

The spatial distribution of *Schistosoma mansoni* infection before and after chemotherapy in the Jequitinhonha Valley in Brazil

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*Schistosomiasis prevalence and egg counts remained low one year after chemotherapy in most households in a hyperendemic rural area in northern Minas Gerais but several distinct spatial patterns could be observed in relation to IgE levels and to a lesser extent to exposure risk (TBM) and type of water supply. An inverse relationship between pre-treatment household prevalence and egg counts on the one hand and post-treatment IgE levels on the other were noted in two of the five communities. Low exposure risk was associated with the low pre-treatment infection rates in the central village but did not contribute to the decline of infection rates after chemotherapy in the study area, as indicated by the significant increase in water contact during the posttreatment period ($p < 0.0001$). Distance between households and the streams and socioeconomic factors were also unimportant in predicting the spatial distribution of infection. These results are consistent with the production and antiparasitic effect of high levels of IgE in *Schistosoma mansoni* infection.*

Key words: schistosomiasis - chemotherapy - spatial clustering - IgE antibodies - exposure risk - nursing - Brazil

Geographical Information Systems (GIS) are increasingly being used in epidemiological and ecological studies of schistosomiasis. Most studies, many of them using GIS in combination with remote sensing techniques, have been carried out at the regional level (Bavia et al. 1999, Brooker & Michael 2000a, Malone et al. 2001, Handzel et al. 2003). The World Health Organization (WHO) recently emphasized the need for studies at the community level in areas where schistosomiasis is gradually being controlled. This includes Brazil, where the national program is making inroads into the prevalence, intensity and pathology of schistosomiasis mostly through chemotherapy and safe water supplies, and where further environmental measures as well as health education have been called for (Katz 1998, WHO 2001).

Schistosomiasis tends to cluster within communities, at both the neighborhood and household levels, due to the characteristic focality of risk behavior and transmission (Barreto 1991, Kloos et al. 1998, Bethony et al. 2004). At the household level, spatial information on schistosomiasis can provide information on socioeconomic, behavioral, and genetic factors in parasite transmission and treat-

ment because of the socioeconomic cohesion and associated exposure risk and health-seeking behavior of families (Berman et al. 1994, Cairncross et al. 1996, Bethony et al. 2002). In addition, intensity of schistosomiasis infection has been found to decline with increasing distance between households and infective water sources (Kloos et al. 1998, McClennon et al. 2004).

The effect of chemotherapy on the spatial distribution of schistosomiasis is poorly known. Different treatment strategies have been associated with fairly predictable reductions in pretreatment infection intensities and transmission levels in Kenya (Butterworth et al. 1991, Sturrock et al. 1994). Similarly, in Côte d'Ivoire, egg output and cure rates were associated by different investigators with pretreatment levels, but additional factors such as age, multiple infections and humoral responses also influenced treatment outcomes (Raso et al. 2004). In a small town in Brazil, Kloetzel (1989) found no significant difference in the spatial distribution and overall level of mean egg counts among 5-14 year olds in different neighborhoods between pre-treatment and 11 months after mass chemotherapy.

The objective of this study is to describe and analyze the spatial distribution of *Schistosoma mansoni* infection, immunological responses and exposure risk in Virgem das Graças, Minas Gerais, Brazil, during pre- and post-treatment.

MATERIALS AND METHODS

Study area and population - Virgem das Graças is a rural area located in the Jequitinhonha Valley in northern Minas Gerais, a semi-arid, poor region of outmigration

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(Goza 1992). According to our census in 2001, 589 people lived in the Virgem das Graças study area in 158 households. This population lived in four dispersed hamlets (Cardoso 1, 2, 3, and Suçuarana) along the main Cardoso Stream and its tributaries and in a central village. The local population depends on subsistence farming based on the staples corn and manioc, cattle husbandry, and remittances from migrants working in cities. The median age was 24 years and 75% of the population were 49 years or younger. This isolated mountainous area, which can be reached only by an unpaved road, ranges in altitude from about 750 to 1000 m (Fig. 1).

GPS maps - Every house, the streams and commonly used water contact sites in Virgem das Graças were mapped using a differential Global Positioning System (DGPS) (AgGPS132, Trimble Navigation Limited, Sunnyvale, CA, US). DGPS is used to remove much of the error associated with ordinary Global Positioning System (GPS) mapping methods. GPS error was computed by comparing GPS readings at a known location for all available GPS satellites with the location's true coordinates. This error term was used to calibrate the field mapping work, reducing horizontal errors to an average of less than 3 m. The TSC1 Asset Surveyor with Pro XRS receiver was used to enter the data and PathFinder software (v. 2.90) was used for data transfer and differential correction (Trimble Navigation Ltd, Sunnyvale, CA, US).

Parasitological examination and antibody characteristics (laboratory methods) - A parasitological survey was carried out in January 2001 and a second one in May 2002, one year after chemotherapy. All study members received three containers to deposit one fecal sample per day during three consecutive days and return the containers immediately to the collection points, where the samples were stored at 4°C. Slides were made using the Kato-Katz thick smear technique (Katz et al. 1972), with

two slides being prepared from each day's stool sample, for a total of six slides per individual. Three laboratory technicians carried out the microscopic examination at a field laboratory in Virgem das Graças. Intensity of infection was determined from the mean *Schistosoma mansoni* egg counts (epg) of each individual from the six slides.

IgE - Five millimeters of blood were collected in March 2001 and again in June 2002 in vacuutainers containing heparin for indirect ELISA by two trained technicians from Universidade Vale do Rio Doce - Univale. The sera collected were stored in appropriate boxes and sent to the Cellular and Molecular Immunology Laboratory of the Centro de Pesquisas René-Rachou-Fiocruz, where immunological assays were performed.

Levels of Total IgE were determined in the patients' plasma by indirect ELISA to evaluate the concentrations of total IgE. One hundred microliters of anti-IgE monoclonal antibody in 0.05 M carbonate-bicarbonate buffer, pH 9.6 at the concentration of 5 µg/ml was added to each well of a polystyrene 96 wells plate (Maxisorb; Nunc, Roskild, Denmark). Each plate had its independent Standard curve using monoclonal IgE antibodies obtained commercially. The plates were incubated overnight at 4°C. After incubation the plates were washed with PBS 0.15 M (pH 7.2) and blocked with PBS containing 0.5% of Tween 20 (PBS-T) (Sigma, St Louis, MO), and 10% bovine fetal calf serum. After blockage the plates were again washed with PBS-T and the serum samples at a dilution of 1:100 added to the wells. The plates were incubated for 1 h, washed with PBS-T and 100 µl of streptavidin horseradish peroxidase (Amersham, Piscataway, NJ) at 1:1000 dilution and incubated for 90 min at room temperature washed with PBS-T and 100 µl of o-phenylenediamine (OPD) (Sigma) containing 0.03% hydrogen peroxidase added to each well. Optical density was measured at 490 nm and the concentration determined based on the standard curve.

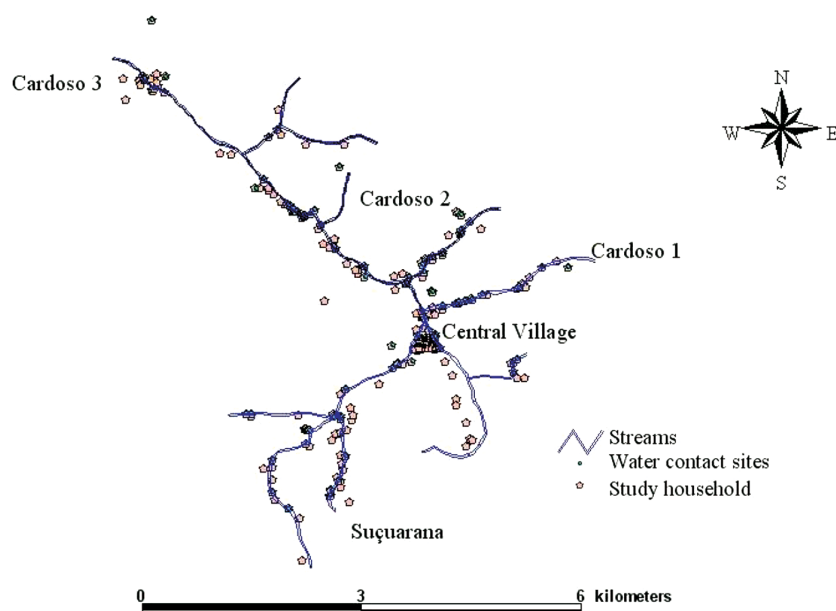


Fig. 1: the Virgem das Graças study area: study households, streams, and water contact sites.

Chemotherapy - All individuals infected with *S. mansoni* were treated on May 2001 with a single oral dose of 50 mg/kg of praziquantel and those infected with *Ascaris lumbricoides*, *Trichuris trichura* or hookworm with a single oral dose of 400 mg of albendazol. A registered nurse of the project was responsible for the treatment that was done either in the Field Laboratory or in the patients' houses in cases of elderly patients or when they live far from the village. A parasitological exam was performed 40 to 50 days after treatment to evaluate the effectiveness of the drugs using again the same parasitological methods.

Exposure risk (TBM) and water contact sites - Using a combination of direct observations and household surveys, we collected information on three exposure parameters: frequency, duration and intensity of contact with water in any water bodies where *Biomphalaria glabrata* had been found (streams, canals, ponds, and unprotected springs) (Kloos et al. 2004). These water contacts were designated as unsafe and used to develop a water exposure index (TBM, or total body minutes), as described by Gazzinelli et al. (2001). A total of 106 household water contact sites and other frequently used sites were identified during community-wide snail surveys and during direct observations of water contact (Figs 1, 4) (Kloos et al. 2004, 2006). Both unsafe TBM and frequency of water contact were mapped.

Mapping of household infections, antibody levels, and exposure - Prevalence and intensity of infection, the IgE antibody response to infection, as well as TBM were mapped in the search for spatial patterns. Average household values were calculated by summing infection levels and antibody responses of all tested persons in a given household and then dividing the sum by the total number of tested household residents. To evaluate the impact of mass treatment for schistosomiasis, we monitored a cohort of persons during both the pre- and post- periods on the three different types of parasitic infection.

Statistical methods - Univariate differences in proportions (Ag and antibody prevalence) were analyzed using the Mantel-Haenszel chi-square or the Fisher exact tests. Univariate differences in continuous measures between groups (antibody level and average distance to the residence an Ag-positive person) were compared using pooled t- and Kruksal-Wallis tests. Univariate analyses on paired data (pre- and post-MDA values) used Wilcoxon matched-pair signed rank tests for continuous variables and McNemar's test for binary variables. All p-values were computed in a manner to account for sample size.

Multiple linear regression conducted on the cohort was used to investigate the association of distance to the nearest household water source, TBM and of different types of water exposure on the measures of infection intensity and reactivity. The distance was computed using a standard formula that measures arc length and therefore accounts for the curvature in the earth's surface. Since multiple people from a single household could be included in our analyses, we used generalized estimating equation methodology. We assumed an exchangeable correlation

structure, which assumes a constant correlation between all persons within a given household. Results were adjusted for age group and log transformation was used on continuous variables, including egg counts and distance. The SAS (v9.1), SAS Institute Inc., Cary, NC, US) procedure PROC GENMOD was used for this modeling.

Contour maps and spatial cluster assessment - The observed household means are shown as dots which are proportionate in size to the magnitude of the infection or antibody measure size. These measures were based on quintiles using the pre-treatment program data. To facilitate assessment of the impact of the treatment program, the contour categories quintiles based on pretreatment values are used for contours for both pre and posttreatment maps. To provide a better descriptive representation of the data, a smoothed contour map was created by using linear inverse density weighting of the distance for the eight nearest neighbors for each household to create the contours. Each color shade represents an approximate quintile for the smoothed data. To facilitate assessment of the impact of the treatment program, the contour categories quintiles were based on pretreatment values are used for contours for both pre and posttreatment maps. ArcView (v3.3) and the Spatial Analyst Extension (v2.0) were used to produce IgE antibody level maps (Environmental Systems Research, Inc., Redlands, CA, US). Spatial clustering was assessed using SatSCAN (v. 5.0; National Cancer Institute, Bethesda, MD, US) (Kulldorf 1997, Kulldorf and Information Management Services Inc 2005); the number procedure PROC GENMOD was used for this modeling. The number of events in an area was compared to the number expected based on a Poisson distribution with a known underlying population at risk

RESULTS

Schistosomiasis prevalence - Prior to the treatment program, the prevalence of *S. mansoni* infection was very high. In the 177 households and 589 people participating in the pre-treatment study, the average household prevalence was over 60%. One group of households, in Cardoso 3 hamlet, had an average prevalence of over 90%. Several other small areas, in Suçuarana and in Cardoso 2, had average prevalences between 75 and 90%. A high-prevalence cluster was found in Cardoso 3, where all 18 persons tested in the seven homes were positive. This represented a significant cluster ($p = 0.029$) (Fig. 2a). The prevalence of above-average egg counts was 1.5 times higher than expected. Three smaller groups of households with high prevalence rates in Cardoso 1 and 2 were not statistically significant. Only a few areas, mostly at the heads of streams and in the central village, had prevalences of less than 33% (Fig. 2a).

The overall prevalence after treatment was 15.7%, when only 5 of the 177 (3%) households had schistosomiasis prevalences equal to or above the pretreatment average. The prevalence for 94% of the posttreatment households was in the lowest pretreatment quintile. The pretreatment cluster was eliminated and there were no new clusters (Fig. 2b).

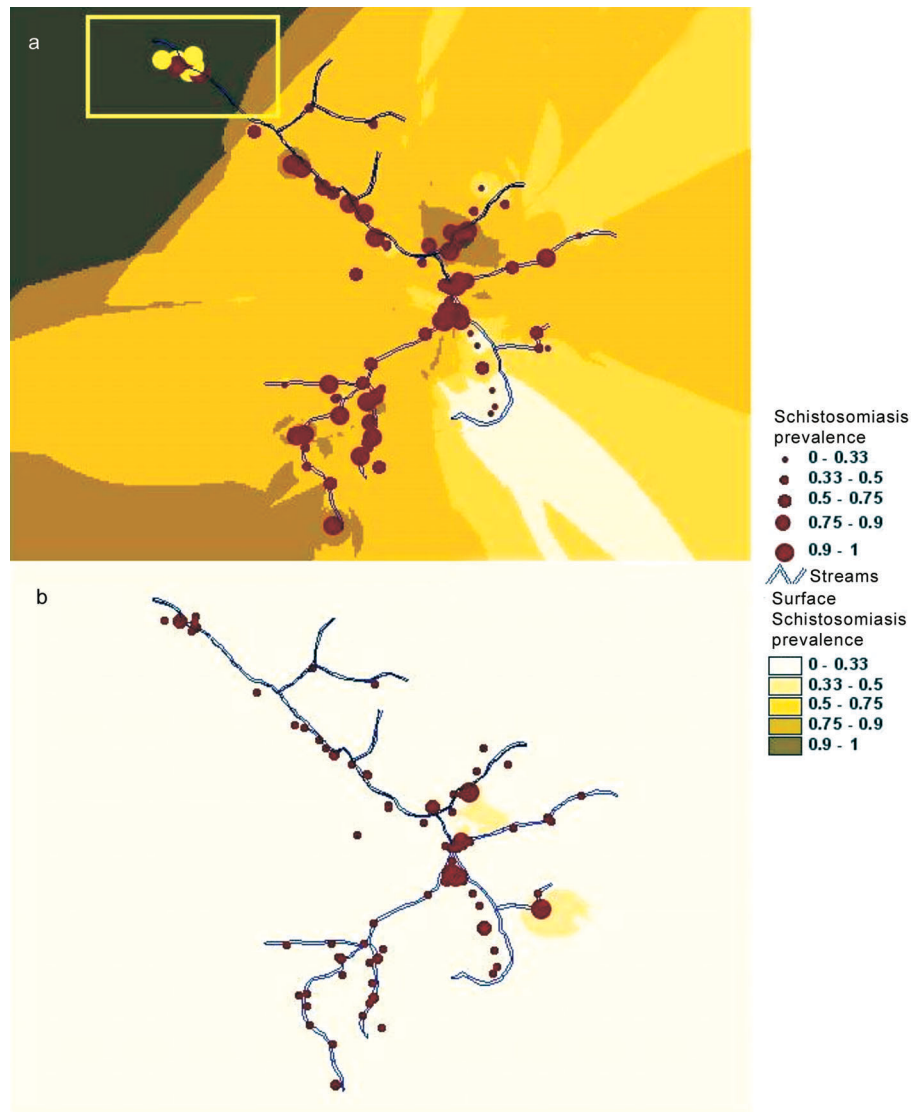


Fig. 2: schistosomiasis prevalence in Virgem das Graças. a: pre-treatment prevalence with a high prevalence cluster ($p = 0.029$); b: post-treatment prevalence.

Egg counts - Before the treatment program, the overall geometric egg count for the 277 people with schistosomiasis was 61.3 (50.4-74.5). The cluster of high egg counts in Cardoso 3 includes 10 households instead of just seven for schistosomiasis prevalence ($p = 0.007$). The prevalence of above-average egg counts was more than 5 times the expected value for this cluster (Fig. 3a). There was also a cluster of individuals in the central village and in the lower part of Cardoso 1 with below-average egg counts ($p = 0.005$) (Figs 3a, b, 4). The prevalence of above-average egg counts in this cluster was 75% less than what was expected.

One year after chemotherapy, the overall egg count was 97% lower than prior to treatment ($p < 0.0001$), with a mean count for infected individuals of 1.95 (S.D. 4.51, $n = 45$). There was a cluster of residents in 10 homes in Cardoso 1 and Cardoso 2 just north of the central village who had above average egg counts after treatment ($p = 0.007$). The

prevalence of these above- average egg counts was more than 8 times the expected value (Fig. 5).

IgE - The average pre-treatment level of IgE was 7.79 (S.D. 10.77, $n = 446$). The distribution of pre-treatment IgE levels contrasted with that of schistosomiasis prevalence. For example, a large cluster of 21 households with above median IgE levels extended from some households in Cardoso 1 to most households in Cardoso 2 ($p = 0.021$), but not to the major cluster of high pre-treatment prevalence in Cardoso 3 (Fig. 6a). The average IgE level in Virgem das Graças after treatment was 3.94, representing an overall reduction by 49.4%. Most household mean IgE values declined between 0 and 10% but posttreatment IgE distributions were spatially related to pretreatment egg counts. For example, IgE levels in the high pretreatment egg count cluster in Cardoso 3 were generally lower than in the low egg count cluster in the central village (Figs 3a, 4, 6a). This relationship is clearly demonstrated when subtract-

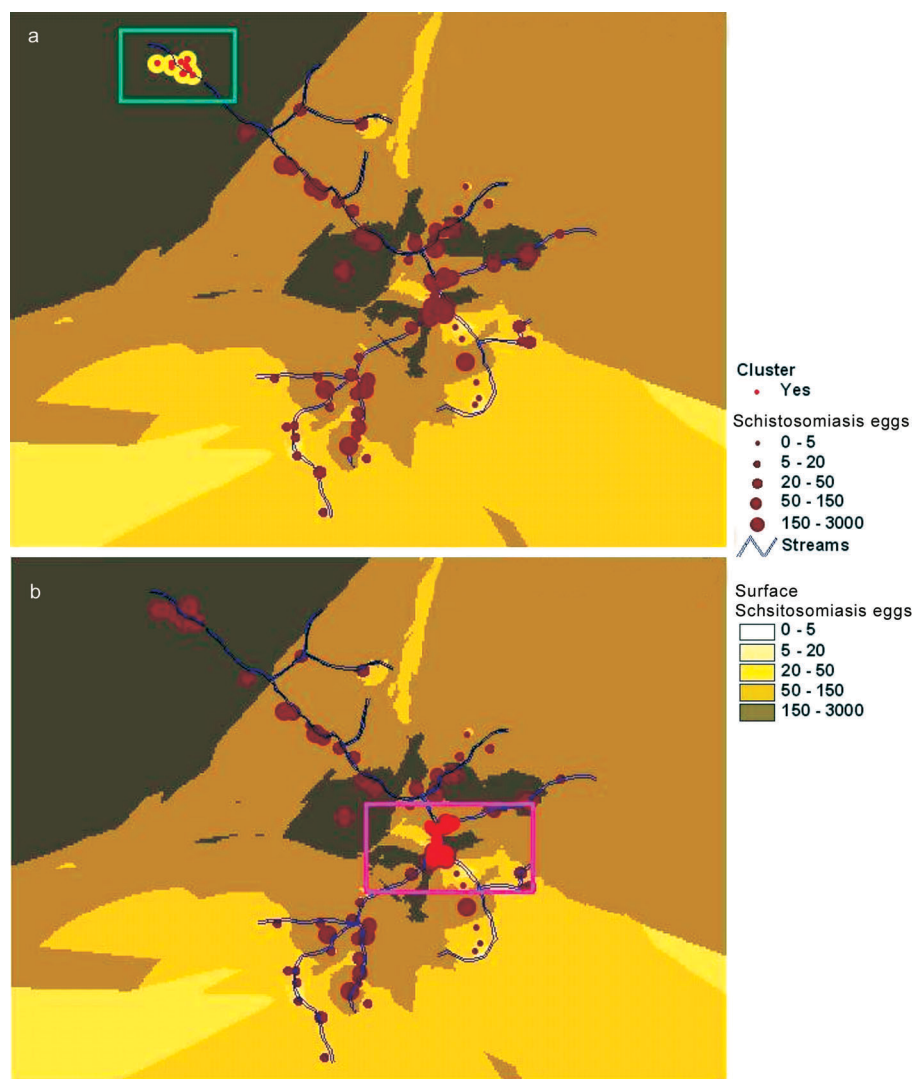


Fig. 3: *Schistosoma mansoni* egg counts in Virgem das Graças. a: pre-treatment above-average counts ($p = 0.001$); b: below-average counts ($p = 0.005$).

ing pretreatment IgE values from posttreatment values (Fig. 7).

TBM - Exposure to potentially infective water varied considerably among households throughout the study area, with more than half of all households in the lowest and highest quintiles (Fig. 8a). In the central village, too, mean TBM varied considerably among households (Fig. 8b). Mean duration of water contacts per household was more uniformly distributed throughout the study area (data not shown). Mean TBM in the study area declined from 49.9 (SD 85.6) prior to treatment to 34.2 (SD 55.4) after treatment ($p < 0.0001$).

Distance - During the pre-chemotherapy survey, people living further away from the nearest household water contact sites were less likely to have schistosomiasis ($p = 0.016$). This relationship disappeared after treatment. No relationship was found between distance and

IgE antibodies before or after treatment or between distance and TBM at the study area level. All households in the central village and in part of Cardoso 1 receiving piped water from a central source, some of them located within 50 m of a stream, were part of the low egg count cluster (Fig. 4).

DISCUSSION

We examined the spatial distribution of schistosomiasis before and after mass chemotherapy in a community with hyperendemic schistosomiasis. We selected an area with high *S. mansoni* infection rates that had not been treated previously by the national program and that lacked a medical facility. This facilitated the examination of the relationship between infection rates, IgE mediated immune responses and exposure risk. The major finding of this study is that although infection rates were significantly lower 12 months after chemotherapy, declines varied spa-

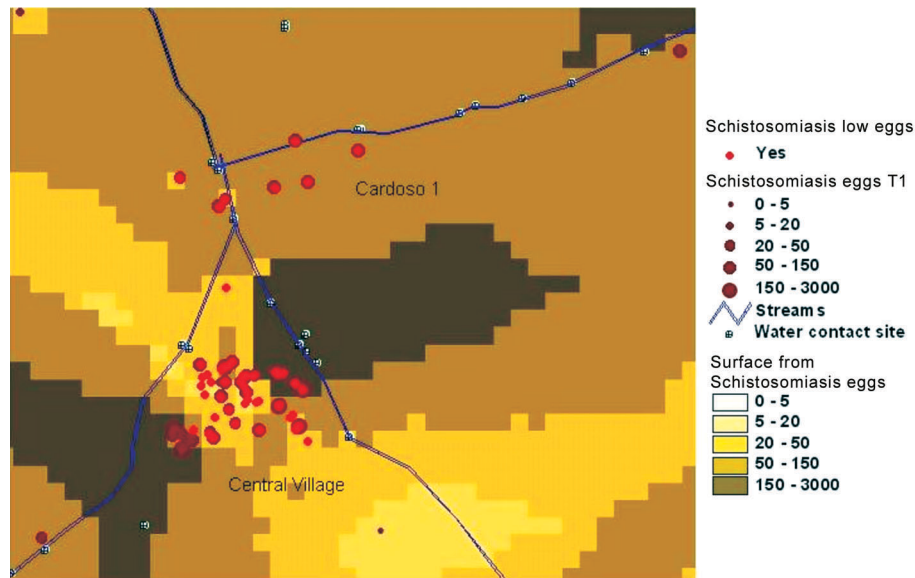


Fig. 4: pre-treatment below-average *Schistosoma mansoni* egg count cluster in the central village and lower Cardoso 1.

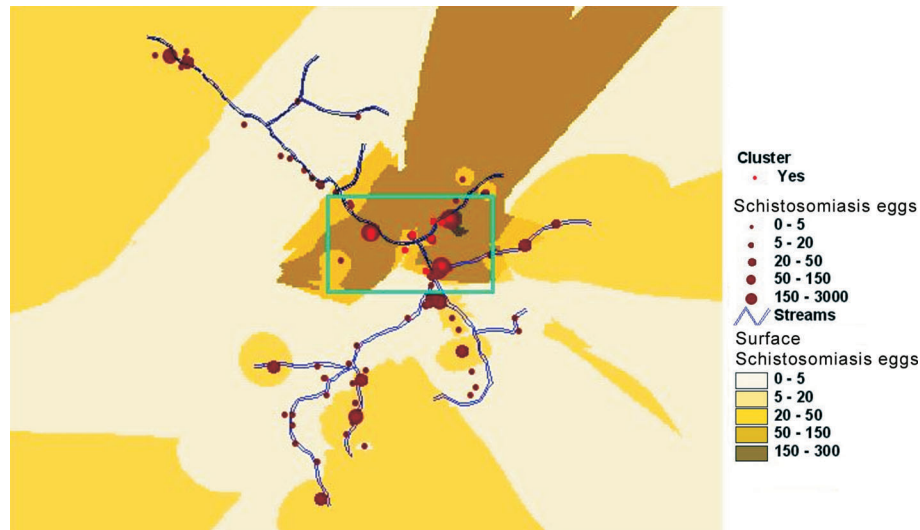


Fig. 5: post-treatment *Schistosoma mansoni* egg counts in Virgem das Graças with high count cluster ($p = 0.007$).

tially and were associated mainly with IgE levels and to a lesser extent to exposure risk and distance.

Previous studies by our group (Hagan et al. 1991, Correa-Oliveira et al. 2000, Caldas et al. 2000, Gazzinelli 2000) indicated that anti-parasite IgE levels confer resistance to *S. mansoni* infection and reinfection. Although it is well known that helminthic infections induce high levels of IgE, previous studies focused only on the analysis of cohorts that were treated and followed for reinfection, without consideration of the spatial distribution of infection and IgE responses. This is the first study to use a spatial approach to study IgE levels and their relationship with infection in an area endemic for schistosomiasis mansoni. By quantifying Total IgE and using GPS and GIS tools, we were able to determine whether infection clusters are correlated with different levels of this antibody isotype. In this paper we demonstrate that elevated

pretreatment levels of this antibody isotope correlate with clusters of low *S. mansoni* infection one year after chemotherapy.

The significant increase in TBM between the pre and posttreatment surveys indicates that water contact behavior did not contribute to the decline in infection rates. The spatial distribution of infection rates was influenced by the use of apparently safe piped spring water for domestic needs, mostly in the central village. The safe water effect is illustrated by the cluster of low egg count households in the central village and in seven households in Cardoso 1, all of which received piped water from the central spring source (Fig. 4). It appears that the spatiality of exposure risk and socioeconomic status in Virgem das Graças did not contribute significantly to the observed distribution of infection aside from the central village. First, the recovery of *B. glabrata* snails from 55% of the 106

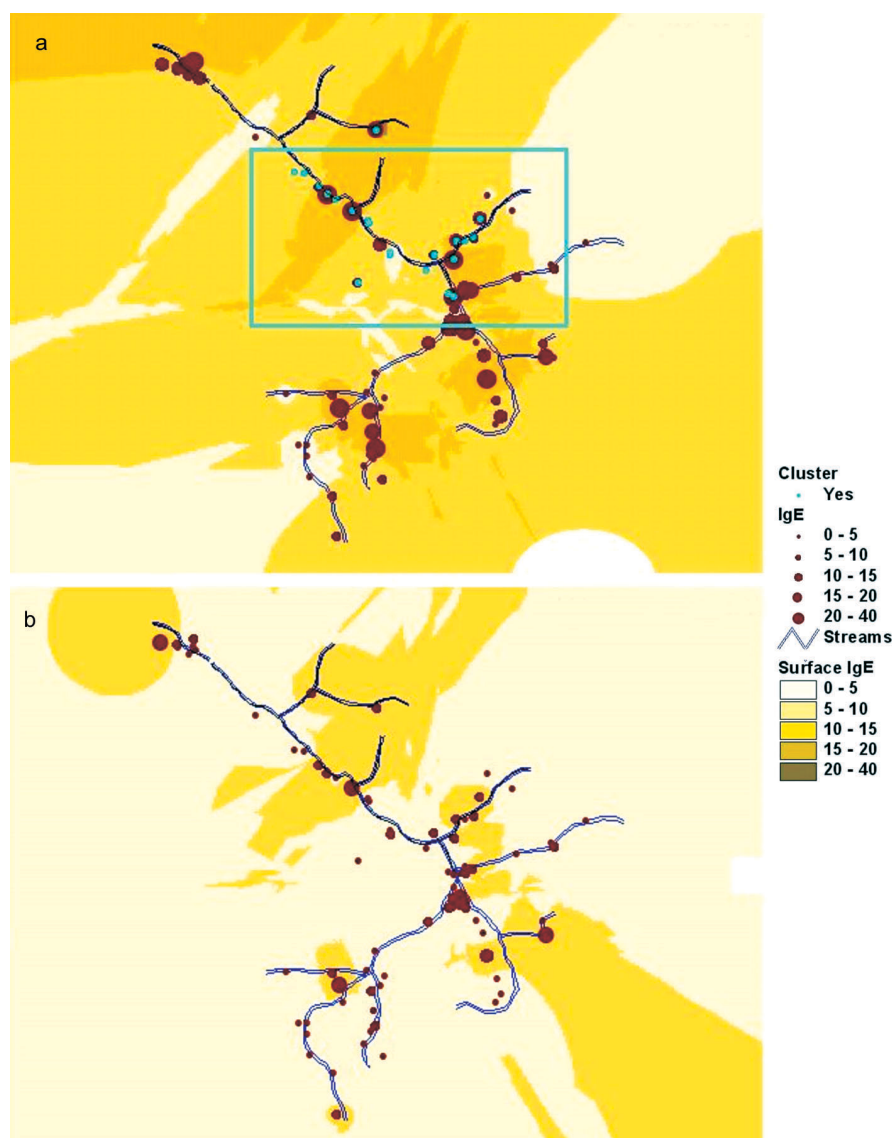


Fig. 6: IgE levels in Virgem das Graças. a: pre-treatment IgE with a cluster of above-average levels ($p = 0.021$); b: post-treatment IgE.

snail collection sites in all 11 habitats surveyed and infected snails from seven habitats (Kloos et al. 2004) indicates that transmission was widespread in the study area. Second, none of the major socioeconomic indicators were associated with *S. mansoni* infection at the household level, apparently due to the uniformly poor living and sanitary conditions in the study area, a situation that was also reported from another poor rural community in the hyper-endemic schistosomiasis area of northeastern Brazil (Moza et al. 1998). Third, the significant increase of TBM during the posttreatment period is in contrast with the observed decrease in infection after chemotherapy.

The inverse relationship between distance and schistosomiasis prevalence in the hamlets prior to but not after chemotherapy and lack of an association with *S. mansoni* egg counts or IgE levels suggests that the short distance between the great majority of houses in the hamlets and

potentially infective water bodies (less than 60 m) did not reduce exposure risk. This is also indicated by the lack of a distance relationship with TBM and IgE levels and the inclusion of the 8 households with the reportedly highest TBM values in the central village (Fig. 7b) in the low egg count cluster (Fig. 4). Our distance results corroborate with those by Lima e Costa et al. (1997) and Silva et al. (1997), who used a 100 m threshold, but contrast with studies in Egypt and Kenya (Kloos et al. 1998, McClennon et al. 2004), where distance was strongly associated with schistosomiasis. These differences may be due to the relatively shorter distances in our study area.

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Fig. 7: difference in IgE levels between pre and post-treatment in Virgem das Graças.

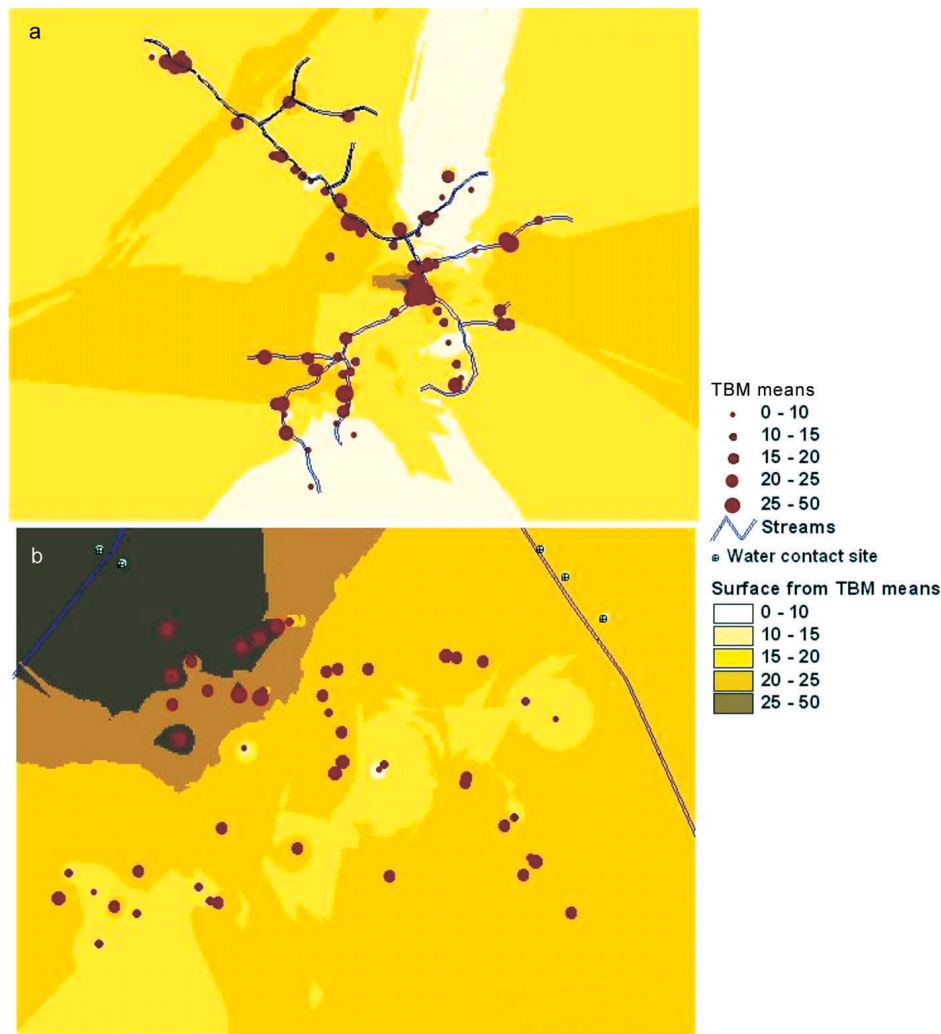


Fig. 8: mean TBM exposure. a: in Virgem das Graças; b: in the central village.

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