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Repeated praziquantel treatments remodel the genetic and spatial landscape of schistosomiasis risk and transmission

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ABSTRACT

Repeated treatments with praziquantel reduce schistosomiasis prevalence and morbidity, but transmission persists and populations often recover within a few years. To identify factors associated with persistence, we surveyed and treated all identified *Schistosoma mansoni* infections in two rural Brazilian communities (Jenipapo and Volta do Rio) in 2009, 2012 and 2013. Eggs were collected from all infected individuals and genotyped with 11 microsatellite markers to evaluate parasite differentiation and diversity. After successive rounds of community-wide treatment, prevalence decreased from 45% to 24% then 16%. Intensity of infection decreased by 57% over this period, and the number of eggs transmitted to the environment decreased by 92%. During all time periods the majority of eggs were excreted by those >15 years of age. The incidence was 23% in 2012 and 15% in 2013, consistent with a decrease in transmission. There was little immigration or gene flow over a distance of 6 km. On reinfection, infrapopulations were moderately differentiated indicating that pretreatment multilocus genotypes were not fully reacquired. The effective population size responded to census population decline more rapidly than differentiation. Reinfection was concentrated in the downstream portion of Jenipapo, consistent with the observed increased human fecal contamination. At this scale and in this area *S. mansoni* infections exist on a fragmented landscape with a highly focal pattern of transmission that may facilitate future elimination.

1. Introduction

Despite advances in the treatment and prevention of schistosomiasis, it remains a major public health problem in Brazil and much of the world (World Health Organization (WHO), Schistosomiasis: Fact sheet N°115, 2014, http://www.who.int/mediacentre/factsheets/fs115/en/). In Brazil, estimates of those infected with *Schistosoma mansoni* range from 2.5 to 12 million, and more than 30 million are at risk of infection (Katz and Peixoto, 2000). Control strategies focusing on treatment with antiparasitic drugs were initiated in the 1970s. This reduced overall prevalence in endemic areas from 25% to approximately 5% by 2011, with consequent steep reductions in morbidity (Carmo and Barreto, 1994; Coura and Amaral, 2004; Amaral et al., 2006) and mortality (Ferreira and Tabosa e Silva, 2007), but without eliminating transmission. There is no sterile immunity to schistosomes, thus reinfection is common.

As emphasis shifts from control to elimination, it is important to better understand the parasite's local population dynamics under pressure from antiparasitic treatments. Population genetics offers tools that can uncover many of the forces that modify parasite populations and may be useful for understanding parasite persistence despite repeated treatments. Population genetics has particularly been applied to problems of conservation in order to plan for the maintenance and health of organisms and ecosystems (Spielman et al., 2004; Frankham, 2005). By contrast, in the management of pathogens, extinction is the desired outcome, and identifying predictive markers and tracking progress toward this end are important objectives.

To date, few studies have tracked schistosome populations longitudinally following treatment and these report differing results.

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Significant parasite allele frequency differences were found in children in Tanzania before and after school-based treatment, suggesting that a single round of treatment produced a significant genetic bottleneck (Norton et al., 2010). In Senegal, a cohort of children was treated up to five times over 2 years (Huyse et al., 2013). After an initial decrease in prevalence, infection recurred with generally higher intensity in subsequent surveys. No change was found in parasite allelic richness, heterozygosity, inbreeding coefficient or fixation index. A 4 year mass drug administration program in Kenya showed no change in parasite genetic diversity in children with no decline in infection intensity (Lelo et al., 2014). These studies may not be contradictory since the dynamics of transmission vary between sites. Each study followed up school-based treatment and all examined small samples of parasites from small samples of infected individuals. While these are useful and provocative initial studies, they do not address the effect of community-wide treatment, the problem of reinfection versus persistence and in one case (Norton et al., 2010) does not reflect the result of individual reinfection. More and diverse sites need to be studied given the heterogeneity in worldwide human and parasite populations as well as the variation in environmental contexts. We propose that using "reverse" conservation genetics can contribute to planning and understanding the requirements for parasite elimination.

In 2009 we began studies on a rural focus of *S. mansoni* transmission in two small communities in Brazil. We registered host epidemiologic characteristics and parasite allele frequencies, and treated all of those infected (Barbosa et al., 2013). This population was then followed for two additional rounds of treatment over a 4 year period, and the longitudinal effects of treatment on parasite population dynamics and structure were analyzed.

2. Materials and methods

2.1. Study sites and written consent

The study was conducted in two rural villages, Jenipapo and Volta do Rio, along the Jiquiriçá River in the northeastern Brazilian state of Bahia. The communities each have 300–400 residents, are 6 km apart by road, share schools and health services, and they are each 10–15 km from larger towns (Fig. 1). This relative isolation, together with the prevalence of schistosomiasis in the area, were the main reasons for choosing these sites. The two communities have been described previously (Blanton et al., 2011; Barbosa et al., 2013).

Written consent was obtained from all participants or their guardians, and the study adhered to procedures approved by the Committee on Ethics in Research of the Oswaldo Cruz Foundation of Salvador, Bahia, Brazil, the Brazilian National Committee on Ethics in Research and the Institutional Review Board for Human Investigation of University Hospitals Case Medical Centre, Cleveland, Ohio, USA.

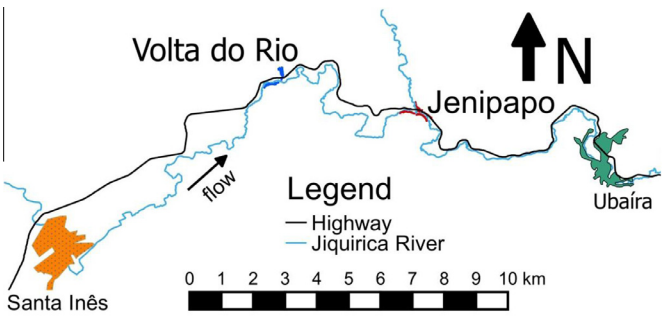

Fig. 1. Map of Ubaíra and Santa Inês areas in Brazil. Jenipapo and Volta do Rio are discrete communities 6 km apart, and each is 9 km distant from urbanised centres.

2.2. Study design

Epidemiologic and parasitologic surveys were conducted in both communities for all inhabitants ⩾1 year old who agreed to participate in 2009 and 2012 (Blanton et al., 2011; Barbosa et al., 2013). Only Jenipapo was studied in 2013 (Fig. 2). Information collected during each year of the study was double entered into the program Epi Info version 7 (Centers for Disease Control, Atlanta, GA, USA). For the parasitological survey, three stool samples were collected on different days from each resident and examined by the Kato-Katz technique for *S. mansoni* infection status and intensity recorded as eggs per gram (epg) of feces. Residents were directed to provide a whole stool for each examination. Infected individuals were treated with a single oral dose of praziquantel (50 mg/kg) according to Brazilian Ministry of Health guidelines (Ministério da Saúde, 2010). The first use of praziquantel in these communities was in 2009, and all subsequent treatments were directly observed. Those found to have soil-transmitted helminths received treatment with mebendazole. Four weeks after treatment, three stools collected on different days were again examined from *S. mansoni*-positive individuals, and those still positive were re-treated with mebendazole.

2.3. Microsatellite genotyping

All procedures and primers were previously described (Blank et al., 2009; Barbosa et al., 2013). Briefly, *S. mansoni* eggs were concentrated from the whole stool by selective sieving and sedimentation, and extracted DNA was PCR-amplified with a panel of primers for 11 microsatellites (Supplementary Table S1). The amplification products 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, USA) was used to determine peak heights from which relative allele frequencies were calculated. Successful PCRs were defined as those in which there was at least one peak >500 pixels and measured peaks fell within the size range expected for a given marker. Peaks <100 pixels were excluded from analysis. If both duplicate samples amplified, their mean allele frequency was used. Subsequent population analyses were limited to those samples for which at least eight of 11 markers were successfully genotyped.

2.4. Data analysis

2.4.1. Reinfection rates and statistical analyses

Reinfection rates were calculated for individuals who were successfully treated in the previous year. Categorical data were

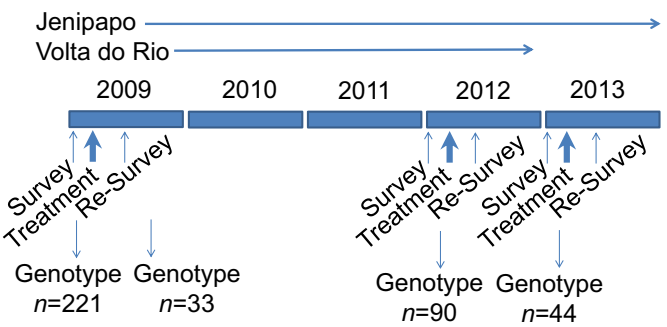

Fig. 2. Study design. Demographic and parasitologic surveys were conducted in 2009, 2012 and 2013. Those with stools positive for *Schistosoma mansoni* eggs were treated with praziquantel and re-examined after 4–6 weeks. Those with persistent infections were retreated. In 2012 and 2013 there were two and zero persistent infections, respectively.