# Final Report

# Evaluation of PCR Assay for Detection of *Schistosoma mansoni* DNA in Human Stool Samples

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## INTRODUCTION

Schistosomiasis is mostly a tropical disease caused by the blood fluke, a group of flat worms that reside in the blood vessels in the human hosts. It presents as an acute, but mostly chronic illness. Due to its geographic and demographic distribution, the disease is listed as one of the neglected tropical diseases (NTDs). Schistosomiasis comes after malaria among parasitic diseases as regards number of people infected and those at risk of infection.[1]

Five species, namely, *Schistosoma haematobium*, S. *mansoni, S. japonicum, S. intercalatum and S. mekongi*, can infect humans, but the three with public health importance are *S. haematobium* (mostly endemic in Africa and the Middle East), *S. mansoni* (common in the tropics and subtropics) and *S. japonicum* (mainly found in the People's Republic of China and the Philippines). *S. haematobium* causes urogenital schistosomiasis, while *S.mansoni* and *S. japonicum* cause intestinal schistosomiasis.[2]

Globally, it is estimated that around 800 million people are at risk of acquiring the infection and 207 million people are already infected.[3] Children, women and farmers in rural communities without access to adequate sanitation or clean water and who depend on water contact for recreational, domestic or occupational activities are most vulnerable to the infection. [3-5]

Schistosomiasis prevalence and morbidity is highest among school children, adolescents and young adults.[6] Thus, the negative impacts on school performance and the debilitation caused by untreated infections demoralize both social and economic development in endemic areas.[7]

Numerous factors act to determine the transmission of schistosomiasis, directly or indirectly, by affecting the transmission cycle of the schistosome parasite. Each case of schistosomiasis, transmission is enabled by the interrelated effects of broader environmental, climatic, biological, political, demographic, economic, social and cultural trends.[8]

The use of the Kato-Katz (KK) technique to examine a single or multiple stool specimens remains the gold standard for the diagnosis of *S. mansoni*. [9] The technique is both specific and sensitive and able to quantify eggs into different intensity levels. It also has low operational cost and can be used in settings with minimal infrastructure.[10] However, sensitivity of parasitological diagnosis by microscopy decreases considerably when egg excretion is low, particularly in low endemicity areas or after chemotherapy.

Recently, schistosome-specific DNA has been successfully detected in *S. mansoni* from feces, serum.[11, 12] The amplification reaction showed no cross reactivity with other related helminthes, and always showed high sensitivity and specificity. This diagnostic technique depends on the polymerase chain reaction (PCR) and so far has not been extensively used in human schistosomiasis detection.[13] It is now important to demonstrate the usage of PCR as a surveillance tool to monitor schistosomiasis prevalence in high and low endemic areas.

Prevention and control of schistosomiasis is based on preventive treatment, snail control, improved sanitation and health education. Praziquantel (PZQ), is a broad spectrum, safe and easy-to-administer medicine immediately became the treatment of choice for schistosomiasis.[14] Factors which have contributed to the drug's usefulness include its excellent pharmacological properties, particularly its effectiveness after only

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one orally administered dose, its lack of toxicity, [15, 16] and substantial reductions in cost.[17]

The relative lack of efficacy of PZQ against juvenile schistosomes is a potentially significant deficiency and may be a factor in the poor cure rates and treatment failures observed in some patient group.[18] A protocol involving administration of two courses of PZQ separated by a short interval (2 to 8 weeks) was advocated for such situations and adoption of this approach has indeed resulted in high cumulative cure rates (CR) with *S.mansoni*.[19] In the laboratory, exposure of schistosomes to sub-curative doses of PZQ over generations resulted in drug resistant schistosomes. [20]

The development of resistance against PZQ was cited as one of the main reasons for the observed low drug performance. Other reasons given were the very high pretreatment intensity of infection observed in Senegal, presence of immature worms less susceptible to PZQ and rapid acquisition of a large number of new infections immediately following treatment. [1]

The role of this study was to estimate the prevalence of *S.mansoni* infection by KK and validate the conventional polymerase chain reaction (cPCR) in diagnosis of *S.mansoni* and to evaluate PZQ resistance among the school aged children in addition to providing health education sessions in order to maintain their adherence to preventive control measure.

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## OBJECTIVES

This study aimed at:

1. To determine the prevalence of *S.mansoi* in a single stool sample collected from the study group by Kato-Katz and cPCR.
2. To evaluate the validity of and performance of cPCR in detection of *S.mansoni.*
3. To determine the prevalence of *S.mansoni* after conventional therapy with PZQ.

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**Study Setting:** The study was conducted in Arab El-Mahdar village, Metobes, Kafr El-Sheikh Governorate.
**Study design:** Cross-sectional design.
**Target population:** School children.
Inclusion criteria:
- Aged 5 – 15 years old (registered in the school from the 1st primary grade to the 3rd preparatory grade)
- Living in Arab El-Mahdar village.
- Not currently on anti-schistosomiasis or other anti-parasitic treatment.
- Their parents agreed to their participation in the study.

Exclusion criteria:
- Refusal to participate in the study despite guardians' approval.
- Disabled children.
- Have chronic debilitating disease.

**Sampling:**
Sample size: The sample size was calculated using Epi Info 7.2.0.1, 2016. Based on the prevalence of 70% of infection among children in this village and 5% confidence limit, the minimal calculated sample size at 95% confidence level was 300.[21] It was increased to 320 to compensate for drop outs. The actual collected samples were 347 samples.

Sampling method: A multistage stratified random sample was adopted.
- Arab El-Mahdar School included two main educational levels, namely; primary and preparatory.
- Each level was divided into grades (6 primary grades and 3 preparatory grades).
- Each grade was divided into classes.
- Half of the classes were randomly selected and children were proportionately allocated according to the number of children in each grade.

**Data collection tools and laboratory methods:**

**1. Interviewing structured questionnaire: (Appendix I)**

A pre-designed interviewing structured questionnaire was used to collect data from the children regarding the following items:

a. Socio-demographic data.
b. Medical history: previous *S.mansoni*. infection or treatment.
c. Risk factors for *S.mansoni* and other hook-worms infection.

Two workshops were held at the High Institute of Public Health to train the data collectors on the field work and modify the questionnaire. Role play was adopted at the end of the workshop to ensure that the agreed data collection tool would be utilized efficiently. A pilot study was conducted prior to starting the field work in order to:

1. Estimate the duration of the filed work that yielded the pre-defined sample size.
2. Test the data collection tools.
3. Estimate the average time needed to fill the questionnaire and obtain the samples from the target population.
4. Test the efficiency of the data collectors in using the tool.
5. Identify the cultural context related to the village of the study.

The feedback was the following:
- The average time needed to fill in the data collection tool ranged from 10-15 minutes,
- Using more simple language to be easily understood by the target population.

**2. Laboratory methods:**

**a. Wet mount technique: (Appendix II)**

Field microscopic examination of 237 fresh stool samples was done. The other 110 samples were not fresh.

**b. Kato Katz (KK) cellophane fecal thick smear technique: (Appendix III)**

- This procedure was carried out in the Lab of Tropical Health Department, High Institute of Public Health, Alexandria University.
- Four-slides KK were examined:
  - At the beginning of the study (347 stool samples).
  - After the infected patients received the 1st dose of treatment (66 samples).
- Nine-slides KK were examined except for two samples one examined on eight slides and another one examined on three slides only:
  - After the 2nd dose of treatment (91 samples).

The intensity of infection was calculated according to Chees Brough method; first the number of eggs of *S. mansoni* per slide was identified then multiplied by 5, as the number of eggs in each slide equals to that in 1/5-gram stool i.e. the number of eggs per gram stool = the total number of eggs present per slide X 5.

- Low intensity: 1-99 eggs/gram stool.
- Moderate intensity: 100-399 eggs/ gram stool.
- Heavy intensity: ≥400 eggs/ gram stool.

**c. Conventional PCR: (Appendix IV)**

- This procedure was carried out in the Lab of Molecular Medical Parasitology (LMMP), Department of Medical Parasitology, Kasr Al-Ainy Faculty of Medicine, Cairo University.
- Initially, it was carried out on 100 random stool samples (100 out of 347). Stool samples were selected by systematic random sampling method.
  - The sampling interval was calculated using the following equation:

  Total number of samples / the desired sample size= 347/100 =3.47.
  - From a randomly selected starting point, every third patient was selected until the required sample size was reached.
- As the results were unsatisfactory, after the children were fully treated (received 2nd dose of PZQ) stool samples, taken to measure the cure rate (CR), were analyzed for *S. mansoni* using cPCR (91 samples).

**d. Blood sampling:**

- Blood sample: Blood samples were taken from all children and divided onto two tubes:

(1) K-EDTA tubes: for complete blood count (CBC) analysis.

(2) Clot-activator gel containing tubes: after centrifugation, the serum was separated for the following analyses:

- Liver enzymes (SGPT, SGOT) using Integra 400 auto analyzer (Roche diagnostic Inc., India).
- ELISA for Hepatitis B Surface Antigen (HBs Ag).
- ELISA for HCV antibodies (HCV-Ab).

**3. Treatment of infected children:**

All infected children were invited to receive two doses of PZQ (40 mg/kg), 4 weeks apart and the response was:

- 1st dose response: 100 out of 106 children received the 1st dose.
- 2nd dose response: 96 out of 106 children received the 2nd dose, all of them received the 1st dose.
- For drug efficacy assessment, cure rate (CR; formula: [number of children excreting S. mansoni eggs/ number of children with confirmed infection before treatment] x 100) and egg reduction rate (ERR; formula arithmetic mean AM: 1 – [AM egg count post-treatment / AM egg counts pre-treatment] x 100).[22]

**4. Health Education: (Appendix V)**

Before receiving the 1st dose, all infected children together with their guardians attended health education sessions about the modes of transmission of *S.mansnoi* and how to prevent its transmission. Information about the modes of transmission, prevention and complications of infection were reinforced before receiving the 2nd dose and 4 weeks after.

**Data management and analysis:**

1) Data processing: Data entry was performed by the statistics team using Statistical package for the Social Sciences (SPSS) version 21, 2014 followed by processing and analysis of data. To ensure that all questions had valid codes, range checking was done by using frequency distributions and cross tabulation. Data processing also included recoding of variables and computation of new variables.
2) Data analysis: SPSS was used for data analysis.
   - Descriptive statistics were used for summarization of data utilizing frequency distribution tables and graphs.
   - For quantitative variables, means and standard deviations were calculated.
   - For qualitative variables: Pearson's chi – square test, Fisher's exact test as well as Monte Carlo Exact probability were calculated.
   - Diagnostic accuracy test:
     a) Sensitivity: probability of positive test in population with the disease.
     b) Specificity: probability of negative test in population without the disease.
     c) Positive predictive value (PPV): probability of the person having the disease when the test is positive.
     d) Negative predictive value (NPV): probability of the person not having the disease when the test is negative.
     e) Positive likelihood ratio (PLR): Sensitivity/ (1-Specificity)
     f) Negative likelihood ratio (NLR): (1-Sensitivity)/ Specificity.
     g) Diagnostic efficiency: (true positives + true negative) / total sample.

**Monitoring and quality control:**

a. For data collection: Three field supervisors were assigned to revise the questionnaire after each interview with the children.
b. For stool sample collection and analysis: checklist was used during sample collection for blood, stool samples, and filling the questionnaire. The quality of the used reagents and instruments were checked by the parasitology consultant, specialists and laboratory technicians. The specimens were also checked by serial number, quantity, precaution of specimen collection, examination and transportation to the assigned laboratory.
   1) For the KK technique: Two parasitology specialists double-checked the results of the positive results and randomly double-checked the negative ones.
   2) For the cPCR technique: before starting the analysis, the reagents and primers were tested over both positive and negative controls.
c. For the blood samples: the quality of the kits and the equipment were checked by the researchers and laboratory technicians. The blood samples were checked for serial number, quantity, precaution of sample collection and transportation to the assigned laboratory.

**Ethical considerations:**

- The approvals of the Ethics Committee of the High Institute of Public Health (Appendix VI) and Central Administration for Communicable and Endemic diseases (Appendix VII) were sought for conducting the research.
- The research team complied with the International Guidelines for Research Ethics.
- A written informed consent was taken from all the guardians of the study participants after explanation of the purpose (Appendix VIII)
- Confidentiality was assured.

## 1. Prevalence of S. mansoni and their associated risk factors.

The prevalence of *S.mansoni* after examination of single stool sample of 347 individuals aged (5-15 years old) based on four slides
KK was (106/347) 30.5%. (**Figure 1)**

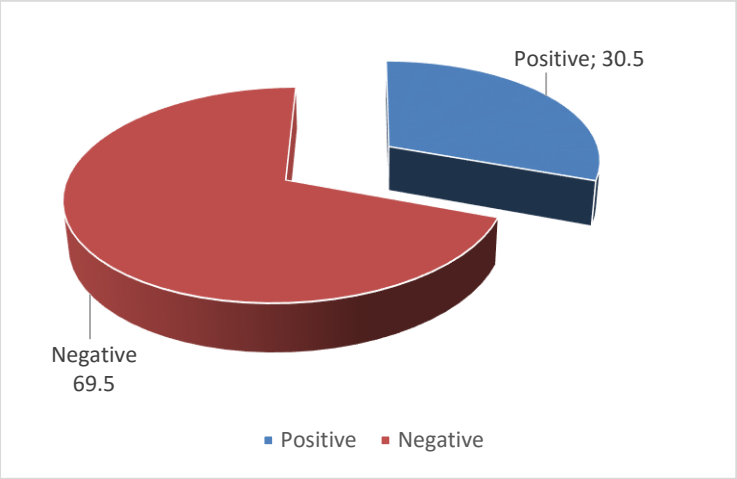


**Figure 1: Prevalence of S.mansoni among children aged 5-15 years old in Arab El Mahder village Kafr Elsheikh**

The intensity of infection is shown in **figure 2;** 87.7% of infected children were of low intensity meanwhile, high and moderate infection represented 5.7% and 6.6% respectively**. Table I** shows that, neither age nor number of family members affected the prevalence of *S. mansoni* infection, meanwhile, male sex significantly increased the prevalence of infection (p-value $\leq$ 0.05). Child order was not associated with increased prevalence of *S.mansoni*. Parents education and mother occupation did not affect the prevalence of *S. mansoni* infection. Fathers' job as a farmer was associated with higher prevalence of infection among their children. Family income did not significantly affect the prevalence of *S.mansoni* infection (p value =0.237).

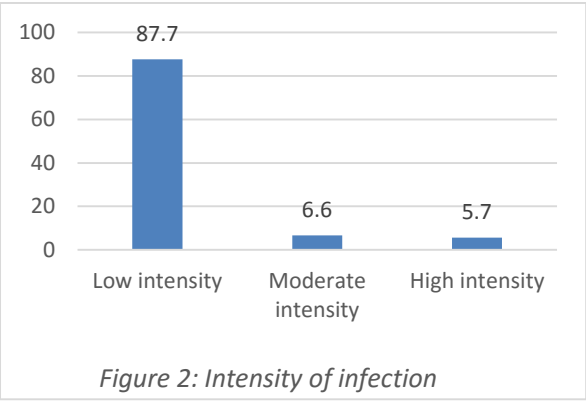


*Figure 2: Intensity of infection*

**Table II**: the method of sanitary disposal did not significantly affect the prevalence of *S. mansoni*. Water accumulation inside homes did not affect the prevalence of infection. Presence of a nearby water canal significantly increased the prevalence of *S. mansoni* (p-value= 0.037). Site of contact with water (central/peripheral) canal, water unavailability, frequency of water supply unavailability, and co-infection with other parasitic infestations significantly increased the prevalence of infection.
History schistosomiasis, family history of schistosomiasis, contact with water canal, frequency of water canal contact, toileting in water canal and history of previous *S.mansoni* treatment did not significantly increased the prevalence of infection. Source of water did not significantly increase the prevalence of *S.mansoni* infection p-value was near the significant level, p-value= 0.072.

**Table III** shows the significant risky behaviors adopted by children living in Arab El-Mahder Village; of infected children; 50 children used to wash clothes in water canal, 37 children were swimming in water canal, 18 children used water canal to wash vegetables, and 24 children were cutting grass with bared feet.

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**Table I: Socio-demographic characteristic of children aged (5-15 years) infected with *S. mansoni* in Arab El-Mahder, Kafr El sheikh 2017**

| | | S. Mansoni infection | | | | P value |
|---|---|---|---|---|---|---|
| | | Negative | | Positive | | |
| | | no | % | no | % | |
| **Age category** | 5-10 | 99 | 70.71 | 41 | 29.29 | 0.722 |
| | >10 | 142 | 69.00 | 65 | 31.40 | |
| **Sex** | Male | 120 | 64.86 | 65 | 35.14 | ***0.047*** |
| | Female | 121 | 74.69 | 41 | 25.31 | |
| **Child order** | 1-2 | 118 | 67.05 | 58 | 32.95 | 0.323 |
| | >3 | 123 | 71.93 | 48 | 28.07 | |
| **Number of family members** | 1-5 | 54 | 65.85 | 28 | 34.15 | 0.418 |
| | 6- | 187 | 70.57 | 78 | 29.43 | |
| **Father education** | Illiterate | 125 | 67.57 | 60 | 32.43 | 0.139 |
| | Read and write | 84 | 70.00 | 36 | 30.00 | |
| | Primary | 7 | 63.64 | 4 | 36.36 | |
| | Preparatory | 9 | 81.81 | 2 | 18.18 | |
| | Secondary | 13 | 86.67 | 2 | 13.33 | |
| | University graduate | 2 | 66.67 | 1 | 33.33 | |
| | Died | 1 | 50.00 | 1 | 50.00 | |
| **Mother education** | Illiterate | 219 | 69.30 | 97 | 30.70 | 0.670 |
| | Read and write | 16 | 69.57 | 7 | 30.43 | |
| | Primary | 2 | 100.00 | 0 | 0.00 | |
| | Preparatory | 2 | 66.67 | 1 | 33.33 | |
| | Secondary | 1 | 100.00 | 0 | 0.00 | |
| | University graduate | 1 | 100.00 | 0 | 0.00 | |
| | Died | 0 | 0.00 | 1 | 100.00 | |
| **Father job** | Farmer | 168 | 71.49 | 67 | 28.51 | ***0.037*** |
| | Fisher | 18 | 69.23 | 8 | 30.77 | |
| | Fisher/farmer | 0 | 0.00 | 4 | 100.00 | |
| | Clerk | 7 | 100.00 | 0 | 0.00 | |
| | Others | 43 | 63.24 | 25 | 36.76 | |
| | Not working | 2 | 100.00 | 0 | 0.00 | |
| | Died | 3 | 60.00 | 2 | 40.00 | |
| **Mother job** | Farmer | 108 | 67.08 | 53 | 32.92 | 0.566 |
| | Housewife | 126 | 72.00 | 49 | 28.00 | |
| | Died | 7 | 63.64 | 4 | 36.36 | |
| **Family income** | More enough | 4 | 80.00 | 1 | 20.00 | 0.232 |
| | enough | 108 | 70.71 | 55 | 29.29 | |
| | not enough | 67 | 68.60 | 25 | 31.40 | |
| | not enough and burrow | 62 | 64.86 | 25 | 35.14 | |

**Table II: Risk factors of children aged (5-15 years) infected with schistosma mansoni in Arab El-Mahder, Kafr El sheikh 2017**

| | | Schistsoma infection | | | | P-value |
|---|---|---|---|---|---|---|
| | | Negative | | Positive | | |
| **Waste disposal** | Sanitary system | 2 | 50.00 | 2 | 50.00 | 0.410 |
| | Well | 230 | 69.07 | 103 | 30.93 | |
| | Water canal | 7 | 87.50 | 1 | 12.50 | |
| | Don't know | 2 | 100.00 | 0 | 0.00 | |
| **Water source** | Water system | 132 | 66.67 | 66 | 33.33 | 0.072 |
| | Water storage | 75 | 70.09 | 32 | 29.91 | |
| | Water pump | 1 | 100.00 | 0 | 0.00 | |
| | Absent | 6 | 75.00 | 2 | 25.00 | |
| | 1 and 2 | 27 | 81.82 | 6 | 18.18 | |
| **Water unavailability** | Yes | 206 | 67.76 | 98 | 32.24 | ***0.001*** |
| | No | 35 | 71.31 | 87 | 29.69 | |
| **Frequency of water unavailability** | Daily | 118 | 64.84 | 64 | 35.16 | ***0.02*** |
| | Weekly | 73 | 71.57 | 29 | 28.43 | |
| | Monthly | 6 | 60.00 | 4 | 40.00 | |
| | Rare | 10 | 90.91 | 1 | 9.09 | |
| | No water sys | 34 | 80.95 | 8 | 19.05 | |
| **Distance to water canal** | <15minutes | 142 | 66..40 | 72 | 33.60 | ***0.037*** |
| | 15-30minutes | 95 | 76.60 | 29 | 23.40 | |
| | >30 | 4 | 44.40 | 5 | 55.60 | |
| **History schist** | Yes | 106 | 100.00 | 51 | 0.00 | 0.331 |
| | No | 126 | 60.00 | 48 | 40.00 | |
| **Site contact** | Center | 69 | 65.70 | 36 | 34.30 | ***0.046*** |
| | Peripheral | 119 | 73.90 | 42 | 26.10 | |
| | Both | 20 | 54.10 | 17 | 55.90 | |
| | No | 35 | 71.40 | 14 | 28.60 | |
| **Comorbid infestation** | No | 198 | 72.80 | 74 | 27.20 | ***0.01*** |
| | Yes | 43 | 69.50 | 32 | 30.50 | |

**Table III: Risky behaviors adopted by infected children livining in Arab El-Mahder Village Kafer El-sheikh**

| Cause of contact | Schistsoma infection | | | | P- value |
|---|---|---|---|---|---|
| | Negative n= 241)( | | Positive n=106)( | | |
| | No | % | No | % | |
| **Washing clothes** | 86 | 63.20 | 50 | 36.80 | ***0.044*** |
| **Swimming** | 50 | 57.50 | 37 | 42.50 | ***0.005*** |
| **Washing vegetables** | 21 | 53.80 | 18 | 46.20 | ***0.025*** |
| **Cutting grass** | 26 | 52.00 | 24 | 48.00 | ***0.004*** |

## 2. Validation of wet mount to Kato Katz technique:

Table IV shows that 51.5% of children were males and 48.5% were females. Their mean age was 9.83 ± 2.60 years, with 62% aged from 5 to less than 10 years and 38% aged from 10 to15 years.

**Table IV: Distribution of the study sample according to their sex and age in Arab El –Mahder Kafr El sheikh, 2017**

| (n=237) | | No. | % |
|---|---|---|---|
| **Sex** | Female | 122 | 51.5 |
| | Male | 115 | 48.5 |
| **Age in years** | 5-10 | 147 | 62.0 |
| | 10-15 | 90 | 38.0 |

Mean age was 9.83 ± 2.60 years

The prevalence of *S.mansoni* using wet mount was 2.1% which significantly increased to 15.2% using KK technique (one slide) ($X^2$= 23.896, P = 0.0001), **table V.** The wet mount had a sensitivity and PPV of 5% and 40%, respectively, and had a specificity and NPV of 98.5% and 85.5%, respectively. Cohen's Kappa coefficient (k) was 0.05 indicates poor agreement between wet mount and KK test **(table VI)**. It could be noticed that the more KK slides examined the higher the number of cases detected. The cases detected by the first, second, third and fourth slide were 15%, 19%, 26% and 27%, respectively. This difference was statistically significant (p< 0.05)**. Table VII**

**Table V: Prevalence of *S. mansoni* children according to the diagnostic test used in Arab El –Mahder Kafr El sheikh, 2017**

| Technique | Positive | Negative | $X^2$ | p value |
|---|---|---|---|---|
| | Number (%) | Number (%) | | |
| **Wet mount** | 5(2.1) | 232( 97.9) | 23.896 | ***0.0001**** |
| **Kato Katz** | 36(15.2) | 201 (84.8) | | |

* Significant (p<0.05)

**Table VI: Performance of Wet mount test in diagnosis of *S.mansoni* Arab El – Mahder Kafr El sheikh, 2017**

| | | Kato Katz technique | | Total | $\kappa$ agreement (p) |
|---|---|---|---|---|---|
| | | Positive | negative | | |
| **Wet mount** | Positive | 2 | 3 | 5 | 0.05 (0.118) |
| | Negative | 34 | 198 | 232 | |
| Total | | 36 | 201 | 237 | |
| Sensitivity (95%CI) | | 5.6 | | (0.68% to 18.66%) | |
| Specificity (95%CI) | | 98.5 | | (95.70% to 99.69%) | |
| PPV (95%CI) | | 40.0 | | (10.35% to 79.38%) | |
| NPV (95%CI) | | 85.3 | | (84.30% to 86.33%) | |
| PLR(95%CI) | | 3.7 | | (0.64 to 21.50) | |
| NLR(95%CI) | | 95.8 | | (0.88 to 1.04) | |
| Accuracy | | 84.4 | | | |

$\kappa$, Kappa agreement; NPV, Negative predictive value; PPV, Positive predictive value; PLR, Positive likelihood ratio; NLR, negative likelihood ratio; CI, confidence interval

**Table VII: Distribution of *S.mansoni* by Kato Katz technique according to the number of examined slides Arab El –Mahder Kafr El Sheikh, 2017**

**Positive cases n= (64)**

| Slide number | No. | Prevalence % | $X^2$ | p. value |
|---|---|---|---|---|
| Sample slide 1 | 36 | 15 | 13.07 | ***0.005**** |
| Sample slide 2 | 46 | 19 | | |
| Sample slide 3 | 62 | 26 | | |
| Sample slide 4 | 64 | 27 | | |

* Significant if p< 0.05

## 3. Validation of PCR to Kato Katz in diagnosis of S.mansoni

**Table VIII** shows the comparison between the results of the (cPCR) and KK (4 slides of single stool sample) of the initially taken samples i.e. before treatment. The cPCR had a sensitivity and PPV of 25.7% and 100%, respectively, and had a specificity and NPV of 100% and 71.4%, respectively. Cohen's Kappa coefficient (k) was 0.31 which indicates poor agreement between cPCR and KK technique.

**Table VIII: Validation of PCR as a diagnostic tool before treatment at Arab El Mahder Village, 2017**

| Item | | Kato Katz | | | |
|---|---|---|---|---|---|
| | | Positive | Negative | Kappa | P-value |
| **cPCR** | Positive | 9 | 0 | 0.31 | 0.001 |
| | Negative | 26 | 65 | | |
| **Sensitivity** | 25.7% | | CI: 95%: 12.49% to 43.26% | | |
| **Specificity** | 100.0% | | CI: 94.48% to 100.00% | | |
| **NPV** | 71.4 | | CI: 67.29% to 75.24% | | |
| **PPV** | 100.0% | | | | |
| **PLR** | | | | | |
| **NLR** | 0.74 | | CI: 0.61 to 0.90 | | |
| **Accuracy** | 74% | | CI: 64.27% to 82.26% | | |

$\kappa$, Kappa agreement; NPV, Negative predictive value; PPV, Positive predictive value; PLR, Positive likelihood ratio; NLR, negative likelihood ratio; CI, confidence

**Table IX** shows the comparison of the results of cPCR with the KK (9 slides) analysis of the stool samples collected after treatment with two doses of PZQ. The KK diagnosed 16 cases out of 91 treated individuals, meanwhile, all treated patients were negative by cPCR.

**Table IX: Validation of PCR as a diagnostic tool after two doses treatment at Arab El Mahder Village, 2017**

| Item | | Kato Katz | |
|---|---|---|---|
| | | Positive | Negative |
| **cPCR** | Positive | 0 | 0 |
| | Negative | 16 | 75 |

## 4. Cure rate of praziquantel after two doses

**Table X: Cure rate after treatment with praziquantel of children aged 5-15 years at Arab El Mahder Village, 2017**

| | Number received PZQ | Stool collected | Cure rate | | p-value |
|---|---|---|---|---|---|
| | | | No | % | |
| 1st dose | 100 | 66 | 44/66 | 66.7 | ***0.037*** |
| 2nd dose | 96 | 91 | 75/91 | 82.4 | |

After screening using KK technique, 106 cases were diagnosed. The cure rate was 66.7% among 66 children who provided samples (100 children received the 1st dose). After the 2nd dose of treatment, the cure significantly increased to 82.4% (p = 0.037). **Figure 3, Table X.** The mean egg count is significantly reduced after two doses of PZQ p-value was 0.001. The egg reduction rate was 92.75. **Table XI**

**Table XI: Mean egg count reduction after treatment with praziquantel of children aged 5-15 years at Arab El Mahder Village, 2017**

| item | Before treatment | After treatment | P-value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | 0.0001 |
| Egg count | 16.00±29.21 | 1.16±5.28 | |

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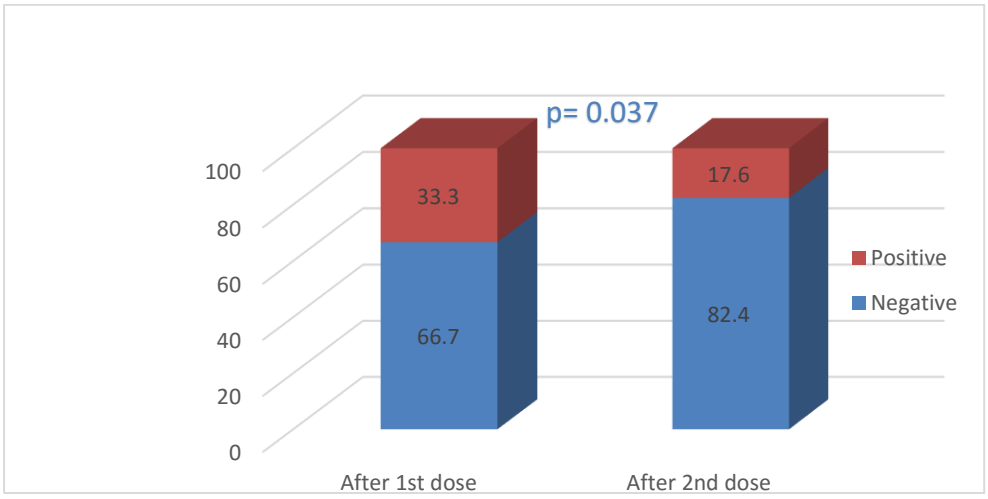


**Figure 3: Cure rate after treatment with praziquantel**

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## 5. Prevalence of Hepatitis C antibody and Hepatitis B surface antigen

**Table XII: Sero-prevalence of HCV Ab among children aged 5-15 years old (Arab el Mahder village, 2017)**

| Item (n=338) | | no | % | HCV Ab prevalence | | p Fischer exact |
|---|---|---|---|---|---|---|
| | | | | Number | % | |
| **Age** | 5-10 | 155 | 60 | 0 | 0 | 0.128 |
| | 10-15 | 183 | 40 | 4 | 2.19 | |
| **Sex** | Males | 177 | 52.4 | 3 | 1.69 | 0.384 |
| | Females | 161 | 47.6 | 1 | 0.62 | |

The prevalence of HBs Ag was 0%, meanwhile, the prevalence of HCV Ab infection was 1.18%, **figure 4**. **Table XII** shows that all HCV positive cases were among children aged 10-15 years, representing a prevalence of 2.19% among this age group, compared to none of children aged 5-10 years. This difference was not statistically significant (p = 0.128). The prevalence of HCV Ab was higher among males than females (1.69% and 0.62%, respectively) but this difference was not statistically significant.

Regarding risk factors, **table XIII** shows that 6.2% and 10.7% of the studied children had chronic diseases and jaundice respectively. None of the cases had such histories. Close contact family members were infected with HCV in 10.7% of studied children. More than one fifth (21.9%) of the children had history of surgical intervention. A family member with HCV infection and history of surgery was noticed among 25% of positive cases. About 86% of girls and none of cases had their ear pierced by non-health care workers (NHCWs). Circumcision was done by NHCWs in 45.9% of included children. Half of the cases were among this group. Sharing barbers tool and nail cutting tool was observed among 23.7% and 86.4 respectively. All cases shared nail cutting tools, while 25% shared barbers tools. Liver enzymes was elevated in 9.8% of children. All cases had elevated liver enzymes. Exposure to repeated injections, tattooing, reuse syringes and blood transfusion were noticed among 6.2 %, 25.8%, 1.8%, 6.2% and 5.6% of studied children, respectively. None of the cases had such exposures. Regarding dentistry intervention, 59% of the studied population and 25% of cases had different dental manipulation.

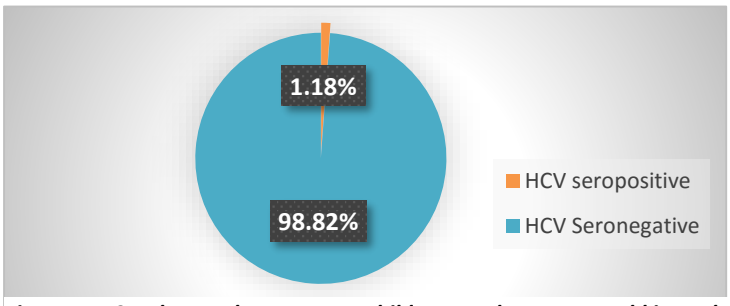


*Figure 4: HCV Ab prevalence among children aged 5-15 years old in Arab El Mahder Village Kafr El-sheikh governorate*

**Table XIII: Prevalence of HCV Ab among children aged 5-15 years old according to certain risk factors (Arab el Mahder village, 2017)**

| Item | | no | % | HCV seropositive | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **no** | **%** | Case 1 | Case 2 | Case 3 | Case 4 |
| **History of chronic disease** | | 21 | 6.2 | 0 | 0 | | | | |
| **History of jaundice** | | 36 | 10.7 | 0 | 0 | | | | |
| **Family member of HCV** | | 47 | 13.9 | 1 | 25 | | | √ | |
| **History of surgery** | | 74 | 21.9 | 1 | 25 | | | √ | |
| **Ear piercing by NHCWs (n= 161)** | | 139 | 86.3 | 0 | 0 | | | | |
| **Circumcision by NHCWs** | **Males** | 125 | 45.9 | 2 | 50 | √ | | √ | |
| | **Females** | 30 | | 1 | 25 | | √ | | |
| **Sharing nail cutting tools** | | 292 | 86.4 | 4 | **100** | √ | √ | √ | √ |
| **Sharing barbers tools** | | 80 | 23.7 | 1 | 25 | √ | | | |
| **Elevated liver enzymes** | | 33 | 9.8 | 4 | **100** | √ | √ | √ | √ |
| **Dentistry intervention** | | 198 | 59 | 1 | 25 | | √ | | |
| **Exposure to repeated injections** | | 87 | 25.8ssss | 0 | 0 | | | | |
| **Tattoo** | | 6 | 1.8 | 0 | 0 | | | | |
| **Reuse syringes** | | 21 | 6.2 | 0 | 0 | | | | |
| **Blood transfusion** | | 21 | 5.6 | 0 | 0 | | | | |

NHCWs, non-health care workers; HCV, Hepatitis C virus

**Prevalence**:

In the present study, the overall infection with S. mansoni among students of primary schools and preparatory school in Arab El-Mahder Village, Kafr El-Sheikh Governorate was 30.5 %. The prevalence rate in the present study was higher than those reported from Ghana (19.8 %)[23] and Ethiopia (24%).[24] This high prevalence emphasizes the need of dealing with other neglected essential components of *S. mansoni* control like provision of clean water, sanitary facilities, health education and personal hygiene. Other attributing factors could be non-treatment of community members (adults) who are potentially infected and lack of control of the intermediate hosts (snails).

**Sex:**

It was observed that prevalence of infection was higher among male child significantly. This could probably be due to boys are more adventurous than girls and they may come into contact with freshwater bodies infested with cercariae that have been released by intermediate host snails while playing or swimming unlike girls who are culturally restricted. This corroborates with a study conducted in Mbita, western Kenya, which reported that male children were highly infected with *S.mansoni* compared to female children due to their play habits.[25] On the other hand, Kumbu et al**,** reported that both males and females prevalence was the same.[26]

**Age:**

In this study, the prevalence of schistosomiasis did not rise with age. The prevalence of infection for the 5-10 and 11-15 age groups were nearly the same. This disagrees with previous reports in other schistosomiasis endemic foci of Ethiopia.[27] The high infection rate in the 5-10 age group is explained by high water contact activities.

**Intensity:**

The intensity infection showed that the largest number of infected children (87.7%) had light intensity. The findings are similar to those reported in the Democratic Republic of the Congo.[28] However, these results are opposite to those found in Ethiopia.[29] This low intensity may be attributed to mass drug administration (MDA).

**Farming activity of the parents:**

Farming activities of the fathers was associated with schistosomiasis, ($p$ = 0.037) not for mothers ($p$ = 0.566); these results are similar to those found in Ethiopia.[30] However, other studies found the opposite, as in Yemen.[31]

**Water source and sanitation:**

In this study, one third (33.3%) of infected cases did not have safe water supply, meanwhile the other two thirds either used water pump, storage system or had no access to safe water supply. The majority (98/106, 92.5%) of infected children complained of water unavailability, of them (64/106, 60.4%) reported daily water unavailability. Water unavailability was a highly significant factor that increased the prevalence of *S. mansoni* infection. Sanitary disposal was not associated with increasing the prevalence of infection. On the same line, Jacket et al, [32] concluded that people with safe water had significantly lower prevalence of schistosoma infection, as did those with adequate sanitation.

**Contact with water canal:**

- Cause of contact: infected children adopted risky behaviors like, washing clothes in water canal, swimming in water canal, using water canal to wash vegetables, and were cutting grass with bared foots.
- Frequency of contact: either daily, weekly, or monthly did not affect the prevalence of *S. mansoni* infection. Similar results was reported by Ismail et al.[33]

Oppositely, higher prevalence of infection was reported in endemic regions where humans frequently contacted water canals.[34]

- Site of contact: The prevalence of infection was higher among those dealing the periphery of water canal than those reaching its center. This may be explained by the prolonged contact of children with the periphery of canals to feed their domestic animals, wash their clothes, or toileting in the canal. Similar result was reported in a study conducted in Tanzania in univariate but not in multivariate analysis.[34]
- Time to reach water canal: More than two thirds (72/106, 67.9%) of children infected with *S mansoni* lived in a nearby place to the water canal that they spent less than 15 minutes to reach it. This was similar to results reported in Yemen.[35]

**Co-morbid infestations:**

The current study showed that the co-infection with other parasites were significantly associated with *S.mansoni* infection. This result agreed with those reported by Fleming *et al*, [36] and Raso *et al*.[37]

**Assessment of the validity of cPCR as a screening tool for the diagnosis of *S.mansoni* versus Kato-Katz examination as the gold standard:**

Polymerase chain reaction (PCR) has been employed in the diagnosis of *S.mansoni* for the first time by Pontes et al.[12] It has demonstrated high specificity and sensitivity in the detection of *S.mansoni* DNA in stool samples from patients in endemic areas.

The present study showed that cPCR technique detected less positive cases (9 cases) a sensitivity of 25.7% and specificity of 100%, compared to the KK that detected 34 cases with a Cohen's Kappa coefficient was 0.31 which indicates poor agreement between cPCR and KK. This result agreed with Allam et al, who reported that PCR failed to detect one positive case detected by KK technique only. They concluded that there must be additional diagnostic test to verify the results obtained by cPCR.[38] Similar results were detected by Pontes et al,[13] who reported that amongst 194 infected patients, no eggs were found in the stools of 16 infected individuals with a positive cPCR, whereas two patients with eggs in their stools diagnosed by KK technique were PCR negative. They concluded that missed cases after PCR were certainly misdiagnosed by the DNA amplification assay due to many factors such as; inhibition of the amplification reaction by fecal components, DNA degradation during transportation from the field to the lab, as well as variation in the daily egg output and uneven egg distribution in faeces which may have happened in the current study.

On the other hand, these results did not agree with the results reported by Carvalho et al, [39] and Lodh et al [40] who reported that that cPCR detected more positive cases than the KK technique.

**Treatment with PZQ:**

The over-dispersion of worm population among the infected children has important epidemiological and public health implications; heavily infected individuals are at the same time at highest risk of morbidity and the major source of environmental contamination. According to Hotez et al, [41] the underlying cause of such predisposition may be attributable to factors such as exposure to infection or differences in susceptibility to infection and the ability to mount effective immunity, among others. Hence, targeting of chemotherapy to school age children, population segment that contains heavily infected individuals, has the two-fold advantage of eliminating morbidity and blocking transmission

The efficacy of the drug is considered as sufficient when the cure rate (CR) is 60–90 % and egg reduction rate (ERR) is > 90 %.[42] Most of the time population treatment with PZQ produces CR of over 70 %. However, efficacy of treatment is

influenced by a number of factors, such as the epidemiological situations and expands on the prevailing ecological conditions which may affect PZQ efficacy.[43] Efficacy of PZQ decreases with pre-treatment intensity of infection, number of pre-patent infections, diagnostic sensitivity and age of the treated individuals. Moreover, poor drug quality and poor patient compliance may also negatively impact the effectiveness of the treatment, and even optimal timing at which treatment is evaluated also affects the outcome of the treatment.[44] similarly, in this research, the egg reduction rate of two doses of PZQ (40 mg/kg body weight two weeks apart) against S. mansoni infection in the study area was 92.75%, meanwhile, the cure rate was 82.4%.

**Limitation of the study:**

The potential weakness of this study may be the fact that only one stool sample was collected. The accuracy of the KK technique in identifying individuals with *S. mansoni* infections is limited by day-to-day variation in egg excretion and sensitivity is greatly reduced when intensity of infections is low. [5]

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# POLICY RECOMMENDATIONS

From the present study the following could be concluded:

1. The national-wide utilization of Kato-Katz (KK) technique in the primary health care units for diagnosis of *S.mansoni* instead of the wet mount technique which is currently adopted in all primary and family health care units is highly recommended.
2. Based on the findings of these studies, the molecular techniques, cPCR has shown to be non-superior to the KK technique. This may be due to the following reasons:
   - The financial burden that may result from the high cost of the materials used in the cPCR compared to the KK technique.
   - The cPCR needs qualified and well-trained personnel to run the technique appropriately, which may also represent a financial burden to train all laboratory personnel on nation-wide scale.
   - It may have low diagnostic accuracy with low intensity of infection with irregular egg shedding.
   - Kato Katz still has high reliability and validity even in the presence of low infectivity.
3. Recommended intervention strategy for the control of *S.mansoni* in the study area:
   Arab El-Mahdar village is a high-risk community with high prevalence of *S.mansoni* infection of low intensity (less than 100 epg stool).
   A multi-dimensional interventional program should be implemented simultaneously in order to stop the cycle of transmission of *S.mansoni* in this village. These dimensions are:
   a. Improvement of the social determinant of health; environmental sanitation and provision of safe water supply as well as sanitary sewage disposal system.
   b. Behavioral modification: this can be implemented in schools, informing children about the risk of exposure to contaminated water canal and risk and complications of getting *S.mansoni* infection.
   c. Mass drug administration (MDA) with PZQ without prior diagnosis (two doses, 40mg/kg body weight) for both adults and school-age children. The drug will be dispensed door-to-door to ensure that the drug reached all individuals in the village. Not only door-to-door strategy, but also directly observed swallowing of the drug should be adopted. Provision of PZQ in a suspension form will help to increase the compliance to treatment among children.
   d. Periodic screening of high risk groups, at least twice yearly, and treatment of infected individuals.
   e. Information, education and communication (IEC) tools are mandatory to improve the population's knowledge of the modes of transmission, prevention and control of *S.mansoni*. Community participation in the control program is a must to influence the faulty behaviors and modify the beliefs of the population. Mass media (television and radio), posters, flyers and leaflets should continuously address the problem and the disease-causing habits. School teachers and physician as well as primary health care workers should reinforce the information related to schsitosomiasis modes of transmission, prevention and control.
   f. Snail control in all water canals in the village, as these canals provide the shelter for the reservoir (snails) that play an important role in the cycle of infection with *S.mansoni.*

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