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DETECTION OF IgM ANTIBODIES TO *Schistosoma mansoni* GUT-ASSOCIATED ANTIGENS FOR THE STUDY OF THE DYNAMICS OF SCHISTOSOMIASIS TRANSMISSION IN AN ENDEMIC AREA WITH LOW WORM BURDEN

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SUMMARY

For a period of 2 years, five follow-up measures of prevalence and incidence rates were estimated in a prospective study of *S. mansoni* infection in a group of schoolchildren who were living in a rural area of the Municipality of Itariri (São Paulo, Brazil), where schistosomiasis is transmitted by *Biomphalaria tenagophila*. Infection was determined by the examination of three Kato-Katz stool slides, and the parasitological findings were analyzed in comparison to serological data. In the five surveys, carried out at 6-month intervals (March-April and September-October), the prevalences were, respectively, 8.6, 6.8, 9.9, 5.8 and 17.2% by the Kato-Katz, and 56.5, 52.6, 60.8, 53.5 and 70.1% by the immunofluorescence test (IFT). Geometric mean egg counts were low: 57.8, 33.0, 35.6, 47.3 and 40.9 eggs per gram of feces, respectively. Of the total of 299 schoolchildren, who submitted five blood samples at 6-month intervals, one for each survey, 40% were IFT-positive throughout the study, and 22% were IFT-negative in all five surveys. Seroconversion from IFT negative to positive, indicating newly acquired *S. mansoni* infection, was observed more frequently in surveys carried out during March-April (after Summer holidays), than during September-October. Seasonal trends were not statistically significant for detection of *S. mansoni* eggs in stool. The results indicate that the use of IgM-IFT is superior to parasitological methods for detection of incidence of *S. mansoni* infection in areas with low worm burden.

KEYWORDS: *Schistosoma mansoni*, Epidemiology, Immunodiagnosis, Seroconversion.

INTRODUCTION

In the State of São Paulo, Brazil, *S. mansoni* infection is endemic, in some known areas, but rates of transmission and worm burden are low. Therefore, detection of infection using only one fecal examination, as is usual in epidemiologic studies, has been shown to be insensitive[4,11].

In our experience with seroepidemiologic studies in different regions of São Paulo State, Brazil, we have found that *S. mansoni* seroprevalence rates are 2-4 times higher than prevalence determined by fecal parasitological methods. These differences seem to be mainly because of the poor sensitivity of the parasitological methods to detect patients with low egg counts[5,21,22]. Seroprevalence values may also be higher because of the incapacity of the serological tests to discriminate between present and past infections[3,17,26,27]. Our experience suggests that, in such areas of low endemicity, serologic results more closely approximate the true situation than do parasitologic results[6, 14,16]. For schistosomiasis control programs, it is important to use the method that best estimates true schistosomiasis prevalence. This is important not only for a better epidemiological understanding, but also for more efficient coverage of chemotherapy in control programs.

The detection of IgM antibodies against gut-associated antigens by immunofluorescence test (IFT) on worm paraffin sections has been shown to be very sensitive and specific for the diagnosis of both acute and chronic *S. mansoni* infections[28]. Also, the detection of IgA antibodies to the gut antigens has been demonstrated to be valuable for differential diagnosis of acute schistosomiasis[18]. The usefulness of the IgM-IFT on worm paraffin sections, by using blood samples collected on filter paper, has been tested for epidemiologic purposes and showed to be very efficient and practical. Slides with paraffin sections can be easily prepared and maintained at room temperature for many months without loss of antigenicity[16].

In the present study we have examined the applicability of the

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IgM-IFT to understanding the dynamics of the schistosomiasis transmission in an area of low endemicity. The serological data were analyzed in comparison with the results of the fecal parasitological examination, following, for a period of 2 years, a group of about 600 schoolchildren living in a rural area of Municipality of Itariri (Sao Paulo, Brazil).

MATERIAL AND METHODS

Description of the study area and population

The climatic and geographical aspects of the study area have been described in a previous paper[7]. The landscape is hilly, with many small streams and plentiful vegetation. The climate is hot and wet, with a mean temperature at 18 °C in the coldest month (July), and temperatures varying from 25 to 35 °C during the day in the hottest months, with a mean temperature higher than 22 °C (December and January).

This project was carried out in a rural village of the Municipality of Itariri, with an area of 294 km^2 and population of about 11,400 inhabitants, in the Vale do Rio Ribeira de Iguape, near to the Southeast coast of the São Paulo State, in Brazil. According to the data on epidemiologic classification by site of infection[24], as collected by SUCEN (Superintendência do Controle de Endemias) of the São Paulo State Health Department, about 68% of the *S. mansoni* cases can be classified as autochthonous, with the infection being acquired in one of the municipalities around Itariri. The autochthonous schistosomiasis prevalence in the municipality of Itariri was 3.1%, ranging from 0 to 9.2% in the different villages, according to the parasitological survey carried out in 1990, when about 4,900 fecal samples were examined. The only intermediary host in the area is the snail *Biomphalaria tenagophila*. From April of 1991 to April of 1993, children from four primary schools were followed by parasitological and serological diagnostic methods. In São Paulo, Brazil, primary school education takes 8 years. The school year starts in February and ends in November, with a period of Summer Holiday from December to February, and Winter Holiday in July. Five surveys were carried out, one for each semester, with a 6-month interval between them. In each survey, one fecal sample was collected from all of the children who were registered in the four schools selected for this study; also capillary blood was collected from fingertip onto a filter paper. Informed consent was obtained from all individuals participating in the study and for children the consent of their parents was obtained.

The 1st, 3rd and 5th surveys were carried out respectively in 1991, 1992 and 1993, between March and April of each year, *i.e.*, soon after the Summer Holiday. The 2nd and 4th surveys were carried out in the second semester, between September and October, respectively of 1991 and 1992, *i.e.*, after the Winter Holiday.

All *S. mansoni* egg positive children, after each positive stool, were treated with Oxamniquine, in a single dose of 20-25mg/kg-body weight.

Immunofluorescence test (IFT)

IgM antibodies to *S. mansoni* gut antigens on Rossman's solution fixed adult worm paraffin sections were detected by IFT, according to the technique previously described [28]. Several drops of capillary blood from a fingertip were collected from each individual onto a Whatman #3 filter paper, dried at room temperature and stored in a plastic bag at -20°C. For the IFT, the dried blood spot discs were cut from the filter by a punch with 8 mm diameter. The paper discs were eluted in 120 µl of 0.01 M pH7.2 PBS (Phosphate buffered saline), and incubated overnight at 4 °C. The serum dilution in each eluate after removal of the paper disc was estimated to be 1:16 [12].

Fecal examination

In each survey, the Kato-Katz technique[19] was performed on one fecal specimen from each schoolchild. *S. mansoni* egg counts were expressed in eggs per gram (epg), using the average of the egg counts obtained from three slides for each individual. Geometric mean of the egg counts was calculated for each survey for the *S. mansoni* egg positive samples only. Eggs of other helminth species were also recorded, and the results published elsewhere[16].

Statistical analyses

Data from the five surveys were entered into a microcomputer using the Epi-Info database package[2] and the same program was used to analyze the results from each survey. Incidence and prevalence rates were compared by the Chi square test using Yate's correction.

RESULTS

A total of 987 schoolchildren, 5 to 18 years old, were enrolled in the study. The number of stool and blood samples examined for each survey and the percentage positive for both methods are shown in Table 1. The number of blood specimens collected exceeds the number of stool specimens for all surveys. Geometric mean egg counts were low: 57.8, 33.0, 35.6, 47.3 and 40.9 epg, respectively for the 1st to 5th surveys. The percentages of children positive for *S. mansoni* infection by stool examination were 8.6% and 9.9% for the 1st and 3rd surveys (after the summer), compared with 6.8% and 5.8% for the 2nd and 4th surveys, respectively (after the winter). The difference between these two groups was statistically significant ($p = 0.004$). In addition, the prevalence of infection in the 5th survey, 17.2%, was significantly greater than that of the previous four surveys ($p<0.000001$). Similarly, seroprevalence was higher for the 5th survey than for the previous four surveys ($p < 0.000001$).

Of the 987 children who participated in the study, 299 were followed from the 1st to the 5th survey, submitting five blood samples to the serological test; from them 296 submitted both fecal and blood specimens to all five surveys (Table 2). Parasitologic and serologic prevalence data obtained for these 296 children (Table 2) were similar to the prevalence data obtained for all the children who were registered in the four schools and examined each semester (Table 1).

TABLE 1

Number of children enrolled in the study and percentage of positive results (95% confidence interval) by parasitological (Kato-Katz) and serological (IgM-IFT) methods for the five surveys carried out on the schoolchildren from four schools of the municipality of Itariri (S.Paulo - Brazil).

Survey	Number of children[a]	Kato-Katz			IgM-IFT		
		Total[b]	Positives		Total[c]	Positives	
			No.	%		No.	%
1st	628	513	44	8.6% (6.4-11.4)	627	354	56.5% (52.5-60.4)
2nd	599	482	33	6.8% (4.8-9.6)	591	311	52.6% (48.5-56.7)
3rd	623	485	48	9.9% (7.5-13.0)	612	372	60.8% (56.8-64.6)
4th	593	485	28	5.8% (3.9-8.3)	564	302	53.5% (49.3-57.7)
5th	618	512	88	17.2% (14.1-20.8)	605	424	70.1% (66.2-73.7)

a = Number of children, enrolled in each survey.
b = Total number of fecal samples submitted to Kato-Katz, in each survey.
c = Total number of blood samples submitted to IgM-IFT, in each survey.

Of the 299 children, who submitted five blood samples for serologic testing, 121 (40.5%) remained IFT-positive throughout the study, and 65 (21.8%) had negative IFT results in all five surveys (Table 3). A total of 63 children seroconverted from IgM-IFT-negative to positive (with no subsequent IFT-negative specimens), indicating new acquisition of *S. mansoni* infection. Of the total of 87 (65+22) children, with negative results for IgM-IFT in the first four surveys, 22 (25.3%) seroconverted between the 4th and 5th surveys. Of the 122 (65+22+3+32) children, who were IgM-IFT negative in first two surveys, 32 (26.2%) seroconverted between the 2nd and 3rd surveys. These rates of seroconversion were observed during the summer months, and they were higher ($p < 0.000001$) than the rates for the other two intervals observed during the winter months: 4.7% and 3.3 %. The former value correspond to 6 of 128 (65+22+3+32+6) children, who seroconverted between the 1st and 2nd surveys, and the latter to 3 of 90 (65+22+3) children, who seroconverted between the 3rd and 4th surveys. Fifty children (3+39+8) had one or more positive specimens followed by one or more negative specimens in a variety of patterns; 39 (78%) of these children were positive on both, the initial and final specimens (Table 3).

As shown in Table 4, of the 160 schoolchildren who submitted fecal samples to all five surveys, only 57 (35.6%) had at least one fecal sample positive for *S. mansoni* eggs, and 103 children (64.4%) remained parasitologically negative throughout the follow-up study. No differences in the incidence rates by the parasitologic Kato-Katz method was observed in the different surveys, except for the 5th survey.

Among the 103 children who consistently tested negative on Kato-Katz examination of stool, 45 (43.7%) had IgM antibodies present at the time of the 1st survey, indicating previous contact with *S. mansoni* or possible infection not detected by the Kato-Katz technique. All of the nine Kato-Katz-positive children in the 1st survey and five positive in the 2nd survey were IgM-IFT-positive in the 1st survey. Among the children who were Kato-Katz-positive in the 3rd, 4th and 5th surveys, respectively, three, two and 9 children had no IgM antibodies at the time of the 1st survey, but all seroconverted during the follow-up. All of the children with two or more fecal samples positive for *S. mansoni* eggs, except one (who was Kato-Katz positive in the 4th and 5th surveys), were IgM-IFT-positive in the 1st survey.

TABLE 2

Prevalences by parasitological (Kato-Katz) and serological (IgM-IFT) methods obtained for the schoolchildren who were submitted to both diagnostic tests in each survey, and participated of the study since the 1st survey.

Survey	Total	Kato-Katz Positives		IgM-IFT Positives	
		No.	%	No.	%
1st	512	44	8.6%	288	56.3%
2nd	452	28	6.2%	233	51.5%
3rd	356	37	10.4%	235	66.0%
4th	344	16	4.7%	209	60.8%
5th	296	54	18.3%	223	75.3%

TABLE 3

Number and percentage of schoolchildren for each possible combination of results of the serological test (IgM-IFT) carried out on 299 schoolchildren, who participated in the project from the 1st to 5th survey and submitted five blood samples, one for each survey.

Results of IgM-IFT in each survey					Number of children		%
1st	2nd	3rd	4th	5th			
P	**P**	**P**	**P**	**P**		121	40.5%
N	N	N	N	N		65	21.8%
N	N	N	N	**P**	63	22	7.4%
N	N	N	**P**	**P**		3	1.0%
N	N	**P**	**P**	**P**		32	10.7%
N	**P**	**P**	**P**	**P**		6	2.0%
P	N	N	N	N	3	1	0.3%
P	**P**	N	N	N		0	0
P	**P**	**P**	N	N		0	0
P	**P**	**P**	**P**	N		2	0.7%
P	**P**	**P**	N	**P**	39	15	5.0%
P	N	**P**	**P**	**P**		11	3.7%
P	N	**P**	N	**P**		6	2.0%
P	N	N	N	**P**		4	1.3%
P	**P**	N	**P**	**P**		2	0.7%
P	**P**	N	N	**P**		1	0.3%
N	N	**P**	N	**P**	8	4	1.3%
N	N	**P**	N	N		2	0.7%
N	N	**P**	**P**	N		1	0.3%
N	**P**	N	N	**P**		1	0.3%
TOTAL						299	100%

N = negative and P = positive result for the IgM-IFT

In nine non-treated children, selected because of their positive result for the IgM-IFT but negative for the Kato-Katz technique in the first survey, new fecal samples were collected to confirm the *S. mansoni* infection. In one case, the infection was confirmed by the finding of one egg on one of three Kato-Katz slides of a second fecal sample. In six children, negative for all three Kato-Katz slides of the second fecal sample, the confirmation was possible only by the spontaneous sedimentation technique, when one to 60 slides had to be examined to find at least one *S. mansoni* egg. In the last two children, *S. mansoni* eggs were detected only by the spontaneous sedimentation technique in a third fecal sample.

DISCUSSION

In the present study, immunofluorescence test (IFT) was performed on Rossman's fixed adult *S. mansoni* worm paraffin sections for detection of IgM antibodies to gut-associated polysaccharide antigens[25], in a group of schoolchildren living in a village of the municipality of Itariri (São Paulo State, Brazil).

TABLE 4

Number of schoolchildren for each possible combination of results of the parasitological (Kato-Katz) method carried out on 160 schoolchildren, who participated in the project from the 1st to 5th survey and submitted five fecal samples, one for each survey.

Results of Kato-Katz in each survey					Number of children	
1st	2nd	3rd	4th	5th		
N	N	N	N	N		103
P	N	N	N	N	45	9
N	**P**	N	N	N		5
N	N	**P**	N	N		6
N	N	N	**P**	N		4
N	N	N	N	**P**		21
N	N	N	**P**	**P**	12	4
P	N	N	N	**P**		4
P	N	**P**	N	**P**		1
P	**P**	**P**	N	N		1
N	**P**	N	**P**	N		1
N	N	**P**	N	**P**		1
TOTAL						160

N = negative and P = positive result for *S. mansoni* eggs in Kato-Katz

This study was planned in order to evaluate the potential of the IgM-IFT, compared to the parasitologic method, as a tool for studying the dynamics of schistosomiasis transmission in an area of low endemicity for schistosomiasis. In previous papers[18, 28], the IgM-IFT was shown to be very sensitive for detecting both acute and chronic *S. mansoni* infections, and also very specific when tested on clinically healthy individuals. When compared to an ELISA for detection of IgG antibodies to total worm protein antigens, although detecting different classes of antibodies against different antigenic structures of the *S. mansoni* worms, the IgG-ELISA and the IgM-IFT showed similar diagnostic potential for both acute and chronic forms of schistosomiasis[29]. When applied in field studies, the IgM-IFT on worm paraffin sections displayed a good specificity in an area with high prevalence of other helminth infections, and proved useful for mapping and differentiating areas with and without active transmission of *S. mansoni*[16]. The IgG-IFT, as used in previous epidemiological studies, on frozen sections of *S. mansoni* worms[6] or egg granulomata[21], has some limitations related to the difficulties for the storage and transportation of the antigen sections, which must be conserved frozen to keep their antigenic activity. The high stability of the Rossman's fixed adult *S. mansoni* worm paraffin sections, at room temperature, and the possibility of detecting IgM antibodies in blood samples collected on filter paper, with good sensitivity and specificity, support the use of the IgM-IFT for epidemiologic purposes.

In this paper, the significantly higher IgM-IFT prevalence rates obtained in comparison to the parasitological data, in all five surveys (Table 1), might actually indicate an area with low worm burden, and not necessarily low endemicity. The geometric mean

egg counts, varying from 33.0 to 57.8 epg, obtained by the examination of three Kato-Katz slides for each stool sample, also suggest a low worm burden in the infected individuals. If greater number of slides for each stool sample were examined[11], the mean egg count in the studied area would probably be lower than 20 epg. The limit of sensitivity of the method, as shown by KATZ et al.[20], is at that level. The comparative analysis of the results of the serologic test and the parasitologic follow-up (Table 4) suggests the superiority of the serological test and this lack of sensitivity of the Kato-Katz method for detecting individuals with low worm burden, as already reported by different authors[1,4,6,11].

The tendency to significantly higher parasitologic prevalence rates observed for the post-Summer surveys, when compared to the post-Winter surveys (Table 1) indicates some seasonal influence on *S. mansoni* transmission in the area. The occurrence of seasonal transmission was clearly detected by the serological follow-up study, since the results of the IgM-IFT showed significant differences of the seroconversion taxes in the post-Summer and post-Winter surveys, statistically higher in the former (Table 3). The positive IgM-IFT result in a schoolchild who showed negative result in the previous survey is undoubtedly indicative of recently acquired infection. In experimentally infected baboons, IgM antibodies to *S. mansoni* gut antigens were detected by IFT around the second week after infection, thus, many weeks before the appearance of *S. mansoni* eggs in the feces (data not published). The possibility of detecting *S. mansoni* infection in such a very early phase after contact with the cercariae can provide a sensitive method to determine incidence of the disease, certainly earlier than the parasitologic method. For this method, we must wait for seven weeks or more for the eggs to be found in the feces. Also, the number of eggs in the feces in this early phase is very small, and the parasitologic methods can fail to detect them. In our study, the parasitologic follow-up data (Table 4) did not show different incidence rates in the post-Summer and post-Winter surveys as did the serological data (Table 3), except for the 5th survey. The increase in incidence during this last period of the study was unexplained. Some exceptional climatic condition must have favored the transmission of schistosomiasis in the area. Therefore, the parasitologic techniques are not as sensitive as the serologic methods for determining schistosomiasis incidence rates, and the IgM-IFT must be certainly more efficient than Kato-Katz technique in *S. mansoni* surveillance programs, especially in endemic areas with low worm burdens.

In different epidemiologic studies carried out in schistosomiasis endemic areas of the São Paulo State[5,6,21], or outside of Brazil[8,10,13,15], the authors have obtained higher prevalence indices by serologic than parasitologic methods. The lack of sensitivity of the parasitologic Kato-Katz method for detecting individuals with low egg output cannot fully explain the exceptionally higher serological prevalence rates, in comparison to the parasitologic ones, observed in the present investigation. Rather, the high sensitivity of the IgM-IFT to detect very low infections, which would not be detected by fecal examination, might be an important explanation for such a great difference observed between the serologic and parasitologic methods in this study. Also, the potential of the IgM-IFT to detect recent infection, even before the appearance of eggs in the feces, probably contributes to this difference. The repeated negative results of fecal examinations in some serologically positive children showed how difficult the parasitological confirmation of the *S mansoni* infection can be in residents of endemic areas with low worm burdens. Also, the possible presence of gut-specific IgM antibodies in some previously infected and self or drug-cured individuals must be considered. *S. mansoni* infection has been reported since 1953 in the municipality of Itariri, and selected treatment with Hycanthone or Oxamniquine has been performed. In fact, about 20 % of the children enrolled in this project had received previous treatment with one of the above-mentioned drugs, and the serologic prevalence was higher in the previously treated children than in those who had never received treatment[16]. Serologic tests based on the detection of circulating antigens would not be subject to these limitations, but such tests have not so far proved to be sufficiently sensitive for diagnosis of individuals with low worm burden[9]. Other factors like contact with cercariae of other parasite species must also be considered and better investigated in the area. The possibility of cross reactivity with other helminth antigens seems unlikely, given the good specificity demonstrated by the IgM-IFT when applied on a rural population of a different village of Itariri. In this village autochthonous schistosomiasis cases had never been detected, but the prevalence rates for other helminth species were as high as the village where the present study was carried out[16].

According to the data in Table 3, a very few individuals (1%) showed definitive seroconversion from IgM-IFT positive to negative status; on the other hand, 39 of them (13%) who showed seroconversion from positive to negative IgM-IFT, returned to positive status in following surveys. These results might suggest that, in our studied area, the children are repeatedly stimulated by a continuous contact with the infective cercariae; but, because of their resistance to new infections, there is no development of mature worms, and consequently no egg production. The higher number of seroconversions from negative to positive IgM-IFT results in post-Summer surveys, when compared to post-Winter surveys, can be explained by the higher chance of children to being exposed to the risk of contact with cercariae during the hot days of Summer holidays. MARÇAL JR. et al.[23], studying the risk factors for *S. mansoni* infection, concluded that recreational activities like swimming, playing and fishing in the river were the main reasons for schistosomiasis transmission in the area, and these activities are more related to the Summer season.

The high sensitivity of the IgM-IFT to detecting schistosomiasis in a very early phase after infection can be used as a powerful tool for studying the dynamics of schistosomiasis transmission in endemic areas with low worm burden.