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ORIGINAL ARTICLE

Prevalence of *Schistosoma mansoni* infection among School Children attending Primary Schools in Menoufia Governorate, Egypt

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ABSTRACT

Background*: Currently, getting rid of schistosomiasis, which is classified as tropical ignored parasitic disease and endemic in Egypt, is a national goal.* ***Objectives:*** *Our work was constructed on detection of Schistosoma mansoni (S. mansoni) among school children using multi-stage survey in Menoufia Governorate, Egypt which is known to be endemic for the disease.* ***Methodology;*** *Formol ether concentration technique was applied as a golden test for stool samples and was compared to Kato-katz procedure, IHA, and FDA (Fast dot ELISA for antigen detection) being the available kits currently used in diagnosis.* ***Results:*** *From our survey; we found that schistosomiasis mansoni accounted for 1.5% of the studied population. Infections were higher in Boys than in girls, 2.0% and 1.0 % respectively. S. mansoni infections were associated with anemia in 62% of children. The percent of reinfection of treated children was 0.4%. Infections were significantly higher among children with high rate of contact with canal water.* ***Conclusion****: From our survey, we found that schistosomiasis mansoni is still represented in Menoufia Governorate with susceptibly to reinfection after MDT, which rises the alarm to the use of different combined methods for elimination of this endemic disease. We should offer another rapid detection method instead. FDA test was the least sensitive test in diagnosis.*

INTRODUCTION

Out of 220 million persons affected by schistosomiasis worldwide, about 2.5 million of them acquired infection in Egypt from 1989 up to 1996.The disease was classified by the World Health Organization (WHO) among one of seventeen ignored tropical endemic diseases[1,2].

Infection with *Schistosoma mansoni* or *S. haematobium* occur when cercaria, which comes out from freshwater snails, penetrate intact skin or mucous membrane[3]. School children are more affected especially with swimming habit in water canal and may suffer from alternation in hemoglobin level, malnourishment, diarrhea, low performance in school, and lack of fitness[4]. Elimination of schistosomiasis was set as a goal at 2025 by WHO[5].

Despite presence of eggs of *S. mansoni* in stool specimens under microscope, the routine method used for diagnosis, this approach shows some difficulties being poor approach in sensitivity in people with acute infection, in areas with low infection rate, presence of little infection level and requiring skilled personnel[6,7]. Low sensitivity of direct stool examination may be also attributed to variability of egg shedding from one time to another which may also misdiagnose active infection and rate of prevalence[8,9]. Based on this, serological antibody testing (indirect haemagglutination test) represented a tool which is sensitive in poor intensity infection and may be the sole approach for diagnosis[10], However, loss its of ability to differentiate between present and past infection limited the role of such approach[11].

Cercarial antigens (CA) have been applied particularly in early stage of infection, where high level of antibodies appear in response to CA rather than egg nor worm antigens, setting the base for highly sensitive approach in diagnosis of early phases of infection[12].

Standing on parasite burden is a must in order to eliminate this catastrophic disease from Egypt and this cannot be applied with false-negative results that may accidentally occur with the use of survey kits. So, we aimed in this work to detect the prevalence of schistosomiasis *mansoni* in Menoufia Governorate, evaluate previous MDT programs and evaluate current commercial approaches for diagnosis of *S. mansoni* in primary school children.

METHODOLOGY

Patients and methods:

We conducted this work on 1100 primary school children (503 boys and 597 girls) aging from ten to twelve years old in Menoufia Governorate (which is situated in the middle of the Nile Delta region of Egypt), and well-known as endemic area for *Schistosoma mansoni* infection.

Survey layout:

A cross-sectional survey involving a multistage random sampling technique was performed. We partitioned the Governorate into two sections. The first one included centers with abundant water canals, where the risk of getting infection was expected to be high, and the second section involved centers with less or no water canals, with low risk of infection. This partition represented the first stage sampling. From each section, four Districts were chosen randomly (second stage sampling) and from each District, one town was randomly selected (third stage sampling), and then a primary school was chosen randomly (fourth stage sampling). Finally, children were randomly selected from each school (fifth stage sampling).

Survey field:

One thousand and one hundred children were selected as a random sample from different villages in Menoufia Governorate. The school children (aged 10-12 years old), who contributed to this study, were from Shebin El-Kom, Ashmoun, Quesna, Tala, Menouf, El-Shohadaa, Berket El-Sabaa, El-Bagour and their surrounding villages with the numbers 101, 217, 85, 119, 116, 135, 101 and 100 respectively.

Ethical consideration:

The current survey was performed after endorsement from the Ministry of Health and written consent from children's parents. Faculty of medicine, Menoufia University ethics committee have blessed our study. Parents were asked to answer some questions about employment, guardian income, way of water consumption, canal water contact and having treatment for schistosomiasis before start of the study.

Parasitological work up:

About 10 gm of fresh stool uncontaminated with urine or water, 10 ml of uncontaminated urine and 5ml blood specimens were collected into a clean, dry, leak proof wide-mouthed disposable stool containers, urine containers and test tubes respectively from each child. All samples were labeled with the student's name, reference number, residence and time of collection. Samples were prepared and proceeded according to the method of examination. Collected stool samples were subjected to examination by using direct smear, formol ether concentration approach (FEC) and Kato-Katz methodology.

In direct smear test, a small portion of collected stools (weighing about two grams) was put on a slide with a drop of saline or iodine as an emulsifier for loose and semi-solid specimens respectively. The prepared slides microscopically examined at low and high power[13].

In formol ether concentration approach, another portion of stool samples (about 0.5g) was mixed with 10 ml of normal saline in an appropriate tube. The contents were strained into a centrifuge tube of 15ml after placing layers of gauze into a funnel, followed by adding formaldehyde in 10% concentration (2.5ml) and ether (1ml). The mix was centrifuged and sediment was examined microscopically for the presence of parasites[14-16].

Guided by Katz *et al.*[17], Kato Katz smears were prepared for microscopic examination after preparation of three slides (41.7 mg each) utilizing fresh stool samples, screens made of nylon and the templates were plastic made. Stool specimens were not allowed for examination after one week.

Immunological workup:

IHA testing:

In accordance with the manufacturer's guidance, IHA testing using Fumouze kits (Levallois-Perret, France) was applied. A titer above 1/80 was considered positive.

***Fast dot ELISA*:**

In guidance to Attallah *et al*. methodology, and according to manufacturer's instructions, rapid detection of *S. mansoni* circulating antigen (CSA) by the use of specified monoclonal antibodies in samples from serum and urine was done (purchased from ABC Diagnostics (Industrial Zone, New Damietta, Egypt). The test was evaluated by the presence of violet color around control dot (considered positive), absence of violet color (considered negative) or absence of control dot (considered invalid test)[18].

RESULTS

We enrolled 1100 children from primary schools in the present survey. None of the studied children was infected with *S. hematobium* infection. Using formol ether concentration technique which was considered our golden test, 16 children were positive for schistosomiasis *mansoni* representing about 1.5% of the whole studied population. Kato Katz approach revealed 14 cases only (1.3%) (Fig. 1D), direct smear method was positive in 11 children (1.0%) (Fig. 1A), IHA test was truly positive in 14 cases and false positive in 5 cases with total positivity in 19 cases out of 1100 examined child (1.7%) (Fig. 1B). Finally Fast-Dot-Eliza (FDE) showed positive reaction to one case only in urine and serum specimens (0.09%) (Fig. 1C) (Table. 1).

Table 1. Prevalence of *S. mansoni* according to each diagnostic method

- Exact 95% confidence interval.

Test method	N tested	N positive	% positive (CI)
Formol-Ether	1100	16	1.5 (0.21, 0.49)
Direct smear	1100	11	1.0 (0.77, 0.96)
Kato-Katz	1100	14	1.3 (0.85, 0.99)
IHAT	48	19	39.6 (0.72, 0.93)
Fast-Dot ELISA	48	1	2.1 (0.54, 0.81)

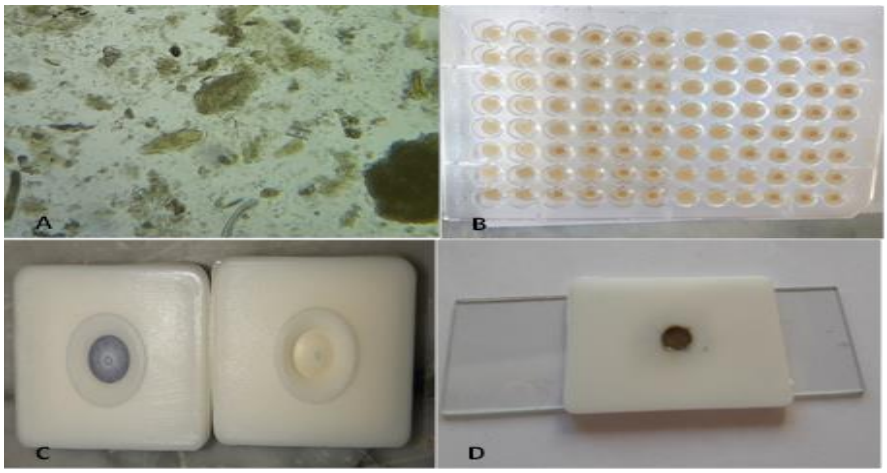

Fig. 1: A: showing egg of *S. mansoni* in unstained stool preparation (X 400).B: IHAT plate with different titre. C: Positive and negative FDE antigen blocks with control dot in the center. The purple color around the control dot means positivity while no color means negativity. D: Kato Katz template to adjust 50 gms of stool on a glass slide.

Demographic information showed that boys had a higher rate of infection with *S. mansoni* than girls. Infections account for 2.0% of boys and 1.0% of girls. However, this difference was not statistically significant .

(Fig. 2a). There was statistically significant difference in infection rate among children contacting water 2.4% compared to 0.9% in those who didn't have this habit (Fig. 2b)

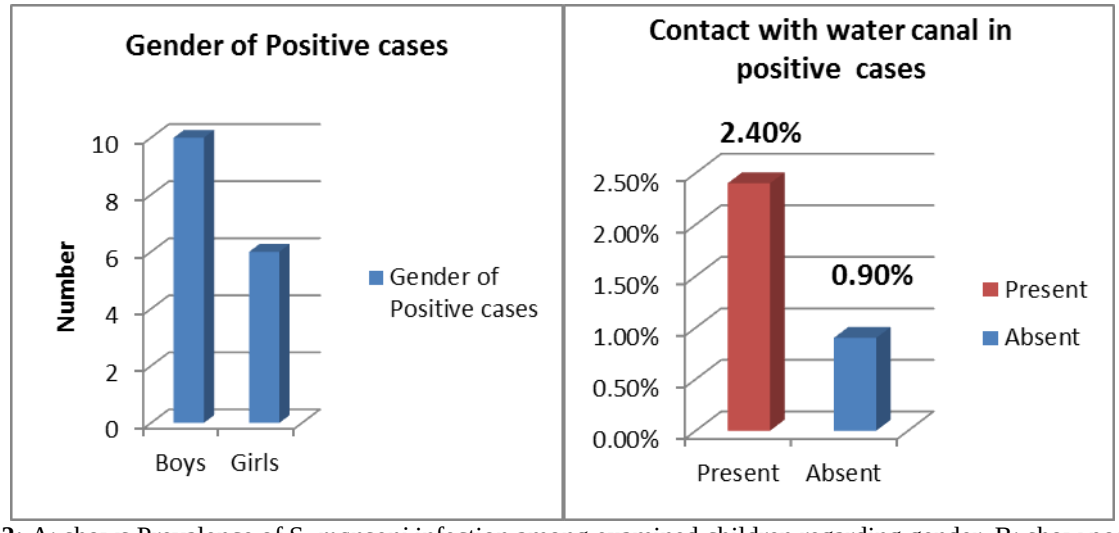

Fig. 2: A; shows Prevalence of S. *mansoni* infection among examined children regarding gender. B; show positive cases of schistosomiasis distributed in relation to contact with water canal.

Sensitivity and specificity of different techniques used in diagnosis of *S. mansoni* were evaluated where Kato-Katz test and IHA test gave almost the same sensitivity (87.5%), while FDE test had the least sensitivity (6.25%). Regarding specificity, IHA test had the least specificity (84.4%) and the least Positive predictive value (73.7%) while, Kato-Katz test has the highest negative predictive value (94.12%), and FDE test has the lowest value (68.0%) (Table 2).

Table 2: Comparison between direct smear test, Kato-Katz thick smear test, indirect haemagglutibation test (IHAT) and Fast-Dot ELISA in serum test regarding sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Test	Sensitivity	Specificity	PPV	NPV
Direct smear test	68.75%	100%	100%	86.49%
Kato-Katz test	87.5%	100%	100%	94.12%
IHAT	87.5%	84.4%	73.7%	93.1%
FDE test	6.25%	100%	100%	68.0%

Herein, anemia was evaluated in the studied children and measured by Hb level. Children who presented with parasitic infections were 41, with mean Hb level of 10.8±0.9 gm whereas, control children number was 51, with mean Hb of 11.2±0.8 and the difference between the two groups was statistically significant (P=0.01). on the contrary, when level of Hb, a representative measure of anemia, was studied in children with schistosomiasis, no significant difference was observed; the mean Hb in cases (16) was 10.8±0.8 while, in the control group the mean level was 11±0.8 with P=0.25 (Table 3).

Table 3: Comparison between anemia among parasitic infections and the control group and among schistosomiasis and the control group regarding hemoglobin level

Parasitic infections	Anemia					
	Mean	SD	NO.	Median	Mann Whitney test	P-value
Positive results (cases group)	10.8	±0.9	41	10.5 (9.5-13)		
Negative cases (control group)	11.2	±0.8	51	11.4 (9.4-12.8)	2.6	0.01
Total	11.04	±0.9	92	11 (9.4-13)		
Schistosomiasis						
Positive results (cases group)	10.8	±0.8	16	10.5(10-12.5)		
Negative cases (control group)	11	±0.8	32	11(9.4-12.2)	1.14	0.25
Total	10.9	±0.8	48	10.8(9.4-12.5)		

Eggs of other parasites were revealed in stool samples during examination. We detected *Entrobius vermicularis* (*E. vermicularis*) eggs (Fig. 3b), which was found alone in 13 children (1.2% of examined stool samples) and combined with other parasites such as *Hymenolepis nana* (*H. nana*) in two children (0.2%), *Taenia* in one case (0.1%) and *S. mansoni* in one case (0.1%). *Taenia* eggs (Fig. 3a) and *H. nana* (Fig. 3c) were also revealed alone in two (0.2%) & seven cases (0.6%) respectively (Table 3).

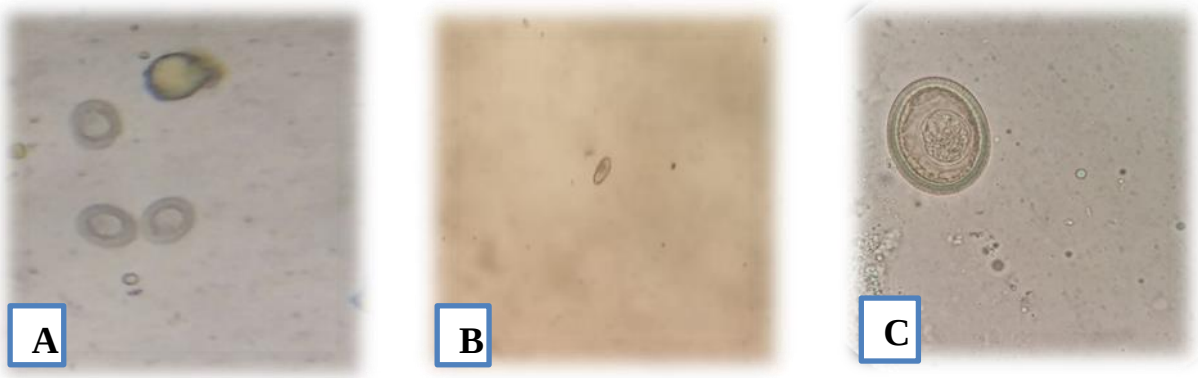

Fig. 3: Shows the following: A: Eggs of H. nana in unstained stool preparation (X 400).B: Egg of E. vermicularis in unstained stool preparation (X100). C: Egg of Taenia in unstained stool preparation (X1000).

Table 4: Prevalence of parasitic infections among examined children.

Species of parasites	Total number examined (1100)	
	No. +ve	% +ve
E. vermicularis	13	1.2%
E. vermicularis - H. nana	2	0.2%
E. vermicularise – Taenia	1	0.1%
Taenia	2	0.2%
H. nana	7	0.6%
S. mansoni	14	1.3%
S. mansoni E. vermicularis	1	0.1%
S. mansoni-H. nana	1	0.1%
Total infected	41	3.7%

DISCUSSION

Schistosomiasis plagued Egypt for a very long period. A huge effort has been applied by the Ministry of Health to eliminate present infection with this parasite. Egypt succeeded after administration of over twenty million doses of praziquantel to diminish the prevalence rate to less than 0.2 in *S. hematobium* and *S. mansoni*[19].

To the best of our knowledge, there are few published data concerning prevalence of human schistosomiasis mansoni in Menoufia Governorate in the last 10 years. Therefore, the present study represents an attempt to highlight the real prevalence of schistosomiasis *mansoni* among school children in Menoufia Governorate and gives a hint about its impact on the general condition of the children and different available methods for diagnosis.

In the present study, the prevalence of *S. mansoni* was 1.5% with a higher rate of infection among males than females 2:1. In accordance with this, in a study conducted in Berket El Sab district, Menoufia Governorate, Bahbah and colleagues reported that the prevalence of *S. mansoni* infection was 0.8% revealing a higher infection rate among males than females as the study showed that the male to female ratio was 7:1[20]. Similarly, Ahmady *et al.*[21] reported that the prevalence of infection among children aged from 6 to 11 years old in Zagazig district, Sharqia Governorate was 0.74% with significant higher infection rate among males than females. Boys to girls infection rate was 39.62% compared to 14.85% respectively as revealed by Abd ELLAH *et al.*[22]. Furthermore, Haggag and colleagues studied the prevalence of *S. mansoni* infection in, Sharqia, Kafr El Sheikh, Dakahlia, Behira, and Qalyubia governoates. They reported prevalence rates of 3.5%, 1.7%, 0.5%, 0.4% and 0.03% respectively[23].

As regards contact with canal water, the present work showed that the prevalence of infection was significantly higher in children with high water contact than those with low water contact. Bahbah & El Shikhsalem[20] reached to similar results. They found *S. mansoni* infection rate among children whose parents were farmers (1.7%) was significantly higher than children whose parents were non-farmers (0.2%), and also reported that the prevalence of infection among children with non-sanitary sewage disposal (1.4%) was significantly higher than children with sanitary sewage disposal (0.2%).

Stool, urine and serum samples were collected and examined for *S. mansoni* infection parasitologically using formol ether sedimentation method, Kato-Katz thick smear test and, direct smear test and, immunologically employing Indirect Haemagglutination Test (IHAT) and Fast-Dot ELISA test. Ova were found in 1.5%, 1.3% and 1.0% of cases respectively. These results were in contrast to those obtained by El Sharazly *et al.*[24] who used nearly the same techniques for detection of schistosomiasis. They found a prevalence rate of 0.9%, by using formol ether sedimentation

method, 1.8% by Kato-Katz thick smear method and 0.0% by using direct smear test.

Antibodies were detected in 14 true positive cases and 5 false-positive cases giving sensitivity of 87.5% and specificity of 84.4%. Fast-Dot ELISA test detected only one positive case out of 16 cases giving a sensitivity of 6.25% and specificity of 100%. However, El-Shahawy *et al*.[25] reported that IHAT sensitivity was 85.7% and specificity was 100% when comparing IHAT with ELISA in diagnosing *S. mansoni* infection. On the other hand, Nagy *et al*.[26], reported that sensitivity of Fast-Dot ELISA test was 71.4% and specificity was 76.0%. This discrepancy in sensitivity and specificity may be attributed to the use of different kits from different suppliers.

Apart from schistosomiasis, eggs of other parasites were revealed in 2.4% of subjects namely *Entrobius vermicularis* eggs, *Hymenolepis nana* eggs, and *Taenia* eggs which were detected in 1.5%, 0.9% and 0.3% of students respectively.

Mahmoud and colleagues conducted a study in El Khalige village, Dakahlia Governorate and reported that the prevalence of parasitic infections among the age group 6-12 years was 37% including *H. nana & G. lamblia* (7.8%), *E. vermicularis* (1.9%) and *S. mansoni* (1.9%). Generally, single infections were about 3% and mixed infections were about 1% in the whole study including *S. mansoni* and other parasitic infections. It has been noticed that the age group which was most affected by parasitic infections was from 6 to 15 years old which could be explained by the fact that this age group have habits of contact with water with poor personal hygiene[27] .

Regarding anemia, the present study showed that the prevalence of anemia among *S. mansoni* infection group was 62.5% with Hb level (mean± SD) (10.8 ± 0.8) which is insignificantly higher than the control group which was 46.9% . Similar results were reported by Ahmady *et al.*[21] and El Shikhsalem & Bahbah[28], who reported that there was non-significant difference between anemia and *S. mansoni* infection among the studied group. Furthermore, Abd ELLAH and colleagues reported that the prevalence of anemia among *S. mansoni* cases was 57.35% which was significantly higher than the control group with anemia prevalence of 49.01%[22].

The present study also showed the state of anemia among the all parasitic infected group and the non-infected group showing that the prevalence of anemia among the infected group was 61% and among the non-infected group was 29.4% with Hb level (mean± SD) (10.8±0.9) with significant difference between both group. Similar study was conducted by Mahmoud *et al*.[27] in El Khalige village, Dakhalia Governorate who reported that the prevalence of anemia within infected group was 52.6% and within non-infected group was 50% with Hb level (mean± SD) (11.12±10.35) with insignificant difference between both groups.

While the prevalence of *S. mansoni* in the Nile Delta was 33% in 1935[29] compared to 1.5% in Menoufia Governorate in 2019 in the current survey. This decreasing rate is thought to be due to the efforts exerted by the national plan, Ministry of Health, to curb the spread of infection by the application of some control measures, including treatment of schoolchildren as well as communities, snail control, environmental rehabilitation, and health education, with a focus on those areas most affected. Furthermore, the transmission of the disease may occur in some areas not targeted by these procedures.

Children with *S. mansoni* were treated with praziquantel. It was used in this study because it is the drug of choice for treating schistosomiasis owing to its efficacy and safety. It was used with a dose of 40 mg/kg bodyweight single oral dose. Moreover, health education about the disease and preventive measures was carried out in the survey field to raise the awareness of all stuff members in the studied schools in general and children with recent infections after previous treatment and their families in particular.

CONCLUSION

Despite the efforts to eradicate *S. mansoni*, there is still some areas of low prevalence rates in Menoufia Governorate among other areas in Egypt. These data should keep the authority responsible for control program vigilant and closely and continuously monitor the situation. Based on data of the present study, formol ether concentration method of stool analysis is the golden method for diagnosis of schistosomiasis while the use of fast dot ELISA method employed in the present study needs further investigation to establish its sensitivity and specificity.

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Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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