changes in allele frequency. **Genetics**, v. 121, n. 2, p. 379-391, feb. 1989.

WEBSTER, J. P.; DAVIES, C. M. Coevolution and compatibility in the snail-schistosome system. **Parasitology**, v. 123 Suppl, n., p. S41-56 2001.

WEGNER, D. H. The profile of the trematodicidal compound praziquantel. **Arzneimittelforschung**, v. 34, n. 9B, p. 1132-1136 1984.

WHO. **Schistosomiasis:** Schistosomiasis, 2013. Disponível em: <http://www.who.int/mediacentre/factsheets/fs115/en/index.html>. Acesso em: 5 oct.

ANEXO A - Comprovante de submissão do artigo para a *International Journal for Parasitology*

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Manuscript Draft

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Manuscript Region of Origin: BRAZIL

Abstract: Urbanization is an increasing trend across the globe, and diseases once considered rural can now be found in urban areas either due to the migration of population from rural endemic areas, the inability of city services to cope with rapid population growth or a combination of both factors. We investigated a focus of transmission of Schistosoma mansoni in Salvador, the third largest urban area of Brazil, and examined all inhabitants in 3 demarcate geographic areas to identify risk factors and understand the dynamics of urban transmission. We determined microsatellite allele frequencies for parasite infrapopulations and for component populations composed of individuals with the same epidemiologic characteristics. Many features of the urban infection were similar to those of rural areas. The peak age for intensity and prevalence was 15, and males were more affected than females. No differences were noted for parasite populations infecting individuals with differing epidemiologic characteristics, such as age or sex. There was little day-to-day differentiation in the allelic composition of excreted eggs. The pairwise infrapopulation differentiation (Jost's D 0.063) and the effective allele number (3.36) were also similar to values observed in a rural district >200 km from Salvador. Eggs from stool samples taken 7 years earlier showed little differentiation from the current population. This all indicates that the parasites being transmitted here represent a single stable population. This urban focus is primarily the result of local transmission rather than migration indicating municipal improvements in sanitation have been insufficient to interrupt transmission.

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Cover Letter

SCHOOL OF MEDICINE

CASE WESTERN RESERVE UNIVERSITY

Center for Global Health and Diseases

August 22, 2013

Dear Editors International Journal for Parasitology:

We submit for your consideration the manuscript entitled: Sources of Schistosomiasis Urbanization in Salvador, Bahia, Brazil. This work incorporates basic epidemiologic approaches with parasite population genetic analysis to understand the origin and transmission characteristics of an urban focus of schistosomiasis in Brazil. Urban disease is more and more becoming the focus of public health efforts if for no other reason than that is where most of the people are. We find their histories that most people probably acquired the infection locally and further supported this by finding infrapopulation differentiation in the city similar to that of a rural area (higher would be expected if migration was a significant factor) and importantly, very little differentiation from a sample collected 7 years ago. Parasite populations showed little host preference based on demographic characteristics, such as sex or age. There are important implications for public health given this pattern of transmission. Control in this urban area at least should be long lasting and little influenced by new immigration from other endemic areas with only local improvements in sanitation.

Yours,

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ANEXO B - Genetic Population Structure of Cercariae from an Urban Foci of *Schistosoma mansoni*

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

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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.[1] Since 2008, it has been responsible for 400–500 hospitalizations per year and a highly prevalent but poorly measured chronic burden of disease.[2–4] 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 km^2 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

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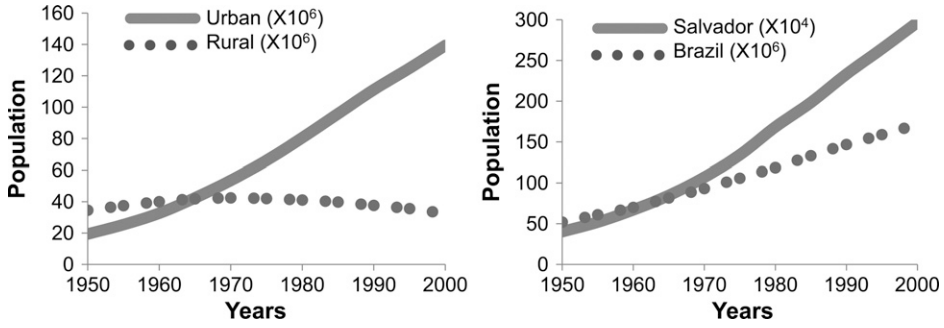

FIGURE 1. Urban growth in Brazil and Salvador. **A**, Rural urban population growth in Brazil, 1950–2000. **B**, Salvador, Brazil population growth, 1950–2000. Source: United Nations Department of Economic and Social Affairs/Population Division *World Urbanization Prospects: The 2005 Revision*, available at: http://www.un.org/esa/population/publications/WUP2005/2005WUP_DataTables12.pdf.

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 = 6.5×10^{-5} pg of DNA/cercaria; Rio do Cobre = 5.6×10^{-5} pg of DNA/cercaria; and Pituaçu = 7.5×10^{-5} 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.[16] 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 each contains 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 × *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 $1/\sum_{i=1}^{n} p_i^2$ where p_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[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}. Jost's D for the São Bartolomeu infrapopulations in

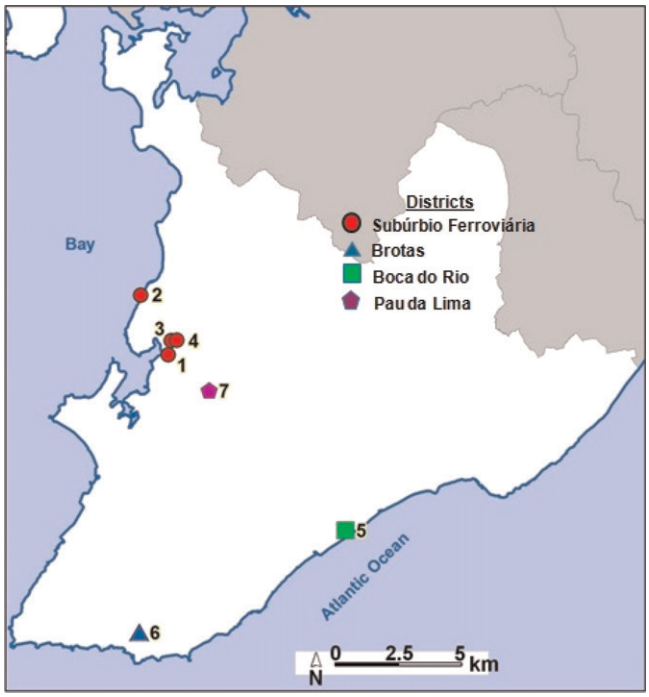

FIGURE 2. Infected snail locations, Salvador, Bahia, Brazil. *Schistosoma mansoni*–infected snail sites, Salvador, Brazil. Greater metropolitan Salvador is show in white. 1 = Dique do Cabrito; 2 = Itacaranhas; 3 = Rio do Cobre; 4 = Parque São Bartolomeu; 5 = Parque Pituaçu; 6 = Avenida Vasco da Gama; 7 = Lagoa do Urubú.

humans was determined by using the allele frequency from the six infections in each microarea weighted by the intensity of infection.[14] 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 Pituaçu. 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.[17] We also observed that

TABLE 1
Schistosoma mansoni sample characteristics, Brazil

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

*Estimated cercarial count by a quantitative polymerase chain reaction.
†Samples no longer available for counting.

TABLE 2

Schistosoma mansoni population genetic diversity, Brazil*

	Cercariae from field collections					Eggs from human infections				Cercariae	Worms	
Locus	1	2	3	5	7	8	9	10	11	12	13	Overall
SMMS2	1 (1.00)	–	3 (1.18)	4 (1.89)	–	3 (1.30)	2 (1.77)	2 (1.73)	2 (1.58)	2 (1.02)	4 (1.99)	4 (1.50)
SMMS16	3 (1.98)	2 (1.88)	5 (2.03)	6 (2.69)	3 (2.52)	6 (4.24)	6 (4.35)	7 (3.87)	7 (4.10)	3 (2.02)	6 (3.41)	7 (3.01)
SMMS3	2 (1.36)	–	6 (3.95)	7 (4.80)	–	–	12 (6.35)	10 (5.41)	12 (6.56)	5 (1.90)	7 (3.53)	12 (4.23)
SMMS17	3 (1.10)	–	4 (2.05)	5 (3.05)	–	4 (2.55)	4 (2.45)	4 (2.34)	5 (2.39)	2 (1.05)	6 (2.51)	5 (2.17)
SMMS18	5 (1.70)	2 (1.60)	8 (1.51)	9 (3.20)	–	7 (4.86)	9 (3.99)	11 (4.41)	9 (4.23)	11 (1.22)	12 (3.85)	12 (3.06)
SMMS21	3 (1.62)	1 (1.00)	3 (1.08)	3 (1.33)	–	4 (1.86)	5 (1.75)	5 (1.62)	5 (1.79)	4 (2.24)	4 (1.97)	5 (1.63)
SMDA23	8 (2.07)	2 (1.01)	9 (2.40)	7 (1.52)	–	9 (3.24)	12 (3.88)	11 (3.75)	11 (3.18)	8 (3.11)	11 (4.71)	12 (2.89)
1F8A	5 (1.11)	3 (1.98)	6 (2.92)	7 (1.97)	–	7 (3.84)	8 (3.31)	5 (4.02)	8 (3.75)	5 (1.38)	7 (3.75)	8 (2.80)
13TAGA	7 (2.84)	5 (2.15)	6 (2.44)	5 (1.50)	–	8 (8.46)	9 (2.83)	9 (3.24)	9 (3.37)	9 (3.03)	11 (2.99)	11 (3.29)
SM13-410	3 (2.03)	3 (2.11)	5 (2.78)	5 (3.03)	–	4 (2.35)	5 (2.14)	4 (2.04)	5 (2.24)	4 (2.10)	4 (2.14)	5 (2.30)
SMU31768	10 (2.00)	12 (4.28)	13 (3.64)	12 (4.55)	–	12 (5.25)	13 (4.15)	13 (4.04)	13 (4.81)	10 (3.01)	12 (3.87)	13 (3.96)
15J15A	8 (2.73)	5 (2.22)	7 (2.74)	8 (4.72)	–	8 (4.32)	9 (3.93)	9 (3.91)	9 (3.78)	8 (3.29)	5 (2.42)	9 (3.41)
29E6A	7 (1.73)	5 (2.36)	8 (2.63)	6 (2.96)	4 (2.83)	6 (4.06)	6 (3.90)	6 (3.72)	8 (3.90)	7 (2.22)	7 (3.77)	9 (3.10)
SM13-478	9 (2.83)	4 (2.45)	7 (2.31)	7 (2.55)	2 (1.98)	8 (5.17)	12 (5.00)	11 (4.66)	12 (5.19)	9 (2.69)	5 (1.57)	12 (3.31)
Total	74 (1.86)	44 (2.10)	90 (2.41)	91 (2.75)	9 (2.44)	86 (3.96)	112 (3.56)	107 (3.48)	115 (3.63)	87 (2.09)	101 (2.95)	124 (2.90)

*Values indicate observed number of alleles (effective number of alleles). Sample numbers are as in Table 1; samples 4 and 6 did not amplify by polymerase chain reaction. The total for each site indicates the sum of the number of observed alleles and the average of the effective allele number.

samples from humans in the same community (Table 3). São 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 characteristic 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*

Location	Cercariae from field collections				Eggs in human infections				Laboratory life cycle of worms	
	Dique do Cabrito	Itacaranhas	Rio do Cobre	Pituaçú	São Bartolomeu†	MA1	MA3	MA6	Feira de Santana	CWRU
Dique do Cabrito	–	0.61 (0.60–0.63)	0.50 (0.50–0.51)	0.37 (0.36–0.37)	0.34 (0.33–0.35)	0.31 (0.31–0.32)	0.37 (0.36–0.37)	0.34 (0.34–0.34)	**0.02 (0.01–0.02)**	0.45 (0.44–0.46)
Itacaranhas		–	0.52 (0.50–0.54)	0.31 (0.28–0.33)	0.35 (0.33–0.37)	0.36 (0.35–0.38)	0.33 (0.31–0.35)	0.34 (0.32–0.36)	0.60 (0.59–0.62)	0.50 (0.47–0.52)
Rio do Cobre			–	0.30 (0.30–0.31)	0.26 (0.25–0.26)	0.26 (0.26–0.27)	0.28 (0.27–0.28)	0.25 (0.25–0.25)	0.51 (0.51–0.51)	0.36 (0.35–0.37)
Pituaçú				–	0.16 (0.15–0.17)	0.18 (0.17–0.18)	0.17 (0.17–0.18)	0.17 (0.17–0.18)	0.37 (0.37–0.38)	0.31 (0.30–0.32)
São Bartolomeu					–	**0.02 (0.02–0.02)**	**0.03 (0.02–0.03)**	**0.01 (0.01–0.01)**	0.33 (0.32–0.34)	0.22 (0.21–0.23)
MA1						–	**0.02 (0.02–0.03)**	**0.01 (0.01–0.01)**	0.30 (0.30–0.30)	0.22 (0.21–0.22)
MA3							–	**0.02 (0.02–0.02)**	0.36 (0.35–0.36)	0.23 (0.23–0.24)
MA6								–	0.33 (0.33–0.33)	0.23 (0.22–0.24)
Feira de Santana									–	0.44 (0.43–0.44)
CWRU										–

*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.

Damietta, Egypt, in which transmission was low because of intensive control efforts, the effective allele number (3.22, as calculated from expected heterozygosity) was also much lower than those for sites in Kenya.[29]

The methods used in these studies were also different from those used in our study. In the study of cercariae from Lake Victoria, genotypes were determined on pools of cercariae used to infect mice and obtain adult worms for DNA from discrete organisms. The study of cercariae in Egypt used total shed cercariae from individual snails for genotyping. In our study, we genotyped the pool of all cercariae from all snails at each site. Although the prevalence of infection was relatively high in the sites with infection in Salvador, we still are likely to have sampled fewer unique snails than the study in Kenya (where up to 5,000 snails were collected at a single site), resulting in our undersampling and greater apparent differentiation in the sample from Brazil.

Because the methods used to survey were not intended to be exhaustive, our results likely underestimated the number of infected sites. Infected snails were found at only 6% of sites with *B. glabrata.* Snail collections were not performed at the same time of year for all areas, and there is great variability in the snail population depending on seasonal climatic conditions. During the period of fieldwork, access to some locations was not possible because of flooding, and certain neighborhoods were occasionally inaccessible for other safety reasons. Methodologically, the snails collected tended to be larger, older individuals and these are notoriously more refractory to infection[30] and may represent self-cured snails. Approximately 30 years ago, *B. glabrata* in some areas of Salvador was extensively studied by multiple groups and appeared particularly resistant to infection with allopatric strains of *S. mansoni.*[7,30] However, the prevalence of infection for infected sites in this study was surprisingly high and likely contributes to maintenance of the parasite in many areas of the city. The active transmission of *S. mansoni* in the city implies that efforts must continue and expand to find and treat those infected and to prevent human fecal contamination of fresh water supplies.

In evaluating parasite population structure, different answers may be obtained depending on the developmental stage sampled. A comprehensive collection of *S. mansoni* eggs or miracidia can represent adult worm genotypes present in the human population.[31] However, cercariae collected at one time point reflect the population in snails over approximately the past three months. Cross-sectional collection of snails at one time point combined with the limited capacity of a single snail to accept more than a few individual miracidia make it unlikely that this stage will represent the human population very well. In addition, Sturrock and others[32] showed that snail infections probably occur in pulses and not as a continuous flow of miracidia, further intensifying an irregular temporal distribution of parasite genotypes. Despite heavy snail infections, our results show decreased parasite diversity in cross-sectional populations in snail hosts relative to human hosts.

The differentiation between snail infections in different regions of the same city contrasts strongly with the stability and similarity of human infections in one neighborhood. Jost's *D* was notably small between the São Bartolomeu microareas in 2011 and the pooled sample from children in São Bartolomeu collected seven years earlier. The small size of the sample, the combination of adults and children, the greater diversity in infrapopulations in humans, and the fact

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.

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REFERENCES

1. Katz N, 1998. Schistosomiasis control in Brazil. *Mem Inst Oswaldo Cruz 93 (Suppl 1):* 33–35.
2. Assis AM, Prado MS, Barreto ML, Reis MG, Conceicao Pinheiro SM, Parraga IM, Blanton RE, 2004. Childhood stunting in northeast Brazil: the role of *Schistosoma mansoni* infection and inadequate dietary intake. *Eur J Clin Nutr 58:* 1022–1029.
3. Brito LL, Barreto ML, Silva Rde C, Assis AM, Reis MG, Parraga IM, Blanton RE, 2006. Moderate- and low-intensity co-infections by intestinal helminths and *Schistosoma mansoni*, dietary iron intake, and anemia in Brazilian children. *Am J Trop Med Hyg 75:* 939–944.
4. Stephenson L, 1993. The impact of schistosomiasis on human nutrition. *Parasitology 107 (Suppl):* S107–S123.
5. Ministério da Saúde, 2007. *Vigilância e Controle de Moluscos de Importância Epidemiológica: Diretrizes Técnicas: Programa de Vigilância e Controle da Esquistossomose (PCE)*. Brasilia: Editora MS Documentação e Informação.
6. Guimaraes IC, Tavares-Neto J, 2006. Urban transmission of schistosomiasis in children from a neighborhood of Salvador, Bahia [in Portuguese]. *Rev Soc Bras Med Trop 39:* 451–455.
7. Barbosa FS, Barreto AC, 1960. Differences in susceptibility of Brazilian strains of *Australorbis glabratus* to *Schistosoma mansoni*. *Exp Parasitol 9:* 137–140.
8. Barbosa CS, Araujo KC, Sevilla MA, Melo F, Gomes EC, Souza-Santos R, 2010. Current epidemiological status of schistosomiasis in the state of Pernambuco, Brazil. *Mem Inst Oswaldo Cruz 105:* 549–554.
9. Coura-Filho P, 1997. *Schistosomiasis mansoni* in urban territory. 2. A theoretical approach to the accumulation, concentration, and centralization of capital and the production of disease [in Portuguese]. *Cad Saude Publica 13:* 415–424.
10. Enk MJ, Amorim A, Schall VT, 2003. Acute schistosomiasis outbreak in the metropolitan area of Belo Horizonte, Minas Gerais: alert about the risk of unnoticed transmission increased by growing rural tourism. *Mem Inst Oswaldo Cruz 98:* 745–750.
11. Firmo JO, Lima Costa MF, Guerra HL, Rocha RS, 1996. Urban schistosomiasis: morbidity, sociodemographic characteristics and water contact patterns predictive of infection. *Int J Epidemiol 25:* 1292–1300.
12. Kloos H, Correa-Oliveira R, dos Reis DC, Rodrigues EW, Monteiro LA, Gazzinelli A, 2010. The role of population movement in the epidemiology and control of schistosomiasis in Brazil: a preliminary typology of population movement. *Mem Inst Oswaldo Cruz 105:* 578–586.
13. Ximenes RA, Southgate B, Smith PG, Guimaraes Neto L, 2000. Migration and urban schistosomiasis. The case of Sao Lourenco da Mata, northeast of Brazil. *Rev Inst Med Trop Sao Paulo 42:* 209–217.
14. Blanton RE, Blank WA, Costa JM, Carmo TM, Reis EA, Silva LK, Barbosa LM, Test MR, Reis MG, 2011. *Schistosoma mansoni* population structure and persistence after praziquantel treatment in two villages of Bahia, Brazil. *Int J Parasitol 41:* 1093–1099.
15. IGBE, 2010. *National Census: Total Population of Bahia (in Portuguese)*. Accessed 2011. Available at: http://www.ibge.gov.br/home/estatistica/populacao/censo2010/tabelas_pdf/total_populacao_bahia.pdf.
16. Souza C, Lima L, Konovaloff L, 1997. Freshwater mollusks of the Belo Horizonte, MG microregion with emphasis on parasite vectors [in Portuguese]. *Rev Soc Bras Med Trop 3:* 449–456.
17. Blank WA, Reis EA, Thiong'o FW, Braghiroli JF, Santos JM, Melo PR, Guimaraes IC, Silva LK, Carmo TM, Reis MG, Blanton RE, 2009. Analysis of *Schistosoma mansoni* population structure using total fecal egg sampling. *J Parasitol 95:* 881–889.
18. Gomes AL, Melo FL, Werkhauser RP, Abath FG, 2006. Development of a real time polymerase chain reaction for quantitation of *Schistosoma mansoni* DNA. *Mem Inst Oswaldo Cruz 101 (Suppl 1):* 133–136.
19. Kimura M, Crow JF, 1964. The number of alleles that can be maintained in a finite population. *Genetics 49:* 725–738.
20. Weir BS, 1996. *Population Structure. Genetic Data Analysis II*. Sunderland, MA: Sinauer Associates, 161–198.
21. Jost L, 2008. G_{ST} and its relatives do not measure differentiation. *Mol Ecol 17:* 4015–4026.
22. Chao A, Shen TJ, 2010. *SPADE (Species Prediction And Diversity Estimation). Program and User's Guide*. Available at: http://chao.stat.nthu.edu.tw.
23. Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P, 2010. Calculations of population differentiation based on GST and D: forget GST but not all of statistics! *Mol Ecol 19:* 3845–3852.
24. Leng L, Zhang DE, 2011. Measuring population differentiation using GST or D? A simulation study with microsatellite DNA markers under a finite island model and nonequilibrium conditions. *Mol Ecol 20:* 2494–2509.
25. Amaral RS, Tauil PL, Lima DD, Engels D, 2006. An analysis of the impact of the Schistosomiasis Control Programme in Brazil. *Mem Inst Oswaldo Cruz 101 (Suppl 1):* 79–85.
26. Steinauer ML, Mwangi IN, Maina GM, Kinuthia JM, Mutuku MW, Agola EL, Mungai B, Mkoji GM, Loker ES, 2008. Interactions between natural populations of human and rodent schistosomes in the Lake Victoria region of Kenya: a molecular epidemiological approach. *PLoS Negl Trop Dis 2:* e222.
27. Ritchie LS, Hemandes A, Rosa-Amador R, 1966. Biological potential of *Australorbis glabrata*, life span and reproduction. *Am J Trop Med Hyg 15:* 614–617.
28. Steinauer ML, Hanelt B, Agola LE, Mkoji GM, Loker ES, 2009. Genetic structure of *Schistosoma mansoni* in western Kenya: the effects of geography and host sharing. *Int J Parasitol 39:* 1353–1362.

29. Lotfy WM, Hanelt B, Mkoji GM, Loker ES, 2011. Genotyping natural infections of *Schistosoma mansoni* in *Biomphalaria alexandrina* from Damietta, Egypt, with comparisons to natural snail infections from Kenya. *J Parasitol 97:* 156–159.
30. Michelson EH, DuBois L, 1978. Susceptibility of Bahian population of *Biomphalaria glabrata* to an allopatric strain of *Schistosoma mansoni*. *Am J Trop Med Hyg 27:* 782–786.
31. Blank WA, Test MR, Liu SF, Lewis FA, Blanton RE, 2010. Long-term genetic stability and population dynamics of laboratory strains of *Schistosoma mansoni*. *J Parasitol 96:* 900–907.
32. Sturrock RF, Karamsadkar SJ, Ouma J, 1979. Schistosome infection rates in field snails: *Schistosoma* mansoni in *Biomphalaria pfeifferi* from Kenya. *Ann Trop Med Parasitol 73:* 369–375.