Detection of *Schistosoma mansoni* Antibodies in a Low-Endemicity Area Using Indirect Immunofluorescence and Circumoval Precipitin Test

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Abstract. Parasitological diagnostic methods for schistosomiasis lack sensitivity, especially in regions of low endemicity. The objective of this study was to determine the prevalence of *Schistosoma mansoni* infections by antibody detection using the indirect immunofluorescence assay (IFA-IgM) and circumoval precipitin test (COPT). Serum samples of 572 individuals were randomly selected. The IFA-IgM and COPT were used to detect anti-*S. mansoni* antibodies. Of the patients studied, 15.9% ($N = 91$) were IFA-IgM positive and 5.1% ($N = 29$) had COPT reactions ($P < 0.001$ by McNemar's test). Immunodiagnostic techniques showed higher infection prevalence than had been previously estimated. This study suggests that combined use of these diagnostic tools could be useful for the diagnosis of schistosomiasis in epidemiological studies in areas of low endemicity.

INTRODUCTION

Schistosomiasis is a major public health problem, with an estimated 200 million people infected worldwide. As a consequence of effective schistosomiasis control programs based on mass or selective chemotherapy, basic sanitation measures, and use of molluscicides, the prevalence of human schistosomiasis has decreased considerably in the Americas and endemic regions in Asia¹; consequently, the World Health Organization (WHO)² has established strategies for schistosomiasis control for the low-transmission area.³

On the basis of experiences with endemic schistosomiasis control in Venezuela, Alarcón de Noya and others⁴ defined low-endemicity areas (LEAs) and characterized clinical cases of schistosomiasis in those areas as case prevalence in up to 25% of the examined population, low infection intensity (< 100 eggs per gram of feces), and prevalence of asymptomatic individuals with continued *Biomphalaria* spp. mollusk presence.

In Brazil, schistosomiasis affects 19 federal units.⁵ Approximately 6 million individuals are infected and 25 million are at risk of infection, with prevalence rates varying by state.⁶

Traditionally, schistosomiasis *mansoni* prevalence is determined by parasitological examination of feces using the Kato-Katz technique,⁷ which is the gold standard for estimation of infection intensity in field studies.

Studies point to a reduction in diagnostic sensitivity in infections with low parasite load.^{8,9} To improve sensitivity, it is necessary to increase the number of fecal samples analyzed; however, obtaining more than one fecal sample from an individual hinders fieldwork. In addition, using the Kato-Katz method, samples from different days showed only a 6% positivity increase compared with the first sample.¹⁰

We are thus faced with a challenge both in the individual and collective context, because this is a chronic infection that, even without progressing to severe forms, triggers debilitating sequelae secondary to parasitism, especially in childhood.

Anemia and chronic malnutrition from infections cause growth and developmental retardation.¹¹ Also of concern is continued transmission by asymptomatic carriers who transmit the parasite in areas without adequate basic sanitation.¹² Thus, LEAs require close surveillance to prevent increased endemicity.⁴

Within this context, immunodiagnostic techniques have been introduced into the schistosomiasis control programs of some countries, such as Venezuela¹³ and China.¹⁴

Studies have shown that an indirect immunofluorescence reaction, using paraffin-embedded sections of adult worms, allows for the detection of immunoglobulin M (IgM) antibodies against antigens of the parasite's digestive tract, making it a highly sensitive method for the diagnosis of acute and chronic schistosomal infections. The specificity of this reaction has also proved to be adequate.^{15,16}

The periovular reaction has a high sensitivity and specificity for the detection of antibodies against the secretion or excretion antigens of viable *Schistosoma mansoni* eggs.^{4,13,17,18} Although it is a very laborious technique, it is the only antibody detection technique that has been shown to correlate with parasite activity.¹⁹

Although these techniques are of limited use for individual diagnoses, they are informative when used for epidemiological studies of parasite prevalence.

This study was performed in Barra Mansa, Rio de Janeiro, Brazil, an area endemic for *S. mansoni*, with an estimated prevalence of 1%.²⁰ Historical data series taken from the National Notification System²¹ from 2001 to 2009 revealed that the neighborhoods with greatest infection prevalence are Siderlândia, Santa Clara, São Luiz, and Nova Esperança. The most common intermediate mollusk host is *Biomphalaria tenagophila*, but *Biomphalaria straminea* and *Biomphalaria glabrata* have also been reported.²²

The objective of this study was to systematically determine the prevalence of *S. mansoni* infection using the indirect immunofluorescence assay (IFA-IgM) and circumoval precipitin test (COPT) techniques in serum samples from the study population in the areas surrounding Barra Mansa, Rio de Janeiro, Brazil. Parasitological techniques described by Hoffman²³ and Katz⁷ were used as references.

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METHODS

A cross-sectional study was conducted from April to December 2011 in the districts of Siderlândia, Cantagalo, São Luiz, Nova Esperança, and Santa Clara. These localities, together, have ~7,000 inhabitants and are located on the outskirts of Barra Mansa, Rio de Janeiro, Brazil.

We used probabilistic sampling to systematically select households (one in six) and randomly selected individuals through a draw among those who agreed to participate in the study. The inclusion criteria were > 5 years of age and not having been treated for *S. mansoni* infections in the previous year.

Statistical analysis. Statistical analysis was performed using SPSS for Windows, version 15.0 (SPSS, Inc., Chicago, IL) and Microsoft Excel 2003 (Microsoft Corp., Redmond, WA). Test significance levels were fixed by accepting a type 1 error of 5% ($\alpha = 0.05$).

Population characteristics were described using absolute and relative frequencies and calculation of average age and standard deviations.

The proportion of positive results for each infection test (prevalence) was evaluated for each diagnostic technique.

Each *S. mansoni* infection measurement technique was compared and marginal associations verified using the McNemar test. Pairwise concordance between results was assessed using the Cohen kappa index (K) and 95% confidence interval.

Associations between *S. mansoni* infection and age range, sex, neighborhood, river water use, and history of schistosomiasis was assessed for each technique using the χ^2 test. Fisher's exact or likelihood ratio tests²⁴ were used when the sample number was insufficient for a χ^2 test.

We compared the accuracy (sensitivity, specificity, likelihood ratio, and predictive values) of serological techniques to parasitological techniques, and also compared results among techniques to determine which were most effective in diagnosing *S. mansoni* in areas with a similar epidemiological profile to the target area of this study.

Ethical aspects. In accordance with the rules governing human subject research, informed consent was obtained to meet the recommendations of Resolution no. 196/96 of October 10, 1996 repealed by Resolution no. 466 from December 12, 2012 of the National Council of Health. This research project was approved by the Research Ethics Committee of the Department of Infectious and Parasitic Diseases of the Faculty of Medicine of the University of São Paulo and the Research Ethics Committee of the Hospital das Clínicas (CAPPesq) of the Faculty of Medicine of the University of São Paulo (approval no. 0405/09).

Ethical aspects of animal experiments. The experimental research projects met Laws 6.638/79 and 9605/98, Decree

24.645/34, the Ethical Principles of Animal Experimentation, the Principles for Research Involving Animals (Geneva, 1985), and other directives governing animal research. The work began after approval of research project no. CEP-IMT 2011/096 by the Ethics Animal Research Committee of the Institute of Tropical Medicine of São Paulo, University of São Paulo, Brazil.

Methods of laboratory diagnosis. The Family Health Program and agents of the Municipal Schistosomiasis Control Program collected 572 randomized feces and serum samples from inhabitants of five peripheral neighborhoods of Barra Mansa, Rio de Janeiro.

The serum samples obtained were aliquoted and stored at -20°C , and then transported to São Paulo in thermal boxes containing dry ice and stored at -20°C in the laboratory until tested.

Parasitological methods. Fecal samples were processed and evaluated using techniques based on Kato and Miura²⁵ and modified by Katz and others⁷ (KK) and Hoffman and others²³ (HH). Four slides were prepared for every participant: two slides each for the KK and HH techniques, respectively.

A Helm Test Kit (Biomanguinhos, Fiocruz, Rio de Janeiro, Brazil) was used to perform the KK technique.

Laboratory maintenance of the *S. mansoni* experimental cycle. The *S. mansoni* cycle is maintained through periodic infection of hamsters (*Mesocricetus auratus*) and *B. glabrata* mollusks (strain BH). Each week, five animals are subcutaneously infected with 200 to 300 cercariae and slaughtered after 49 to 56 days to collect adult worms and parasite eggs. *Schistosoma mansoni* eggs are collected from liver granulomas.

Periovar reaction. A 10 μL aliquot of a purified egg suspension, adjusted to contain 300 viable eggs in 0.9% saline solution, and 50 μL of each serum sample were added to 2 mL Eppendorf tubes (Eppendorf do Brasil Ltd., São Paulo, São Paulo, Brazil) and incubated at 37°C for 48 hours. Subsequently, a 30 μL aliquot of each mixture (serum + viable egg suspension) was placed on a slide and covered with a coverslip (22×22 mm). Assessment was performed on a binocular Olympus-CX41 microscope (Olympus Corporation, Tokyo, Japan), with a $10\times$ or $40\times$ objective lens. The number of reactive eggs per 100 viable eggs and the periovar precipitate morphology were used for assessment. *Schistosoma mansoni* eggs were considered reactive if they contained globular precipitates of variable size and form, or if they appeared as small or long septated chains similar to *Taenia* segments (Figure 1). Sera were positive if at least 9% of the mature eggs were reactive in the presence of different precipitates.^{13,18,26}

Detection of IgM antibodies against antigens of the *S. mansoni* digestive tract. The IFA-IgM was used to detect anti-antigen polysaccharide IgM antibodies in the digestive

FIGURE 1. Reactive *Schistosoma mansoni* eggs showing different precipitate types observed on circumoval precipitin test: (A) small globular precipitates; (B) non-septated long globular precipitates; (C) long septated precipitates, similar to *Taenia* segments. 240×91 mm (300×300 DPI).

tract of adult *S. mansoni* worms on paraffin sections, according to the technique described by Reference ²⁷.

Obtaining adult worm. Infected animals were anesthetized and killed, with a longitudinal thoracoabdominal incision for visceral exposure. After sectioning the portal vein, 50 mL of 0.85% saline solution with ethylenediaminetetraacetic acid were infused into the left ventricle using a 20 mL syringe. Adult worms were obtained after perfusion of the portal system, removed from the abdominal cavity, and numbered and stored at -20°C .^{28,29} Adult male worms were separated for "particulate" antigen processing in paraffin sections for IFA-IgM analysis.

Slide preparation for IFA-IgM. Approximately 60 adult male worms were placed on an end-joint 200 μm mesh screen (Tecmolín, PA-6-212/XX, São Paulo, Brazil), immersed in a Rossman's solution fixative for 2 hours at room temperature, and then immersed in 90% ethanol three times for 2 hours. The sample was then immersed in absolute alcohol for 15 hours, incubated in methyl benzoate for 4 hours, xylol at 60°C for 15 minutes, 50% xylene/paraplast for 15 minutes, and 100% paraplast (Sigma-Aldrich, Saint Louis, MO, USA) at 60°C for 30 minutes. The material was embedded in an L-shaped aluminum frame, placed at room temperature for 12 hours, and subsequently stored at the same temperature for processing of histological sections. Serial sections of 3 μm were made using a microtome, with 10 sections per slide.

The slides with paraffin sections were subjected to dewaxing and rehydration, through successive baths of xylene and ethanol at different concentrations, with a final bath in phosphate buffered saline (PBS), Buffer pH = 7.2.³⁰ The slides were stored at room temperature until use.

IFA-IgM. Serum samples were diluted 1:10 in PBS solution (pH 7.2) and deposited onto the paraffined sections of adult worms. After incubation at 37°C for 50 minutes in a humid chamber, slides were washed in 0.01 M PBS pH 7.2 baths for 10 minutes. Anti-human IgM fluorescent conjugate (anti-human IgG γ -chain-specific fluorescein isothiocyanate antibody produced in goat) was added (Sigma-Aldrich, St. Louis, MO) in accordance with its optimal use titer (1 of 320) in PBS, Buffer pH = 7.2 containing 1% Evans blue solution (Bio-Rad Laboratories, Hercules, CA). After further incubation and washes, slides were dried and mounted with glycerol and cover glass. Positive standard serum was used for 1/10, 1/40, 1/160 dilutions. Negative standard serum was used for 1/10 dilution.

The IFA-IgM reading was performed using an Olympus BX-FLA fluorescence microscope (Olympus Corporation, Tokyo, Japan) equipped with an epi-illumination system, with $100\times$ and/or $200\times$ amplification.

A sample was positive when fluorescence was present only in structures related to the parasite digestive tract. Fluorescence detected in membranes or the parasite parenchyma was considered nonspecific. The results were expressed as reactive (presence of fluorescence in the digestive tract) and non-reactive (absence of fluorescence) sera (Figure 2).

Antibody detection of *S. mansoni* egg antigens. The COPT was used to detect antibody reactions against excretion and secretion products of *S. mansoni* eggs using previously described techniques.^{13,18,26}

Isolation and purification of *S. mansoni* eggs. *Schistosoma mansoni* eggs were isolated and purified as described by Dresden and Payne³¹ and Pinto and others³² with some modifications.

FIGURE 2. Serialized paraffin section of male *Schistosoma mansoni* worms. Reactivity of the intestinal lining of the digestive tract (polysaccharide antigen) with human IgM-class antibodies.

Livers from three infected hamsters were cut into small pieces and incubated in a water bath at 37°C for 20 minutes in 4 mg % pepsin and 0.7% hydrochloric acid solution. After incubation, the peptic solution was discarded and the tissue fragments were added to 150 mL of 0.9% ice-cold saline solution containing 50 μL of Triton 100. The material was homogenized using several drive pulses on a domestic blender (Walita Philips, Amsterdam, Netherlands) until the fragments were completely ruptured. The material was then filtered in 4-fold gauze and the screen processed under negative pressure in a series of metallic sieves with mesh numbers 100 (0.150 mm), 200 (0.075 mm), and 400 (0.038 mm) (Granutest, Telastem, peneiras para análise Ltd., São Paulo, Brazil).

Eggs retained on the last sieve were removed by successive washing with ice-cold 0.9% saline solution and concentrated to 1 mL volume by centrifugation at 1,000 rpm for 15 seconds. From this concentrated suspension, a 10 μL aliquot was placed between the blade and the coverslip and evaluated using a microscope (Olympus-CX41, Olympus Corporation, Tokyo, Japan) at $100\times$ amplification. The number of eggs in the whole extension of the coverslip was counted. Suspensions exceeding 20% of cell debris in relation to the number of eggs found were reprocessed in 200 and 400 meshes. For COPT, the egg suspensions were adjusted to contain 300 viable eggs per 10 μL of 0.9% saline solution and stored at 4°C for 4 hours until reaction preparation.

RESULTS

A total of 572 paired serum and feces samples from volunteers were evaluated using COPT and IFA-IgM serological techniques and KK and HH parasitological techniques. The majority of subjects in the study population were females. The average age of that population was 40–41 years. The Siderlândia neighborhood had the highest number of cases (243/42.5%), although Santa Clara had a smaller number of cases (32/5.6%). Approximately 4.2% of the participants reported a history of schistosomiasis.

TABLE 1

Schistosoma mansoni-positive infections for each diagnostic technique in samples collected from individuals in Barra Mansa/RJ, 2011*

Method	Positive/total	%
KK-HH	5/572	0.9
COPT	29/572	5.1
IFA-IgM	91/572	15.9

*KK = Kato-Katz technique; HH = Hoffman technique; COPT = circumoval periovular test; IFA = indirect immunofluorescence assay.

The IFA-IgM identified the greatest number of *S. mansoni* infections (91 of 572, 15.9%), followed by COPT (29 of 572, 5.1%). The parasitological techniques (KK-HH) identified the fewest infections (5 of 572, 0.9%; Table 1).

The results of parasitological tests using the KK and HH techniques were 0.7% ($N = 4$) and 0.8% ($N = 5$), respectively (Table 2). Other helminth infections had a lower prevalence in relation to *S. mansoni* infections, but protozoan infections such as *Endolimax nana* and *Blastocystis* spp. were higher, with infection rates of 17.4% and 10.8%, respectively.

Table 3 shows the rates of sensitivity, specificity, positive predictive value, and negative predictive value of the diagnostic techniques compared with the parasitological techniques. The COPT showed a higher sensitivity and specificity when compared with parasitological techniques.

Table 4 shows that COPT positivity is statistically different from the IFA-IgM and HH and KK techniques ($P < 0.001$ and $P < 0.001$, respectively). The results of the COPT technique generally agree with those of the other techniques, with the concordance being higher with IFA-IgG ($K = 0.332$) and lower with the parasitological techniques ($K = 0.224$).

Table 5 shows that positivity by IFA-IgM is significantly higher in men ($P < 0.001$) between 20 and 49 years of age ($P < 0.001$) in Santa Clara ($P = 0.022$) who reported previous schistosomiasis ($P < 0.001$).

Table 5 shows that the COPT positivity is significantly higher in male individuals ($P < 0.001$) in Santa Clara ($P < 0.001$) who use river water ($P = 0.003$) and have a history of schistosomiasis ($P < 0.001$).

DISCUSSION

Clinical cases of *S. mansoni* infection in Brazil have been identified and classified using criteria based on clinical manifestations.³³ Alarcon de Noya and others (2006)⁴ suggested instead that identification of cases in areas of low endemicity should be based on laboratory techniques followed by clinical classification.

Immunodiagnostic techniques are already used in schistosomiasis control programs in China^{34,35} and Venezuela.^{4,13}

TABLE 2

Associations between parasitological tests positive for *Schistosoma mansoni* infection and immunodiagnostic techniques of the population sampled from Barra Mansa, Rio de Janeiro in 2011*

Sample	A	Sex	KK S1	KK S2	HH S1	HH S2	COPT	IFA-IgM
31C	46	M	1 egg	0	SM	SM	—	+
208E	52	M	8 eggs	19 eggs	SM	SM	+	+
251E	42	M	6 eggs	2 eggs	SM	SM	+	—
132E	21	M	6 eggs	1 eggs	—	SM	+	+
7C	19	F	0	0	SM	0	+	—

*A = age; KK = Kato-Katz technique; HH = Hoffman technique; S1 = first slide; S2 = second slide; COPT = circumoval periovular test; IFA = indirect immunofluorescence assay; SM = positive for *Schistosoma mansoni*.

TABLE 3

Description of sensitivity, specificity, positive, and negative predictive values for all diagnostic techniques compared with the HH and KK reference techniques in individuals of Barra Mansa Rio de Janeiro, 2011*

Method	Measure	Estimate (%)	CI (95%) Lower	CI (95%) Higher
COPT	Sensitivity (%)	80.0	28.4	99.5
	Specificity (%)	95.6	93.6	97.1
	Likelihood ratio (+)	18.1	10.1	32.5
	Likelihood ratio (—)	0.2	0.0	1.2
	Positive predictive value (%)	13.8	3.9	31.7
	Negative predictive value (%)	99.8	99.0	100.0
	Accuracy (%)	95.5	95.1	95.9
IFA-IgM	Sensitivity (%)	60.0	14.7	94.7
	Specificity (%)	84.5	81.2	87.4
	Likelihood ratio (+)	3.9	1.8	8.1
	Likelihood ratio (—)	0.5	0.2	1.4
	Positive predictive value (%)	3.3	0.7	9.3
	Negative predictive value (%)	99.6	98.5	99.9
	Accuracy (%)	84.2	83.1	85.3

*CI = confidence interval; COPT = circumoval periovular test; IFA = indirect immunofluorescence assay.

In Brazil, several techniques have been tested in LEAs, such as São Paulo.^{12,15,36,37}

In Barra Mansa, as a result of implementation of a special program for controlling schistosomiasis (Programa Especial de Controle da Esquistossomose) in 1976, schistosomiasis prevalence was around 1%.²⁰

The aim of this study was to compare and evaluate the accuracy of the COPT, IFA-IgM, and parasitological diagnostic techniques in the identification of *S. mansoni* infections, by means of epidemiological inquiry of randomized fecal and serum samples from individuals living in areas peripheral to Barra Mansa, Rio de Janeiro.

The results showed that, compared with the KK and HH parasitological methods, IFA-IgM and COPT serological techniques were more sensitive in the detection of *S. mansoni* infections in this study population. The positivity rates obtained for IFA-IgM, COPT, and KK and HH parasitological techniques were 15.8% ($N = 91$), 5.1% ($N = 29$), and 0.9% ($N = 5$), respectively. This comparison showed a statistically significant difference ($P < 0.001$) in detection between parasitological and serological techniques.

Socio-epidemiological variables such as male sex, residing in the Santa Clara neighborhood, river water use, and a history of schistosomiasis and diagnostic techniques used showed statistically significant differences ($P = 0.001$) for the KK, HH, IFA-IgM, and COPT techniques. There was also a statistically significant association with age (20–49 years) with IFA-IgM. These variables may indicate risk factors and may be involved in a causal chain.³⁸

Mello SG (2011)³⁹ observed that human infection with *S. mansoni* occurs in all age groups, with a higher prevalence between 7 and 17 years of age (34%) and 18 and 28 years of age (28%) in both women and men.

Alarcon de Noya and others (2007)⁴⁰ reported higher positivity using KK in age groups ranging from 11 to 16 years and 16 to 20 years, and declined with age, in schistosomiasis-endemic areas of Venezuela, which has a prevalence of 6.1%.

Epidemiological studies estimate the risk of a given outcome from a specific exposure. Even if a risk factor does not cause the disease, its presence allows us to predict the probability that the disease will occur.⁴¹

TABLE 4

Agreement between the positive results obtained with the COPT technique and those presented by other techniques, in feces and serum samples collected from the sampled population of Barra Mansa, Rio de Janeiro, 2011*

COPT										
Test	Negative		Positive		Total		<i>P</i> McNemar	Kappa	CI (95%)	
	n	%	n	%	n	%			Lower	Higher
KK-HH							< 0.001	0.224	0.038	0.410
Negative	542	94.8	25	4.4	567	99.1				
Positive	1	0.2	4	0.7	5	0.9				
Total	543	94.9	29	5.1	572	100				
IFA-IgM							< 0.001	0.332	0.222	0.442
Negative	475	83.0	6	1.0	481	84.1				
Positive	68	11.9	23	4.0	91	15.9				
Total	543	94.9	29	5.1	572	100				

* KK = Kato-Katz technique; HH = Hoffman technique; CI = confidence interval; COPT = circumoval periovular test; IFA = indirect immunofluorescence assay.

The COPT positivity showed a statistically significant difference compared with parasitological techniques ($P < 0.001$). The level of agreement between COPT ($K = 0.224$) and parasitological techniques was higher than that with IFA-IgM.⁴²

The higher COPT positivity compared with parasitological techniques may be caused by oviposition occurrence, where eggs deposited in the deepest layers of the intestine can be detected even in the absence of eggs in feces, especially in chronic infections in patients over 40 years of age⁴³ and with low parasite load.¹³

However, in this study, COPT was positive in four of the five individuals who were positive for *S. mansoni* using parasitology techniques. This serological technique was able to identify one case⁴³ that tested negative by KK in two slides and positive by HH in one slide, which would typically be considered an undetectable parasite load. In this case, the samples were also negative by IFA.

The negative COPT and positive parasitological results can be explained by factors associated with the sample quality, such as the presence of hemoglobin in the blood due hemolysis and storage time of sera at -20°C .⁴⁴ Furthermore, one should

take into account that the intensity of the reaction depends on the parasite load.⁴³

The COPT positivity rate was lower than that by IFA-IgM, probably because the techniques test antigens with different evolutionary forms. The COPT detects antibodies against excretion and secretion products of miracidia, the fluid that surrounds the parasite,¹⁷ whereas IFA-IgM reactions detect antibodies against antigens of the internal lining of the adult worms' digestive tract.⁴²

The antibodies detected by COPT appear early, matching the beginning of egg dissemination into feces.^{43,45,46} In this way, the antibodies are detected only when the infection is caused by worms of both sexes.^{46,47} However, IFA-IgM techniques may be positive in unisexual infections.⁴⁸

The serological positivity detected by IFA-IgM in this study may be related to individuals previously infected with *S. mansoni* but properly treated and cured⁴⁹ or those who were exposed to very low loads of cercariae or unisexual infections.^{48,50,51} Cross-reactivity with cercariae antigens from organisms that infect other species of animals are possible,²⁹ as are cross-reaction with other parasites.^{15,36,52}

TABLE 5

Association between the diagnosis of *Schistosoma mansoni* infection using COPT and IFA-IgM and socio-demographic characteristics of the population sampled from Barra Mansa Rio de Janeiro, 2011*

Variable	Total	COPT			IFA-IgM		
		Negative n	Positive n (%)	<i>P</i>	Negative n (%)	Positive n (%)	<i>P</i>
Age group (years)							
1 to 9	23	23	0 (0)	0.244†	23	0 (0)	< 0.001†
10 to 19	123	119	4 (3.3)		108	15 (12.2)	
20 to 49	217	203	14 (6.5)		166	51 (23.5)	
50 to 90	209	198	11 (5.3)		184	25 (12)	
Sex							
Female	347	341	6 (1.7)	< 0.001	316	31 (8.9)	< 0.001
Male	225	202	23 (10.2)		165	60 (26.7)	
Neighborhood							
Cantagalo	46	44	2 (4.3)	< 0.001†	40	6 (13)	0.022
Nova Esperança	162	161	1 (0.6)		142	20 (12.3)	
Santa Clara	32	27	5 (15.6)		21	11 (34.4)	
São Luiz	89	88	1 (1.1)		78	11 (12.4)	
Siderlândia	243	223	20 (8.2)		200	43 (17.7)	
River water use							
No	416	400	16 (3.8)	0.003‡	354	62 (14.9)	0.061
Yes	33	27	6 (18.2)		24	9 (27.3)	
Schistosomiasis							
Yes	24	18	6 (25)	< 0.001‡	9	15 (62.5)	< 0.001‡
No	451	434	17 (3.8)		388	63 (14)	

* COPT = circumoval periovular test; IFA = indirect immunofluorescence assay; Chi-square test result.

† Likelihood ratio test result.

‡ Fisher's exact test result.

Among five individuals who tested positive by parasitological techniques, two were negative by IFA-IgM. The KK technique did not detect any eggs in one individual; another one had four eggs (average number of *S. mansoni* eggs in two KK slides). The negative results of this serological technique can be related to low immune response by the host^{53,54} and a decrease in sensitivity in infections with low parasite load.^{35,36}

The Kato and Miura technique,²⁵ modified by Katz and others⁷ has become the international reference standard by the WHO. However, we can observe a decrease in sensitivity in this technique in LEAs and after specific treatment,^{55,56} a fact that undermines sensitivity assessment of new *S. mansoni* diagnostic techniques when used as a reference under these low-endemicity conditions. Other studies have used other tests as reference for the diagnosis of schistosomiasis mansoni.^{18,57,58}

Parasitological techniques, mainly, the KK technique, have brought a substantial benefit to helminthiasis control.⁷ The presence of LEAs can be considered an indicator of positive outcomes of the schistosomiasis control programs. However, without surveillance and supervision, these areas have the potential for increased endemicity.⁴

Alarcon de Noya and others (2006)⁴ proposed strategies and goals for schistosomiasis mansoni control according to endemicity: areas with high and medium endemicity should focus on morbidity control, whereas areas with low endemicity should focus on transmission control.

In this way, a reflection is necessary to develop a proposal for controlling helminthiasis in LEAs, promoting equal surveillance actions for the local populations, and a path for schistosomiasis elimination.

In this study, positivity rates were highest using IFA-IgM compared with parasitological techniques and COPT. Therefore, we could use it as a primary approach to screen a large number of suspected cases of schistosomal infection in epidemiological inquiries, in combination with the KK and HH techniques, taking into account the statistically significant associations of socio-demographic variables. Confirmation of IFA-IgM-positive cases with COPT techniques would be beneficial, as it is a more sensitive and specific technique that should be considered a gold standard in the serological diagnosis of active *S. mansoni* infections in LEAs, according to Noya and others (2002).²⁶

Further studies are required to identify the most accurate and representative method for detection in these areas, but this study points to the potential of combined diagnostic tools to diagnose schistosomiasis mansoni in LEAs.

It remains a challenge for researchers and society to eliminate the transmission of helminthiasis and improve the quality of life for affected populations.

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