

Evaluation of various techniques used for the diagnosis of *Schistosomiasis*

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Abstract:

The main objective of the study was to determine the rates of prevalence for both species of *Schistosoma* amongst the population with special emphasis on school children. Moreover, the study aimed at the determination of sensitivity of various techniques for the diagnosis of each of the two *Schistosoma* species.

1000 specimens were, in total, collected; 500 samples of urine and 500 samples of stool from the population in the area around Sinnar city. The surrounding environment and social behavior of the communities were taken into consideration, especially the habits that aid the spread of infection. For stool detection, direct faecal smear, formal ether centrifugation technique, formal detergent quantitative technique, ordinary kato technique, and modified kato-katz technique were used. For urine detection, sedimentation technique by centrifugation, sedimentation technique by gravity, and filtration technique were used.

The overall rate of prevalence of *S. mansoni* was 24.8%, while *S. haematobium* was 43.0%. Amongst the diagnostic methods for the examination of the samples, formal ether technique proved to be the most sensitive (24.8%) for detecting and determining the mean egg count of *S. mansoni*, while filtration technique proved to be the most sensitive (43.8%) for detecting and determining the mean egg count for *S. haematobium*. Other methods showed relatively less sensitivity and quantitative efficiency

Introduction:

Schistosomiasis is considered to be one of the major public health problems in Sudan, and also is considered to be the most important tropical disease next to malaria. Christopherson ⁽¹⁾ in 1918 held the view that schistosomiasis was introduced into the Sudan by the Egyptians. Human schistosomiasis or bilharziasis is caused by three main species; *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*, which infect 10% of the world population. *S. haematobium* causes urinary schistosomiasis, *S. mansoni* and *S. japonicum* cause hepatosplenic and intestinal

schistosomiasis. The estimated global total annual loss from reduced productivity amounted to 642 million US dollars. This sum did not include the cost of public health programs, medical care, or compensation for illness ⁽²⁾.

The simplistic equation: man + snail + water = schistosomiasis, is valid because of high reproductive potentiality of life cycle ⁽³⁾.

The peak age of infection is in the first two decades of life. In the population, the prevalence at this age approaches 100% ⁽⁴⁾. Schistosomiasis causes chronic morbidity, with mortality estimated at 2-10%. It is predominantly a rural disease, commonly infecting farmers, and is

acquired through contact with contaminated water ⁽⁵⁾. Children in particular, with their high infection level indiscriminate habits of excretion, and predilection for playing in water, are very important in the transmission of the disease ⁽⁶⁾. The distribution of *S. haematobium* has been reviewed in a World Health Organization questionnaire. It is endemic in all countries in Africa, except Rwanda and Burundi, and occurs in islands off the African mainland including Zanzibar and Madagascar ⁽⁷⁾. The parasite is often endemic in only a part of a country where it occurs - reflecting the distribution and population dynamics of the snail host and variation in mass contamination of, and contact with, the water bodies where these snails are present ⁽⁸⁾. Nevertheless, in a few studies, surprisingly low mean egg counts have been found, in association with significant levels of prevalence, perhaps, due to technical factors such as the time of specimen collection ⁽⁹⁾.

A considerable number of studies have been carried on *S. haematobium* infection in the Sudan. In 1933 ⁽¹⁰⁾, urinary schistosomiasis was reported in Kordofan and in Darfour regions.

Over the years, repeated surveys showed the gradual establishment of the disease in the Gezira. Stephenson ⁽¹¹⁾ reported a prevalence rate of 21% among adults and 45% among children for *S. haematobium*, and suspected a fairly high non-documented prevalence for *Schistosoma mansoni*. Omer, et al ⁽¹²⁾ reported an over all prevalence of *S. mansoni* infection, on Elgalgala village in northern Gezira of 50% and a peak of 80% in the age group 10 – 20 years. Only 0.2% of cases were suffering from *S. haematobium*. Amin and Fenwick ⁽¹³⁾ reported a prevalence of

70 – 86% for *S. mansoni* and only 1.13% for *S. haematobium*. They noticed the ever increasing prevalence of *S. mansoni* and the dwindling incidence of urinary schistosomiasis. This is in spite of the widespread distribution of both types of snails.

This study aimed to compare the efficiency of various methods used for the diagnosis of *S.mansoni* and *S.haematobium*; and also to determine the prevalence of *S.mansoni* and *S. haematobium* in Sinnar Province.

Materials and methods:

Study area: The crescent around Sinnar town that lies about 280 km south from the capital, Khartoum was chosen as the survey area. The samples were collected from sporadically scattered places in the vicinity of Sinnar town, Wad Hashim village, Al-muraffaa Umda, Hillat Albeer, Sinnar Junction, and Mahala village

Collection of specimens: Samples of urine and stool were collected from 1000 randomly selected individuals of various sexes and mainly school children of 5-16 years of age (500 faecal and 500 urine samples). The persons from whom samples were collected were also requested to respond to a questionnaire comprising beside the personal particulars (name, age and occupation etc), the environmental factors and surroundings that could most probably lead to infection.

Collection of faeces samples: Faecal specimens were collected in clean, dry wide-mouthed plastic containers with tightly fitted lids.

Collection of urine samples: The urine samples were collected between 10a.m-2p.m. 30 ml volume of urine was taken. When it was not

possible to examine the fresh specimen within the first hour of collection, it was preserved with formalin (1ml per 100ml of urine) to avoid hatching of eggs.

Methods:

Stool samples:

- **Direct faecal smear:** using standard method published by Engels et al ⁽¹⁴⁾.
- **Formal ether centrifugation technique:** using method published by Katz et al ⁽¹⁵⁾.
- **Formal detergent quantitative technique:** using the method published by Borel et al ⁽¹⁶⁾.
- **Ordinary Kato technique:** using the method published by Ebrahim et al ⁽¹⁷⁾.

- **Modified Kato-Katz technique:** using standard method published by Erko et al ⁽¹⁸⁾.

Urine samples:

- **Sedimentation technique by centrifugation:** using method published by Fisher et al ⁽²⁰⁾.
- **Sedimentation technique by gravity:** using standard method published by Gutierrez ⁽²¹⁾.
- **Filtration technique:** using standard method published by Kean et al ⁽²²⁾.

Results:

S. mansoni

The examined fecal samples in this study were 500 samples. 24.8% of them were positive for *S.mansoni* (table 1).

Table (1): The overall prevalence rate of *S. mansoni* in the surveyed individuals:

Number of samples	Number of positive samples	Percentage
500	124	24.8%

The positive samples for *S.mansoni* in males' prevalence percentage (23.9%) than females faecal samples showed insignificant less (25.3%) in this study (table 2).

Table (2): Prevalence of *S. mansoni* according to sex of school children:

Sex of children	Examined	Positive	Percentage	P value
Boys	394	94	23.9%	>0.05
Girls	87	22	25.3%	

Out of 124 positive stool samples, the highest sensitive technique used for detection of *S. mansoni* was formal ether (100% sensitive) which was increased significantly compared to other techniques. The mean number of egg count obtained by this technique has also showed the highest number. While the lowest sensitivity was shown by the wet preparation (24.2%) which decreased significantly compared to other techniques (table 3 and figure 1).

Table (3): The number found positive by the respective techniques and their sensitivity:

Techniques	Wet preparation	formal ether	formal detergent	modified kato	ordinary kato	P value
Positive	30	124	110	103	100	0.08
Relative sensitivity	24.2%	100%	88.7%	83.1%	80.6%	

Insignificant changes

Figure (1): Mean number of egg count obtained by different techniques applied for stool examination:

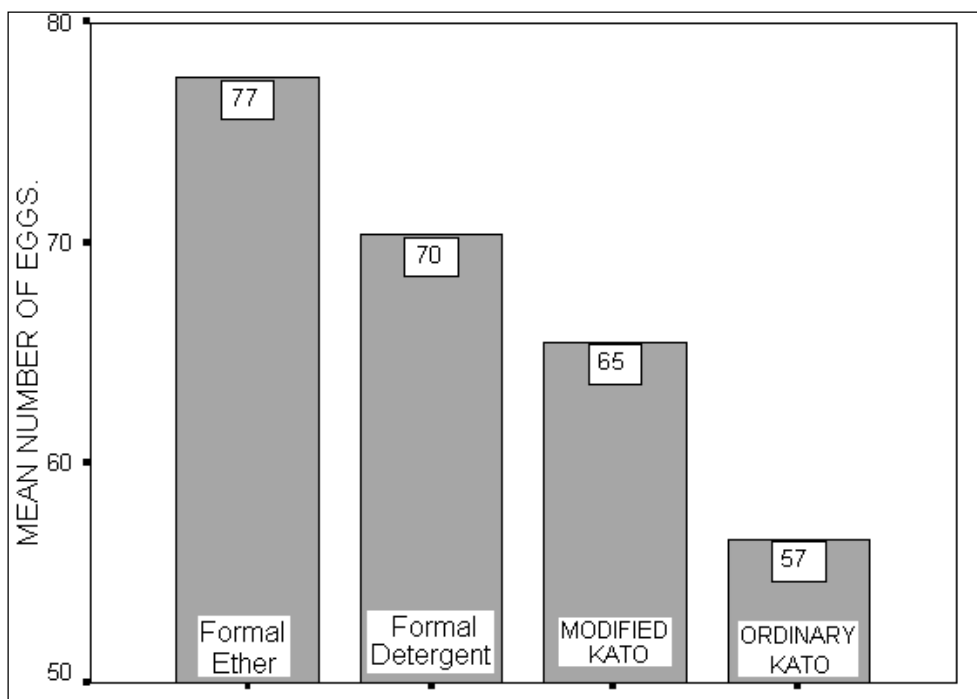


Figure (1): Mean number of egg count obtained by different techniques applied for stool examination:

***S. haematobium*:**

The examined fecal samples in this study were 500 samples. 43% of them were positive for *S. haematobium* (table 4).

Table (4): The overall prevalence rate of *S. haematobium* in the surveyed population:

Number of samples	Number of positive	Percentage
500	216	43%

The positive samples for *S. haematobium* in males' faecal samples showed insignificant increase prevalence percentage (45.9%) than females (34.6%) in this study (table 5).

Table (5): Prevalence of *S. haematobium* according to sex of school children:

Sex of children	No examined	No positive	Percentage	P value
Boys	399	183	45.9%	>0.05
Girls	78	27	34.6%	

With no significant variations, out of 216 positive urine samples (table 6), the highest sensitive technique used for detection of *S. haematobium* was the filtration technique (100% sensitive), while the lowest sensitivity was shown by the sedimentation with gravity technique (97.2% sensitive).

Table (6): The number found positive by each technique and their sensitivity:

Technique	Filtration	Sedimentation with centrifugation	Sedimentation with gravity	P vale
Positive	216	213	210	>0.05
Relative sensitivity	100%	98.6%	97.2%	

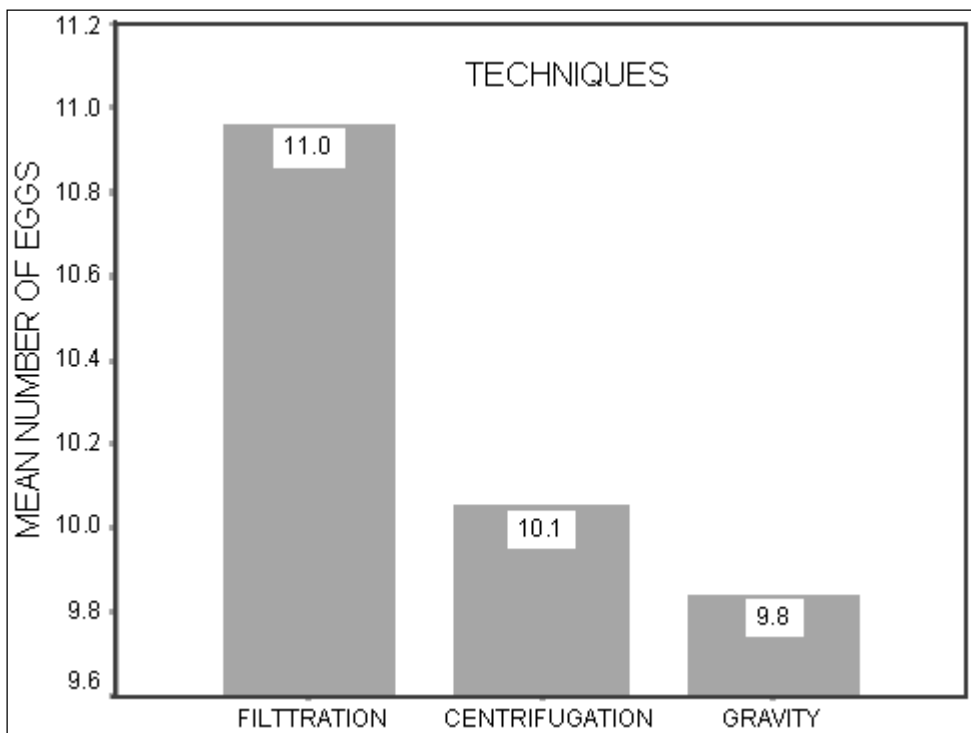


Figure (2): Mean number of eggs count obtained by different techniques applied for urine examination:

Discussion:

The results from the present study show that, from 500 collected samples for each of *S. mansoni* and *S. haematobium* detection, the overall prevalence was 24.8 % and 43% respectively. This reveals that several factors could be held responsible for the transmission of the disease. Among these factors: the social behavior, non-hygienic community practices, improper disposal of human waste and lack of health care.

Stephenson ⁽¹¹⁾ had reported a very high prevalence of *S. mansoni* (35 %) and *S. haematobium* (40%) from the northern province. This finding was in agreement with the findings obtained in this study.

This study concentrated particularly upon school children in the age range of 5 to 16 years. The rate of positive cases resulting from the present study, for *S. mansoni* among boys and girls was

23.8% and 25.3% respectively. The prevalence rate of *S. haematobium* for boys and girls was 45.9% and 34% respectively. This difference between infection in boys and girls is, perhaps, attributed to the more frequent visits paid by boys to the contaminated water supply sources.

El Alamy and Cline ⁽⁷⁾, accounted for the epidemiology of *S. haematobium* that it differs from that of other species in respect to relationship between age and the prevalence and intensity of infection. *S. haematobium* infection characteristically has peak intensity and prevalence of infection in the 5 to 16 years old age group, after which there is a fall to much lower levels of intensity and prevalence in middle age.

For the detection of *S. mansoni*, the present results proved that formal ether yielded the highest relative sensitivity value of 100% in the examined samples, whereas other methods

showed less sensitivity. For instance, formal detergent method could recognize 88.7% of the examined samples. The wet preparation technique proved the least reliable in this respect. It showed only 24.2% of the positive samples. Yet, though the wet preparation technique is not considered a quantitative method, for egg count, it is distinguished by being a quick and simple method and requires small amount of sample. It is not efficient in detecting light and chronic infections. Ordinary Kato and modified Kato Katz gave considerable and reproducible results, 80.6% and 83.1%, respectively. The mean egg count showed varied results among these methods considerably. The mean egg count with the Formal ether was 77, formal detergent 70, modified Kato Katz 65, whereas with the ordinary Kato was only 57. This denotes that great quantity of sample contributes to the reliability of detection, since the quantity taken for formal ether detection weighs up to 1g of stool sample. Formal detergent detection requires 0.33g of stool sample. Meanwhile, for modified Kato Katz 25mg and ordinary Kato, only 50 mg of stool sample is prescribed. Engels et al ⁽¹⁴⁾, stated that modified Kato Katz gave better results than the wet preparation method for detection of helminthes, and that it is better than ordinary Kato. Ebrahim et al ⁽¹⁷⁾ made a comparison between formal ether technique and modified Kato Katz. He found that the overall sensitivity of a single modified Kato Katz smear was 70.8%, whilst the sensitivity of the formal ether sedimentation technique was 83.3%. He concluded that formal ether method gave better results than modified Kato Katz in slight infections. The above findings correspond with

the results of the present study. In a comparison made by Borel et al ⁽¹⁶⁾, they concluded that the principal advantage of formal detergent method over modified Kato Katz technique was its better sensitivity to detect light infections resulting from larger amounts of stool processed.

In the present study, the techniques used for the detection of *S. haematobium* in urine samples showed highly sensitivity without significance between them ($p>0.05$). Out of 216 positive urine samples for *S. haematobium*, the sensitivity of filtration, sedimentation by centrifugation and sedimentation by gravity techniques were 100%, 98.6% and 97.2% respectively. This shows, indisputably, the higher relative sensitivity of the filtration technique. As for the mean egg count is concerned, almost similar results were procured, since a mean egg count 11.0 resulted from the filtration method, 10.1 resulted from sedimentation by centrifugation and 9.8 resulted from. In South Africa, Schutte et al ⁽²⁴⁾ reported that the filtration technique was the most sensitive in detecting ova in urine. Furthermore, there was little agreement between the mean egg count obtained using the filtration technique and those obtained with the other two techniques.

Richards et al ⁽²⁵⁾ reported that sedimentation by centrifugation gave statistically higher values than all other techniques, including filtration, as for the number of eggs recovered in the preserved urine. This is mainly attributed to the fact that the preserved urine, unlike fresh urine, frequently caused obstruction of the polycarbonate and Whatmain No. 1 filters.

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