Evaluation of microscopical and serological techniques in the diagnosis of Schistosoma mansoni infection at Sennar State, Central Sudan

Abdelbasit Mohamed Ibrahim, Mutasim Elhadi Ibrahim

1Department of Parasitology and Medical Entomology, Faculty of Medical Laboratory Sciences, Khartoum University, Khartoum, Sudan
2Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Khartoum University, Khartoum, Sudan

1. Introduction

Schistosomiasis is a tropical parasitic disease endemic in many developing countries. It affects over 200 million people worldwide and about 90% of them are found in sub-Saharan Africa[1,2]. Different species of the genus Schistosoma known to cause the disease in the humans such as Schistosoma mansoni (S. mansoni), Schistosoma haematobium, Schistosoma japonicum and Schistosoma intercalatum[1]. S. mansoni is a causative agent of intestinal schistosomiasis, and it is endemic in over 70 countries and widely distributed in Africa, South America, the eastern Mediterranean regions and the Caribbean Sea[2,3]. People are at risk of infection due to agricultural, domestic and recreational activities which expose them to infested water[2]. The highest rates of schistosomal infections are
commonly found among children and young adults and in recognition of these schoolchildren are the main target of schistosomiasis control programmes[4].

The Kato–Katz microscopic smear is a standard laboratory method recommended for diagnosing of intestinal schistosomiasis in the field study because it is quantitative, relatively inexpensive, simple and fast[3]. In addition, the semi–quantitative egg counts can be performed by this method to determine the intensity of the infection[5]. Despite the specificity is very high, the sensitivity of Kato–Katz in single stool sample examination is becoming not satisfied, especially when the number of worms is low or the test is done after the eggs were eliminated from the body[1,6], which leads to measurement error in estimating the presence of infection[1]. Nevertheless, there is a possibility to increase the sensitivity of Kato–Katz smear through examination of multiple samples[5], but this is a limiting procedure for field study[7]. Moreover, this microscopic method is unsufficient in the areas of low endemicity, in post-treatment situations, and in the control of transmission[8,9]. Therefore, other diagnostic assays like detection of parasite–specific antibodies have been shown to be more sensitive than the parasitological examination and are needed to plan and monitor control measure[3,9].

Various serodiagnostic methods have been developed to detect anti–schistosomal antibodies, such as indirect hemagglutination assays (IHA), and enzyme-linked immunosorbent assays (ELISA) using different type of antigens[10,11]. In Sudan, the high endemicity of S. mansoni infections have been reported in many parts of the country[12–14]. Therefore, the first approach for prevention and control is to diagnose the disease through applying different laboratory methods. The present study aimed to determine the prevalence of S. mansoni infection among schoolchildren in Sennar State, central of Sudan, to evaluate the sero–diagnostic techniques of the IHA and ELISA in comparison with Kato–Katz smear examinations, in order to be applied and adopted as to improve the diagnosis and assist in the control of schistosomiasis.

2. Materials and methods

2.1. Study design and setting

This was a descriptive cross–sectional study conducted at the Sennar State (300 km south of Khartoum capital), central of Sudan, during the period from August, 2011 to January, 2012. In this state, the Sennar Dam distributes water through canals for irrigation purpose. Although most villages have a chlorinated water supply, the water contact takes place along the untreated canals for recreation purposes or for domestic uses (washing utensils, bathing and watering animals)[15].

2.2. Study population

The study population was comprised of schoolchildren of both genders those were selected from three basic schools of Huzaifa Ibn Alyaman School, Al khansaa Basic School and Alkeila Basic Co-educated School at Sennar State. The age groups of the subjects were ranged from 6 to 16 years with the mean age of 11 years old.

2.3. Sample size ethical considerations

The sample size was obtained as recommended by the World Health Organization[16]. For this purpose, a total of 214 stool and blood samples were collected from the study subjects. Before the onset of the sample collections, informative meetings were held and the aim of the study was discussed with the headmasters and teachers of the selected schools and then a lecture about the disease was introduced to the students in each school. A written consent was obtained from each student or his/her parents after informing them about the importance of the study. The study was approved by a Committee of Research Council of Faculty of Medical Laboratory Science, University of Khartoum.

2.4. Collection of samples

To obtain the stool samples, each student was given a wide dry and clean container and informed him or her to provide at least 10 g of a stool sample. Whereas about 5 mL of venous blood sample was extracted from each subject using sterile disposable syringe. Only data from individuals who provided the recommended samples were included in the final analysis.

2.5. Microscopic examination of S. mansoni egg’s

Upon receiving the stool sample, it was immediately processed in the study field using Kato–Katz technique for the detection of S. mansoni eggs[17]. Each sample was pressed through a sieve and the amount of 41.7 mg sieved stool measured by a standard template was transferred to a microscope slide where it was pressed by another slide. The slides were then examined microscopically within 15 min. Intensity of infection was categorized according to the eggs count per gram of stool (epg): light (1–99 epg), moderate (100–399 epg) and heavy (≥400 epg)[18].

2.6. Immunological diagnosis of S. mansoni

2.6.1. IHA method

IHA assay was performed for the detection of S. mansoni antibodies using erythrocytes coated with specific adult worm antigen as described by God et al[10]. The IHA test kit (Fumouze Diagnostics Company, Paris, France) was prepared following the manufacturer’s instructions. An exactly 50 µL of phosphate buffer solution was delivered to all eight wells of the microplate, then 50 µL of serum stock dilution was added to the first well, mixing it well with the buffer solution, and then 50 µL from the first well was transferred to the second well. Then similar action was repeated for all wells up to the well number six. Then the last 50 µL from the well number six was discarded as to obtain serial dilutions of 1:80, 1:160, 1:320, 1:640, 1:1280 and 1:2560. Then 50 µL of the stock serum dilution was added to the well number seven, mixed and 50 µL was aspirated and discarded, to get 1:80 dilutions constituting the serum control. The well number eight was left only with the buffer solution to serve as reagent control. Then carefully, one drop of the sensitized red blood cells was delivered into the first six wells and to the well number eight. One drop of un–sensitized red blood cells was added to the seventh well number seven (serum control). Very carefully, the Wells content was homogenized
and allowed to remain motionless protected from vibration, for 2 h. The plate was examined for any agglutination. Each sample yielded agglutination reaction equal to/or more than 1:160 was considered as a significant reaction.

2.6.2. ELISA method

The test method of ELISA was carried out for detection of *S. mansoni* IgG class antibodies as previously described[10]. ELISA reagent kits (Nova Tec Immundiagnostica GmbH Technologic & Waldpark Company, Dietzenbach, Germany) were prepared according to the manufacturer’s instructions. The microtitre strip was pre-coated with *S. mansoni* specific antigen. The strip was designed as follows: first well (A1) for substrate buffer, second well (B1) for the negative control, third and fourth wells (C1 and D1) for the cutoff control and the well number five for the positive control. An exactly 100 µL of the positive and negative controls and the diluted samples were dispensed on their corresponding wells, then the strip was covered with a foil and then incubated in an ELISA incubator at 37 °C for 1 h. The foil was removed, aspirated the content of all wells and washed five times with a 300 µL diluted washing solution and the remaining solution was removed carefully by tapping the plate on a tissue paper. Then 100 µL of protein a conjugate was added to all wells except the blank well and then the plate was covered with a foil and incubated at room temperature for 30 min. By the end of the second incubation, the plate again was washed five times with the diluted washing buffer, and then 100 µL of the solution was dispensed to all wells and then incubated for 15 min at room temperature in dark. Then a 100 µL of stop solution was added into all wells. The plate was examined on ELISA reader at a wavelength of 450 nm. The result was considered to be positive when the absorbance value was higher than 10% over the cutoff titre.

2.7. Quality control

A total of 20 blood samples which were collected from healthy non-infected subjects and free of schistosomiasis from non-endemic area were applied as a quality control measure. Control samples were examined for the presence of disease by ELISA and IHA methods when every batch of patient samples was carried out.

2.8. Data analysis

Data were analyzed using the Statistical Package for Social Sciences (IBM SPSS for windows, version 16 software). Considering the Kato–Katz methods as a reference method, the results of serological tests of IHA and ELISA were tabulated and calculated their sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV). Proportions of the prevalence of schistosomiasis between boys and girls were compared using Chi-square test with *P*-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Prevalence and intensity of infection

A total of 214 schoolchildren (124 boys and 90 girls) were recruited from three basic schools at Sennar State, central of Sudan. Forty five (21%) were found to be positive for *S. mansoni* infections using Kato–Katz as a reference method. Of these 45 positive cases, 33 were boys while 12 were girls. The frequency of *S. mansoni* infection was significantly higher (*P*-value 0.007) among boys (33/124; 26.6%) than girls (12/90; 13.3%) (Figure 1).

![Figure 1. Frequency of *S. mansoni* infections according to the schoolchildren gender's at Sennar State, central Sudan.](Image)

The intensity of infection was expressed according to the number of egg’s count by Kato–Katz method. Among the 45 infected individuals, 38 (84.4%) were having light infections, 3 (6.7%) with moderate infections and 4 (8.9%) with heavy infections.

3.2. Screening of infection using Kato–Katz, IHA and ELISA methods

Table 1 summarizes the distribution of positive results determined by all the three applied test methods of Kato–Katz, IHA and ELISA. The majority of the positive cases were detected by ELISA (56.1%; 120/214) followed by IHA (33.2%; 71/214) and Kato–Katz (21%; 45/214). Out of the 45 positive cases by Kato–Katz smear, 42 and 38 cases were found to be positive by ELISA and IHA, respectively. Of the total 214 cases, 78 (36.4%) were recorded as false positive results by ELISA, whereas 33 (15.5%) false positive were detected IHA.

| Test method | Number of positive |
|-------------|--------------------|
| Kato–Katz   | 45 (21%)           |
| IHA         | 71 (33.2%)         |
| ELISA       | 120 (56.1%)        |

3.3. Comparative analysis of the three applied methods

Considering Kato–Katz results as a true positive, the sensitivity, specificity, positive predictive value and negative predictive value of the ELISA and IHA were evaluated (Table 2). Of the 45 true positive cases by Kato–Katz, 42 and 38 were positive by ELISA and IHA, respectively. On the other hand, out of 169 negative cases by Kato–Katz method, 78
were positive and 91 were negative by ELISA, while 33 were positive and 136 were negative by IHA method (Table 2). On the basis of these results, the sensitivity of the ELISA was 93.3% compared to 84.4% given by IHA. Furthermore, the specificity was reduced to 53.8% in ELISA compared to the 95.1% detected by IHA. Moreover, the PPV was increased in IHA than ELISA (53.5% vs. 35%, respectively), while both tests were given a similar percentage of NPV (95.1%; 96.8%, respectively).

When we evaluated the combination of positive results of both ELISA and IHA, the sensitivity was high 93.3%; the specificity and PPV were increased to 85.8% and 55.1%, respectively compared to the ELISA method (Table 2).

**Table 2**
Comparison between IHA and ELISA assays in diagnosis of *S. mansoni* in term of sensitivity, specificity, PPV and NPV.

| Methods          | Sensitivity (ELISA) | Specificity (ELISA) | PPV (ELISA) | NPV (ELISA) |
|------------------|---------------------|---------------------|-------------|-------------|
|                  |                     |                     |             |             |
| IHA              | 38/45 (84.4%)       | 136/169 (80.0%)     | 38/71 (53.5%) | 136/143 (95.1%) |
| IHA & ELISA      | 38/45 (93.3%)       | 145/169 (85.8%)     | 38/69 (55.1%) | 145/152 (95.4%) |

4. Discussion

In consider of microscopic examination using Kato–Katz smear as the gold standard method for the diagnosis of intestinal schistosomiasis, the prevalence of *S. mansoni* infection among schoolchildren in Semnar State, central of Sudan was 21%. In recent studies carried out in many parts of Sudan, different prevalences of *S. mansoni* infection have been recorded, e.g. in Wadi Halfa (ancient Nubia) was 26.1%[12], in central Sudan was 13% prevalence among pregnant women[13] and was 36.7% among schoolchildren[19]. Furthermore, worldwide studies have been determined the prevalence of *S. mansoni* infection. For example, the prevalence of *S. mansoni* infection was up to 62.2% among the schoolchildren in Tanzania[20], 60.5% in Kenya[21], 24.5% in Southern Sudan[22], 20.3% in Côte d’Ivoire among preschool-aged children[7], 23.7% in Brazil[23] and 26.3% in Ethiopia[24]. These results with our finding reflecting that there were various geographical distributions of *S. mansoni* infections, indicating the needs to implement eradication programs in many endemic areas.

In this study, the important causes of this high prevalence of schistosomiasis in Semnar area might be due to the habit of playing, swimming and using canal water for domestic uses. Moreover, we noticed that the water canals are surrounding the villages and most of the population daily cross these canals to reach either their school or their farms. However, they can easily access to this full infected water. In a previous study carried out in Kenya, the specific water-related activities such as swimming and fishing have been documented as risk factors for *S. mansoni* infections[25].

In out settings, despite of a relatively high prevalence of infection, we found 84.4% of infected children with light intensity infection, indicating of low endemicity of *S. mansoni* among the schoolchildren. Similar findings have been reported by other researchers[22,25]. In this study, the prevalence of *S. mansoni* infection was found to be significantly higher among the boys (33%) than that of in the girls (12%). This might he due to difference in exposure status. In our setting, the variations in the prevalence of infections between both sexes could be attributed to social and traditional habits which give the boys freedom to find more chances to play outside than girls. Similar findings have been reported among schoolchildren in the Al Gunaid area in Central Sudan[19]. In contrast, higher prevalence in females than males has been reported by others[25]. In a study carried out in Tanzania, no significant differences of infections were detected between boys and girls[20]. Likewise, in ancient Nubia, Northern Sudan, Hibbs et al. have reported that no statistically significant differences were in prevalence between males and females[12].

In comparison between microscopic and serological methods, we found higher positive results detected by serological tests of ELISA (56.1%) and IHA (33.2%) than Kato–Katz microscopic smear (21%). These findings could be compared with a survey conducted in Brazil, where the stool samples were examined using the Kato–Katz and serum samples were tested by IgG–ELISA. Of the total screened individuals, 49% showed positive serological test results. Of these, 16 (6%) had positive results in stool examination in the first sample batch[26]. Burlandy et al. reported that the high difference between serologic and parasitologic prevalence data is undoubtedly due to the low diagnostic sensitivity of the parasitologic methods[27]. Therefore, microscopic method is not satisfied, especially when the number of worms is low or the test is done after the eggs were eliminated from the body[6]. In such cases, we should increase the number Kato–Katz smear per sample or run multiple Kato–Katz tests in interval days[26].

In this study, we found high proportions of positive results that detected by the serological methods of the IHA and ELISA in spite of negative Kato–Katz examinations. In a study carried out by da Forta et al., a single Kato–Katz smear detected only 12% of the 25 infections[25]; this increased to 44% (three smears, one stool sample), 84% (five smears, three stool samples) and 96% (six smears, four stool samples). In our study, since we examined a single Kato–Katz slide from each patient sample, the false positive results which detected by the serological tests should be followed up and confirmed by increasing the number of Kato–Katz smears per sample or repeating the stool sample in interval of days. However, these cases could be due to previous infections[11], or due to the cross reactions with other parasites[5]. Also it could be useful to determine whether there is cross–reactivity with antibodies against other helminthes infections[7].

For a diagnostic tool, it should be both sensitivity and specificity of the applied technique should be evaluated, especially when used for human diagnosis[28]. In this study, we assessed the sensitivity and specificity of the IHA and IgG–ELISA techniques were by comparing them to the Kato–Katz reference method. We found a good sensitivity of IgG–ELISA (93.3%) comparable to the 84.4% given by IHA method, while the specificity of ELISA was relatively lower (53.3%) than IHA (80%). Earlier in a study in Ethiopia, the sensitivity and specificity of the ELISA test were 97.6% and 30.3%, respectively[29]. Despite the serological tests like ELISA are not able to discriminate between previous contact with the parasite and active infections[30], the high sensitivity ELISA has indicated this technique as one of the most successful serological tests for epidemiological studies to detect parasite burden[30,31]. In areas of endemicity, other researchers have suggested to carry out serological tests.
In estimating the presence of infection. Other diagnostic techniques have been required. For the diagnosis of imported schistosomiasis, however, high sensitivity is important, and a combination of two or more serological tests seems more useful[11]. This has become obvious in our finding, when we combined the both results of IHA and ELISA the specificity was increased to 85.8%. Gool et al. reported that the combined use of these two tests enabled the serological diagnosis of schistosomiasis to be achieved with very high degrees of both sensitivity and specificity[10].

In a clinical diagnostic method, PPV is a useful measure of the proportion of patients with positive test results that are correctly diagnosed[28,32]. In our setting, where 45 out 214 individuals were diagnosed microscopically with schistosomiasis, serological assays of ELISA and IHA have good predictive value for the absence of the infection but positive results have a low predictive value. Thus it will be useful to apply a valuable alternative reference method like polymerase chain reaction which may obtain a more accurate assessment whether the infection is present[3,33].

The present study concluded that the prevalence of S. mansoni infection in Sennar State, central of Sudan was found to be relatively high (21%) with low infection intensities. Therefore, in such setting screening of anti-schistosomal antibodies followed by a multiple stool sample examinations for each suspected individuals are required to detect more positive cases. Although IHA and ELISA tests yielded high sensitivity, the specificity rates of each assay alone were found to be low. However, it could be useful to perform a combination of such methods or to use more reliable molecular assay.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors express a gratitude to all administrations of participating schools. Special thanks go to Mr. Adam Alfaki Badawi, Medical Laboratory Technologist at National Health Laboratory for the technical assistance. This study was partially supported by the Faculty of Medical Laboratory Sciences and Medical and Health Studies Board, University of Khartoum as a Master Degree requirement to Abdelbasit Ibrahim (Grant No. UK/MHSB/Lab.Sc.13–2011/I0).

Research frontiers

This research determined the prevalence of S. mansoni infection among schoolchildren. In addition, it evaluated the sero–diagnostic techniques of the IHA and ELISA in comparison with Kato–Katz smear examinations, in order to be applied and adopted as to improve the diagnosis and assist in the control of schistosomiasis.

Related reports

Similar studies have been done by others in many part of the world. The performance of the serological tests in the diagnosis, using the Kato–Katz method as a diagnostic reference has been established (Carneiro et al., 2012). Gool et al. (2002) reported the combined use of these two tests which enabled the serological diagnosis of schistosomiasis with very high degrees of both sensitivity and specificity. This in agreement with the finding in present study.

Innovations & breakthroughs

In developing countries, there are limitations in diagnostic tools that investigate schistosomiasis. This paper focused on different diagnostic methods that could help and confirm the presence of the disease with a high accuracy.

Applications

Standard parasitological method as well as sero-immunological methods such as IHA and ELISA assays can be used for the diagnosis of schistosomiasis.

Peer review

In this study, authors focused on how to diagnose an important neglected disease using different laboratory methods, selecting in principle the cost–effective ones. These applied diagnostic methods have been compared to a reference method and the sensitivity and specificity of these methods were determined which aim to reach a feasible conclusion. These findings are useful to improve the diagnosis and assist in the control of schistosomiasis.

References

[1] Tchuenté LA, Fouodo CJ, Ngassam RI, Sumo L, Noundem C, Kenfack CM, et al. Evaluation of circulating cathodic antigen (CCA) urine–tests for diagnosis of Schistosoma mansoni infection in Cameroon. *PloS Negl Trop Dis* 2012; 6(7): e1758.
[2] World Health Organization, Schistosomiasis. Geneva: World Health Organization; 2013. [Online] Available from: http://www.who.int/mediacentre/factsheets/fs115/en/index.html. [Accessed on 26th September, 2013]
[3] Pontes LA, Oliveira MC, Katz N, Dias–Neto E, Rabello A. Comparison of a polymerase chain reaction and the Kato–Katz technique for diagnosing infection with Schistosoma mansoni. *Am J Trop Med Hyg* 2003; 68(5): 652–656.
[4] Smith H, Doenhoff M, Aitken C, Bailey W, Ji M, Dawson E, et al. Comparison of *Schistosoma mansoni* soluble cercarial antigens and soluble egg antigens for serodiagnosing schistosome infections. *PloS Negl Trop Dis* 2012; 6(9): 1815.
[5] Carneiro TR, Painheiro MC, da Oliveira SM, Hanemann AL, Queiroz JA, Bezerra FS. Increased detection of schistosomiasis with Kato–Katz and SWAP–IgG–ELISA in a Northeastern Brazil low–intensity transmission area. *Rev Soc Bras Med Trop* 2012; 45(4): 510–513.
[6] Erik MJ, Lima AG, Drummond SC, Schall VT, Coelho PM. The
effect of the number of stool samples on the observed prevalence and the infection intensity with *Schistosoma mansoni* among a population in an area of low transmission. *Acta Trop* 2008; 108(2–3): 222–228.

[7] Coulibaly JT, N’Goran EK, Utzinger J, Doenhoff MJ, Dawson EM. A new rapid diagnostic test for detection of anti-*Schistosoma mansoni* and anti-*Schistosoma haematobium* antibodies. *Parasit Vectors* 2013; 6: 29.

[8] Oliveira EJ, Kanamura HY, Lima DM. Efficacy of an enzyme–linked immunosorbent assay as a diagnostic tool for schistosomiasis mansoni in individuals with low worm burden. *Mem Inst Oswaldo Cruz* 2005; 100(4): 421–425.

[9] da Frota SM, Carneiro TR, Queiroz JA, Alencar LM, Heukelbach J, Bezerra FS. Combination of Kato–Katz faecal examinations and ELISA to improve accuracy of diagnosis of intestinal schistosomiasis in a low–endemic setting in Brazil. *Acta Trop* 2011; 120(Suppl 1): S138–S141.

[10] Van Gool T, Vervoort T, Weitzen J, Overbosch D. Serodiagnosis of imported schistosomiasis by a combination of a commercial indirect hemagglutination test with *Schistosoma mansoni* adult worm antigens and an enzyme–linked immunosorbent assay with *S. mansoni* egg antigens. *J Clin Microbiol* 2002; 40(9): 3432–3437.

[11] Kinkel HF, Dittrich S, Bäumer B, Weitzel T. Evaluation of eight serological tests for diagnosis of imported schistosomiasis. *Clin Vaccine Immunol* 2012; 19(6): 948–953.

[12] Hibbs AC, Secor WE, Van Gerven D, Armelagos G. Irrigation and schistosomiasis in Gezira area Central Sudan and analysis of cytokine profiles. *Obstet Gynecol Surv* 2012; 67(6): 343–347.

[13] Khalid A, Abdelgadir MA, Ashmaig A, Ibrahim AM, Ahmed AA, Adam I. *Schistosoma mansoni* infection among prenatal attendees at a secondary–care hospital in central Sudan. *Int J Gynaecol Obstet* 2012; 116(1): 10–12.

[14] Eltayeb NM, Mukhtar MM, Mohamed AB. Epidemiology of schistosomiasis in Gezira area Central Sudan and analysis of cytokine profiles. *Asian Pac J Trop Med* 2013; 6(2): 119–125.

[15] Omer AH. *Schistosoma mansoni* infection in the Sudan: the magnitude of the problem and the means whereby this disease may be controlled. In: Wood C, Rue Y, editors. *Health policies in developing countries*. London: Royal Society of Medicine Press; 1980. p. 141–146.

[16] Deganello R, Cruciani M, Beltramello C, Duncan O, Oyugi V, Montresor A. *Schistosoma hematobium* and *S. mansoni* among children, Southern Sudan. *Emerg Infec Dis* 2007; 13(10): 1504–1506.

[17] Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thicksmear technique in schistosomiasis mansoni. *Rev Inst Med Trop Sao Paulo* 1972; 14(6): 397–400.

[18] Montresor A, Crompton DW, Hall A, Bundy DA, Savioli L. Guidelines for the evaluation of soil–transmitted helminthiasis and schistosomiasis at community level. A guide for managers of control programmes. Geneva: World Health Organization; 1998. [Online] Available from: http://medискин.сн/uploadfiles/фile/20130424/2013042412528_1123.pdf. [Accessed on 26th September, 2013]

[19] Ahmed AM, El Tash LA, Mohamed EY, Adam I. High levels of *Schistosoma mansoni* infections among schoolchildren in central Sudan one year after treatment with praziquantel. *J Helminthol* 2012; 36(2): 228–232.

[20] El Scheich T, Hofer L, Kaatano G, Foya J, Odhiambo D, Igogote J, et al. Hepatosplenic morbidity due to *Schistosoma mansoni* in schoolchildren on Ukerewe Island, Tanzania. *Parasitol Res* 2012; 110(26): 2515–2520.

[21] Odiere MR, Rawago FO, Ombok M, Secor WE, Karanja DM, Mwini PN, et al. High prevalence of schistosomiasis in Mbira and its adjacent islands of Lake Victoria, western Kenya. *Parasit Vectors* 2012; 5: 278.

[22] Ashton RA, Stewart BT, Petty N, Lado M, Finn T, Brooker S, et al. Accuracy of circulating cathodic antigen tests for rapid mapping of *Schistosoma mansoni* and *S. haematobium* infections in Southern Sudan. *Trop Med Int Health* 2011; 16(9): 1099–1103.

[23] de Assis EM, de Oliveira BC, Moreira LE, Pena JL, Rodrigues LC, Machado–Coelho GL. [Prevalence of intestinal parasites in the Maxakali indigenous community in Minas Gerais, Brazil, 2009]. *Cad Saude Publica* 2013; 29(4): 681–690. Portuguese.

[24] Mekonnen Z, Haileslassie M, Medhin G, Erko B, Berhe NE. Schistosomiasis mansoni focus in Mekele City, northern Ethiopia. *Ethiop Med J* 2012; 50(4): 331–336.

[25] Handzel T, Karanja DM, Addiss DG, Hightower AW, Rosen DH, Colley DG, et al. Geographic distribution of schistosomiasis and soil–transmitted helminths in Western Kenya: implications for anthelmintic mass treatment. *Am J Trop Med Hyg* 2003; 69(3): 318–323.

[26] Gonçalves MM, Barreto MG, Peralta RH, Gargioni C, Gonçalves T, Igreja RP, et al. Immunoassays as an auxiliary tool for the serodiagnosis of *Schistosoma mansoni* infection in individuals with low intensity of egg elimination. *Acta Trop* 2006; 100(1–2): 24–30.

[27] Burlandy–Soares LC, de Souza Dias LC, Kanamura HY, de Oliveira EJ, Giarruvo RM. Schistosomiasis mansoni: follow–up of control program based on parasitologic and serologic methods in a Brazilian community of low endemicity. *Mem Inst Oswaldo Cruz* 2003; 98(6): 853–859.

[28] Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of soil–transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis* 2008; 2(1): 331.

[29] Eltiro F, Ye–ebiyo Y, Taylor MG. Evaluation of an enzyme linked immunosorbent assay (ELISA) using *Schistosoma mansoni* soluble egg antigen as a diagnostic tool for *Schistosoma mansoni* infection in Ethiopian schoolchildren. *J Trop Med Hyg* 1992; 95(1): 52–56.

[30] Pinto PL, Kanamura HY, Silva RM, Rossi CR, de Andrade Júnior HF, Amato Neto V, Dot–ELISA for the detection of IgM and IgG antibodies to *Schistosoma mansoni* worm and egg antigens, associated with egg excretion by patients. *Rev Inst Med Trop Sao Paulo* 1995; 37(2): 109–115.

[31] da Silva RM, Kanamura HY, Camargo ED, Chiodelli SG, Nakamura PM, Gargioni C, et al. A comparative study on IgG–ELISA, IgM–IAT and Kato–Katz methods for epidemiological purposes in a low endemic area for schistosomiasis. *Mem Inst Oswaldo Cruz* 1998; 93(Suppl 1): S279–S282.

[32] Stothard JR, Kabaterine NB, Tukaheluwa EM, Kazibwe F, Mathieson W, Webster JP, et al. Field evaluation of the Meade Readview handheld microscope for diagnosis of intestinal schistosomiasis in Ugandan school children. *Am J Trop Med Hyg* 2003; 69(5): 949–955.

[33] Oliveira LM, Santos HL, Gonçalves MM, Barreto MG, Peralta JM. Evaluation of polymerase chain reaction as an additional tool for the diagnosis of low–intensity *Schistosoma mansoni* infection. *Diagn Microbiol Infect Dis* 2010; 68(4): 416–421.