ADDIS ABABA UNIVERSITY

SCIENCE FACULTY

SCHOOL OF GRADUATE STUDIES

EPIDEMIOLOGY OF INTESTINAL SCHISTOSOMIASSIS IN HAYK TOWN, NORTHEAST ETHIOPIA

BY

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Acknowledgments

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Abstract

Background: The endemicity of intestinal and urinary schistosomiasis has long been established in Ethiopia, and new foci have also been continuously discovered.

Objective: The objective of this study was to determine the transmission and magnitude of schistosomiasis in Hayk area, Northeastern Ethiopia.

Methods: A cross sectional parasitological study involving 384 school children was undertaken to determine the prevalence of intestinal schistosomiasis between January and March 2010 in two primary schools in Hayk area, Northeastern Ethiopia. Stool samples from 384 school children were processed for microscopic examination using Kato-Katz technique. Malacological survey and observation on human water contact activities were also carried out. As a malacological survey snails were checked for schistosome infection by exposing lab-bred mice to the cercariae shed from *Biomphalaria pfeifferi en masse*.

Results: The prevalence and intensity of intestinal schistosomiasis among school children in Hayk Number 1 and Hayk Number 2 Primary Schools was found to be 45% and 161 epg, respectively. The prevalence of infection had relationship with age and gender. Males are more infected than females. Children in the age group 15-19 years had the highest infection rate, followed by 10-14 and 5-9 years age groups. Schistosome infection in *Biomphalaria pfeifferi* was 3.2%. Schistosome infection was also established in laboratory-bred mice and adult *Schistosoma mansoni* worms were harvested from exposed mice after 6 weeks of maintenance.

Conclusion: The observed intestinal schistosomiasis with prevalence of 45% among young children, collection of schistosome infected *Biomphalaria pfeifferi*, and the establishment of lab infection in mice showed that transmission of intestinal schistosomiasis is taking place in the area. Since the community represents a high risk community, schistosomiasis control should be immediately put in place.

List of Abbreviations and Acronyms

DALYs	Disability adjusted life years
epg	Egg per gram
Masl	Meters above sea level
P-value	Probability value
STHs	Soil transmitted helminths
WHO	World Health Organization

1. Introduction

Schistosomiasis is a chronic water- related parasitic disease caused by blood flukes of the genus *Schistosoma*. It is the most important disease in terms of its public health and socioeconomic impact next to malaria and is still a major helminth infection at the beginning of the 21st century in many developing countries of the tropics. The disease is endemic in 74 tropical developing countries (Chitsulo *et al*., 2000). People become infected when coming in contact with water containing schistosome-infected snails. There are five species of schistosomes that cause disease in humans, namely *S. mansoni, S. intercalatum, S. haematobium, S. japonicum and S. mekongi* (Gurarie and Seto, 2009). Among these, *S. mansoni*, *S. japonicum* and *S.haematobium* cause significant public health problems (McManus and Loukas, 2008). *S. mansoni*, *S.japonicum* and *S. intercalatum* cause intestinal schistosomiasis whereas *S. haematobium* is responsible for urinary schistosomiasis. These worms deposit eggs in blood vessels surrounding the gut or bladder of the infected hosts. There are also other minor non- human species that may also cause accidental infections, or cercarial dermatitis (bird-infecting schistosomes e.g. *Trichobilharzia* sp.). Other schistosomes of veterinary importance include *S. bovis, S. mathei*, *S. hippopotami*, *S. sprinallis* and *S. rohhaini* (Okpala *et al*., 2004).

In an attempt to measure the global burden of schistosomiasis, various estimates have been made. Recent systematic review integrating results of several independent studies by Steinmann *et al.* (2006) suggests that 779 million people are at risk of schistosomiasis and 207 million people are infected worldwide. According to Steinmann *et al.* (2006), earlier estimates of an annual loss of between 1·7 and 4·5 million DALYs due to schistosomiasis is underestimated and this figure could be many times greater in view of recent meta-analysis. Although, infection with schistosome parasite is mostly associated with morbidity, it also has considerable mortality effect resulting in death (King *et al.*, 2005). It is probably responsible for hundreds of thousands of deaths annually (Bergquist, 2001).

As many other tropical diseases, schistosomiasis is found much concentrated in Africa. It is endemic in 46 countries in the continent (Boelee and Madsen, 2006). In Africa again, the great majority (80-85%) of schistosomiasis is found in sub-Saharan Africa (Bergquist, 2002), where *S. haematobium, S. intercalatum* and *S. mansoni* are endemic. However, the two main causative species of schistosomiasis are *Schistosoma haematobium* and *Schistosoma mansoni*. In this sub-continent, approximately 393 million people are at risk of infection from *S. mansoni,* of which 54 million are infected. For *S. haematobium* an estimated number of 436 million at risk, of which 112 million are infected (van der Werf *et al*., 2003). However, this estimate is higher compared to the figure of the world mentioned above and this shows the inconsistency of various estimates. Overall, annual mortality rate might exceed 200,000 people in Africa, mainly due to bladder cancer or renal failure caused by urinary schistosomiasis, and liver fibrosis and portal hypertension caused by intestinal schistosomiasis (van der Werf *et al.*, 2003). However, much of the morbidity associated with infection can be reversed with the use of effective drug treatments (Keiser and Utzinger, 2008).

Schistosome infection during childhood causes substantial growth retardation and anemia (Olds *et al.*, 1996). Infected children may also have cognitive impairment and memory deficits (Nokes *et al.*, 1999; Savioli *et al*., 2004). According to Nokes *et al*. (1999), the cognitive impairment and memory deficits that may happen in infected children have been shown to impair their school performance. The disease is linked with anemia perhaps due to blood and nutrient loss in either the stool or urine. More serious and very common complications include bladder or ureter calcification in urinary schistosomiasis and an enlarged liver and spleen in intestinal schistosomiasis.

Prevalence and intensity are related and, generally, populations with high prevalence of infection tend to have high intensity (Hoffman *et al.,* 1979). Schistosomiasis mansoni morbidity (prevalence of hepatomegaly and splenomegaly) is directly correlated to the prevalence and intensity of infection and explains the morbidity variation between areas better than the prevalence of infection (Barreto and Loureiro, 1984).

Transmission potential of an area can be estimated through various techniques which focus on monitoring the components of the human-snail-water interface, where transmission occurs. These surveillance techniques include monitoring schistosome egg output by humans (Vercruysse *et al*., 2001), human water contact activities (Chandiwana and Woolhouse, 1991) and snail infection rates (Sturrock, 2001).

1.1. The Biology of Schistosomes and Snail Intermediate Hosts

1.1.1. The Schistosomes

Unlike many other trematodes, schistosomes exhibit sexual dimorphism and have distinct separate sexes. Moreover, their thread like appearance is not typical for flukes which are usually flat and leaf like. The more slender female is held permanently in a groove in the front of the male’s body. Male and female adult worms remain joined together for life. In intestinal schistosomiasis the worms attach themselves to the blood vessels that line the intestines; in urinary schistosomiasis, they live in the blood vessels of the bladder.

The basic life-cycle (Figure 1) of schistosomiasis is complex involving an alternation of generations with the sexual generation of adult schistosomes in the definitive vertebrate host and an asexual multiplicative stage in a molluscan host (Webbe, 1981). The life cycle begins when sexually pairing adult worms within the blood vessels of the host mate and excrete eggs in feces (or urine in the case of *S. haematobium*). Eggs are produced only by mated females. Upon contact with water the eggs hatch and free-swimming miracidium larvae released. The purpose of miracidium larvae is to find and penetrate an appropriate snail in which to develop. After a period of multiplicative asexual reproduction, numerous large tailed free-swimming larvae called cercariae leave the snail and swim in water where they actively seek an appropriate vertebrate host. When the definitive host and the cercaria converge in water medium, cercaria can penetrate the intact skin of the host and infect it. The cercariae utilize a proteolytic enzyme, produced in specialized glands in the head region, to penetrate the skin of humans or, in the case of *S. japonicum*, other mammals (buffaloes, pigs, dogs, etc.), which act as reservoirs for human transmission(Ross *et al*.,2001). This process sometimes causes itching, but most people do not notice it. The cercariae shed their bifurcated tails and transform their tegument into a form adaptive to a mammalian environment and this is called schistosomula. Once in the blood, the schistosomulae travel long journey in different organs of the definitive host through the blood circulation. They subsequently mature into adult worms in the liver. Paired adult worms migrate to mesenteric venules and mate to complete the cycle. The life span of the adult in definitive host is substantially longer (3-6years) (Brooker, 2007). The complicated life cycle is often difficult to break.

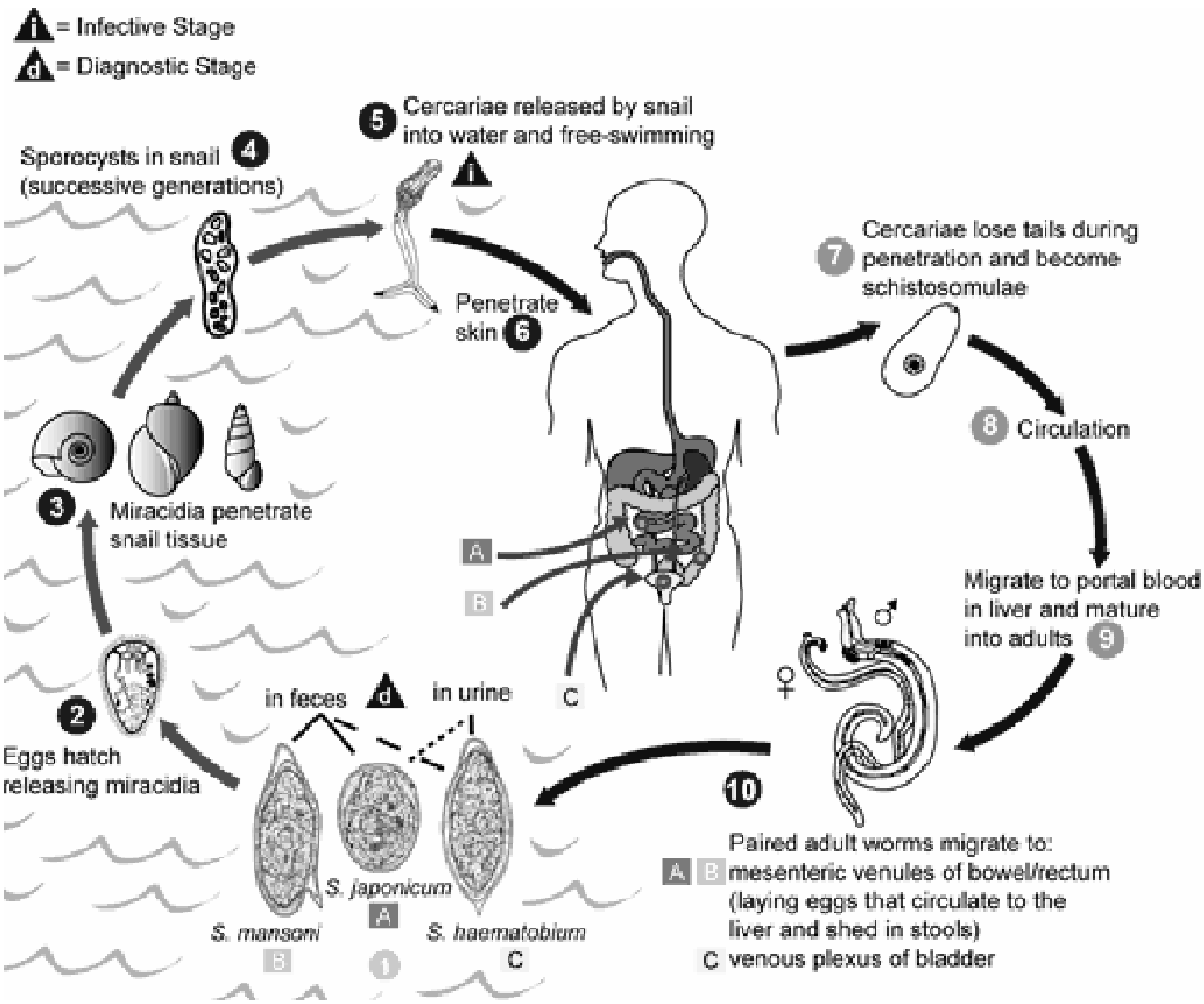

Figure 1. The life cycle of the three common human Schistosomes *(CDC. http: /dpd. Cdc. gov/ dpdx)*

Like other helminths, schistosomes do not directly reproduce within the human host but rather the female worm produces several eggs, which are either voided in the host's excreta or become trapped within tissues. While *S. mansoni, S. japonicum, S. mekongi,* or *S. intercalatum* excrete their eggs with feces, *S.haematobium* excretes its egg through urine. These species that infect man may produce egg that can fall in one of the following category of egg character. Egg with lateral spine (e.g. *S. mansoni*), egg with terminal spine (e.g. *S. heamatobium* and *S. intercalatum*) and egg that are round and minutely spined (e.g. *S.japonicum* and *S. mekongi*) (WHO, 1984).

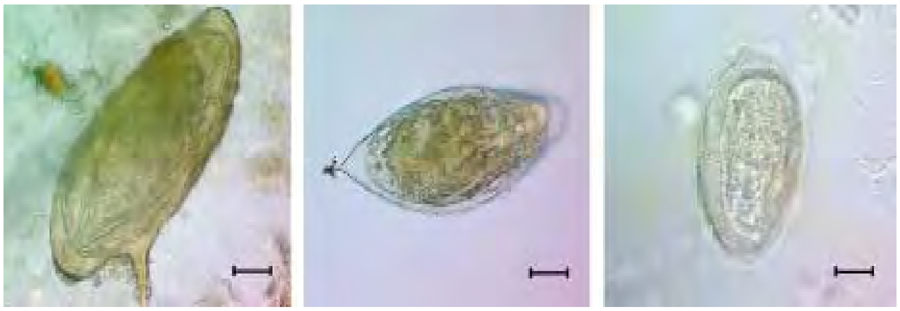

Figure 2. Schistosome eggs (eggs of *S. mansoni,* S. *haematobium and* S. *japonicum* from right to left) **(** adapted from Gryseels *et al*.,2006)

Epidemiological and other related studies have established that egg output reflects intensity of infection (worm burden), being measured in the field either as eggs per gram of faeces (*S. mansoni, S. japonicum*) or eggs per unit volume of urine (*S. haematobium*) (webbe, 1981). This is on the notion that egg output is closely related to the number of worm pairs that the host carries. By a recent estimate, a moderately infected individual with a typical parasite burden of 80–100 worm pairs would excrete 12,000–15,000 eggs daily (Wilson *et al*., 2006).

1.1.2. Snail intermediate hosts

The intermediate hosts of schistosomes in Africa are freshwater pulmonate snails, which belong to the Planorbidae family. The species belong to two genera, namely *Biomphalaria,* host for *S. mansoni,* and *Bulinus,* host for *S. haematobium* and *S. intercalatum* (Sturrock, 1993). All species of *Biomphalaria* and *Bulinus* are hermaphrodite, possessing both male and female organs in single organism.

1.2. Epidemiology and Ecology of Schistosomiasis

For schistosomiasis transmission to take place, the ecologies of the schistosomes, the aquatic snail intermediate host and the human definitive host must converge in space and time in suitable water bodies. By affecting the transmission cycle of schistosome parasite directly or indirectly, numerous factors act to determine the transmission of schistosomiasis (Cox, 1993). These factors include biotic and abiotic factors, behavioral and socioeconomic factors. Ecological changes resulting from water resource development and/or population movement are also important determinants of the epidemiology of schistosomiasis.

Biotic factors such as aquatic vegetation and algal cover, presence and prevalence of the pathogenic worms and host snails largely influence the distribution of schitosomiasis. In addition to its role as main sources of nutrition, aquatic vegetation and algae also create favorable habitats for intermediate snail hosts (e.g. provide suitable surface for depositing egg masses (Boelee and Madsen, 2006).

The abiotic factors such as climatic (temperature and rainfall), physical (e.g. water current velocity and water turbidity) and chemical factors, which affect the survival and development of the schistosome parasite and the snail intermediate host in turn affect transmission dynamics. For instance, various species of schistosomes have different water temperature, current, turbidity, and desiccation tolerances. Both *Biomphalari*a and *Bulinus* species prefer gradual changes in the water level, little turbidity and partial shade (Birley, 1991). Moreover, *Bulinus* and *Biomphalaria* spp. prefer water velocities below 0.3 m/s (Birley, 1991). Hence, transmission foci involving snail of these genera tend to be limited to still or slow flowing water bodies (Southgate and Rollinson, 1987). In addition, many intermediate snail hosts thrive on organic material and firm mud substrate.

Population dynamics of snails and transmission is correlated more closely with climatic factors, especially rainfall and temperature, rather than with physicochemical factors and schistosome transmission usually is seasonal, primarily due to the variation in temperature and the water cycle (WHO, 1993).

1.2.1. Rainfall and water availability

Rainfall in determining habitat stability or habitat permanence defines the pattern of schistosomiasis. Like temperature, seasonal changes in rainfall and/ or water level cause marked fluctuations in snail population densities and transmission rates in most areas. On one hand, rainfall could enhance transmission of schistosomiasis by increasing the amount of surface water which can be suitable environment for breeding of snail intermediate host. On the other hand, rainfall may denote the cessation of active transmission either in dry season when no or little rainfall or when there is too much raining that can wash the infected snails away from the transmission sites. Hence, foci are common where the transmission is negatively inhibited as a result excessive rain or prolonged dry season. Most aquatic snail species die when stranded on dry land in the dry season. However, a proportion of some snail species are able to withstand desiccation for months while buried in the mud bottom.

1.2.2. Temperature

Temperature affects the distribution of schistosmiasis by its effect on the parasite development and the snail survival. The different developmental stages in the transmission cycle of the parasite are susceptible to thermal variation. The intra-molluscan stage of the parasite development, for instance, has been identified as the most sensitive to the effects of temperature (Michelson, 1961).

Moderately high temperature create short pre-patent period of intra-molluscan stage of development and hence enhance transmission rates. However, temperature below and above the optimum level is not often useful for the survival and development of the larval stage in mollusk or for the survival and breeding of mollusk it self. In this regard, different temperature limits beyond which transmission is inhibited have been described. For instance, below 15^{o}c larval development in general is inhibited while above 35^{o}c, snail and parasite death occur rapidly and these situations may interrupt transmission (Anderson and May, 1979).

Snails are, however, less sensitive to low temperature than schistosome parasite. Un-infected snail can therefore be found at high altitude, where low temperature inhibits the development of the parasite in snail. Hence, suitable snail hosts do occur with out establishment of the disease. (Anderson and May, 1979; Brooker, 2007).

1.2.3. Behavioral and socio-economic factors

Socioeconomic status has a significant influence on the transmission and spread schistosomiasis. In addition, behavioral activities determine transmission rate of schistosomiasis by affecting the exposure level of the local people to the schistosome parasite in endemic areas. As a behaviour related disease, the risk of infection with schistosomiasis is associated to age, gender, and occupation of individuals.

Human water contact studies to date have estimated exposure at the population level with either direct observational studies or interviews (Ross *et al*., 2001). These studies have revealed that intensity of infection is influenced considerably by many aspects of human contact with infective water including frequency, duration or time of day of contact. For example, water contact is greatest during the middle of the day or early afternoon, which is just the time that cercariae are released from the snail intermediate host (Azim *et al.*, 2008).

Generally, exposure levels drop with rise in age (Scot *et al*., 2003). According to Ross *et al*. (2001) there is a general trend for adolescents (<21 years) to have greater exposure levels which occurred in the afternoon. Ross and his colleagues added that studies from Africa have reported higher degrees of water contact in young (10 to 20 years) females. The contrasting exposure patterns are ultimately a reflection of social, economic, and ecological variation from one area to another (Huang and Manderson, 1992).

Hence, in most schistosomiasis endemic areas, prevalence and intensity of infection are higher in children than adults (Davis, 2003). The pattern of distribution that the disease is more prevalent in children is not only for schistosomiasis, but also for soil- transmitted helminthic infection. According to the epidemiological studies of soil- transmitted helminthic infections, the prevalence and intensity of *Ascaris lumbricoides, Trichuris trichiura,* hookworms and *Strongyloides stercoralis* is highest among children 4-15 years of age (Azim *et al., 2008*). Difference in the peak age-related prevalence of disease is probably due to the gradual development of immunity and differences in the extent of exposure (Barbosa, 2006).

Schistosomiasis is a disease of the less developed society characterized by poverty, lacking basic services, and lack of information and instruction. Particularly, the scarcity of latrines enhances transmission probabilities through indiscriminate defecation habits. Under poor hygienic condition, faeces and urine often enter water body occurring near human habitations and this enhances transmission. Indiscriminate defecation either increase the chance of the schistosome parasite to reach in to the water body, which is the critical point in the life cycle of the parasite or improve the water habitat as habitat moderately polluted with organic matter create suitable conditions for the snail intermediate host.

Overall, schistosomiasis transmission depends, unlike the transmission of malaria and other insect-transmitted diseases, on the active role of the human host in the transmission process, through excretory contamination of snail habitats and direct contact with infective water (Kloos and David**,** 2002).

1.2.4. Water resource development

Water resources development takes place in most parts of the world, at different scales and at a rapid pace to meet the growing demand of energy and food which emanate from population increase and/or development. The resulting hydrologic and land use changes that occur from the water development project, usually dam and its reservoir, mix in a complex way to affect health (Seto *et al*., 2008). A typical dam, for example, can be modified in a way appropriate for the creation of habitat ideally suited to the breeding of snails that serve as an intermediate hosts for the parasite. This may be through increasing areas of irrigated agriculture. Irrigation canals often become places where major water contact occurs. Increased habitat for snails, coupled with lack of adequate sanitary facilities offer great opportunity for the spread of schistosomiasis.

1.2.5. Population movement

It has been documented that population movements which may occur for various reasons have led to an important increase in the spread of schistosomiasis. Infected individuals carry parasites with them when they travel. Transmission of schistosomiasis may become more intense in man-made water bodies than natural water bodies due to population movement and the resulting higher human population density. Migration does not only intensify infection in endemic areas but also introduce new infection to previously non indemic areas given the presence of susceptible intermediate snail hosts (Steinmann *et al*., 2006).

Generally, climate and the distribution of surface waters suitable for snail intermediate hosts and the free-swimming parasite are crucial in the macrogeographic (world-wide, regional, national and district level) distribution of schistosomiasis. Microgeographic variations in the physical environment, human settlement pattern and degree of exposure to cercarial infected water determine the local (community level) distribution of schistosomiasis.

1.3. Ecology and Epidemiology of Schistosomiasis in Ethiopia

In Ethiopia, human schistosomiasis due to *S. mansoni* is widely distributed (Kloos *et al*., 1988). Nevertheless, the distribution is highly focal and discontinuous (Erko *et al.,* 1997). In the country, it is generally established that *S.mansoni* is largely confined within an altitudinal limit of 800m-2200m above sea level. Cold water temperature, which may occur above 2200m, could be a hindrance for the intermediate host whereas, hot water temperature become a limiting factor below 800m (Kloos *et al*., 1988). An experimental study by Joubert *et at*. (1986), for example, demonstrated that *B. pfeifferi* when infected with *S. mansoni* dies at temperatures below 16 and above 30 °C. Conversely, *S. haematobium* is found in low lands below 800m because the *Bulinus* spp. in Ethiopia favors warm water temperatures (Kloos *et al*., 1988).

Two species of the genus *Biomphalaria, B.sudanica* and *B.pfeifferi,* are known to transmit *S. mansoni* in Ethiopia (Erko *et al.,* 2006). Unlike, *B. pfeifferi,* which is known to have a wide geographical distribution, *B. sudanica* has very limited distribution in Ethiopia. Its presence has so far been reported from only three areas in Rift Valley, Ziway and Abaya Lakes and the interface between Tikur Wuha River and Awassa Lake (Birrie *et al.*, 1995). It seems, therefore, that *B.pfeifferi* has ubiquitous distribution, while *B. sudanica* is limited in its distribution.

Bulinus abyssinicus and *B.africanus* are the only bulinid species found naturally transmitting *S. haematobium* in Ethiopia, even though about 10 bilinus species are expected to occur (Lo *et al*., 1988). From the previous studies it is established that *B. abyssinicus* is the intermediate host for *S. heamtobium* in Awash valley, whereas *B. africanus* transmit the disease in kurmuk (an area locating near to Sudan) (Kloos *et al.*, 1988).

Generally, three main ecological settings (irrigation ecology, stream ecology and lake ecology) where transmission of schistosomiasis occur are recognized in Ethiopia (Birrie *et al*., 1993a). Since streams create more favorable habitats for the breeding of the snail intermediate host, it constitutes the major transmission ecology. Besides, it is evident that the majority of *S. mansoni* endemic areas occur in agricultural communities residing along stream (Birrie *et al*., 1993a). Growing evidences also suggest that irrigation ecologies are becoming malacologically suitable areas and these areas are sites of intense transmission for *S. mansoni* (Kloos *et al*., 1988).

1.4. Statement of the problem

In Ethiopia, both intestinal and urinary schistosomiasis is endemic and cause considerable health and economic impact (Kloos *et al*., 1988). An estimated number of 29.89 million people are at risk and of these 4 million are estimated to be infected in the country (Chitsulo *et al*., 2000). The presence schistosomiasis is realized in the country since 1950s after Ayad (1956) presented a review on the survey of intestinal and urinary schistosomiasis for some east African countries including Ethiopia. Since then, numerous local epidemiological studies have been conducted in different parts of the country and various epidemiological reports are available.

Kloos *et al*. (1988) reviewed previous studies between 1961 and 1986 and reported that from among 365 communities assessed for schistosomiasis mansoni, cases were reported from 225 (62%), and in 85 (23%) the prevalence ranged from 10 to 92%. Regarding urinary schistosomiasis, cases have been reported from 30 of the 54 communities studied, 17 of them with infection rates between 14% and 75%. Furthermore, other studies have also shown the occurrence of intestinal schistosomiasis in various magnitudes. These included southern Ethiopia (e.g. Erko *et al*., 2001; Erko *et al*., 2002), northwestern Ethiopia (Teklehaimanot and Fletcher, 1990), north Ethiopia (Jemaneh, 2000) and north east Ethiopia (Birrie, 1986; Birrie *et al*., 1998) The disease has also been reported in Addis Ababa (Erko, 1996). Generally, foci of intestinal schistosomiasis have now reached fifty (Erko, 2010).

New transmission foci are being discovered in different parts of the country over time. The reasons for the spreading of the disease to new localities seem to be an extensive population linkage and water resource development (Erko, 1996). Additionally, in this era of global warming and climatic change, the epidemiology of temperature-dependent infectious diseases could be changing implying the possibility of new transmission area in the world in general and in Ethiopia in particular.

The endemicity of intestinal schistosomiasis has long been established in Northeastern Ethiopia. Borkena River Basin, including Harbu, Kemise and Bati (Birrie, 1986; Birrie *et al*., 1998) are for instance the endemic localities in this geographical region. The present epidemiological study was, therefore, conducted in Hayk area, Northeastern Ethiopia, to determine the transmission and magnitude of intestinal schistosomiasis in Hayk area.

1.5. Significance of the study

Finding from the study can provide information about the burden of intestinal schistosomiasis to concerned governmental and non-governmental bodies to guide intervention measures. The study also raises awareness of community about schistosomiasis and this would also contribute towards the disease control. Furthermore, the findings can serve as reference baseline data for further research to be done in the area.

2. OBJECTIVES

2.1. General objective

- To determine the transmission and magnitude of intestinal schistosomiasis in Hayk area.

2.2. Specific objectives

- To determine the prevalence and intensity of infection of *Schistosoma mansoni* in lake Hayk area.
- To identify the risk factors for transmission of the disease.
- To identify snail intermediate hosts of schistosomiasis in the area.

Hypothesis

The altitudinal measure of Lake Hayk area is within the proposed optimum transmission altitudinal range of schistosomiasis with two main water bodies (Lake Hayk and Kete stream). There also occur frequent cases of the disease in the health center of this area. Hence, it was hypothesized that schistosomiasis transmission takes place in this area.

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Abstract

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Objective: The objective of this study was to determine the transmission and magnitude of schistosomiasis in Hayk area, Northeastern Ethiopia.

Methods: A cross sectional parasitological study involving 384 school children was undertaken to determine the prevalence of intestinal schistosomiasis between January and March 2010 in two primary schools in Hayk area, Northeastern Ethiopia. Stool samples from 384 school children were processed for microscopic examination using Kato-Katz technique. Malacological survey and observation on human water contact activities were also carried out. As a malacological survey snails were checked for schistosome infection by exposing lab-bred mice to the cercariae shed from *Biomphalaria pfeifferi en masse*.

Results: The prevalence and intensity of intestinal schistosomiasis among school children in Hayk Number 1 and Hayk Number 2 Primary Schools was found to be 45% and 161 epg, respectively. The prevalence of infection had relationship with age and gender. Males are more infected than females. Children in the age group 15-19 years had the highest infection rate, followed by 10-14 and 5-9 years age groups. Schistosome infection in *Biomphalaria pfeifferi* was 3.2%. Schistosome infection was also established in laboratory-bred mice and adult *Schistosoma mansoni* worms were harvested from exposed mice after 6 weeks of maintenance.

Conclusion: The observed intestinal schistosomiasis with prevalence of 45% among young children, collection of schistosome infected *Biomphalaria pfeifferi*, and the establishment of lab infection in mice showed that transmission of intestinal schistosomiasis is taking place in the area. Since the community represents a high risk community, schistosomiasis control should be immediately put in place.

3. Materials and methods

3.1. Study area

Hayk area is located about 430 km from Addis Ababa in the northeast of Ethiopia. The area has altitudinal ranges of 1480 to 1900 masl. The main all weather road extending from Addis Ababa to Mekele, pass across Hayk town. There is also another all weather road in the area running by the side of lake and connects this town to other small town (Bistima) founding to the east of Hayk town. The climate is moderately hot with an annual average temperature ranging from 15-21°C and annual rainfall is of approximately 1030 mm. The main rainy season is from the beginning of June to the end of October. The inhabitants earn their living by mixed farming, cultivating crop and rearing livestock (mainly cattle and camel). Agriculture largely depends on rain with two growing seasons and very limited irrigation based agricultural practice. The major crops grown in the area are "teff" and sorgum. Chat (*Catha edulis*) is the major cash crop cultivated in the area. Some inhabitants also earn their living as small scale merchants and government employees. According to the 2001.E.C census, the population is over 25,000 most of whom are Muslims. Hayk town is an administrative center for many farmers' associations.

There are three schools and one health centre in Hayk town. The two primary schools from which the stool samples were collected were Hayk Number-2 and Hayk number-1. Two main fresh water bodies are found in the area, a Lake called Lake Hayk (after which the town is named) and a permanent stream called "Kete wenz". Considering the distance of the two schools from the respective water body and the associated water contact activity, Hayk Number-2 is found very near to the stream and it seems that students of this school have major contact with the stream.

The surrounding rural area of Hayk town is relatively mountainous and full of grooves and ridges. The natural vegetation cover of the surrounding rural area appears deciduous acacia forest. The rural dwellers had no access to piped water and used the stream or the lake for domestic purposes. Primarily, due to lack of toilet, most people were observed defecating in open field in groove, nearby bushes or at the shore of the water body.

Compared to the villagers living around the town, some of the town dwellers had toilet. However, still many dwellers of the town were lacking toilet and indiscriminate defecating like the villagers was also observed in town dwellers. The residence of the town used piped water and the existence of modern public water supply reduce the risk to be infected by water born and water related diseases compared to the village dwellers of the surrounding area.

The lake is found to the east of the Hayk town. It lies relatively deeper (1500 masl) compared to the occurrence of the surrounding land mass (1900masl) and appears like a “cup”. The maximum depth of the lake reaches 88m and it covers an area of 23 km^2. Except at some points at the shore, the lake appeared little turbid. One can see no obvious inflow or out flow stream or river in this lake. At one edge of the lake there occurs an attractive historical monastery where big biodiversified trees are found. The local people (villagers) depend on the lake for irrigation, fishing, swimming, bathing and fetching water. Many people from towns like Dessie come to the lake at weekend for the purpose of recreation.

Siltation and eutrophication are well observable in this lake. Some activities are undergoing by the local people with the help of the government and nongovernmental organization to save the lake from these problems.

Kete stream occur southward to the town. It is a small, shallow and perennial stream. The water discharge of the stream gets lowered in the middle of the dry season not only because of a decrease from the source, but also farmers draw water at different sites of the stream for small scale irrigation purposes. The stream captures the water shade from the town

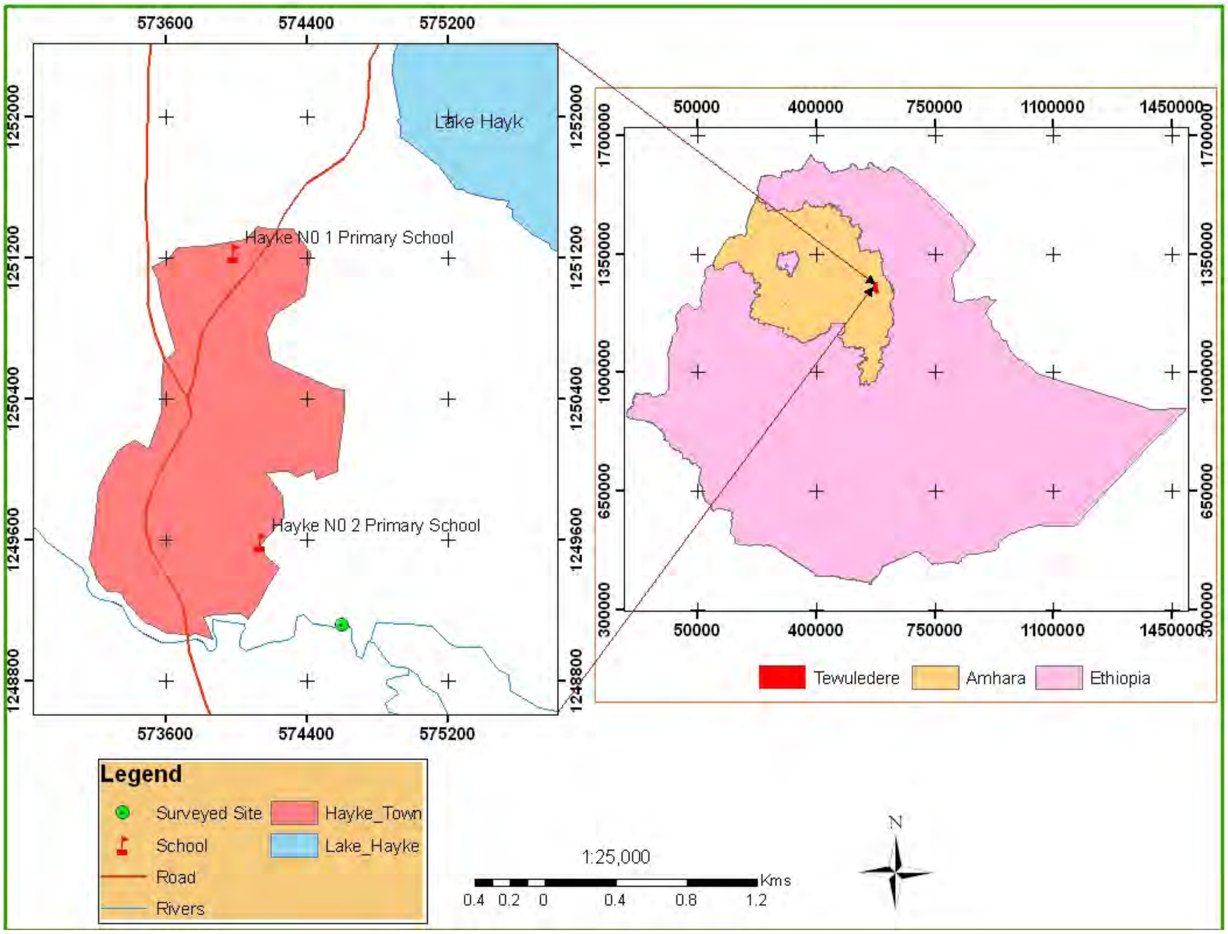

Figure 3. Map of Lake Hayk area.

3.2. Study population

Parasitological survey was conducted in Hayk Number-1and Hayk number -2 primary schools in Hayk area, northeast Ethiopia. Malacological survey was also conducted in Lake Hayk and Kete stream. The entire study was conducted between January and March 2010. Stool samples were collected from school children by simple random sampling**.** This was on the ground that school-age children usually have higher prevalence rates than younger or older population segments (Huang and Manderson, 1992), and appropriately designed studies among school children may also provide data on the overall prevalence and incidence of schistosomiasis in certain endemic area (Mott and Cline, 1980). In addition, parasitological data from primary school children can be used as an index for assessing community prevalence and most data on prevalence have come primarily from school-age children (Guyatt *et al*., 1999).

3.3. Sample size estimation

The sample size (n) was estimated using the statistical formula (Daniel, 1995), $n = \frac{z^2 p (1-p)}{d^2}$,

Where n = sample size; z=z statistic for a level of confidence; d= precision; p= expected prevalence or proportion

Since the overall prevalence rate (p) of intestinal parasites was not known for the study area, p was taken to be 50%. For the calculation, a 95% confidence interval (z) and a 5% margin of error (d) were used. This gave a sample size of 384 (255 in Kete school and 129 in Kekewa). Number of females and males enrolled in the study were 117 and 138 in Hayk number two and 58 and 71 in Hayk number one, respectively.

3.4. Stool collection and examination

Stool samples were collected from children enrolled in Hayk Number-1and Hayk Number-2 Primary schools, in Hayk town. The study objectives were explained to the school directors, teachers and children before stool collection. Only children who assented to providing stool participated in the study. Each subject was given plastic sheet with applicator stick and was instructed to bring sizable stool sample of his own. Slides were labeled with identification number and school name. Information including age, sex, and whether the pupil has been in Hayk or has just come new was also collected at the time of stool collection.

Kato- Katz method was used to process the stool specimen for microscopic examination (WHO, 1991). A single Kato slide was prepared from stool of each child. The Kato slides were transported and systematically examined in the laboratory by an experienced technician within one week of collection. For quality control, 10% of the slides were randomly selected and re-examined by another experienced technician.

The egg of *S. mansoni* was counted and the number of eggs was multiplied by 24 to convert into eggs per gram of stool. The intensity of infcetion was calculated as the geometric mean for infected children and espressed as eggs per gram of feces (epg).

Three infection categories with respcet to intesity were considered. Those egg counts < 100 e.p.g were categoried under light infection, whereas egg counts between 101and 400 e.p.g. and ≥400e.p.g. were considerd moderate and severe infections respectively (WHO,2002).

3.5. Malacological Survey

Intermediate snail hosts were surveyed both in the lake and in the stream at human water contact sites. The likely habitats, vegetation and stones, were examined. Snails were collected by handpicking using forceps and gloves because the stream water was shallow

The collected snails were put in a polyethylene bag that contained a small quantity of water and weeds. Thereafter, snails were transported to Aklilu Lemma Institute of Pathobiology for determination of infection. The snails were then examined for natural trematode infections by shedding method. Each snail was placed individually in the shedding vials with water (de-ionized water) and then exposed to electric light for about one hour. The cercariae shed by the snails were identified to the genus level by their tail morphology.

Lab-bred mice were exposed to the cercariae en masse. This was done by suspending the mice in a way that the tails of the mice was in infected water. The exposed mice were kept until infection was established. Mice were sacrificed and worms were identified after 6 weeks of maintenance (Duval and Dewitt, 1967).

During snail collection, observations were also made on physical characteristics of the habitat such as vegetation abundance, turbidity, the nature of the substrate. In the mean time, water contact activities were also surveyed.

As part of this epidemiological study, water contact survey to evaluate exposure risk levels, through direct observation was carried out at the selected sites of the stream and observations were recorded. Selection criteria of sites surveyed for water contact activities were based on the closeness to the town in general and the school in particular.

3.6. Ethical consideration

This study was reviewed and approved by the Ethical Committee of the Biology Department, Addis Ababa University. The objective of the study was explained to the teachers and students at the time of specimen collection. Positive individuals for schistosomiasis were treated using standard dose of praziquantel(single oral dose of 40 mg/kg body weight).

3.7. Data Analysis

Data were analyzed with SPSS software version 13. Chi-square (X^2) was used to test possible association of infection with gender and age. P-value of less than 0.05 was considered statistically significant.

4. Result

4.1. Parasitological survey

A total of three hundred eighty four stool specimens were collected from school children in Hayk town (255 from Hayk Number-2 and 129 from Hayk Number-1 primary schools) and microscopically examined for intestinal schistosomiasis. The age groups and gender of the study participants are presented in Tables 1 and 2.

The prevalence of *Schistosoma mansoni* infection among children in Hayk Number-2 was 61% (49% in females and 71% in males) while the prevalence in Hayk Number-1 was 13% (7% in female and 19% in male). Over 55% of the school children of both schools were infected with one or more intestinal helminths.

The mean intensity of *Schistosoma mansoni* infection among children in Hayk Number-2 was 179 epg (160 for females and 193 for males) it was 78 epg (48 among positive female students and 81 among positive male students) for Hayk Number-1 primary school. Egg load ranged from 24 to 1608 per gram of stool was observed.

In school children of Hayk Number-2 primary school, 52% had light infections (<100 eggs/gm of stool), 35% had moderate infections (100–399 eggs/gm of stool) and the remaining 13% had heavy infections (≥400 egg/gm of stool) (WHO, 2002). Among school children of Hayk Number-1 who harbored the parasite, 76% were lightly infected and 18% were moderately infected and only 6% were heavily infected. Among female children in Hayk Number-1, 57% were under the category of light infection, whereas 37% and 6% were placed under category of moderate and heavy infections, respectively. Infected females in Hayk Number-1 were all in the category of light infection. Among positive males of Hayk Number-2, 43% had light infections, 41% had moderate infections and the rest 16% had heavy infections. Similarly in Hayk Number-1 primary school 73%, 20%, and 7% males were lightly, moderately and heavily infected respectively.

Gender specific prevalence among Hayk Number-2 children showed that 71% males and 49% females ($P<0.05$) had schistosomiasis mansoni(Table-1). Similar pattern of infection rate among males than females was also observed among school children of Hayk Number-1 (19% in males and 7 % in females ($P<0.05$) (Table-2).

Age-wise, children in the age group 15-19 years had the highest infection followed by the age group 10-14, and 5-9 age groups in both primary schools (Tables 1&2).

Among the soil transmitted intestinal helminths, the prevalence of *Ascaris lumbricoides* infection was 12% (14% in Hayk Number-2 and 9% in Hayk number-1) while the prevalence of *Trichuris trichiura* infection was 4%. Other rare helminth infections (less than 3%) observed were *Hymenolepis nana*, *Entrobius vermicularis* and *Taenia* species infections. Since slides were not examined within 30 minutes of stool collection, the status of hookworm infection was not determined.

4.2. Malacological survey

Snails were surveyed in selected water contact sites of Kete stream and Lake Hayk and were collected by handpicking using forceps and gloves. *Biomphalaria pfeifferi* were collected from Kete stream only. However, no live snail was found from the lake during the survey period, except shell (Figure.4)

Figure 4. Snail shells collected from Lake Hayk, 2010

Biomphalaria pfeifferi were found being attached to the grass abundant at the edge of the stream. To detect natural infection, snails were allowed to shed cercariae individually in vials containing aged water by exposing to electric light. From 31 numbers of *Biomphalaria pfeifferi*, only one shed (3.2%) schistosome cercariae. *S. mansoni* infection was successfully established in the laboratory mice and adult worms were harvested (two male the remaining female) after six weeks of laboratory maintenance.

Observations made on physical characteristics of the stream during snail survey showed that the water was moderately turbid, and the substratum muddy and sandy. The stream had moderate vegetation cover and also algae.

Figure 5. Picture view of shore of Kete stream

4.3. Risk factors

Observations showed that people excreted in open field, particularly in grooves and shades of eucalyptus trees near Kete stream. The residents were also observed to perform such water contact activities as swimming, bathing, playing, cutting grass, drawing water, performing religious rites such as Wudu by Muslims, washing clothes and horses. Students also crossed the stream on their way to and from school. Especially, the surrounding land mass of the stream is arable and farmers draw the water to cultivate vegetable such as carrot.

Figure 6. Photograph showing children taking bath

Figure 7. A woman washing clothes in the stream

Figure 8.Children swimming in back water of the stream

Table 1. Prevalence and intensity of intestinal schistosomiasis among school children in Hayk Number-2 (Kete) primary school, Hayk, Northeast Ethiopia, 2010

Age	Number examined			Number(%) infected		
group	Female	Male	Total	Female	Male	Total
5-9	56	71	127	23(41)	42(59)	65(51)
EPG				166	191	182
10-14	60	62	122	33(55)	51(82)	84(68)
EPG				152	187	170
15-19	1	5	6	1(100)	5(100)	6(100)
EPG				216	240	234
Total	117	138	255	57(49)	98(71)	155(61)
EPG				160	193	179

Table 2. Prevalence and intensity of intestinal schistosomiasis among school children in Hayk Number-1Primary School, Hayk, Northeast Ethiopia, 2010

Age	Number examined			Number(%) infected		
group	Female	Male	Total	Female	Male	Total
5-9	25	30	55	1(4)	4(13)	5(9)
EPG				24	46	48
10- 14	30	37	67	2(7)	7(19)	9(13)
EPG				48	81	80
15-19	4	3	7	1(25)	2(67)	3(43)
EPG				24	84	64
Total	59	70	129	4 (7)	13(19)	17(13)
EPG				48	81	78

5. Discussion

Parasitological and malacological surveys were conducted in Hayk area, Northeastern Ethiopia, to determine the transmission and magnitude of schistosomiasis. The prevalence of intestinal schistosomiasis observed among school children in Hayk Number-2 Primary School was 61% while a prevalence of 13% was observed among school children in Hayk Number-1 Primary School, the overall prevalence for the two schools being 45%. The overall intensity of infection was 161epg (179 in Hayk Number-2 primary school, and 78 in Hayk Number-1primary school). Moreover, schistosome infected *Biomphalaria pfeifferi* were collected from Kete River and the life cycle of *Schistosoma mansoni* was also established in laboratory-bred mice. All these observations confirmed the transmission of intestinal schistosomiasis in Kete steam. This transmission site might have existed for undetermined period of time or could be a newly established one as a result of ecological changes and population movements.

It has been documented that population movements which may occur for various reasons have led to an increase in the spread of schistosomiasis. If infected individuals arrive in malacologically and ecologically receptive areas, new schistosomiasis foci would inevitably be established (Steinmann *et al*., 2006).

The prevalence of infection observed among the school children in Hayk number 2 was high (61%), whereas the prevalence among school children of Hayk Number-1 primary school was medium (13%). According to WHO (2002) classification, communities around Kete River represent high risk communities. Thus, considering the levels of prevalence and/or intensity of infection observed among school children, there is a considerable risk of schistosomiasis in the study area and the situation deserves special attention. In such areas, WHO (2006) recommends the treatment of children and adults at high risks.

The prevalence of *Schistosoma mansoni* infection was much higher among children in Hayk Number-2 primary school than among children in Hayk number-1 (61% versus 13%). This also holds true for other intestinal helminths including *Ascaris lumbricoides and Trichuris trichiura.*

The observed difference in prevalence (higher in Hayk Number-2 primary school) of intestinal schistosomiasis among school children could be attributable to distance from Kete stream where infected snails were collected and people catch the disease and this showed how much focal the transmission was. It is generally true that prevalence rate as well as intensity of schistosomiasis considerably vary with proximity to the infected water bodies (Steinmann *et al.*, 2006). Moreover, this observation reflects the focal nature of schistosomiasis where substantial spatial and temporal variation in transmission of schistosomiasis occurs within human populations (Gryseels *et al.,* 2006*).*

Based on WHO classification of intensity of infection, the majority of the children were lightly infected in both schools (52% in Hayk Number-2 and 76% in Hayk Number-1primary school) followed by moderate infection (35% in Hayk Number-2 and 18% in Hayk Number-1 primary school), and few heavily infected (13% in Hayk Number-2 and 6% Hayk Number-1primary school). This finding reflects the general pattern of schistosomiasis infection that only ~10% of those infected are often heavily infected but most people infected lightly and/or moderately, the effects of which are thought to be minimal or ill defined (Assis *et al* .,1998).

The overall prevalence (45%) observed in Hayk area was higher than the overall prevalence (25%) reported by Birrie (1986) from Borkena river basin, the origin of which is very close to Dessie. On the contrary, higher infection rates were reported by Birrie *et al.* (1998) from Kemise (59%) and Bati (51%), which were also in the Borkena river basin. Nevertheless, these findings cannot be compared due to differences in sampling technique and study population. Moreover, at the local level, disease endemicity is often the product of place-specific factors both in time and space, and hence no two disease transmission foci are the same (Stothard, 2009).

From the study finding it was implicated that more males than females were found to be positive. This was 71% males versus 49% females among Hayk Number-2 children and 19% males versus 7% females among Hayk Number-1 children ($P<0.05$) (table1&2). This result is in agreement with several previous reports in different parts of the country including the report by Birrie (1986) from Borkena river basin, Dejenie *et al*. (2009) from Waja/Tigray/ and Erko and Tedla (1993) from Zege/pensula of Lake Tana. This could be probably due to a consequence of different water contact patterns to water bodies containing schistosome infected snails between males and females. The association of schistosomiasis and sex is well documented (Brooker, 2007).

The study revealed a peak prevalence rate among age group 15-19 years followed by the age group 10-14 and 5-9 years in both schools. This corroborates other previous epidemiological studies of schistosomiasis (Dejenie *et al*., 2009; Erko and Tedla, 1993). On the other hand, other investigators reported peak infection in the age group 10-14 years followed 15-19 and 5-9 years (Erko *et al*., 1991; Birrie *et al*., 1998). The low infection rate in the age group 5-9 years observed in the present study and most other studies might be attributed to the low water contact behavior of children in this age range.

A prevalence of 12% for *A. lumbricoides* and 4% for *T. trichiura* observed in this study was much lower compared to that observed in many other localities. Erko and Medhin (2003) reported prevalence of infection of 83.4% and 86.4% for *A. lumbricoides* and *T. trichiura* infections, respectively. Other studies also reported high infection rates for these helminths. Among other factors, such variations are attributable to differences in transmission ecologies. Hookworm is not considered in the present study because the Kato-Katz slides were microscopically examined after 30 minutes of preparation.

In addition to identifying the presence of school age children passing eggs in the stools, assessing the presence of infected snails with consequent establishing of the life cycle in laboratory using lab-bred mice and surveying the risk factors was performed. This has been worked out because these procedures are crucial aspects in determining a certain locality as new transmission foci for schistosomiasis.

Observation on physical characteristics of Kete stream revealed the habitats are favorable for *B. pfeifferi*. The stream was little turbid, slow flowing, and had dense vegetation (emergent and submerged), back water, water weeds and algae. It has been described that small streams with flow rate of 10–30 cm/s, with slight turbidity, abundant vegetation at the edge and muddy bottoms have been found to be potentially favorable habitats for *B. pfeifferi* and other snails including *Bulinus truncatus* and *Lymnaea natalensis* (Lo *et al*., 1982).

Moreover, observations on the stream bank showed human excreta a few meters from the riverbank under bushes. It was also observed that the local people came into contact with the water via different activities. Some of the activities including bathing, laundering and drawing water for cultivation of vegetables entail spending long periods of time in cercariae infested water. Hence, human behavior is conducive for increased transmission of schistosomiasis.

Measurement of infection rates (monitoring the number of infected local snails) in field populations of snails is one of the basic tools for studies on the epidemiology of schistosomiasis to estimate the transmission potential of an area (Sturrock, 2001). Out of the 31 *Biomphalaria pfeifferi* collected, only one (3.2%) snail was found infected with schistosome cercariae. As Anderson and May (1979) suggested, high rates of infected snail mortality and the duration of the latent period of infection within the snail are closely associated and accounted for the low infection rate in snail populations.

The limitations of this study include small sample taken to represent the age group of 15-19 in Hayk Number-2 primary schools as compared to other age groups and the knowledge level of the pupil (using structured questioner) that would have helped to provide more information on the risk factor of the disease was not assessed. This might have either exaggerated or undermined the age specific infection.

6. Conclusion and Recommendations

The overall intestinal schistosomiasis prevalence of 45% observed among school children in Hayk area indicates that the community represents a high risk community. Collection of schistosome-infected *Biomphalaria pfeifferi* from Kete stream, detection of *S. mansoni* eggs in stool of young children, and the establishment of lab infection in mice confirmed that transmission of intestinal schistosomiasis is taking place in the area.

Gender, age, and proximity of the primary schools to the infected water body (Kete stream) were independently associated with infection in this study area.

Observation on water contact activities showed that the major water contact activities in Kete stream were playing, swimming, bathing, crossing and washing close and domestic animals, and performing religious activities.

Based on WHO (WHO, 2006), communities around Kete stream represent high risk communities, and it is recommended that all school children and adults at higher risk should be treated.

Snail control using endod berries *(Phytolacca dodecandra)* is also required to complement chemotherapy. Health education is also suggested in order to improve hygienic practices and reduce fecal pollution of the environment.

Finally, further study with the objective of elucidating factors influencing the changes in population density of the snail and identifying season of peak transmission is recommended in Kete stream to determine appropriate time for snail control.

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Appendices

Appendix 1: **Kato- katz technique**

Materials:

Spatula,	previously soaked pre-soaked cellophane
Applicator sticks	plastic sheet
Template with hole measuring 41.7g	Microscope slides
Screen with specific mesh size	forceps

Procedure

1. Put a screen over the sizeable stool specimen on the plastic sheet
2. Scrap the stool under the screen with spatula so that stool will be sieved out of the screen
3. collected the sieved stool with spatula and fill it in the holes of templates which each holes form approximately 41.7mg stool sample
4. Remove the template carefully so that the cylinder of feces is left on the slide.
5. Cover the slide with the stool with pre-soaked cellophane.
6. Gently slide over the sample with another slide so that the stool spread evenly.
7. Leave the prepared slide for at least 24 hrs to clear the smear.
8. Examine the slide with microscope for the presence of STH and schistosome egg.
9. Count the number of eggs encountered per each slide.

Appendix 2: Written Consent Form

Here am intending to study on the kind disease known as Bilharzia (schistosomiasis). For this, I need stool sample from your children which would be used only to detect the presence of schistosomiasis. It will be my pleasure, if you are volunteer to let the children participate in this particular study. I also want to inform you that there is no any health related risk in participating. If your children are found positive for schistosomiasis, they will receive standard drugs free of charge. The information in your records is strictly confidential.

Your participation in this study is completely voluntary and you can refuse to participate or free to withdraw yourself from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. Do you understand what has been said to you? If not, you have the right to get proper explanation.

This consent form has been readout to me in my own language, and I understand the content and I am voluntarily consent to participate in the study.

Study Area ______________________________

Name ________________________ Signature ____________________ Date _______________

Wittiness Name _______________________ Signature ________________ Date____________

Investigator Name _____________________________ Signature ____________ Date_________