



**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF BIOLOGY**

**IMPLICATIONS OF INTRA- AND INTER-SPECIMEN FECAL EGG
COUNT VARIATIONS IN DIAGNOSING *Schistosoma mansoni*
INFECTION BY THE KATO-KATZ METHOD, IN WORKIE MADU
VILLAGE, KEMISE, NORTH-EAST ETHIOPIA**

A thesis submitted to the School of Graduate Studies:

In partial fulfillment of
the requirements for the Degree of Master of Science in Biology
(Biomedical Sciences)

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Dedicated to:

*My late Father, Bireda Essa,
With Love*

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List of abbreviations

AAU	Addis Ababa University
ALIPB	Aklilu Lemma Institute of Patho-biology
ANOVA	Analysis of Variance
CNS	central nervous system
Epg	egg per gram of feces
Eps	egg per slide
KHC	Kemise Health Centre
K-K	Kato-Katz thick smear
LSD	Least significant difference
Spp	Species
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization

Abstract

Examination of stool specimens by the Kato–Katz (K–K) technique has been a standard method for field diagnosis of intestinal Schistosomiasis. However, it has been debated that this technique has low diagnostic sensitivity due to intra- and inter-specimen fecal egg count variation. The relative contribution of these two sources of variation among 107 community members of Workie Mado village, northeast Ethiopia, which is known for its high endemicity of *S. mansoni* infection was quantified. The diagnostic yield of examining one, three, or five Kato–Katz thick smears prepared from one stool specimen, using 41.7 mg templates was compared. In a subset of 11 volunteers, who had no demonstrable eggs in their first five K–K thick smears, the advantage of examining two additional triplet K–K thick smears from stool specimens, taken in two subsequent days was assessed. The overall prevalence estimates of infections increased with increasing number of slides examined. Prevalence of *S. mansoni* infection based on single, triplet, and quintet K–K thick smears was 62.6%, 75.7% and 84.1%, respectively. Cumulative prevalence obtained with two additional triplet Kato–Katz thick smears from 2nd and 3rd day stool specimens was 85.0% and 86.0%, which is not significantly different from quintet measurement. Compared to quintet K–K thick smears, single K–K thick smear missed 46.8%, 2.6% and 0% of subjects with light, moderate and heavy infections, respectively, while triplet K–K thick smears missed 19.1% of light infections and 0% of moderate and heavy infections. We conclude that diagnostic sensitivity in such high transmission areas can be maximized by using quintet K–K thick smears from one stool specimen to reduce the number of missing lightly infected individuals, and thereby examining smear-negative individuals with additional triplet K–K thick smears, from subsequent day stool specimens. Moreover, examining only one stool specimen with quintet K–K thick smears can also make reasonable estimates of *S. mansoni* infection intensity, and it would be more feasible and less expensive approach than day-to-day examination of individuals in communities.

Key words: *Schistosoma mansoni*, Kato-Katz technique, egg count, intra-specimen, inter-specimen, Workie Mado village, Ethiopia.

1. Introduction

Schistosomiasis is one of the world's widespread parasitic diseases of man which is second only to malaria in socioeconomic and public health importance, mostly in tropical and subtropical areas. It is mainly found in Africa, South America, the Middle East, East Asia, and the Philippines (Chitsulo *et al.*, 2000). Recent World Health Organization (WHO, 2002) estimates indicate that more than 200 million people are infected worldwide, and tens of millions suffer with debilitating chronic morbidity. Around 600 million people are at risk in 76 countries, particularly in urban areas, refugee camps, and where water resources are being developed (Ross *et al.*, 2002). It is estimated that 85% of all cases of schistosomiasis, and virtually the entire most severe cases, are concentrated in Africa (Engels *et al.*, 2002).

The disease can cause both short-term (acute schistosomiasis) and long-term (chronic schistosomiasis) symptoms. The symptoms of acute schistosomiasis include skin rash, a high fever, and muscle aches whereas symptoms of chronic schistosomiasis include weight loss, persistent diarrhea, and breathing difficulties. Schistosomiasis is associated with renal and bladder dysfunction (*Schistosoma haematobium*) or liver and intestinal disease (*Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, and *Schistosoma intercalatum*), and it also is a contributing cause of anemia and growth retardation (Ross *et al.*, 2002).

Schistosomiasis is acquired through the skin while wading or bathing in freshwater when the human host comes into contact with the infectious, free-living, cercarial larvae that are released by the parasite's intermediate hosts—aquatic or amphibious snails (Ross *et al.*, 2002). Patterns of water supply, sanitation, and human water use are, therefore, critical factors in determining the risk of infection. In addition, the geographic distribution of the different *Schistosoma* species is entirely dependent on the distribution of the distinct snail species that serve as intermediate hosts. Climate, water quality, and other ecologic factors that regulate these snail populations also determine the distribution of schistosomiasis on a district as well as national level (King, 2001).

Transmission occurs in places where people and snails come together at the same water habitat. Hence, schistosomiasis tends to be commonly found in rural communities where contact with freshwater bodies can be a routine and inevitable occurrence. Everyday needs ensure that contact with freshwater is almost unavoidable in many situations. Collecting drinking water, washing of all kinds, bathing and playing bring people to the water, whilst many occupations, including fishing and agriculture, expose individuals to contaminated water on a regular basis.

Schistosomiasis is generally considered a rural disease but, Mott and colleagues (1990) have indicated that, owing to migration, both urban and peri-urban areas of endemic countries in Africa and South America are now foci of transmission. They informed, in the same article, that it is a major public health problem in some African metropolitan cities such as Harare (Zimbabwe), Dar es Salam (Tanzania), Kinshasa (Zaire) as well as industrialized cities like Sao Paulo and Belo Horizonte in Brazil. In Ethiopia, major towns such as Harar, Jimma, Dessie and Bahir Dar are endemic for intestinal schistosomiasis (Birrie *et al.*, 1996).

1.1. The Disease Burden

Infection with intestinal schistosomiasis in Africa results in approximately 8.5 million cases of hepatomegaly, and African urinary schistosomiasis results in about 18 million cases of bladder wall pathology and 20 million cases of hydronephrosis (Van der Werf, 2003). Poverty promotes higher worm burdens, yet poor health induced by schistosomiasis can lead to lower incomes. Poverty attributable to schistosomiasis results from disfigurement or other sequel of long-term illness, impaired childhood growth and cognitive development, and reduced productive capacity (King *et al.*, 2005).

This disease is listed among the 13 diseases classified by the WHO as “Neglected Tropical diseases” (Hotez *et al.*, 2007). They are named so because they persist in the poorest and marginalized people who are often subsistence farmers, essentially living on

no money and stuck in poverty, with no education. Because they arise mainly in rural areas where families depend on subsistence agriculture, they impair agriculture productivity. Despite the severe pain and life-long disabilities they cause, these diseases are given a low priority alongside high mortality diseases (HIV-AIDS, TB and Malaria) known as the “big three” (Hotez *et al.*, 2007).

The neglected diseases often receive less attention by healthcare providers, national governments and international agencies than they merit. That is partly because not everyone infected becomes ill. However the disability caused by their morbidity remains a serious public health problem. Schistosomiasis infection is often asymptomatic and that is why incidence is not known. Prevalence is the only available epidemiological parameter and in most areas, this measure is also incomplete.

1.2. Life Cycle and Transmission of Schistosomiasis

The fact that 200 million people are currently infected with schistosomiasis is ample proof of the success of the schistosome life cycle (Chitsulo *et al.*, 2000). Like all other trematodes, the schistosomes require a molluscan intermediate host in which they undergo development, and freshwater snails from four different genera (*Bulinus*, *Biomphalaria*, *Oncomelania*, and *Neotricula*) form an essential component in the life cycle of the four major schistosome species that are responsible for human schistosomiasis.

The parasite relies on a basic behavioral characteristic of humans and that is the tendency to defecate and urinate in and around water. Much of the schistosome life cycle takes place in the aquatic environment and when eggs, which are excreted in large numbers with urine and/or feces, reach freshwater, they hatch releasing a free-swimming larva or miracidium. The miracidium is a non-feeding, short-lived stage that attempts to seek out and penetrate a suitable intermediate snail host in which to continue development. Those eggs that do not reach water are soon desiccated and play no part in transmission.

The most striking fact relevant to control is that if human behavior could be changed to stop contamination of water bodies with urine and feces containing schistosome eggs, then transmission of the parasite would cease. This fact is central to many longer-term control efforts, including the education of children, to reduce water contamination, together with the provision of piped water and better sanitation. In theory, the breaking of the schistosome life cycle is remarkably easy, whereas in practice, it is extremely difficult at the community level among poor and underserved populations (Rollinson and Johnston, 1996).

Within the snail, the parasite develops and multiplies asexually. This allows the parasite to increase dramatically in numbers enhancing considerably the chances of re-infecting humans. The schistosome has an extremely specific and selective need at this stage in the cycle and will only develop successfully in certain snails (Rollinson and Southgate, 1987)

The adult worms, which mature in the human host, live in a food-rich environment in blood vessels (Fulford *et al.*, 1995). Schistosomes are dioecious and males and females must pair in order to mature and mate and to locate to the blood systems in the body that allow the egress of eggs. Each female worm is capable of laying many hundreds of eggs on a daily basis (Loker, 1983) but many of the eggs fail to leave the body and get trapped in various tissues and organs; it is the eggs rather than the adult worms that are primarily responsible for the pathology associated with the disease. Eggs that escape through the bladder or intestine cause blood loss as capillaries are severed, and those that do not escape end up in organs and tissues such as the liver, causing long-term damage.

Thus the schistosome life cycle and the host parasite interactions involved are complex, involving the parasite moving, surviving, penetrating and developing in quite different environments: mammalian blood, water and intermediate snail hosts. The broad biological processes relating to the life cycle are now sufficiently well understood to help target efforts to control both the disease and its transmission.

Each schistosome species utilises a different intermediate aquatic snail host, which becomes infected by the aquatic larval stage (miracidium) hatching from eggs excreted in urine or feces of infected humans. A period of development in the body of the snail produces the cercariae, the infective stage for the human host, that are released from the body of the snail into the water for several weeks. Skin contact with water containing the cercaria is required for transmission (Figure 1).

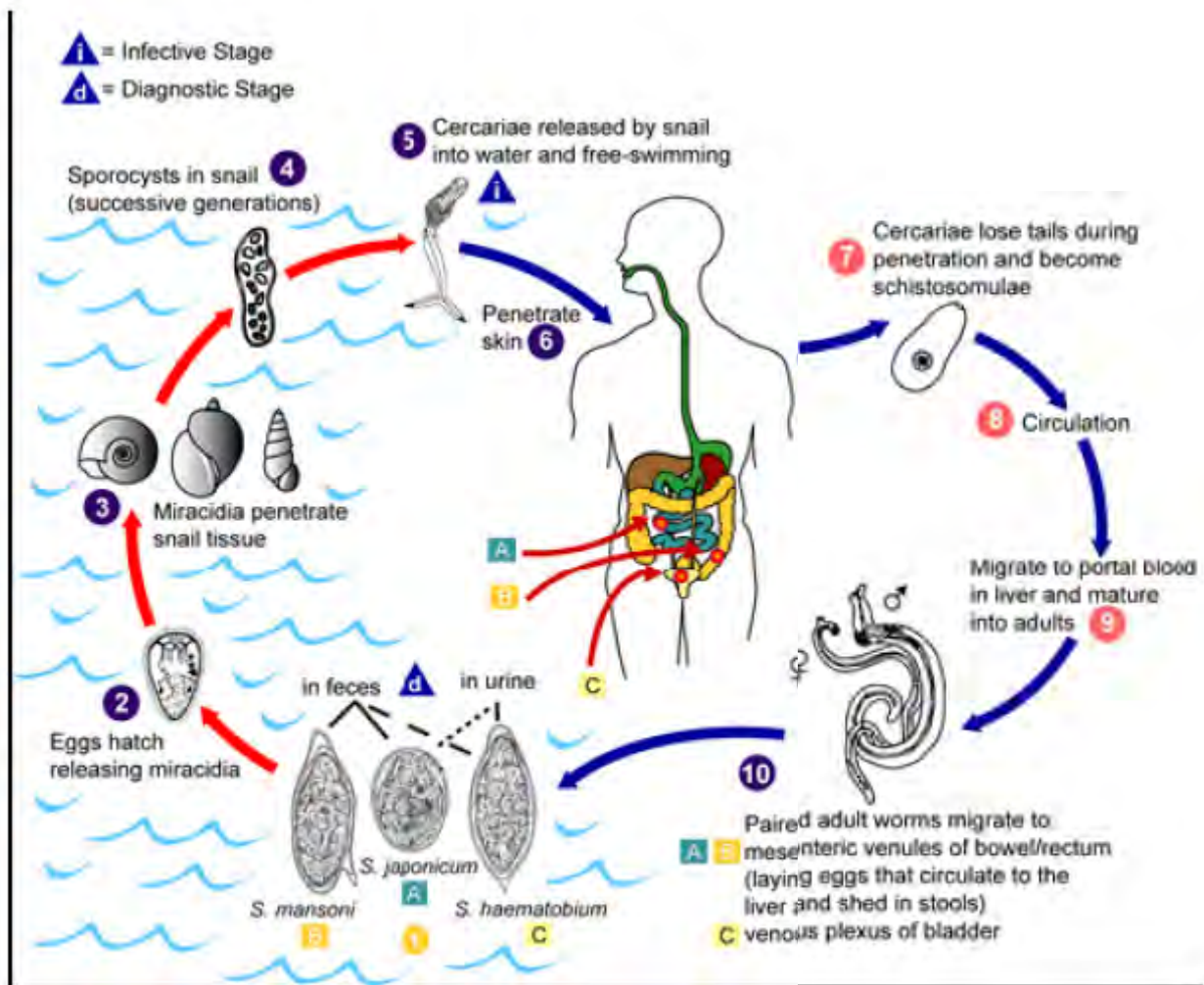


Figure 1: Life Cycle of Schistosomiasis. (Source: www.dpd.cdc.gov/dpdx)

Humans are definitive hosts (i.e. they harbour the adult stages) of schistosome parasites. The final sites of predilection of the flukes are the veins of the bladder (*S. haematobium*) and the intestine (*S. mansoni* and *S. japonicum*). Immature stages may also be

responsible for disease, especially in immunologically naive hosts, before maturity and egg-laying occur. The number of flukes, and therefore the burden of eggs in a patient, is the major determinant of disease severity and outcome, but even a single pair of schistosomes, or a few eggs, in an ectopic site like the spinal cord or brain, may have disastrous consequences.

1.3. Epidemiology of Schistosomiasis

Of the 200 million people infected with schistosomiasis, 120 million are symptomatic and 20 million suffer from severe disease (WHO, 1999). Even though schistosomiasis is most severe, and highly concentrated, in the African continent other countries are also affected. The most affected country in the Americas is Brazil, with 3 million infected people; in Asia, it is China, with nearly 1 million infected, and in the Middle East, Yemen. Population growth and movement in endemic areas, and ecological changes resulting from increasing use of water for irrigation and electricity generation, have contributed to the spread of infection (WHO, 1999).

In endemic areas, schistosome infection is acquired in childhood. Infection increases in prevalence and intensity with age, peaking in the age group of 15 to 20 years. In older people, a drastic decline in intensity, but not in prevalence, has been demonstrated (King *et al.*, 1988; Hagan *et al.*, 1994).

Schistosome infections in the human populations of endemic areas follow an overdispersed pattern, in which most infected persons show low egg counts, and a small percentage (1% to 5%) harbor extremely heavy infection (Abel *et al.*, 1993). Susceptibility to high-intensity of infection may reflect water exposure patterns. The epidemiology of schistosomiasis is further regulated by the duration of the parasite life span (3 to 7 years) and the multiple immunologic and nonimmunologic responses of the host that participate in regulating infection and disease (Abel *et al.*, 1993).

Expression of disease due to schistosome infection is similarly complex. Although adult schistosomes do not replicate in the mammalian host, they produce eggs throughout their life span. These parasite eggs elicit host immunopathologic reactions, which are responsible for most disease manifestations (King, 2001). Pathogenesis may be related, therefore, to intensity of infection and certainly to factors that regulate host response, including genetic influences (Dessein *et al.*, 1999).

1.3.1. The Parasite

Five species of schistosomes infect humans. The three major species are *S. haematobium*, found in Africa and the Middle East; *S. mansoni*, found in Africa, the Middle East, the Caribbean, and South America; and *S. japonicum*, found in China, Southeast Asia, and the Philippines. Human infection also occurs with *S. intercalatum*, which is found in central and West Africa, and *S. mekongi*, which is found only in the Mekong river basin of Southeast Asia.

Species differences are distinguished by differences in morphology, both in the parasite stages and in their eggs. The characteristic feature of the *S. mansoni* egg is its lateral spine; of the *S. haematobium* egg, its terminal spine; and of the *S. japonicum* egg, its limited, inconspicuous spine (Ross *et al.*, 2002). Further species distinction is made by the species of intermediate host snails found to be supporting transmission of the parasite. These are *Biomphalaria* species snails for *S. mansoni*, *Bulinus* species snails for *S. haematobium*, and *Oncomelania* species snails for *S. japonicum* (King, 2001).

Given the geographic differences reported in local susceptibility to antiparasitic drugs, there undoubtedly are *Schistosoma* subspecies, as well as intraspecies strain differences that exist between continents and within individual countries (Thiongo *et al.*, 1997; Brouwer *et al.*, 2003). Epidemiologic studies in Kenya have focused on significant differences in the pathogenicity of *S. mansoni* infection in different areas of the country (Thiongo *et al.*, 1997).

1.3.2. The Snail Intermediate Host

The main molluscan genera used by the human schistosomes are *Bulinus* (*Schistosoma haematobium*), *Biomphalaria* (*Schistosoma mansoni*), *Oncomelania* (*Schistosoma japonicum*) and *Neotricula* (*Schistosoma mekongi*). The snail intermediate host that is responsible for human *Schistosoma mansoni* infection is the genus *Biomphalaria*.

The global distribution of species that act as intermediate snail hosts reflects the distribution of human schistosomiasis. These snails, especially *Bulinus* and *Biomphalaria* thrive in areas frequented by man. Indeed certain species seem to favour habitats polluted with human excreta and the waste of everyday living. The complex relationship between snail and schistosome make this part of the life cycle vulnerable to control activities, and there is a long history of attempted snail control using chemicals (molluscicides) to kill snails (Barnish, 1970; Sturrock, 1995). Biological and environmental manipulation of the habitat to reduce numbers have been considered and targeting of specific intermediate snail hosts is a challenge for the future (Pointier and Jourdan, 2000; Pointier and David, 2004).

Snails have limited powers of dispersal and are unlikely to move far during their lifetime unless carried by freshwater currents. However, when they are introduced into favourable habitats, they colonise new water bodies quickly due to their large reproductive potential. Movement and spread of the parasite is much more likely due to the movement of infected people. Infection from snail to humans depends on water contact, the free-swimming larvae or cercariae released from the snail penetrating directly through areas of the skin in contact with water.

1.4. Geographic Distribution of Schistosomiasis

Africa is the continent most widely affected by the disease, caused mainly by *S. mansoni* and *S. haematobium*, which are the major causes of intestinal and urinary schistosomiasis, respectively (figure 2). *Schistosoma japonicum* is transmitted in several Far Eastern countries (China, Philippines, Indonesia). *S. intercalatum* and *S. mekongi* are geographically highly restricted to small areas of Africa and Southeast Asia, respectively.

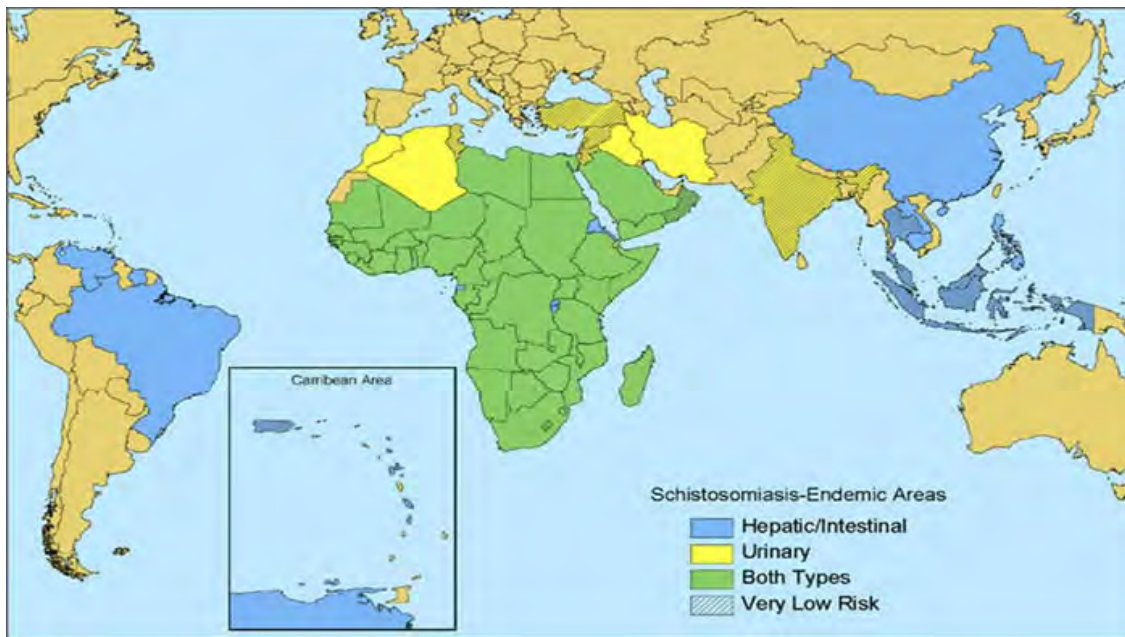


Figure 2: Global distribution of schistosomiasis. (Source: CDC 2007)

The ecology of *S. mansoni* and *S. haematobium*, their snail intermediate hosts, and the human definitive host determine the occurrence of the disease in the Ethiopian population. The topographic/climatic diversity and water resources development with associated labor migrations and poverty are key factors in the geographic distribution of the two forms of schistosomiasis in Ethiopia. *S. mansoni* prevalence rates range from 0 to 94% in communities at different altitudes, characterized by clustering of both low and high levels of infection in different altitudinal zones and parts of the country (Kloos, 1993). *S. haematobium* infections have been reported from the Awash and Wabe Shebele river basins and from Kurmuk at the Ethiopian/Sudan border (Malone *et al.*, 2001).

1.5. Schistosomiasis in Ethiopia

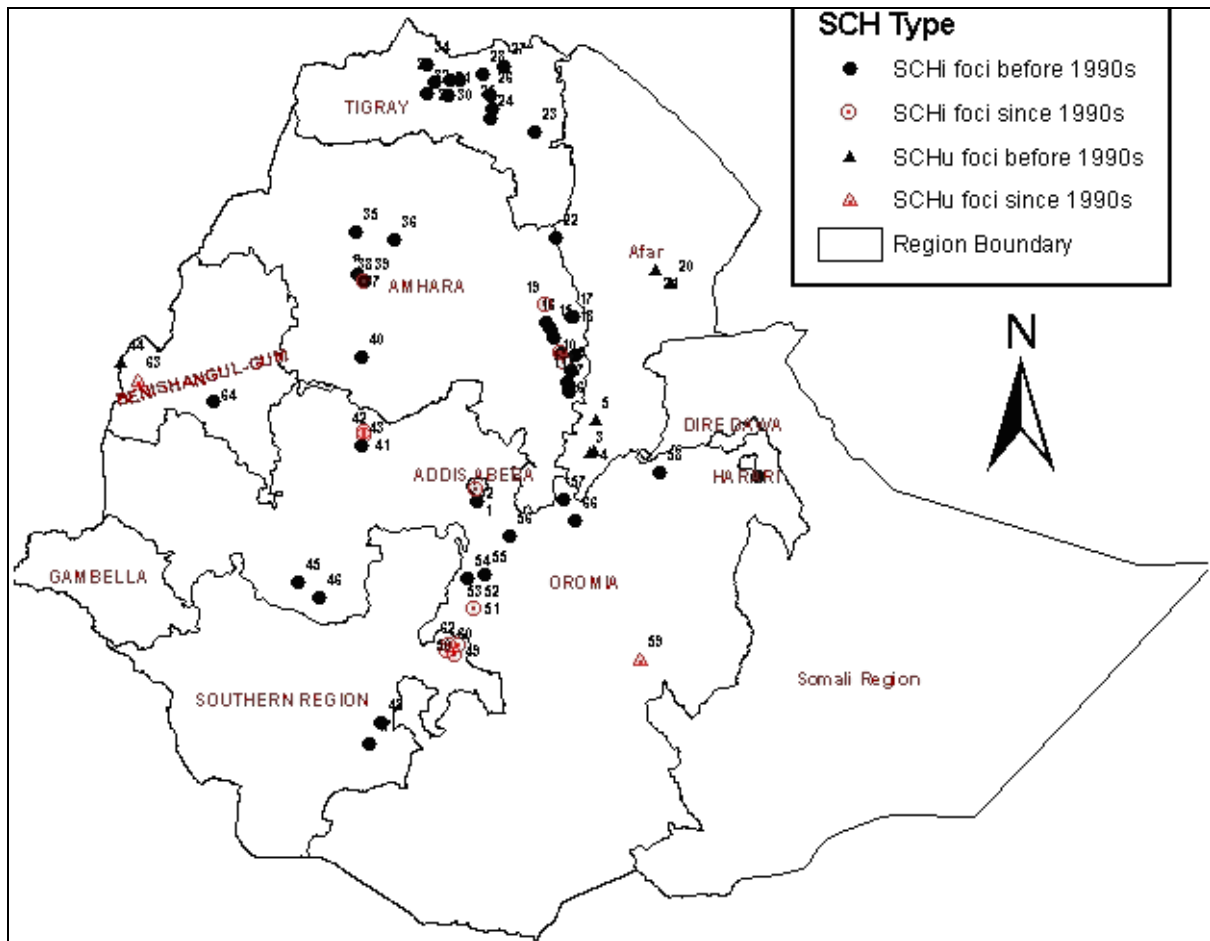
Documented evidence on the endemicity of schistosomiasis in Ethiopia dates back to the 1950s (Ayad, 1956). Both intestinal schistosomiasis caused by *Schistosoma mansoni* and urinary schistosomiasis caused by *Schistosoma haematobium* are endemic. The former is widespread, while the latter is restricted to some lowland areas. *Biomphalaria pfeifferi* and *B. sudanica* are the known intermediate host snails for *S. mansoni* and *Bulinus abyssinicus* and *B. africanus* for *S. haematobium*.

While some investigators, including Ayad, considered schistosomiasis to have been recently introduced to Ethiopia, Tedla and Leykun (1988) & Kloos and others (1978) suggested that the disease is of antiquity. While the occurrence of schistosomiasis in several remote and focal localities of the country argues for a long presence in Ethiopia, the development of commercial irrigation and hydroelectric schemes has resulted in its spread into previously non-endemic areas (Lo *et al.*, 1988; Kloos, 1985).

Schistosoma mansoni is widely distributed and is more rapidly spreading in connection with water resources development and intensive population movements, while *Schistosoma haematobium* has so far been reported from three foci in western and eastern low lands of Ethiopia. The number of known endemic localities for *Schistosoma mansoni* has increased from less than ten in the 1970s (Figure 3) to some 45 at present (Kloos and Birrie, 1988; Erko *et al.*, 1997). Despite its significant public health and socio-economic impact, the control of the disease has not received due priority from health authorities. Control efforts made so far have been limited to few pilot trials towards the development of control strategies.

More studies have been carried out on schistosomiasis in Ethiopia than on any other vector-borne disease, with the possible exception of malaria, largely due to the intensive schistosomiasis research program launched around 1970 by the late Dr. Aklilu Lemma in the Institute of Pathobiology. The fact that Endod grows locally and is biodegradable,

makes it ecologically and economically more acceptable for use in schistosomiasis control compared to synthetic molluscicides. However, with the change in WHO recommended control strategies from snail control to disease control in schistosomiasis in the 1980s, the role of molluscicides diminished relative to that of chemotherapy, with the provision of water supplies and health education becoming more prominent (Jordan *et al.*, 1993). Currently, plant and synthetic molluscicides are used focally rather than for blanket treatments of snail habitats as a more cost-effective measure.



No.	Locality Name	No.	Locality Name	No.	Locality Name	No.	Locality Name
1	Akaki(Fanta stream)	18	Bati	34	Adi Daero	51	Burka Dita
3	Hasoba	19	Hayk	35	Gorgora	52	Enchocho
4	Dahitele	20	Date Bahri	36	Adis Zemen	53	Bishan Gari
5	Geleila Dora	21	Dubti	37	Zege (Lake Tana)	54	Ziway town
6	Gerbi	22	Zobil	38	Bahir Dar(Lake Tana)	55	Herera
7	Senbete	23	Kwiha	39	Bahir Dar (Abay River)	56	Wonji Sugar Estate
8	Ataye	24	Abiyi Adi	40	Jiga	57	Metehera Sugar Estate
9	Chekorty	25	Werkamba	41	Camp 7 (Lemlem Berha)	58	Hirna
10	Cherety	26	Maykinatil	42	Fekere (Lemlem Berha)	59	Dolo Odo
11	Dewe	27	Inticho	43	Finchaa	60	Bushilo/Loke
12	Dolu	28	Adi Abun	45	Agaro	61	Marefia Spring
13	Kemise (Workie River)	29	Wukro Marya	46	Jimma	62	Tikur Wuha
14	Harbu	30	Selekileha	47	Sille	65	Esakoy
15	Kombolcha (Irrigation canal)	31	Debre Kerbe	48	Lake Abaya	66	Abomssa
16	Dese (Borkena)	32	Endaba Guna	49	Eshebe Stream		
17	Silmeni	33	Shire Enida Silase	50	Wosha		

Figure 3: Schistosomiasis endemic foci in Ethiopia (SCHi: intestinal schistosomiasis; SCHu: urinary schistosomiasis)

(Source: www.aau.edu.et/news/inag/Berhanu_Erko_synopsis.doc)

The migratory movements of agricultural workers and the pastoral nomads may be related to the wide spread distribution of schistosomiasis in Ethiopia. The prevalence of both *S. haematobium* and *S. mansoni* among the pastoral population is usually higher than in the groups engaged in permanent agriculture as observed in the Awash valley (Kloos, 1985).

Aklilu Lemma (Kloos, *et al.*, 1978), in his study on *Schistosoma mansoni* distribution in Ethiopia, anticipated that owing to improvement of highways and rapid shift of population in search of work opportunities, it would be a matter of time before the disease would appear in major towns including the city of Addis Ababa itself. He stressed that the migration of infected people to Addis Ababa and the presence of the snail host, *Biomphalaria Pfeifferi*, in some streams within the city would provide adequate opportunity for the possible introduction of the disease into the city. However, transmission has already been confirmed in Akaki Town, a suburb located at about 10 km south of Addis Ababa (Mammo & Assefa, 1989).

Most studies in Ethiopia reported *S. mansoni* and *S. haematobium* prevalence and incidence rates to peak in adolescents, with higher rates in males than females, similar to the pattern reported from other endemic areas (Tedla *et al.*, 1989). Human water contact activities involving snail-infested surface waters are associated with the distribution of both forms of schistosomiasis in the population (Birrie *et al.*, 1998). Domestic, occupational and recreational water contact activities put the majority of the Ethiopian population in endemic areas at risk of acquiring the infection in the absence of safe domestic water supplies.

Activities such as swimming and bathing, cleaning irrigation canals without protective rubber boots, and washing clothes and other articles expose people to schistosomiasis infection (Lemma *et al.*, 1979). Lemma and others, in the same article, also mentioned that children (especially boys), peasants and daily laborers have higher exposure risk than housewives and shop owners, and they reported higher prevalence rates in Muslims than

Christians in Arsi and Harerge regions which may be due to the Islamic practice of ritual ablution before prayer.

1.6. Risk Factors

Schistosomiasis is widespread among the poor populations in less developed countries, who live in conditions that favor transmission and who have no access to proper health care or effective prevention measures. Infection is predominant in school-age children between 10 and 15 years old. Difference in the peak age-related prevalence of disease is due to the gradual development of immunity and changes in the extent of freshwater exposure (Barbosa *et al.*, 2006).

Adults who immigrate to endemic areas are as susceptible to infection as young children. Children who practice swimming are particularly at high risk, because of their prolonged and complete body exposure. These endemic areas are often characterized by low socioeconomic conditions and poor sanitary facilities, erroneous habits of the people as regards urination and defecation in canal water, and exposure to this polluted water by bathing, swimming, washing utensils and clothes, walking bare-foot during irrigation in agriculture or fishing.

Most communities affected by schistosomiasis *mansoni* in Ethiopia are rural farming villages at intermediate altitudes depending on rain-fed agriculture that are located near the many perennial streams (Kloos *et al.*, 1993). Dependence on more than 90% of rural households on these and other potentially infective surface waters in the absence of safe water supplies and sanitary facilities puts the great majority of the population at risk of infection (Malone *et al.*, 2001). The absence of health services providing affordable anti-schistosomal drugs, lack of awareness of schistosomiasis in the population and population movements to endemic communities are additional risk factors, although little is known about their relative importance.

1.7. Water Resource Developments

Developing countries faced with an increasing human population are required to expand agricultural production and in many areas this can only be achieved by intensification of water impoundment and irrigation. However, by increasing the global area under surface irrigation, opportunities are created for greater food production and increased prosperity. The construction of water schemes to meet increasing agricultural and power requirements may have an unfortunate negative impact on health due to increases in water-borne diseases, especially schistosomiasis.

The increase in freshwater habitats associated with dams, either in holding water bodies, irrigation canals or rivers, provides ideal conditions for intermediate snail hosts and facilitates greater water contact and transmission. On the positive side, water development activities such as sinking of wells, provision of clean tap water and adequate latrines, can play a major role in the reduction of transmission by reducing water contact (Jordan *et al.*, 1982b).

There are many examples of increased transmission of schistosomiasis as a result of irrigation, the most dramatic being found along the Nile Valley in Egypt and Sudan and more recently along the Senegal River Basin (SRB), where a serious outbreak of intestinal schistosomiasis in populations with no earlier experience of the disease was associated with a large water development programme (Picquet *et al.*, 1996; Southgate, 1997).

Prior to the water resource development, donor agencies assessed health risks but no means were provided for schistosomiasis control even though some studies warned of a possible extension of schistosomiasis in the SRB. The increase in prevalence and intensity of infection with *S. mansoni* was rapid. High prevalence of infection was observed in all age groups, suggesting that the infection was recent and that the population was immunologically naive. Intensity of infection, measured by the number of eggs per gram (epg) of feces, was particularly high (Gryseels *et al.*, 1994).

In Ethiopia, the current trend of harvesting water to supplement the agricultural productivity is associated with the expansion of schistosomiasis mansoni and other intestinal parasitic infections (Dejene & Asmelash, 2010). Lemma (1969) and Kloos (1985) also observed that the continuing large-scale agricultural use contributes to the spread of schistosomiasis.

On the other hand, the fact that the major human intestinal helminth parasites such as hookworm, *Ascaris* and *Trichuris* larvae and ova require humid environments indicates that water source could still be a factor for transmission of intestinal parasites (Kloos et al., 1981). Due attention should be given to health impacts of such agricultural interventions, and the worm burden can be reduced by proper management of the water and the canal system that would reduce the establishment of the intermediate hosts.

During construction of large-scale water development programmes, simple engineering measures could often be taken to reduce the introduction and growth of snail populations and to provide safe and clean water for the local population. For example, concrete lining and covering of irrigation canals, altering the speed of water flow, regular drainage and reduction of water plants, can all help to minimize snail populations. Adequate piped water, washing areas and latrines need to be part of new and old settlements associated with any new water development projects in order to reduce dangerous water contact and contamination.

1.8. Diagnosis of schistosomiasis

1.8.1. Stool Examination

The gold standard for diagnosis of schistosomiasis infection is the detection of schistosome eggs excreted in stools (*S. mansoni*, *S. japonicum*) and in the urine (*S. haematobium*). The number of eggs excreted determines the intensity of infection and these parameters can be easily measured with limited laboratory equipment in resource-limited settings such as sub Saharan Africa where the fecal thick smear or Kato-Katz (K-K) method (Katz, 1972) is commonly used. The K-K method allows quantification of infections by egg counts, usually expressed as per gram of feces (epg). The determination of average number of eggs per gram of feces reflects the intensity of schistosomal infection.

1.8.2. Ultrasonography

Ultrasonography is used to detect *schistosomal* pathology both at the hospital and field level (Hatz, 2001). It has been established as a safe, rapid non-invasive and relatively inexpensive technique for assessing schistosomiasis –related lesions in individual patients and in community surveys, according to the same author. It can also be used to validate laboratory tests, to measure morbidity, and provides an opportunity to visualize the evolution of pathological lesions after treatment. Ultrasonography has been useful in revealing the fibrotic liver during chronic hepatic schistosomiasis (Uttinger *et al.*, 2000). Schistosomiasis infections are considered to be the most frequent cause of liver fibrosis worldwide (Warren, 1984). Although portal hypertension syndrome (hepatomegaly, splenomegaly, ascites), is commonly said to be cirrhotic, schistosomiasis should be considered too (Doumenge, 1987).

1.8.3. Immunodiagnosis

Many studies have documented the applicability of antibody detection for the diagnosis of schistosome infections both at the level of the individual and in sero-epidemiological studies. Like a parasitological diagnosis, and in contrast to antibody detection, a diagnosis based on antigen detection directly reflects the parasite burden and thus provides quantitative information (WHO, 1999). However, the sensitivity is - like in parasitological diagnosis - influenced by the intensity of infection. The main advantage of antigen detection, is the fact that antigen levels show little fluctuation. A one-point determination therefore provides more reliable quantitative data than in the case of a parasitological diagnosis. The main disadvantages of antigen detection are related to the availability and cost of the reagents, and to the relatively time-consuming and expensive (ELISA) assay, which also does not lend itself well to use outside a laboratory setting.

1.9. Prevention and Control of Schistosomiasis

Given the complex nature of *Schistosoma* life cycle, there are, in theory, a number of different ways to prevent transmission of infection or reduce the likelihood of heavy infection (King, 2001). These include (1) reduction or elimination of intermediate host snails; (2) elimination of snail habitats; (3) sanitation measures to prevent human excreta from contaminating local water sources; (4) provision of safe freshwater supplies to reduce contact with snail-infested water sources (this may include provision of communal baths, laundries, and swimming facilities) (Jordan *et al.*, 1982b); (5) use of protective footwear or clothing, or use of protective medicated salves to prevent cercariae from reaching the skin; and (6) use of periodic drugs to limit infection intensity in exposed populations (Chan *et al.*, 1996). If applied on a population-wide or targeted subpopulation basis to achieve coverage of the most heavy excretors of eggs, this last approach also results in a significant reduction in parasite transmission as a result of a substantial reduction in parasite eggs reaching transmission sites. Each of these approaches has been tested under field conditions.

Long-term eradication of schistosome transmission has been achieved in Japan and Venezuela, where water improvements and sanitation measures associated with development have eliminated transmission of *S. japonicum* and *S. mansoni*, respectively (King *et al.*, 1992). Similarly, water supply projects, including provision of communal swimming pools, were associated with a significant reduction in *S. haematobium* prevalence in southern Iraq (Chitsulo *et al.*, 2000).

A systematic comparison of snail vector control, drug therapy, and water and sanitation measures for control of *S. mansoni* was performed over a 10-year period on the island of Saint Lucia in the Caribbean (Jordan *et al.*, 1982a). Although all modalities were effective in reducing infection, drug therapy was the most effective and least expensive, while provision of piped water was nearly as effective but much more expensive, requiring provision of individual household taps, as well as communal baths and laundries, to achieve comparable levels of control.

The most practical approaches to control appear to be, in the short term, provision of periodic drugs to limit intensity of infection and morbidity (Mahmoud *et al.*, 1983), and in the long term, provision of safe water supplies (El Kholy *et al.*, 1989), with continuing integrated health-care education (Kloos, 1989) to limit high-risk exposure. Repeated drug therapy reduced the prevalence of severe morbidity in longitudinal studies. However, in some cases, particularly with *S. japonicum*, suspension of control measures runs the risk of rapid reemergence of infection prevalence and increased risk of hepatic morbidity (Cioli *et al.*, 1995).

Sustainability is an essential feature of any planned schistosomiasis control program. This may be difficult to achieve without assistance from developed nations owing to the relatively high per capita cost of medication and sometimes the low priority assigned to control of a disease perceived as minimally lethal. Nevertheless, schistosomiasis has a significant impact on chronic morbidity, including malnutrition, anemia, retardation of growth and development, loss of exercise or work capacity, and either urinary tract or intestinal and hepatic disease (Parraga *et al.*, 1996). Recent social science and health-care

delivery research is focusing on developing more effective ways to raise awareness of schistosomiasis as a significant health problem and on developing means to incorporate schistosomiasis control into primary health-care delivery.

Prevention and control essentially need to be targeted on the weakest link in the transmission cycle with the objective of controlling schistosomiasis most cost effectively and permanently, which will require the integration of prevention and control interventions in intersectoral programs. These include: safe water supplies and sanitary facilities, chemotherapy, health education, and snail control through plant or synthetic molluscicides and environmental modification. Most Pilot control programs in Ethiopia have been restricted to a single intervention, mostly the use of *endod*, which could not be sustained in the absence of strong community participation, the production of a secure supply of high-potency *endod* (*Phytolacca dodecandra*) berries, and their systematic application and monitoring of molluscicidal effects.

1.10. Treatment

Praziquantel is currently the treatment of choice for all forms of schistosomiasis. Praziquantel is a well-tolerated, broad-spectrum oral anthelmintic agent that is given as a single dose of 40 mg/kg for treatment of *S. haematobium*, *S. mansoni*, and *S. intercalatum* infections, and as two separate 30 mg/kg doses (spaced at least 3 hours apart) for *S. japonicum* and *S. mekongi* infections (King, 1989). Cure rates in field studies have typically been equal to or greater than 85% and those with infection still remaining typically have their infection intensity reduced by more than 99%, as measured by reduction in parasite egg counts in stool or urine samples (Olds *et al.*, 1999).

Some side effects are directly drug-induced (vomiting, dysphoria, abdominal pain), while others are proportionate to the intensity of the parasite load and appear to be related to the host immune response to the dying parasites (abdominal pain, urticaria, diarrhea). Among younger patients, completion of therapy is associated with reductions in egg output, as well as reduced blood loss, and regression of granulomatous inflammation in the liver (*S.*

mansoni and *S. japonicum* infection) or bladder (*S. haematobium* infection) (King *et al.*, 1991). These latter effects can result in reduction of portal pressure and reversal of hydronephrosis.

In older people with more advanced fibrotic tissue injury, *Schistosoma*-associated lesions may not be able to be reversed. Likewise, in patients with the late findings of esophageal varices or cor pulmonale, therapy may or may not improve their hemodynamic values. Symptomatic “ectopic” infection, as in the CNS, may initially worsen with therapy because it provokes a strong local inflammatory response. Coinfection with human immunodeficiency virus (HIV)-1 does not alter the efficacy of praziquantel therapy for schistosomiasis (King *et al.*, 1991).

1.11. Justification of the Study

Several studies have been carried out in different epidemiological settings that showed poor diagnostic sensitivity upon examination of a single Kato-Katz thick smear due to the high intra- and inter-specimen variations in egg counts. These studies have shown that single K–K thick smear preparation is a poor predictor of the ‘true’ prevalence of *S. mansoni* infections and hence a poor test in assessing the ‘real’ infection status of individuals, particularly when infection intensities are low (Engels *et al.*, 1996, 1997; Utzinger *et al.*, 2001).

Due to important intra-specimen and inter-specimen variation, a parasitologic diagnosis made by a single stool examination is often not an accurate reflection of the real individual infection status and can also considerably bias parameters at the group level. Fecal egg counts as a quantitative measure of infection on *S. mansoni* in epidemiologic studies are the result of inter- and intra-individual variations: the former reflecting egg counts varying from individual to individual caused by differences in worm loads, and the latter reflecting day-to-day egg count fluctuation for an individual with a given worm load and/or variations within a fecal specimen (Engels *et al.*, 1996).

There is evidence that fecal egg counts of *S. mansoni* vary considerably from day to day, which results in poor sensitivity of single stool readings. Intra-specimen variation of *S. mansoni* egg counts may also be considerable, but has previously been considered as the less important component (Utzinger *et al.*, 2001). However, recent studies have revealed that intra-specimen variations appear to have more impact in the assessment of infection status of individuals (Berhe *et al.*, 2004).

Therefore, in this research, we have tried to compare the diagnostic efficiency of repeating smears on the same stool sample or on stools collected on consecutive days, and also to quantify the relative contribution of these two sources of variation among individuals from Kemise town, Workie Mado village, which is highly endemic for *S. mansoni*. The resulting information will be appropriate for strengthening the evidence base of important intra- and inter-specimen variations in egg counts that must be taken into account in the assessment of infection status of individuals and it assists in designing control methods.

2. Objectives of the Study

2.1. General objective:

- To compare the diagnostic efficiency of repeating smears from the same stool sample or from stools collected on consecutive days, and to assess the prevalence and intensity of *Schistosoma mansoni* infection in the study area.

2.2. Specific objectives:

- To assess the relative importance of intra- and inter-specimen fecal egg count variations and recommend cost-effective sampling strategy.

- To evaluate the consequences of the variation for the interpretation of individual and population-based results of fecal screening.

- To investigate the prevalence of other helminthes in the study area.

3. Materials and Methods

3.1. The Study Area

The study was conducted in 'Workie Mado' village, a well known endemic area for *Schistosoma mansoni*. The village is found in Kemise town which is 325 kilometers away from Addis Ababa. The town is the administrative center of the Oromia Zone of the Amhara Region which is a vibrant business center located in North-Eastern part of Ethiopia (10°43'N and 39°30'E) with an elevation of 1424 meters above sea level. The village has got its name from its river called 'Workie River'.

3.2. The Study population

A total of 125 people, with five different age categories (< 10, 11-20, 21-30, 31-40, >40 years of age) and sexes (Yu *et al*, 1998; Berhe *et al*, 2004), randomly selected from Workie Mado village residents' list, were asked to produce fresh stool specimens in the morning. Out of these, 115 people showed up in the health center and only 107 people delivered adequate amount of stool required for the study. Workie Mado village had around 155 households with a population of 657 people. Kemise town had 12,000 population, the majority being Muslims, while the minorities are Christians of different denomination.

3.3. Sample size and sampling technique

3.3.1. Sample size

In this study, sampling unit was residents of Workie Mado village with a total population of 657. In estimating the sample size, prevalence rate of 92% *S. mansoni* infection was assumed as the area is known for its high endemicity for *S. mansoni*. The minimum number of the sample size (n) was determined using the statistical formula of sample size calculation (Daniel, 1995) : -

$$n = z^2 p(1-p)/d^2$$

Where n = The number of the sample size

z = 1.96 at 95% confidence interval

d= Margin of error assumed to be (0.05)

p= Expected prevalence rate of *S. mansoni* infection (92%)

By adding 10% for non-response, a total of 125 subjects were included. However, 18 participants were excluded because of inadequate stool to prepare the required 5 K-K thick smears and a total of 107 subjects were enrolled in the study.

3.3.2. Sampling technique

To select the study subjects, the participants were first stratified according to age. Allocation of subjects was done proportional to the number of age groups. Finally, the study subjects were selected using systematic random sampling by using village resident's list, from earlier studies, as the sampling frame.

3.4. Collection of Stool Samples and Process

3.4.1. Collection of Stool Samples

From each stool specimen, five 41.7mg-thick smears (5 K-K slides) were prepared, according to a modified Kato-Katz method (Peters *et al.*, 1980). Each slide was then labeled A-E and examined for Schistosomiasis and other helminth infections. Schistosome eggs were counted to measure the intra-specimen variation and other helminth infections were examined only for their presence or absence as the focus of this study was on fecal egg count variation in *S. mansoni* infection.

Five slides for each individual's stool specimen were prepared and examined on the first day. And all smear-negative individuals, who had no *S. mansoni* parasite on any of the 5

slides, were selected for further investigation and were asked to provide stool specimens for two consecutive days. This time we prepared only three slides for each day's stool specimen. A sum total of 601 Kato-Katz thick smears (535 on 1st day, 33 on 2nd and 33 on 3rd day) were examined and *Schistosoma mansoni* eggs were counted. For children, consent was obtained from their parents or guardians.

For the 107 participants we took 5 Kato-Katz slides from each individual's stool sample (intra-specimen variation). Out of which 17 individuals were found negative on the first day. From those negative people 11 volunteers showed up on 2nd and 3rd day examination. Therefore, those eleven individuals were used for the analysis of day-to-day (inter-specimen) variation. All individuals who didn't provide sufficient stool for preparing five K-K thick smears were excluded.

3.4.2. Examination of stool specimens

All slides were prepared by an experienced assistant lab-technician and all slides were read by two well experienced laboratory technicians from the Institute of Patho-Biology and all readings were completed inside the Health Centre. To maintain quality control part of the slides were re-examined in the laboratory of the Institute of Patho-Biology, Addis Ababa.

A small amount of fecal material was placed on plastic sheet and a piece of nylon screen was pressed on top so that some of the feces sieved through the screen and accumulated on top. A flat-sided spatula was scraped across the upper surface of the screen to collect the sieved feces. A template was placed on the slide and the sieved feces were added with the spatula so that the hole in the template was completely filled. The spatula was passed over the filled template to remove excess feces from the edge of the hole. The template was removed carefully so that a cylinder of feces was left on the slide. The fecal material was covered with a pre-soaked cellophane strip. The slide was inverted and the fecal sample was pressed firmly against the hydrophilic cellophane strip to spread evenly. The

slide was then placed on the bench with cellophane upwards to enable the evaporation of water while glycerol cleared the feces.

Classification in egg output categories was done on the basis of the total egg output detected in all examined slides of that individual, and output was converted into eggs per gram (epg) according to the amount of stool examined. Intensities of *S. mansoni* egg excretions were categorized as light infections (1-100epg), moderate infections (101-400epg), and heavy infections (> 400epg) (WHO, 2002).

3.5. Data Analysis

Statistical analysis was performed with SPSS software version 15. In order to analysis intensity of infection for STH parasites, the number of eggs were converted into the number of eggs per gram of stool and transformed to log scales for analysis of geometric mean. Analysis of Variance (ANOVA) was used to verify possible variations in egg counts of different age groups of people. Values were considered to be statistically significant when the p-value obtained was less than 0.05.

3.6. Ethical Clearance

The study was reviewed and approved by the Ethical Review Committee of the Biology Department, Addis Ababa University. Ethical considerations were addressed by treating positive individuals for intestinal parasites using the standard drugs. Written consent was obtained from participants and parents or guardians of the selected children.

4. Results

A total of 125 people with different age categories from Workie Mado village, Kemise town (Northeast Ethiopia), were asked to produce fresh stool specimens in the morning. Out of which, 107 individuals showed up in Kemise Health Center (KHC) and delivered the required amount of stool specimen (Table 1). Five Kato-Katz (K-K) thick smears for each individual's stool sample, a total of 535 slides on first day examination, were prepared and examined by two professional laboratory technicians. From these participants, 90 people were found positive for *S. mansoni* infection in this first day examination and 17 individuals were negative.

Table 1: Age and sex distribution of the study population, Workie Mado, Northeast Ethiopia (May 2010)

Age group (years)	Male	Female	Total
<10yrs	6	4	10
11-20	17	9	26
21-30	11	14	25
31-40	11	15	26
>40yrs	12	8	20
Total	57	50	107

All negative individuals were asked to provide another stool specimen for a second and third day. From this subgroup of people eleven individuals showed up in the health center and provided adequate stool specimen on the second and third day examination. Out of these first-day-negatives, one individual was found positive on the 2nd day and another individual on the 3rd day, making the total number of positive cases in the study sample to be 92 (86%). Tables in the following subsections clarified these figures, and would show the intra- and inter-specimen fecal egg count variations in diagnosing *Schistosoma mansoni* infections by the Kato-Katz thick smear method. The prevalence and intensity of infection in the study village were also estimated, and the relative sensitivity of the K-K method assessed.

4.1. Prevalence and intensity of *S. mansoni* among different age groups and sexes.

The prevalence of *Schistosoma mansoni* infection among 107 community members of Workie Mado was 86% (84% in females and 88% in males) (table 2). The mean intensity of *Schistosoma mansoni* infection was 66 epg (61 for females and 71 for males). Egg load ranging from 24 to 600 per gram of stool was observed. Out of the infected subjects 53% had light infections (<100 epg), 42% had moderate infections (101–400 epg) and the remaining 5% had heavy infections (>400 epg).

Intensity of infection was highest in the age group less than 10 years of age, followed by 11-20 years and >40 years. Among the under 10 children, males (346epg) were highly infected than females (86epg). This might be because; boys are allowed greater freedom than girls, particularly in Muslim communities like Workie Mado village.

Table 2: Prevalence and intensity of intestinal schistosomiasis among 107 community members of Workie Mado, Northeast Ethiopia (May 2010).

Age group (years)	Intensity of infection (epg)			No. (%) infected		
	Male	Female	Total	Male	Female	Total
<10yrs Intensity: mean(epg)	346	86	199	6(100)	4(100)	10(100)
11-20	86	105	91	13(76)	7(78)	20(77)
21-30	55	39	46	11(100)	11(79)	22(88)
31-40	32	62	48	9(82)	14(93)	23(89)
>40yrs	59	61	60	11(92)	6(75)	17(85)
Overall	71	61	66	50(88)	42(84)	92(86)

4.1.1. Prevalence of schistosomiasis among different age groups

Among all age groups, the prevalence of schistosomiasis was more than 75% (table 3). The majority (82.3%) of the study group were lightly and moderately infected, 45.8% and 36.5%, respectively. In fact, all children (under 10 years old) were examined positive for schistosomiasis, the majority (90%) being moderately and heavily infected, 70% and 20%, respectively. All heavily infected people were under 20 years and no heavy infection found in individuals above 20 years of age. The majority of people, greater than 20 years, were lightly infected. This might be because of avoidance of water contact behavior of older people.

Table 3: Categorization of intensity of infection with *S. mansoni* by different age groups, Workie Mado, Northeast Ethiopia (May 2010).

Age group (years)	Infection status			
	Light n(%)	Moderate n(%)	Heavy n(%)	Total positive n(%)
<10 (n=10)	1 (10.0)	7(70.0)	2 (20.0)	10 (100)
11-20 (n=26)	9 (34.6)	9 (34.6)	2 (7.7)	20 (76.9)
21-30 (n=25)	13 (52.0)	9 (36.0)	0 (0)	22 (88.0)
31-40 (n=26)	17(65.4)	6 (23.1)	0 (0)	23 (88.5)
>40 (n=20)	9 (45.0)	8 (40.0)	0 (0)	17 (85.0)
Total(n=107)	49(45.8)	39 (36.5)	4 (3.7)	92 (85.9)

N.B: Light=1-100epg, Moderate=101-400epg, Heavy=>400epg

4.1.2. Prevalence of schistosomiasis among different sexes

Disease prevalence was higher on males than females even though there is no significant difference between the two. Table 4 shows that, 87.7% (50 of 57) of males were infected with schistosomiasis where as, among females we found 84 % (42 of 50) to be infected. And cumulative prevalence in the study population was 85.9% after three days count. However, as indicated above, schistosomiasis prevalence in the study population was

84.1% on first-day-count. However, there was no significant difference in disease prevalence of both sexes.

Table 4: Categorization of intensity of infection with *S. mansoni* by different sexes, Workie Mado, Northeast Ethiopia (May 2010).

Infection status	Number (%)		
	Male (n=57)	Female (n=50)	Total (n=107)
Light	24 (42.1)	25 (50.0)	49 (45.8)
Moderate	23 (40.4)	16 (32.0)	39 (36.4)
Heavy	3 (5.3)	1 (2.0)	4 (3.7)
Total	50 (87.7)	42 (84.0)	92 (85.9)

4.2. Prevalence of intestinal parasites other than *S. mansoni*

Based on parasitological examination of stool specimens by the Kato-Katz thick smear method, different types of intestinal parasites other than *Schistosoma mansoni* were identified on the field (Table 5). In the present study eight different species of parasites were identified from the village. Relatively the highest prevalence of infection was due to *S. mansoni* (85.9%) followed by *Hookworm spp.* (17%). The prevalence of infection for one or more of intestinal helminthic parasite was 88.8%, while infection due to *S. mansoni* was 85.9%. The study has shown that the prevalence of *Ascaris lumbricoides* was 1%, *Trichuris trichiura* was 3%, *Hymenolepis nana* was 1%, *Enterobius vermicularis* was 5%, *Taenia saginata* was 4% and that of *Strongyloides spp.* was 2%.

Table 5: Intestinal helminth parasites other than *Schistosoma mansoni* detected in Workie Mado village in Kemise town, 2010.

Intestinal parasites	No. Observed (%) (N=107)
<i>Hookworm spp.</i>	18 (16.8)
<i>Ascaris spp.</i>	1 (0.9)
<i>Trichuris trichiura</i>	3 (2.8)
<i>Hymenolepis nana</i>	1 (0.9)
<i>Enterobious vermicularis</i>	5 (4.7)
<i>Taenia saginata</i>	4 (3.7)
<i>Trichostrongylus spp.</i>	2 (1.9)

Single parasite infection had the highest prevalence followed by double and triple. Overall co-infection was detected in 21% of the study subjects. Among the double parasitic infection, *S. mansoni* and *Hookworm* species comprised the highest proportion. *Ascaris* was found only on one moderately (274epg) infected 10 year old boy. Likewise, *Hamenolepis nana* was investigated only on one heavily (461epg) infected 10 year old boy. However, *Hookworm* species was found on 18 people majority being male adults with *S. mansoni* co-infection.

4.3. Intra-specimen variation

Table 6 shows prevalence and intensity of *S. mansoni* infections stratified by five different age groups. Overall geometric mean *S. mansoni* egg output per gram of stool among 107 study subjects was 66.4epg. Except for subjects in the age groups <10 and 11–20 years, who had highest intensity of *S. mansoni* infection (198.5epg and 91.3epg, respectively), the prevalence estimates using single K–K thick smear preparations were markedly lower than estimates based on triplet or quintet K–K thick smears (Table 6).

Table 6: Individuals in different age categories positive for eggs of *S. mansoni* detected in a single, duplicate, triplet, quadruplet or quintet K–K thick smears derived from a single stool specimen among 107 community members of Workie Mado, Northeast Ethiopia (May 2010).

Age group (years)	Intensity of infection (epg) ^a	K-K thick smear number				
		1 n(%)	2 n(%)	3 n(%)	4 n(%)	5 n(%)
<10 (n=10)	199	10(100)	10(100)	10(100)	10(100)	10(100)
11-20 (n=26)	91	15(57.7)	15(57.7)	16(61.5)	18(69.2)	19(73.1)
21-30 (n=25)	46	13(52.0)	18(72.0)	20(80.0)	21(84.0)	22(88.0)
31-40 (n=26)	48	16(61.5)	17(65.4)	19(73.1)	21(80.8)	22(84.6)
>40 (n=20)	60	13(65.0)	15(75.0)	16(80.0)	16(80.0)	17(85.0)
Overall(n=107)	66	67(62.6)	75(70.1)	81(75.7)	86(80.4)	90(84.1)

^a Based on geometric mean egg load per gram of stool.

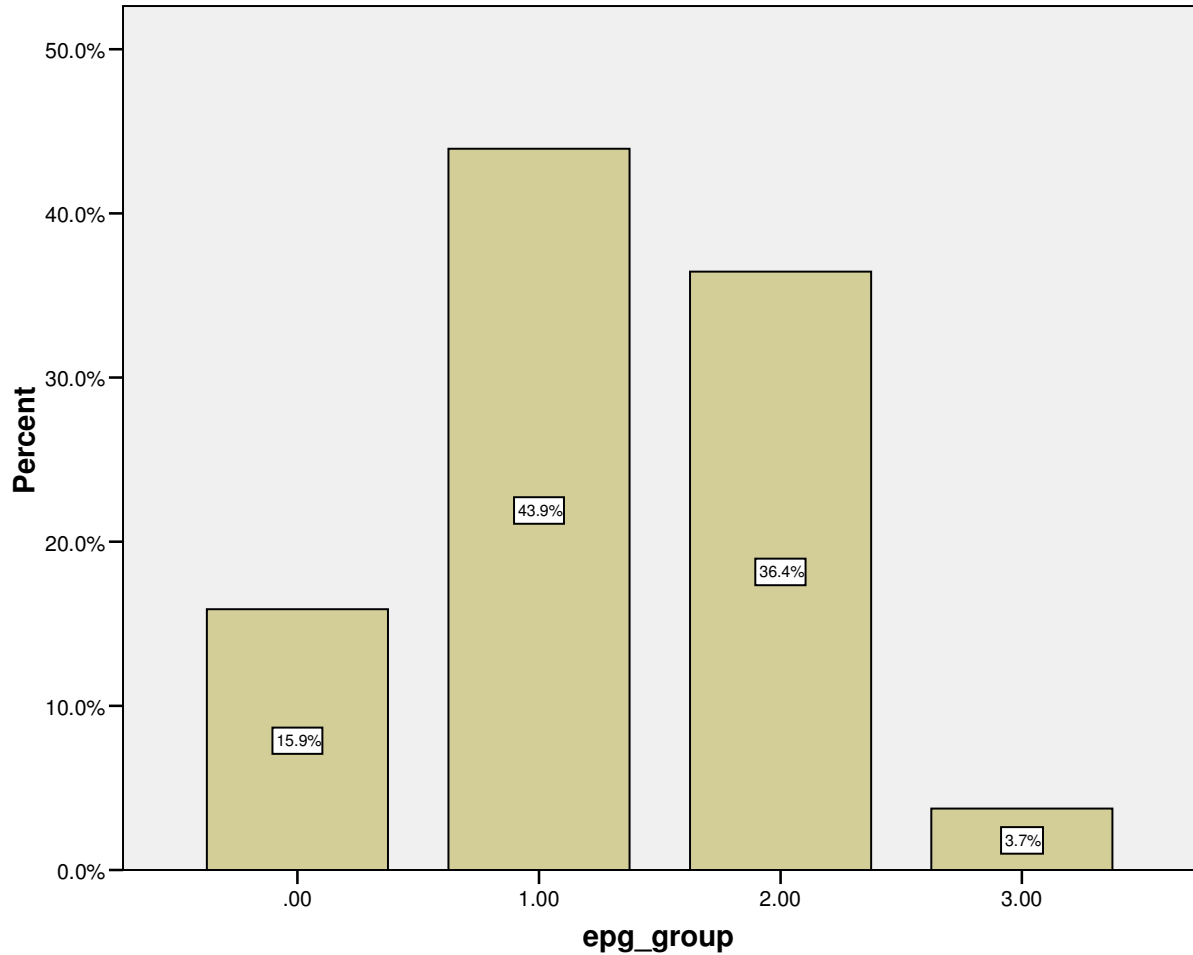
Overall percentage of positives missed when using only one, only two, and only three K-K thick smears was 25.6%, 16.7%, and 10%, respectively (Table 7). The use of a single K-K thick smear, from a single stool specimen of children under 10 years of age, however, didn't miss any positive individuals identified by quintet measurement. This is because this group of participants has been identified to be the highest excretors of schistosoma parasite eggs which made detection very simple with only a single K-K thick smear. All children under this age group were positive, 90% of them being 6-10 years old.

Table 7 also indicates that the percentage of positives missed with single slide preparation was also lower among age categories of <10 and 11-20 years (0% and 21.1%, respectively), who had highest intensity of infection, than individuals above 20 years. In this later group of people, the percentage of positives missed has markedly lowered when using double or triple K-K thick smears compared to using only one K-K.

Table 7: Egg positivity missed, in different age categories, with single, double or triple slides compared to quintet measurement, Workie Mado, Northeast Ethiopia (May 2010).

Age group (years)	Number(%) Positive (quintet KK)	Positives missed		
		Only one K-K n(%)	Only two K-K n(%)	Only three K-K n(%)
<10 (n=10)	10(100.0)	0(0)	0(0)	0(0)
11-20(n=26)	19(73.1)	4(21.1)	4(21.1)	3(15.8)
21-30(n=25)	22(88.0)	9(40.9)	4(18.2)	2(9.1)
31-40(n=26)	22(84.6)	6(27.3)	5(22.7)	3(13.6)
>40 (n=20)	17(85.0)	4(23.5)	2(11.8)	1(5.9)
Overall(107)	90(84.1)	23(25.6)	15(16.7)	9(10.0)

Out of all participants examined on the first day, the vast majority were under the category of light infection (1-100epg) and moderate infection (101-400epg). The percentage of people with light, moderate, and heavy infection were 43.9%, 36.4% and 3.7%, respectively, while the prevalence of negative individuals in the study area was 15.9% (Figure 4).



0=negative, 1=Light infection, 2=Moderate infection, 3=Heavy infection

Figure 4: Proportion of individuals with different infection status detected with quintet K-K thick smears, Workie Mado, Northeast Ethiopia (May 2010).

The sensitivity of a single K-K thick smear examination to detect *S. mansoni* infection was higher among heavy than among light or moderately infected individuals. Point infection, obtained by the examination of a single Kato-Katz slide varied strongly, especially for light infections. Table 8 indicates that, only 53.2% of the lightly infected individuals, who were identified by quintet measurement, were identified in the first single K-K thick smear. This figure was 97% and 100%, respectively, for the moderate and heavy categories of infection.

Table 8: Relative sensitivity of detecting *Schistosoma mansoni* infection compared to quintet K-K thick smears, Workie Mado, Northeast Ethiopia (May 2010).

Slide	Cumulative Number (%) of positives			
	Light	Moderate	Heavy	Total
1	25(53.2)	38(97)	4(100)	67(74.4)
2	32(68.1)	39(100)	4(100)	75(83.3)
3	38(80.9)	39(100)	4(100)	81(90)
4	43(91.5)	39(100)	4(100)	86(95.6)
5	47(100)	39(100)	4(100)	90(100)

In a series of 107 subjects, single, duplicate, triplet, and quadruplet K–K thick smears picked 74.4%, 83.3%, 90%, and 95.6%, respectively, of *S. mansoni* positives that were detected by quintet K–K thick smear preparations (Table 8). These figures would be comparable to the lightly infected 47 subjects, whose same series of slides picked 53.2%, 68.1%, 80.9, and 91.5%, respectively.

The table above shows that, with every single addition of slides, the cumulative prevalence of infection in the study area also increases, especially with the lightly infected group increasing more sharply. However, the moderately and heavily infected groups show, more or less, an overlapping line graph which shows the percentage of detection with a single and multiple slides within this groups is almost closer to 100%.

Table 9 has indicated that the percentage of individuals with presence of eggs of *S. mansoni* detected in a single, duplicate, triplet, quadruplet and quintet K–K thick smears derived from a single stool specimen among 107 community members of Workie Mado, Northeast Ethiopia was 62.6%,70.1%,75.7%, 80.4%, and 84.1% respectively.

Compared to quintet K–K thick smears, single K–K thick smear missed 47%, 3% and 0% of subjects with light, moderate and heavy *S. mansoni* egg excretions, respectively (Table 9). In this study, double and triple K-K thick smears did not miss any moderate and heavy infections, but missed 32% and 19% of subjects with light infection.

Table 9: Comparisons of prevalence and percentage of positives missed based on single, double, triplet, quadruplet and quintet K–K thick smear preparations of 107 study subjects in Worke Mado, Northeast Ethiopia (May 2010).

Study Variable	K–K thick smear number				
	1 n(%)	2 n(%)	3 n(%)	4 n(%)	5 n(%)
Overall prevalence	67(62.6)	75(70.1)	81(75.7)	86(80.4)	90(84.1)
Prevalence of light infection ^a	25(23.4)	32(29.9)	38(35.5)	43(40.2)	47(43.9)
Proportion of subjects with light infection missed by 1 to 4 smears compared to quintet smears	22(46.8)	15(31.9)	9(19.1)	4(8.5)	-
Prevalence of moderate infection ^a	38(35.5)	39(36.4)	39(36.4)	39(36.4)	39(36.4)
Proportion of subjects with moderate infection missed by 1 to 4 smears compared to quintet smears	1(2.6)	0.0	0.0	0.0	-
Prevalence of heavy infection ^a	4(3.7)	4(3.7)	4(3.7)	4(3.7)	4(3.7)
Proportion of subjects with heavy infection missed by 1 to 4 smears compared to quintet smears	0.0	0.0	0.0	0.0	-

^a Based on arithmetic mean egg load per gram of stool.

Table 10 shows that, among all *S. mansoni* positive individuals 75.8% (341 of 450) of their slides were positive. This figure was 54.5% (128 of 235) for the lightly infected, whereas, it was 99% (193 of 195) and 100% (20/20) for the moderate and heavy infections, respectively. In other words, among the 450 slides examined from 90 positive people 24.2% (109 of 450 slides) were missed. This figure was 45.5% among the lightly infected, 1% among the moderately infected, and 0% among the heavily infected people.

Table 10: Relative sensitivity of the Kato-Katz method in detecting *S. mansoni* eggs in individuals with different infection status, Workie Mado, Northeast Ethiopia (May 2010).

Slides	Infection status			
	Light(n=47)	Moderate(n=39)	Heavy(n=4)	Total(n=90)
Number Examined	235	195	20	450
No. Negative (% missed)	107 (45.5)	2 (1)	0 (0)	109 (24.2)
No. Positive (% picked)	128 (54.5)	193 (99)	20 (100)	341 (75.8)

A one-way ANOVA comparing the mean intensity of infection of different age groups was computed. A significant difference was found among the age groups ($p < .01$). LSD was used to determine the nature of the differences between the age groups. This analysis revealed that children under 10 years and young people 11-20 years had a higher mean intensity of infection than the other age groups, and there was no significant difference between the mean egg outputs of age groups 21-30, 31-40 and > 40 years of age. And also, the mean egg outputs between age groups < 10 years and 11-20 years were not significantly different with each other.

Variations in *S. mansoni* egg counts per K–K thick smear were more marked in the age group with highest intensity of egg excretion (Figure 5). Variations in *S. mansoni* egg counts per K–K thick smear were also more marked in the heavily infected group (Figure 6).

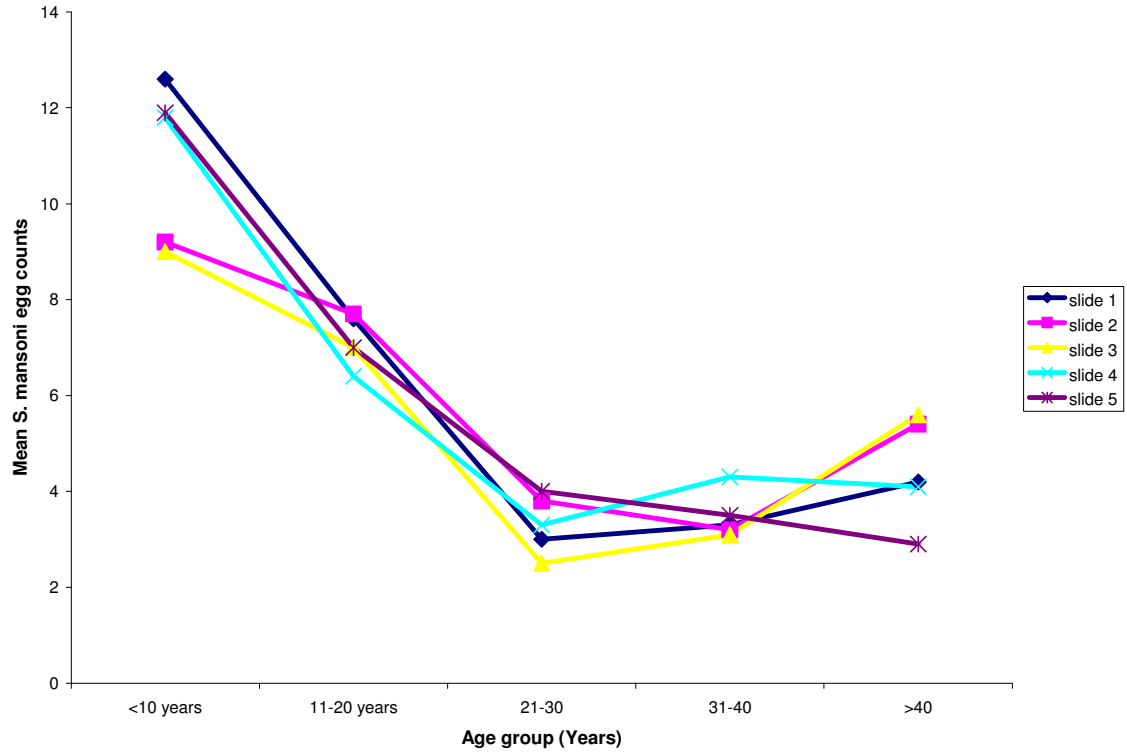


Figure 5: Mean *S. mansoni* egg counts/slide of different age categories in five successive Kato-Katz slides, Workie Mado, Northeast Ethiopia (May 2010).

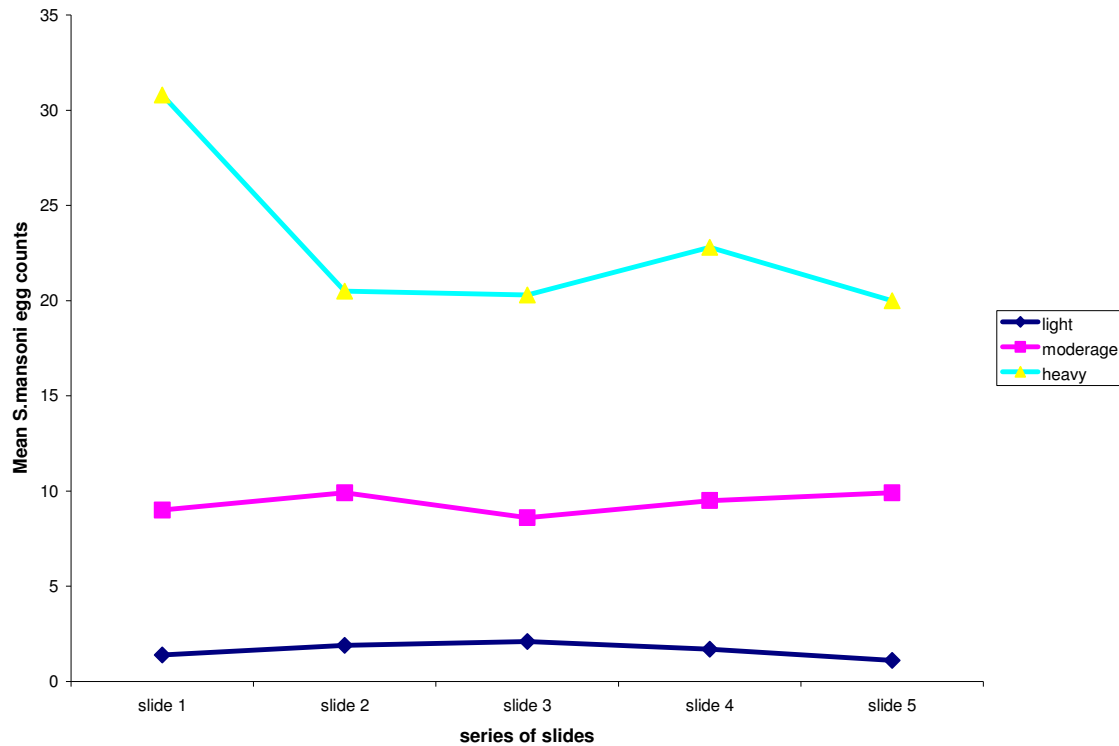


Figure 6: Mean *S. mansoni* egg counts/slide in different infection categories of people, Workie Mado, Northeast Ethiopia (May 2010).

4.4. Inter-specimen variation

Out of the total 92 positive individuals investigated within 3 days, 67 individuals were already identified in the first day single slide (table 11). The first slide (41.7mg Kato-Katz slide) detected 73% (67 of 92 positives) of positive individuals. The second slide (83.4mg) detected 8 more individuals, making the percentage of positives grow to 82% (75 of 92); the third slide (125.1mg) detected 6 other individuals, making the percentage of positives 88% (81 of 92). And the fourth and fifth slides (166.8mg, 208.5mg, respectively) identified 5 and 4 other persons making the percentage of positives grow to 93.5% and 98%. On the second day, one more individual was identified positive. Therefore, 99% of positive individuals were identified on the 2nd day. Lastly, on the third day, we identified one new person as positive (Table 11).

Table 11: Relative sensitivity of detecting *Schistosoma mansoni* infection as compared to three-days-measurement, Workie Mado, Northeast Ethiopia (May 2010).

Variable	Cumulative number of positives detected						
	Day 1					Day 2	Day 3
	Slide 1	Slide 2	Slide 3	Slide 4	Slide 5	(three smears)	(three smears)
No. positive	67	75	81	86	90	91	92
Prevalence (%)	63	70	76	80	84	85	86
Overall sensitivity (%)	73	82	88	94	98	99	100

The above table has shown that a single Kato-Katz smear had a detection rate of 73% and after examining three smears, it reached 88%. Compared to the three days' measurement, detection rate of the Kato Katz method reached 98% on the fifth K-K thick smear. The study shows that, from all sample cases, 84% were positive on the first day and 86% were found positive on total of 3 days, which may further increase day by day (Table 11).

In a subset of 26 subjects whose initial three K–K thick smears had no demonstrable *S. mansoni* eggs, 9 people (34.6%) were positive (geometric mean intensity 8.5epg) after examination of two additional K–K thick smears from the first day sample (Table 12). Further examination of additional three K–K thick smears, from stool samples collected on two consecutive days, picked 2 (18%) positives of the 11 volunteers whose initial quintet K-K thick smears show a negative result. One of these new positives was picked on the second day and, the other, on the third day.

Table 12: Comparison of three-day-measurement with quintet K-K thick smears in detecting new *S. mansoni* positives, Workie Mado, Northeast Ethiopia (May 2010).

Variable	No. Examined	No. positive	% detected
<u>5KK negatives</u> + <u>3KK</u> + <u>3KK</u> (3-days-measurement)	11	2	18
<u>3KK negatives</u> + <u>2KK</u> (quintet K-K)	26	9	34.6

5. Discussion

S. mansoni diagnosis has been the subject of some scrutiny in recent years, with particular emphasis placed on the difficulties faced by health workers when trying to estimate prevalence and mean infection intensities as a prelude to intervention (Utzinger et al, 2001). Different researchers have mentioned that these difficulties are primarily a result of the extreme levels of variation in fecal egg counts that are characteristic of schistosome infections.

The modified Kato-Katz method, because of its many practical advantages for examining large numbers of people at low cost within a short time, has been a standard method for the field diagnosis of *S. mansoni* infection. However, it has been debated that this technique has low diagnostic sensitivity due to intra- and inter-specimen fecal egg count variation. The relative contribution of these two sources of variation among 107 community members of Workie Mado village, northeast Ethiopia, which is known for its high endemicity in *S. mansoni* infection, was quantified.

The result of this study indicates that the prevalence of *S. mansoni* was not significantly different between both sexes, even though it shows a higher percentage of infected males (87%) than females (84%). This finding may contradict with the results of previous studies which (Lemma, 1969; Birrie *et al.*, 1994; Woldemichael and Kebede, 1996) suggested that a significantly higher prevalence is observed among males. Higher prevalence rates among males than females have also been reported from different parts of Ethiopia (Birrie *et al.*, 1998).

This variation in infection may be associated to division of work (not investigated in this study) in different communities. Contradictory to their findings, for example, the prevalence rate for *S. haematobium* among the Afar in the Awash Valley was reported to be twice as high among women as in men; because women collect aquatic plants in infected swamps on the Awash flood plain (Kloos *et al.*, 1978).

Among other intestinal parasites the prevalence of Hookworm infection (17%) was higher compared to other intestinal helminthes in the study area and, also the majority of co-infection with *Schistosoma mansoni* being this parasite. The high prevalence of this blood-sucking intestinal parasite together with the existing high *Schistosoma mansoni* infection would undoubtedly exacerbate the public health situation in the area, especially in association with anemia and diarrhea. Hookworm also causes apathy and malnutrition and, in children, underdevelopment.

In this study, a single K-K smear had a sensitivity of 72.8% and, sensitivity reached 88% after examination of three smears. After examining four smears the percentage was 93.5% and addition of one more slide (quintet measurement) identified 97.8% of all individuals detected within three days' measurement. This result is in agreement with the findings of Ebrahim and others (1997), who mentioned that a single Kato smear had a sensitivity of 70.8% in diagnosing Schistosomiasis. According to their findings, after examining three smears, sensitivity reached 88.5%, and it also increased with each additional slide to reach 91.7% on examining four smears.

Obviously, intense infections are easier to diagnose because higher worm loads will lead to higher densities of eggs in the stool. In this study, 97% of moderately infected and 100% of heavily infected people were detected in the first single slide. As expected, the sensitivity of a single slide (or a few) examinations to detect *S. mansoni* infection is higher among heavy than among light or moderately infected individuals. Therefore, additional stool examinations are more beneficial in the lightly infected group (1–100 epg) than in the moderately (101–400 epg) and intensely infected groups (>400 epg).

This study contradicts the findings previously described by other investigators (Woodstock et al., 1971) who mentioned that there is a homogeneous distribution of *S. mansoni* eggs in stools. According to the present study, this distribution, which can be considered homogeneous in the case of light infections, appears to become more and more heterogeneous as the intensity of infection increases. Such heterogeneity, suggesting clustering of eggs in stool (Yu et al, 1998), is likely to have profound

consequences in the quantitative diagnosis of *S. mansoni* by means of a stool examination. Such clustering makes it necessary to examine multiple slides from one specimen to get a reliable idea of the individual infection status.

In operational circumstances, with the main objective of controlling morbidity, a single stool measurement can still be very useful because most of the pathology is connected with heavy infections (WHO, 1985). However, if control or even eradication of *S. mansoni* infection is the objective, single or a few fecal egg count measurements will never suffice (Yu *et al.*, 1998).

The sensitivity of a qualitative diagnosis can be quite low, especially in lightly infected people. Indeed, despite the fact that the majority of subjects (86%) in our study group were infected, a considerable proportion of all examined slides of positive individuals were negative for *S. mansoni*, especially in this lightly infected group of people. In this study, 24.2% of all examined slides of positive individuals were negative. This percentage of missed positives is highly pronounced in lightly infected groups (45.5%). However, single slide examinations were able to detect most of the moderate infections and all of the heavily infected people. Since it is generally assumed that morbidity is related to intensity of infection, they may therefore be appropriate for interventions in which control of morbidity is the aim and only qualitative diagnosis is required.

The results of the present study, carried out in an area of north east Ethiopia, confirm that *S. mansoni* infections are highly endemic. It also supports the general assumption that the distribution of *S. mansoni* egg counts in a given population is over dispersed, with only a few individuals excreting the bulk of the eggs (Anderson & May, 1985). Out of all examined people, children under 10 years of age, especially 6-10 years old, has excreted a large amount of *S. mansoni* eggs (198.5 eggs per gram), followed by 11-20, and >40 years (91epg, 60epg, respectively).

The use of three K-K thick smears from a single stool specimen missed only 10% of positive individuals identified by quintet measurement. Percentage of positives missed

when using only one, only two, and only three K-K thick smears was 25.6%, 16.7%, and 10%, respectively. This shows that, preparation of three K-K thick smears on the first day is sufficient to diagnose patients in communities with high intensity of infection, and if these slides show a negative result it will be better to call the person for a second day examination.

The results of this study confirm previous reports regarding the underestimation of *S. mansoni* infection prevalence (De Vlas *et al.*, 1997; Utzinger *et al.*, 2000b) and emphasizes the need to examine stools taken on different days, when a definitive diagnosis of infection status is required and the first sample is negative. This recommendation is of particular importance for areas of low infection intensity (Engels *et al.*, 1996). It is likely that day-to-day variation will be more important in such areas, because the clustering of low numbers of eggs within stools (which causes intra-specimen variation) will have a less pronounced impact on egg count variation.

The addition of two slides on triple negative slides detected 35% positive cases, while the addition of two days of stool specimens detected only 18% positive cases. This suggested that the overall gain in identifying new positives by calling negative individuals for several days might not be that much important than examining multiple slides from a single stool. Therefore, the Kato-Katz method, five smears from a single stool specimen, would be particularly appropriate for large-scale surveys because of its simplicity, lower cost, and rapidity.

6. Conclusion

The present study showed that schistosomiasis is highly prevalent in the area (85.9%). High intensity of infection was observed among the age group <10 years old, followed by 11-20 years. Except for this group of individuals, who had the highest intensity of *S. mansoni* infection, the prevalence estimates using single K–K thick smear preparations were markedly lower than estimates based on triplet or quintet K–K thick smears, indicating that multiple slides are necessary in such lightly infected groups of people.

It can be concluded from the results of this study that single slide examinations are able to detect most moderate infections and all heavy infections and may therefore be acceptable for interventions aimed at the control of morbidity. Nevertheless, if the objective is transmission control or elimination of *S. mansoni*, examination of single stool specimens will never suffice.

This study also demonstrates that intra-specimen variation was more important and has noticeably biased operational parameters used to determine the infection status at group level. Point prevalence, obtained by the examination of a single, 41.7-mg Kato-Katz slide, varied strongly, especially for light and moderate infections. This has influenced the total prevalence to be greatly underestimated and therefore, the use of triplet or quintet measurement might provide better estimates of the total prevalence.

In our study, the majority (88%) of positive people (examined within three days) were already detected in the first triple K–K thick smears in diagnosing *S. mansoni* infection, and further thick smear preparations would only be required in the remaining percentage (12%) of people. However, quintet K-K thick smears identified 97.8% of positives examined within three days. Thus, in a health care setting, as single day visit is more cost effective than a two or three day clinic visit, quintet K-K thick smear reading is necessary to identify the lightly infected individuals easily, otherwise calling the person for another day or so. However, for cross-sectional morbidity studies, a quintet K–K thick smear

preparation from one stool sample is enough as it is logistically more feasible than examination for a further day stool sampling effort.

In conclusion, examination of quintet K–K thick smears from one stool specimen, and if these are negative, followed by examination of additional triplet K–K thick smears, from subsequent day stool specimens, can adequately assess individuals for infection prevalence with *S. mansoni*. Therefore, our conclusions may be valid in other areas of similar endemicity. Generally, the present investigation highlights the importance of schistosomiasis among the community living around Workie river and forms the basis for targeting control efforts through effective diagnostic method.

7. Recommendations

Since quintet examinations of one stool requires less time and labor than analyzing consecutive day stool samples, it may therefore be a reasonable compromise in high transmission areas, like in our case, to reduce the number of days of sampling whilst increasing the number of examinations of each stool sample, and further work using a similar intensity of sampling needs to be conducted in areas of low transmission.

We recommend that, in another study, the origin of variation in the stool and examining specimen from different section of the stool should be investigated. There is a need for more population-based studies that carefully figure out the disease situation in the population, and investigation of all possible contributions of risk factors.

Health education to decrease the frequency of water contact together with chemotherapy is essential in the area. In addition, the high prevalence of hookworm co-infection observed in the study area indicates that there is a need to investigate the real impact of schistosomiasis and hookworm co-infection in the overall health of the community.

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Name of the study participant ----- Age ----- Sex -----

Name of Physician ----- Site/Health center -----

I have been informed about a study that plans to investigate the “Implications of intra- and inter-specimen fecal egg count variation in diagnosing *Schistosoma mansoni* by the Kato-Katz method, in Workie Mado village, Kemise town”.

For this study I was requested to give a stool sample for *Schistosoma mansoni* identification. I was informed that I will get proper therapy if I found to be positive for any of intestinal parasites. The investigator has also briefed me that there would be no major risks associated with the sampling procedure. He also informed me that all laboratory results would be kept in secret. Moreover, I was clearly informed that I have a right to withdraw from participating in this study and in so doing there will be no impact on the overall management of my conditions. I was given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave informed consent and cooperate at my will in the course of the conduct of the study.

Name (participant) ----- Signature -----Date -----

Name (investigator) ----- Signature -----Date -----

Name (Witness) -----Signature -----Date -----

Appendix II: Individual egg counts in each Kato-Katz thick smear and other parasite eggs detected in eleven first-day-negative individuals, Workie Mado, Northeast Ethiopia (May 2010).

No.	Age	Sex	<i>S. mansoni</i> egg/slide on			Other Parasites
			Day 1	Day2	Day3	
1	30	F	0-0-0-0-0	0-0-0	0-0-0	-
2	18	F	0-0-0-0-0	0-0-0	1-0-0	Hw.
3	40	F	0-0-0-0-0	0-0-0	0-0-0	T.s.
4	40	M	0-0-0-0-0	0-0-0	0-0-0	E.v.
5	33	F	0-0-0-0-0	1-0-0	0-0-0	-
6	22	F	0-0-0-0-0	0-0-0	0-0-0	-
7	45	F	0-0-0-0-0	0-0-0	0-0-0	-
8	13	M	0-0-0-0-0	0-0-0	0-0-0	-
9	14	F	0-0-0-0-0	0-0-0	0-0-0	-
10	30	F	0-0-0-0-0	0-0-0	0-0-0	-
11	11	M	0-0-0-0-0	0-0-0	0-0-0	-

T.s: *T. saginata*, E.v.: *E.vermicularis*, Hw: Hookworm

Declaration

I, the undersigned declare that this thesis is my original work. It has not been presented for a degree in this or any university and all the source materials used for the thesis have been duly acknowledged.

Name of candidate: Manyawkal Bireda Essa

Signature: _____

Place: Addis Ababa

Date of submission: June, 2011

The work has been done under my supervision

Name: Nega Berhe (PhD.)

Signature: _____

Date: _____

