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***Schistosoma mansoni* infection in a rural area of the Jequitinhonha Valley, Minas Gerais, Brazil: analysis of exposure risk**

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Abstract

This study examines the relative contribution of age-specific total IgE levels, eosinophils and water contact behavior to the prevalence and intensity (geometric mean egg counts) of *Schistosoma mansoni* infection in the poor rural population of Virgem das Graças in northern Minas Gerais State. In bivariate analysis, age was strongly correlated with both prevalence and intensity of infection, while eosinophil levels with prevalence only ($p < 0.0001$); IgE levels and 5 demographic and socioeconomic variables were moderately correlated with prevalence ($p < 0.05$), as were number of persons per room and TBM (total body minutes) with egg counts. In multivariate analysis, after controlling for demographic and socioeconomic factors, only total IgE levels were significantly correlated with both prevalence ($p = 0.0248$, 95% CI = 1.01–1.11) and intensity ($p = 0.0217$, 95% CI = 0.01–0.14) of infection and eosinophil levels with prevalence ($p = 0.0005$, 95% CI = 1.07–1.24). Although any causal relationship cannot be confirmed by a cross-sectional study, we demonstrated an associated decrease in prevalence and intensity of *S. mansoni* infection with increased IgE levels.

Keywords

Schistosoma mansoni; IgE; eosinophils; water contact; age distribution; Brazil

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INTRODUCTION

Transmission of *Schistosoma mansoni* and subsequent infection of human hosts are influenced by a large number of variables that are important not only to maintain the parasite life cycle but also to determine the outcome and severity of the disease. Among these factors are those related to the environment, particularly surface water distribution, presence of the snail intermediate host, and also factors related to the human host such as age, sex, water contact behavior, type of water supply, genetics, immune response and physiology (Bethony et al., 2001, Caldas et al., 2000, Fulford et al., 1998, Kabatereine et al., 2004, Lima e Costa et al., 1991, Ndassa et al., 2007, Pinot de Moira et al., 2007, Silveira et al., 2002). Indeed, several studies have been carried out to understand how epidemiological, socioeconomic and behavioral factors can affect the transmission and prevalence of infection (Bethony et al., 2002, Bethony et al., 2004, Correa-Oliveira et al., 2000, Gazzinelli et al., 2006, Kloos et al., 2006).

The typical convex age distribution curve (peak around childhood) of the prevalence and intensity of *Schistosoma*-infection in human populations residing in endemic areas (Kabatereine et al., 1999) suggests that in older individuals, a combination of worm attrition over time, acquired immunity, and possibly reduced exposure are involved in decreasing infection levels. Among the most commonly studied factors related to infection or reinfection resistance are the levels of anti-parasite IgE response. The role of IgE and eosinophils in age-specific schistosomiasis rates has been studied in cross-sectional and longitudinal studies in endemic areas in the infected as well as in a population of putative resistant individuals. Other studies have focused on immigrant populations and on individuals after chemotherapy, showing that IgE expression is also influenced by infection intensity (Butterworth et al., 1992, Butterworth et al., 1988, Caldas et al., 2000, Correa-Oliveira et al., 1998, Correa-Oliveira et al., 2000, Demeure et al., 1993, Dessein et al., 1992, Dunne et al., 1992, Hagan et al., 1985, Naus et al., 1999, Ouma et al., 1998).

Although immunological responses to schistosome infection have been studied in detail, the controversy over the relative importance of behavioral (exposure to infection) and immunological factors (acquired immunity) in the age distribution of infection is not fully elucidated. Several studies of both *S. mansoni* and *S. haematobium* revealed that following chemotherapy, fewer older individuals are reinfected when compared to young children (Dessein et al., 1992, Kabatereine et al., 1999, Naus et al., 1999). Some of this age-dependent resistance to reinfection is thought to be linked with previous infections, also suggesting the development of acquired immunity (Dunne et al., 1992). In fact, reinfection rates with *S. mansoni* in adults after chemotherapy, in a fishing community on Lake Albert in Uganda, were higher in younger individuals even though adults had significantly more water contact (Kabatereine et al., 1999). These results suggest that adults may be physiologically and immunologically more resistant to post-treatment reinfection than children (Fulford et al., 1998, Kabatereine et al., 1999). Noteworthy, heterogeneities in schistosome transmission reported in five communities of Mali and Zimbabwe were specifically associated with the typically dispersed water contact rates among individuals (Woolhouse et al., 1998).

A study of car washers in Lake Victoria revealed significant heterogeneity in *S. mansoni* infection among similarly exposed adults, suggesting that the development of acquired immunological resistance is age, genetically and exposure dependent (Karanja et al., 2002). Several authors showed the importance of immunological factors in the intensity (egg counts) and prevalence of *S. mansoni* infection in Africa (Hagan et al., 1991, Webster et al., 1996) and in Brazil (Caldas et al., 2000, Correa-Oliveira et al., 1998, Correa-Oliveira et al., 2000, Viana et al., 1995). However, some investigators have questioned the importance of the immune response as a central factor in the decrease of intensity of infection or reinfection with age,

arguing that other factors, such as genetics, physiology and mostly behavior can explain the decrease in infection rates in the older population (Abel et al., 1991, Abel et al., 1997, Gryseels, 1996, Marquet et al., 1996). These and other studies of specific age-linked immune responses and exposure risk in schistosomiasis-endemic communities emphasize the need to consider additional epidemiological factors in schistosomiasis dynamics, such as gender, type of household water supply, and socioeconomic status (Gazzinelli et al., 2006, Lima e Costa et al., 1998, Remoue et al., 2000, Ximenes et al., 2003). The multifactorial nature of the age-linked distribution of *S. mansoni* infection has also been indicated by studies in a rural area of Brazil (Bethony et al., 2002, Silveira et al., 2002).

In this study, we extended the work of previous authors by determining the relative contribution of total IgE levels, eosinophils and water contact behavior on the prevalence of *S. mansoni* infection in a rural population in northern Minas Gerais after controlling for socioeconomic factors. Age was included in the model in order to evaluate possible interaction with the study parameters. Finally, the relationship between total IgE levels, eosinophils, exposure risk, intensity and prevalence of *S. mansoni* infection was explored.

MATERIALS AND METHODS

Study area and population

The study was carried out in Virgem das Graças (Municipality of Ponto dos Volantes), a poor rural area hyperendemic for schistosomiasis in the Jequitinhonha Valley in northern Minas Gerais State. The study population resides in a central village (Taboca) and 4 dispersed hamlets (Cardoso 1, Cardoso 2, Cardoso 3 and Suçuarana). All settlements are located in close proximity of streams in 4 valleys. Previous malacological surveys showed that 1.4% of 1808 snails were infected with *S. mansoni*, all of them *Biomphalaria glabrata* (Kloos et al., 2004).

According to our census in 2001, 589 individuals (48.2% males and 51.8% females) lived in the study area in 141 households. All households and residents present at the time of the survey were registered and assigned unique household identification (HHID) and personal identification numbers (PID). Only individuals older than 6 years of age and those who provided stool and blood samples and participated in the socioeconomic and water contact surveys were included in this study, leaving an effective sample size of 428 individuals.

Using a consent form approved by the National Committee of Ethics in Research (CONEP) of Brazil and the Internal Review Board of the State University of New York at Buffalo, informed consent was obtained from all volunteers, including parental consent for young children and adolescent participation in the study.

Parasitological survey

A cross-sectional parasitological survey was conducted in 2001 and stool specimens were examined for *S. mansoni*, *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* eggs using the Kato-Katz method (Katz et al., 1972). Briefly, all participants received 3 plastic containers for fecal samples. Participants were instructed to deposit 1 fecal sample per day into each container and return the containers immediately to one of the several collection points, where the samples were stored at 4°C. Two slides for each stool sample provided (a total of 6 slides per individual) were prepared within 24 hours of collection, as previously described by Gazzinelli et al. (2006).

Blood collection and eosinophil counting

In 2001, 10 milliliters of blood were collected from each patient after obtaining informed consent from all donors. Plasma samples for indirect ELISA (Enzyme Linked Immunosorbent

Assay) were obtained from heparinized blood after centrifugation (10 minutes, 2500 rpm at room temperature). These samples were further transferred to the Cellular and Molecular Immunology Laboratory at the Centro de Pesquisas René Rachou/FIOCRUZ in Belo Horizonte, where they were stored at -70°C until needed.

Additional blood samples in EDTA (ethylenediaminetetraacetic acid) were collected for hematological analysis. Complete hemograms were determined by microscopic smear analysis in order to obtain the global count of eosinophils/mm³ present in the peripheral blood from all patients.

Total IgE levels

Plasma levels of total immunoglobulin E (IgE) were determined by indirect ELISA. Thus, 100 μL of anti-IgE monoclonal antibody (AbD Serotec, USA) diluted in 0.05M carbonate-bicarbonate buffer (pH 9.6), were coated onto 96-well microplates (MaxiSorp, Nalge Nunc Intl, USA) at a concentration of 5 $\mu\text{g/mL}$. Plates were sealed and incubated overnight at 4°C and then washed 3 times with 0.15 M phosphate buffer (PBS, pH 7.2). Wells were blocked for 90 minutes at room temperature with 250 μL of 0.15M PBS containing 0.5% of Tween-20 (PBST20) and 10% fetal bovine serum. Sera (100 μl) were added at the concentrations of 1:50 and 1:100 (diluted in PBST20) followed by washes. As controls, known quantities of IgE monoclonal antibodies were added for the development of a standard curve. Sera and standards were incubated overnight at 4°C . Plates were then washed and incubated with 100 μL of biotin-conjugated anti-IgE polyclonal antibody (AbD Serotec, USA) for 2 hours at room temperature. 100 μL of streptavidin-peroxidase (Amersham, Piscataway, NJ) diluted to 1:1000 were added to each well after the plates were washed. Finally, the substrate (H_2O_2 , Sigma-Aldrich Co., USA) and chromogen (O-Phenylenediamine, Sigma-Aldrich Co., USA) were added after washing and the absorbance was read on an automatic ELISA microplate reader (SpectraMax 340 PC reader, Molecular Devices, Sunnyvale, CA, USA) at 492 nm with SOFTMax Pro software (Molecular Devices).

Socioeconomic and water contact surveys

Pre-tested questionnaires were administered to all individuals enrolled in the study to obtain information on the study population's water contact with potentially infective sources. All households and their inhabitants had been assigned unique code numbers during the initial census. Information on water contact behavior was obtained from all individuals over the age of 6 years. Questionnaires for children below the age of 10 years were answered by their parents. Contact with streams and other potentially infective sources during the 7 days preceding the survey were recorded. Additionally, rare and seasonal activities (swimming, cleaning streams or fishing) during the preceding 12 months were also recorded using a pre-tested form (Gazzinelli et al., 2001). All water contact was classified as either safe (piped supply in the central village and protected springs) or unsafe (all streams and all other unprotected sources), based on the presence/absence of *Biomphalaria* snails found in previous seasonal surveys (Kloos et al., 2004).

Water contact frequency and TBM (total body minutes) determinations

All participants were asked about the frequency of contact with local water sources for domestic, recreational, and occupational purposes. Contact with all water bodies, including streams, canals, springs, wells, ponds, and swamps were included due to the widespread distribution of *B. glabrata* in the study area (Kloos et al., 2004).

TBM was calculated from both direct observation and questionnaire data using the method described by Gazzinelli et al. (2001). The data was collected in two-stages. First, the frequency, duration and intensity (percentage of body surface exposed) of all water contact activities in

representative households of 3 out of the 5 communities in the study area was recorded for 7 consecutive days during the dry and rainy seasons. The objective was to determine the most frequent contact activities in the study community, together with average duration and intensity values for each activity. Based on these results, questions were formulated to determine the number of activity-specific water contacts for each person during the 7 days prior to the survey. These frequencies were multiplied by the mean intensity (proportion of body surface exposed) and duration values obtained during direct observation of water contacts in the study area. This method was used in another study area in Minas Gerais, as described by Gazzinelli et al. (2001) and Bethony et al. (2004). By combining direct observation with interviews, their shortcomings, such as underreporting of water contacts taking place outside the immediate vicinity of the observer or the designated observation period (Fulford et al., 1996; Kloos et al., 1998; Ofoezi et al., 1998) and the recall problems and information bias in regard to duration and intensity of exposure (Kloos et al., 2006; WHO, 1979; Sama & Ratard, 1994), may be overcome. This was borne out by our earlier studies, which revealed a considerably homogeneous relationship between duration and intensity (percentage of body exposed) of contacts for any given activity. Thus, nearly all domestic contact activities, including washing clothes, washing utensils and fetching water, washing the floor in the house, crossing streams and washing limbs, and bathing, present relatively little variation concerning the duration of these contacts (Gazzinelli et al. 2001). The predominance (over 90%) of single rather than multiple activities during water contacts facilitated reliable measurement of duration and intensity of contacts (H. Kloos, unpublished data), further justifying the combined observation/interview method.

Data Analysis

The final data used for this analysis contain information on 428 individuals and 129 households. Table 1 shows the association between a number of variables including age, occupation, etc. with prevalence of schistosomiasis among all 428 individuals and with intensity of infection among those individuals with schistosomiasis. These estimates, confidence intervals, and p-values were obtained using the survey procedures in SAS v 9.1 and include adjustments for clustering by household (SAS Institute Inc. 2004).

The relationship between the four independent variables (total IgE levels, eosinophils, total TBM, and total frequency of contacts) with both prevalence and intensity of schistosomiasis among the infected subjects was assessed. In a previous study exploring the prevalence/intensity relationship with basic demographic and socioeconomic variables, the important predictors of infection included age group, place of residence, head of household education, head of household occupation, possessions and number of persons per room (Gazzinelli et al., 2006). Therefore, the influence of age on the relationship between independent and dependent variables was also evaluated in addition to duration of stay at the present residence (another covariate that is associated with schistosomiasis, which was introduced for this analysis). These variables were controlled in subsequent analyses.

Initially, single variable analysis was performed using each of the 4 independent variables (total IgE levels, eosinophils, total TBM, and total water contact frequency), controlling for the appropriate demographic and socioeconomic variables. The relationship between each of these variables with both prevalence and intensity of infection controlling for age was explored. A multivariate analysis was then carried out to assess which subset of the four variables best predicted prevalence and intensity of infection, while controlling for the demographic and socioeconomic predictors. Log binomial models were used to assess the relationship between the independent variables and prevalence, producing prevalence ratios, confidence intervals, and type III tests of significance for both main effect and interaction terms (Skov et al., 1998). The modified Poisson approach was used as an alternative method to avoid the

convergence failure observed in some models (Spiegelman et al., 2005). The standard errors for the parameter estimations were corrected using generalized estimating equations with an exchangeable correlation matrix (Liang et al., 1986, Lipsitz et al., 1994). Generalized estimating equations were used to account for additional correlation taken on measurements within a household versus across households. Mixed models were used to measure the relationship between intensity of infection and the four independent variables of interest, producing estimations of the slope, confidence intervals, and type III tests of significance. Again, the clustering by household was captured using an exchangeable covariance matrix. All analyses were done using Statistical Analyses System 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Of the 428 individuals (45.5% males and 54.5% females) living in 129 households that were included in this study, 277 (64.7%, CI 95% = 58.8–70.7) had schistosomiasis. The age range for the population was 6–95 years, with a mean age of 33.9 ± 22.0 years (male: 35.0 ± 22.3 years; female: 33.0 ± 21.8 years). The prevalence of schistosomiasis tended to be higher among poorer people living in the hamlets, especially farmers, and households with family heads lacking education and living in crowded conditions without a safe water supply. Forty-eight percent of the study population lived in the central Taboca village, where *S. mansoni* prevalence was 59.7% (CI 95% = 55.1–64.3). Overall prevalence in the hamlets (69.4%, CI 95% = 62.1–76.6) was similar to those observed in urban areas (59.7%, CI 95% = 50.2–69.2). Although mean egg counts were higher in rural than central village dwellers, these differences were also not statistically significant (Table 1). Overall prevalences of hookworm infection, *Ascaris lumbricoides* and *Trichuris trichiura* were 54.2%, 8.2% and 4.2%, respectively. The prevalence distribution by age for these helminths is presented on Table 2.

The overall geometric mean of egg count in the study area was 61.3 eggs/mg of feces (CI 95% = 46.0–81.7), peaking between the ages of 6–14 years (93.2 eggs/mg, CI 95% = 45.3–191.7). Infection intensity continued to decline with age, reaching the lowest level among individuals aged 65 years and older (22.7 eggs/mg, CI 95% = 10.9–47.1). The prevalence of infection peaked in the 15–29 year old age group (77.1%, CI 95% = 67.7–86.4) and was lowest in individuals older than 65 years (47.8%, CI 95% = 33.6–62.0) (Table 1, Fig. 1). Analysis of the relationship between the 4 parameters evaluated (total IgE levels, eosinophils, total TBM, and total water contact frequency) and prevalence and intensity of schistosomiasis, when controlling for the socioeconomic covariates, showed that none of these relationships was modified by age. Water contact frequency was associated with prevalence, although with a borderline statistical significance (PR = 1.02, $p = 0.0561$). On the other hand, both IgE levels and number of eosinophils were significantly associated with prevalence (PR = 1.06, $p = 0.0248$ and PR = 1.15, $p = 0.0005$; respectively). Therefore, a 10 unit increase in IgE levels would raise the expected prevalence 1.06 times, while a 1000 unit increase in the number of eosinophils would augment the prevalence 1.15 times. When infected individuals were considered separately, only IgE levels were significantly associated with intensity of infection. Thus, a 10 unit increase in the IgE levels would raise the log₁₀ of the egg counts by 0.08 units ($p = 0.0217$).

We considered all 4 independent variables of interest for inclusion in multivariable models for prevalence and intensity, controlling again for age group, place of residence, household head education, household head occupation, possessions, number of persons per room, and duration of residence in the currently occupied house. Since TBM was not significantly associated with prevalence after controlling for preliminary covariates, and considering that TBM was highly correlated with total water contact frequency, only IgE levels, eosinophils, and total water contact frequency were assessed for inclusion in the multivariable model for prevalence. Water contact frequency was removed from the multivariable model due to lack of significance,

leaving IgE levels (PR=1.06, $p = 0.0248$) and eosinophils count (PR = 1.15, $p = 0.0005$), which were indeed significantly associated with prevalence. In the multivariable model for intensity of *Schistosoma* infection, only IgE levels were statistically significant in the assessment of each variable (Table 3). After including other confounding variables, IgE levels remained the only covariate associated with geometric mean of egg counts.

DISCUSSION

Identification of factors that may influence the host-parasite relationship is important to understand the dynamics of *S. mansoni* infection in endemic populations. Our results corroborate earlier studies showing that humoral and cellular immune responses to *S. mansoni* infection might be directly correlated with intensity of infection. Indeed, we showed a clear association between total IgE levels and both the prevalence and intensity of infection, which suggests that total IgE levels may be an important tool to predict infection and worm burden. Several studies have evaluated IgE levels to crude antigen extracts or to purified or recombinant antigens, suggesting that parasite specific IgE levels may correlate with resistance to infection (Hagan et al., 1987, Hagan et al., 1991, Vereecken et al., 2007). Webster et al. (1998) indicated that higher IgE levels in adult individuals living in endemic areas are probably due to a continuous antigen exposure, leading to resistance to reinfections among middle-aged and elderly groups. Moreover, resistance to infection has also been associated with high levels of IgE against crude adult antigen extracts from adult worm (SWAP) (Vereecken et al., 2007). Interestingly, reinfection may occur when elevated levels of IgG4 subclass anti-SWAP are observed (Correa-Oliveira et al., 2000, Hagan et al., 1987, Silveira et al., 2002). Although the *Schistosoma*-specific IgE levels were not measured in the current work, it is likely that increased total IgE levels may partially reflect a specific anti-parasite antibody response. However, the induction of polyclonal IgE production by other helminths, such as hookworms, was not evaluated and may act as a confounder. Nonetheless, despite of lack of specificity, we showed that the measurement of total IgE levels may be useful for epidemiological studies of *S. mansoni*.

Our results also showed that the association between total IgE levels and *S. mansoni* infection present a characteristic age-response profile (Fig. 1A). Noteworthy, we showed that higher levels of total IgE apparently corresponded with lower prevalence rates and worm burden. On the other hand, several studies reported a direct correlation between intensity of infection and age in areas endemic for *S. mansoni* (Caldas et al., 2000), *S. haematobium* (Grogan et al., 1997, Hagan et al., 1991) and *S. japonicum* (Zhang et al., 1997).

A significant association between number of eosinophils and prevalence was observed, but not with intensity of *S. mansoni* infection (Table 3). It has previously been shown that patients with higher eosinophil levels living in endemic areas are less susceptible to *S. haematobium* infections (Hagan et al., 1987, Kimani et al., 1991, Sturrock et al., 1983). These data suggest that older individuals either acquire resistance to infection or are less likely to be exposed to schistosomes, resulting in reduced parasitism and number of eosinophils. Nevertheless, these cells may continue to elicit an effective immune response and result in resistance to infection/reinfection. According to De Andres et al. (1997), eosinophils can contribute to the protective antibody-dependent immune response that is elicited to destroy miracidia and eggs of *S. mansoni*.

In relation to socioeconomic factors, our results show a direct relationship between number of persons per room and prevalence of infection. We also observed that the possession of a car/motorbike was significantly associated with lower prevalence and intensity of infection. These results are similar to those obtained in a previous study in the same community, suggesting that these 2 socioeconomic variables can be used as an indicator of risk for schistosomiasis

infection in rural areas with subsistence economies (Gazzinelli et al., 2006). The association between number of persons per room and *S. mansoni* infection was also reported by Pedrazzani et al. (1988) and Parraga et al. (1996), suggesting that poorer and crowded households are associated with higher risk of infection among their inhabitants.

Unlike previous studies, we evaluated whether total IgE levels considered within the context of age, sex, socioeconomic status as well as water contact can be used as an indicator of infection intensity. We found that total IgE serum levels, number of eosinophils and exposure to water in activities such as washing clothes, fetching water, washing parts of the body, crossing streams, swimming and playing in streams were associated with prevalence of *S. mansoni* infection in Virgem das Graças. It is important to mention that in the multivariable model, IgE levels and water contact were not related to prevalence. On the other hand, total IgE levels and activities such as bathing, washing parts of the body, and fishing among persons 50 years and older were associated with higher *S. mansoni* egg counts.

In this study we demonstrate that while water exposure (TBM) did not significantly vary with age (Fig. 1B), prevalence and intensity of infection tend to diminish according to the age-related increase of total IgE serum levels. These exposure and infection patterns are similar to those observed by various other studies, which show that exposure levels alone cannot explain the typical decrease in *Schistosoma* infection rates (Butterworth et al., 1992, Chandiwana et al., 1991, Guimaraes et al., 1985, Kloos et al., 1983, Scott et al., 2003). The current work corroborates the findings of various laboratory studies showing that immunological markers characteristically rise or decline with age and intensity of infection (Caldas et al., 2000, Dessein et al., 1992, Dunne et al., 1992, Hagan et al., 1985).

In Virgem das Graças, a strong association between socioeconomic variables and infection was not observed. Previous studies on the relationship between socioeconomic factors and schistosomiasis in Brazil revealed higher infection rates in low income population (Bethony et al. 2004, Lima e Costa et al., 1991, Ximenes et al., 2003). Virgem das Graças is characterized by widespread impoverished living conditions, including low family income, nearly total dependence on subsistence agriculture, poor housing conditions and lack of both piped water or sewerage facilities in nearly all households (Gazzinelli et al., 2006), similar to the situation in a poor village in Pernambuco State (Moza et al. 1998).

Finally, this study shows that total IgE levels, number of eosinophils and water contact frequency were associated with the prevalence of schistosomiasis, while only total IgE levels were associated with intensity of *S. mansoni* infection in the rural community of Virgem das Graças. We also showed an age-dependent increase in total IgE levels, demonstrated in groups with lower prevalence and intensity of *S. mansoni* infection, when controlling for socioeconomic variables. Although any causal relationship cannot be confirmed by a cross-sectional study, we hypothesized that humoral immunity might contribute to the reduction of infection levels, as revealed by an associated decrease in prevalence and intensity of *S. mansoni* infection with increased IgE levels.

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Figure 1.

Table 1

Socioeconomic, demographic, exposure and immunological levels in relation to prevalence and intensity of *S. mansoni* infection in Virgem das Graças*

Independent variable	N	Prevalence (95% C.I.)	p-value	Geometric mean egg counts ^f (95% C.I.)	p-value
Age group					
6-14	111	64.9		(45.3 - 191.7)	
15-29	109	77.1	0.0001	(66.7 - 123.9)	<0.0001
30-44	68	76.5		(40.8 - 89.3)	
45-64	94	50.0		(17.0 - 39.1)	
> 64	46	47.8		(10.9 - 47.1)	
Place of residence					
Rural	222	69.4		(47.3 - 109.6)	
Urban	206	59.7	0.1092	(34.2 - 73.4)	0.2027
Education ^{**}					
None	244	68.4		(47.3 - 98.6)	
Some	184	59.8	0.1520	(32.8 - 82.4)	0.3531
Occupation ^{**}					
Agriculture	225	69.8		(46.8 - 94.6)	
Retired/handicapped	119	52.1	0.0480	(22.4 - 77.0)	0.3463
All others	84	69.0		(32.7 - 169.6)	
Possessions					
No car/motorbike	393	66.7		(45.4 - 82.8)	
Have car/motorbike	35	42.9	0.0483	(21.7 - 168.1)	0.9727
No. of persons per room					
0 - <0.60	97	60.8		(23.7 - 54.8)	
0.60 - <0.80	76	47.4	0.0021	(25.9 - 71.3)	0.0440
0.80 - <1.33	141	63.1		(39.4 - 123.1)	
1.33 - 4.00	114	81.6		(48.5 - 156.3)	
Duration in house (years) ^g					
0 - <3	94	73.4		(29.9 - 92.2)	
3 - <9	118	69.5	0.0146	(41.2 - 136.2)	0.6606
9 - <17	106	68.9		(37.6 - 117.9)	
17+	110	48.2		(30.0 - 79.9)	
IgE ^h					
0 - <1.28	107	56.1		(34.3 - 83.3)	
1.28 - <3.46	107	57.0	0.0117	(38.9 - 99.8)	0.0704
3.46 - <10.64	107	71.0		(31.5 - 66.8)	
10.64+	107	74.8		(57.1 - 136.1)	
Eosinophils ^h /mm ³					
0 - <356	103	49.5		(27.3 - 77.0)	
356 - <625.5	104	59.6	<0001	(31.6 - 90.9)	0.3588
625.5 - <1105	101	67.3		(49.8 - 126.4)	
1105+	106	81.1		(43.0 - 115.9)	
TBM ^h					
0 - <92.45	107	57.9	0.3555	(27.6 - 84.9)	0.0431

Independent variable	N	Prevalence (95% C.I.)	p-value	Geometric mean egg counts [†] (95% C.I.)	p-value
92.45 – <119.14	107	64.5		(37.9 – 88.3)	
119.14 – <160.07	107	70.1		(27.8 – 76.4)	
160.07+	107	66.4		(66.4 – 174.0)	
Water contact frequency [‡]					
0 – <35.00	104	59.6		(27.6 – 83.2)	
35.00 – <51.52	110	60.0	0.0998	(33.3 – 79.0)	0.5271
51.52 – <76.01	107	63.6		(43.7 – 121.7)	
76.01+	107	75.7		(46.2 – 118.2)	

* Unadjusted estimates for prevalence and geometric mean egg counts.

[†] Among infected individuals only.

[‡] Used as a continuous variable for analysis.

** All data are for household heads only

Prevalence distribution by age of hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* infections in Virgem das Graças

Table 2

Age groups (years)	N	Prevalence					
		Hookworm			<i>Ascaris lumbricoides</i>		
		%	95% C.I	%	%	95% C.I	<i>Trichuris trichiura</i>
6–14	111	42.3	(33.12 – 51.48)	11.7	7.2	(5.73 – 17.67)	(2.40 – 12.0)
15–29	109	65.1	(56.16 – 74.04)	9.2	4.6	(3.78 – 14.62)	(0.68 – 8.52)
30–44	68	58.8	(47.11 – 70.49)	8.8	4.4	(2.07 – 15.53)	(–0.47 – 9.27)
45–64	94	58.5	(48.54 – 68.46)	6.4	1.1	(1.46 – 11.34)	(–1.01 – 3.21)
65+	46	41.3	(27.8 – 55.52)	0.0	2.2	-	(–2.03 – 6.43)

Table 3
Relationship between IgE, eosinophils, TBM and water contact frequency and prevalence and intensity of *S. mansoni* infection *

Variable	Prevalence			Intensity		
	PR [†]	95% CI	p value	Slope [¶]	95% CI	p value
IgE	1.06	(1.01 – 1.11)	0.0248	0.08	(0.01 – 0.14)	0.0217
Eosinophils	1.15	(1.07 – 1.24)	0.0005	0.02	(-0.09 – 0.12)	0.7604
TBM	1.00	(0.98 – 1.01)	0.8473	0.01	(-0.01 – 0.03)	0.1924
Water contact frequency	1.02	(1.00 – 1.03)	0.0561	0.01	(-0.02 – 0.03)	0.6091

* Controlling for age group, place of residence, head of household education, head of household occupation, possessions, number of persons per room, and time in the house.

[†] Multiple increase in prevalence of schistosomiasis for a 10-unit increase in IgE, total TBM, and total water contact frequency, and for a 1000-unit increase in eosinophils.

[¶] Change in the log base 10 of the egg counts for a 10-unit increase in IgE, total TBM, and total water contact frequency, and for a 1000-unit increase in eosinophils.