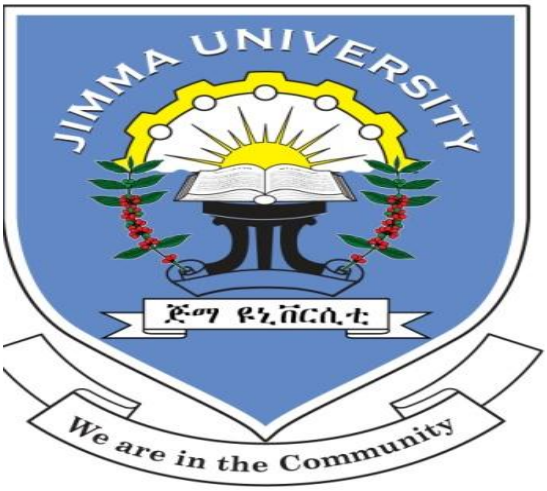
COMPARISON OF INDIVIDUAL AND POOLED STOOL SAMPLES FOR THE ASSESSMENT OF *SCHISTOSOMA MANSONI* AND SOIL-TRANSMITTED HELMINTH INFECTIONS INTENSITY BY KATO KATZ TECHNIQUE IN JIMMA ZONE, SOUTH-WEST, ETHIOPIA



BY: ASHENAFI KURE: (BSc)

A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCES IN MEDICAL PARASITOLOGY

JULY, 2014

JIMMA, ETHIOPIA

JIMMA UNIVERSITY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY

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BY: ASHENAFI KURE: (BSc)

ADVISORS:

1. **DANIEL DANA: (BSc, MSc)**
2. **ZELEKE MEKONNEN: (MSc, PhD scholar)**
3. **BRUNO LEVECKE: (PhD)**

JULY, 2014

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ABSTRACT

BACKGROUND: Schistosomiasis and soil-transmitted helminthiasis (STHs) are the most pervasive types of parasitic infections in the world.

Schistosomiasis or bilharziasis is a tropical disease caused by worms of the genus *Schistosoma*. More than 207 million people are suffering from schistosomiasis and nearly 800 million people are at risk of infection globally and leading to the loss of between 1.7 and 4.5 million disability adjusted life years (DALYs). Schistosomiasis is a public health problem, and its control is advocated by the World Health Organization (WHO) and other international agencies with the strategy based on preventive chemotherapy. Moreover health strategy for the success of effective parasitic disease control programs demand knowledge of magnitude of the disease. Rapid, cost effective and accurate assessment of the prevalence and intensity of infections are lacking. Therefore, pooling of stool samples may be the new possible alternative to assess infection intensity of schistosomiasis in comparison to individual stool examination. In the present study we evaluated the pooling of stool for the assessment of intensity of *Schistosoma mansoni* and STHs (*Ascaris lumbricoides*, *Trichuris trichiura* and hookworms) infections. In addition, we assessed the time required to screen individual and pooled stool samples.

METHODS AND MATERIALS: A cross-sectional school based survey was conducted in 360 children aged between 5 to 18 years from six schools in Jimma Zone (South-west Ethiopia) from February to May 2014. In both individual and pooled stool samples (pools sizes of 5, 10 and 20) the faecal egg counts (FEC) was determined by means of eggs per gram of stool (epg) using the Kato-Katz technique. The agreement between means fecal egg count for individual samples and the pooled samples were evaluated by the Spearman rank correlation coefficient for *S. mansoni* and STHs infections. The confidence interval and the mean difference in FEC were calculated at 95%. The data were analyzed by SPSS window version 20.0.

RESULTS: Out of 360 study participants, 218 (60.5%) of them were found positive for any helminthic (*S. mansoni* and the three STHs) infection. The prevalence rate of *S. mansoni* and STHs infection were 25.3 % (11.7% in females and 13.6% in males) and 48.3% (22.8% in females and 25.5% in males) respectively. *T. trichiura* was the predominant species (30.6 %), followed by hookworms (21.4%) and *A. lumbricoides* (18.1%) among STHs infection respectively.

The arithmetic mean FEC was 2,596.3 EPG, 126.0 EPG, 47.3 EPG and 40.7 EPG for *A. lumbricoides, T. trichiura, S. mansoni* and hookworms, respectively. Except for hookworms, there was a significant correlation (coefficient = 0.53-0.95) between the mean of individual FECs and the FECs of pooled samples for *A. lumbricoides, T. trichiura* and *S. mansoni*, regardless of the pool size. There was no significant difference in FECs between the examination of individual and pooled stool samples, except for hookworms. For these STHs, pools of 10 resulted in a significant lower FECs. The total time to determine individual FECs for 360 samples was 65 hours and 5 minutes, while a pools of 20 only 12 hours and 42 minutes.

CONCLUSIONS AND RECOMMENDATIONS: *S. mansoni* was moderately prevalent in the study areas, while almost half of the study population harbors any STHs. Pooling of stool samples holds promising for a rapid assessment of the infections intensity of *S. mansoni* and STHs by Kato-Katz technique. Employing pooling strategies can reduce time of examination by up to 87% compared to examining individual samples. Preventive chemotherapy and health education should be implemented to reduce the burden of schistosomiasis and STHs in the study areas. Moreover, further research is required to determine how pooling of stool samples applicable for this parasitic infections with different pools size, samples size, diagnostic technique and prevalence study.

Key words: *S. mansoni*, Soil-transmitted helminths, Pooling, School children, Kato Katz, Time, Jimma Zone

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisors, Mr. Daniel Dana, Mr. Zeleke Mekonnen and Dr. Bruno Levecke for their unreserved assistance , valuable comments, critical advice and guidance throughout my work.

I am very grateful to Jimma University for giving me the chance to undertake this post graduate research project.

My great gratitude also goes to all the children, parents and schoolteachers who cooperated with us in this study.

I am grateful to South Nations, Nationalities and Peoples Regional Health Bureau for sponsoring my study at Jimma University.

My sincere appreciation also goes to NTD teams of Jimma University, particularly, Mr. Mio Ayana, Mr. Bizuwork Sharew and Mr. Yesuf Seid , for their kindness, help and keen interest to participate from data collection to examination .

Finally, I would like to acknowledge my families and friends for encouraging me and moral support in all my academic activities.

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LIST OF ACRONYMS

CDC: Center for Disease Control

CI: Confidence Interval

DALYs: Disability Adjusted Life Years

epg: Eggs per Gram of stool

FEC: Fecal Egg Count

KK: Kato Katz technique

MDA: Mass Drug Administration

NTD: Neglected Tropical Diseases

PC: Preventive Chemotherapy

SAC: School Age Children

SPSS: Statistical Package for Social Sciences

STHs: Soil-Transmitted Helminths

WHO: World Health Organization

CHAPTER ONE

1. BACKGROUND

1.1. Introduction

Schistosomiasis and soil-transmitted helminthiasis (STHs) are the most pervasive types of parasitic infections in the world. These diseases have major health and socio-economic impact, and constitute an important public health problem in developing countries (Crompton, 1999; WHO, 2002).

Schistosomiasis or bilharziasis is a disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. There are five *Schistosoma* species (*S. mansoni, S. hematobium, S. japonicum, S. intercalatum* and *S. mekongi)* that cause human schistosomiasis; the first three are the main disease causing species (Gray & Rose, 2011).

The three species of *Schistosoma* have different geographic distributions (Figure 1). Intestinal schistosomiasis due to *S. mansoni* is endemic in 52 countries. It is widespread in African, South America and Asia. *S. mansoni* co-exist with *S. haematobium* in 41 countries of Africa and the eastern Mediterranean, whereas *S. japonicum* is widely distributed in mainland China, parts of the Philippines, and western Indonesia (Boelee & Madsen, 2006; Cheesbrough, 2009). Intestinal Schistosomiasis caused by *S. mansoni* is widespread throughout Africa, where endemic in more than 46 countries (Boelee & Madsen, 2006).

In Ethiopia different report showed that both *S. mansoni* and *S. haematobium* are widely distributed (Birrie *et al*., 1989; Ali *et al*., 2006 ; Deribe *et al*. 2012).

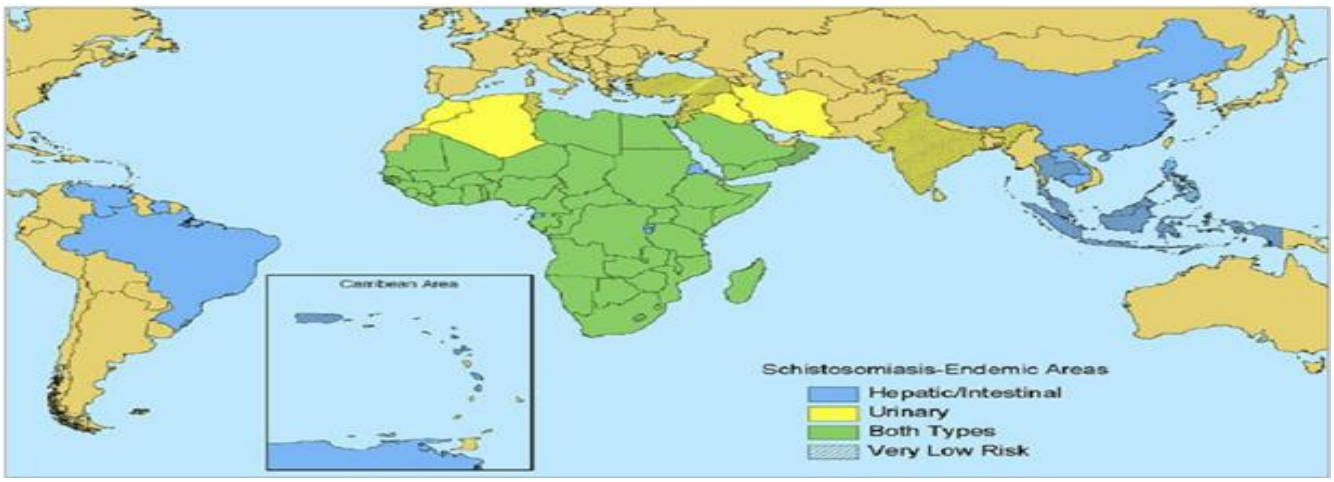

Figure 1: Distribution of schistosomiasis, worldwide in 2011(Data source: WHO Map production: Control of NTDs, WHO, 2012)

Schistosomiasis transmission occurs when larval forms (*cercaria*) of the parasite released by freshwater snails penetrate the skin during contact with infested water (streams, irrigation schemes, and lakes). Development of irrigation schemes, dam construction for hydroelectric power, water conservation for different purposes, human behaviors such as swimming habits, fishing, bathing and improper waste disposal, poverty like use of river water for different purposes and wide distribution of intermediate host identified as the major contributing factors for the increased prevalence and wide distribution of schistosomiasis (WHO, 2002 , 2013).

Life Cycle of the Schistosomiasis

The life cycle of the three main species of schistosome infecting humans, *S. mansoni*, *S. haematobium*, and *S. japonicum*, is described in *(*Figure 2).

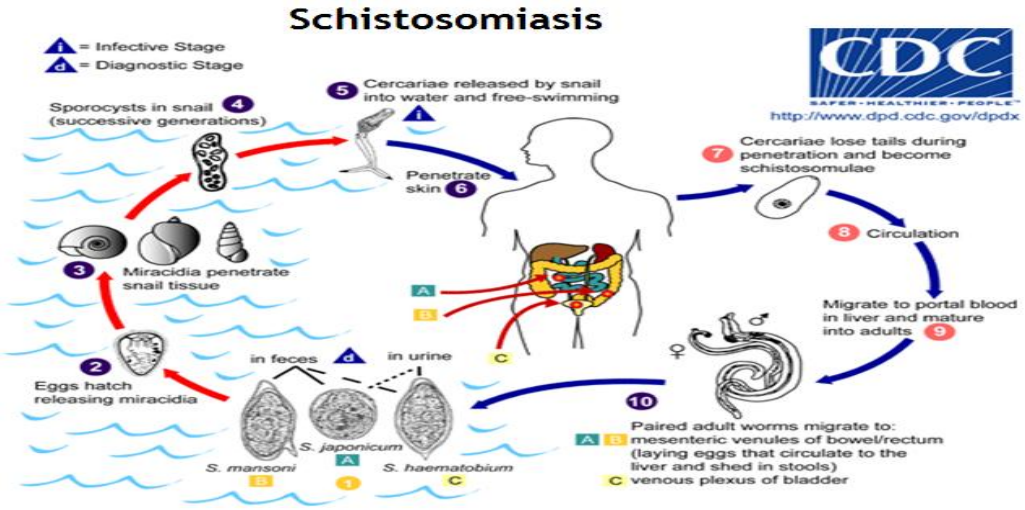

Figure 2: Life cycle of schistosomiasis (Source: CDC, 2012)

Steps 1–3: When people infected with schistosomiasis parasites urinate or defecate in freshwater, parasite eggs pass from the body. Once in freshwater, the eggs hatch and infect freshwater snails that serve as an intermediate host. The parasites develop and multiply inside the snails.

Steps 4–5: During its larval stage, the parasite emerges from infected snails back into fresh water, where they can survive for about 48-72 hours.

Step 6: Free-swimming larva penetrate a person's skin.

Steps 7–10: Once in the body, the larvae develop into adult male and female parasites, which can live, mate, and multiply in blood vessels for as long as 7 years. Female parasites release thousands of eggs, some of which are passed out in the urine, in the case of urinary schistosomiasis, or feces in the case of intestinal schistosomiasis. Some eggs remain trapped in body tissues (CDC , 2012).

A serious acute illness accompanied by fever and lymphadenopathy, known as Katayama Syndrome, can result from heavy schistosome infections. Symptoms of schistosomiasis are caused by the body's reaction to the worms' eggs, not by the worms themselves. Chronic disease is mostly due to perforation of blood vessels and entrapment of eggs by host tissues. The host's reaction to entrapped eggs results in granuloma formation. *S. haematobium* causes bladder wall pathology, leading to ulcer formation, hematuria, and dysuria. Granulomatous changes and ulcers of the bladder wall and ureter can lead to bladder obstruction, dilatation, secondary urinary tract infections and subsequent bladder calcification, renal failure, lesions of the female and male genital tracts, and hydronephrosis. *S. haematobium* is also associated with increased risk of bladder cancer. The morbidity commonly associated with *S. mansoni* infection includes lesions of the liver, portal vein, and spleen, leading to periportal fibrosis, portal hypertension, hepatosplenomegaly, splenomegaly, and ascites (Ross *et al*., 2002; Gryseels *et al*., 2006; Cheesbrough, 2009).

The intensity of infection correlates with severity of infection (Chitsulo *et al*., 2004)**.** Both prevalence and intensity of infection increase with age, peaking in the 5 to 14 years age group (WHO, 2006).

In children the disease contributes to stunted growth, impaired cognitive development, malnutrition, and anemia and disrupts school attendance and even sometimes death, because of their hygiene and play habit in water, and this can also hinder people's ability to work and economic development of the society (WHO, 2011).

The gold standard for schistosomiasis diagnostic is the examination of stool specimens by microscopy to detect the presence of parasite eggs (Feldmeier, 1993). The eggs of intestinal schistosomiasis can be detected in stool specimens through a technique using methylene blue stained cellophane soaked in glycerin or glass slides, known as the Kato-Katz technique (Katz *et al.*, 1972), which is the current widely used diagnostic approach and WHO recommended screening standard for intestinal schistosomiasis (WHO, 1991, 1994). For people from non-endemic or living in low transmission areas, serological, immunological, imaging and molecular tests may be useful in showing exposure to infection and the need for thorough examination and treatment (WHO, 1994).

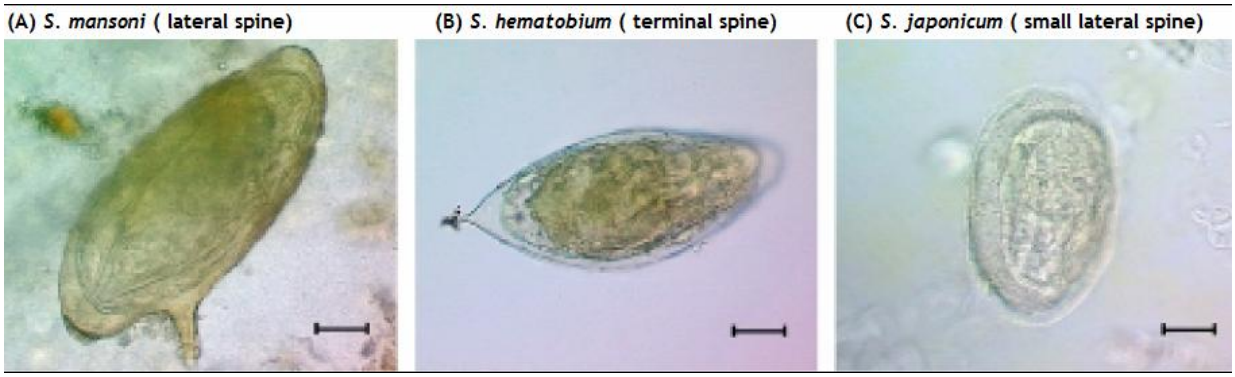

Figure 3 : Schistosome eggs of different species (A) *S. mansoni* (B) *S. hematobium* (C) *S. japonicum. (*Adapted from Gryseels *et al., 2006)*

Schistosomiasis are treatable using inexpensive drugs and/or behavioral change; the challenge in the resource-poor setting of sub-Saharan Africa therefore lies in implementing an effective, wide-scale series of control programs. Praziquantel is the recommended treatment against all forms of schistosomiasis (WHO, 1995, 2006)**.**

The control of schistosomiasis is based on large-scale treatment of at risk population groups with praziquantel, accompanied by access to safe water, improved sanitation, hygiene education and snail control. The current WHO strategy for schistosomiasis control focuses on reducing disease principally through periodic, targeted treatment, through what is called "Mass Drug Administration" (MDA) or simply called preventive chemotherapy (PC) to school age children and other risk groups with praziquantel.

The WHO guidelines identify three treatment strategies based on community prevalence and infection intensity (Egg per Gram, epg) as follows: 1) in communities with a high prevalence (more than 50 %) and heavy intensity (≥400 epg) , where universal treatment is conducted once a year; 2) in communities with a moderate prevalence (≥ 20% but <50%,) and moderate intensity infection (100-399 epg) treatment is once every 2 years; and 3) in communities with a low prevalence (<10%) and low intensity infection (1-99 epg) chemotherapy should be available in nearby health facilities for treatment of confirmed cases (WHO, 2006).

1.2. Statement of the problem

Helminth infections caused by schistosomes and STHs are among the most prevalent infections of humans living in areas of poverty in the developing world (WHO, 2002).

Schistosomiasis is the second most devastating parasitic disease in tropical countries both socio-economically and public health wise (in terms of the number of people infected and those at risk) next to malaria and is the third most prevalent parasitic disease in the world.

More than 207 million people are infected worldwide, with an estimated 800 million people at risk in 78 endemic countries and leading to an estimated annual loss of between 1.7 and 4.5 million DALYs (Lammie *et al*., 2006 ; Steinmann *et al*., 2006; Tchuenté, 2008).

It is one of the Neglected Tropical Infectious Disease (NTDs), which is so called because it primarily persist in the poorest and rural communities of developing countries, and have been largely eliminated and thus forgotten in advanced countries (WHO, 2010; Choffnes *et al*., 2011).

Schistosomiasis is now largely restricted to Sub-Saharan African countries where more than 90% of the estimated 207 million people infected world-wide occur and responsible for the death of more than 300,000 people annually in Africa region alone where *S. mansoni* and *S. haematobium* are widespread (Hotez & Fenwick, 2009).

In Ethiopia, a systematic review and meta-analysis published in 2012 estimated that more than 5 million people are believed to be infected with schistosomiasis (*S. mansoni* and *S. hematobium*) and more than 37 million to be at risk of infection and the overall prevalence of the disease is 25% (Deribe et al., 2012). Intestinal schistosomiasis, due to *S. mansoni* infection has a wide distribution in several localities of the country with varying magnitudes of prevalence as high as 90% in school children (Erko *et al*., 2001; Ali *et al.*, 2006).

Jimma zone is one of a region where *S. mansoni* infection is documented according to the previous survey conducted by different individuals (Mengistu *et al*., 2004 ; Mengistu *et al*., 2011; Bajiro *et al*., unpublished data).

The morbidity caused by STHs and schistosomes is most commonly associated with infections of heavy intensity. School age children (SAC) in the developing world are at highest risk of morbidity

due to intestinal schistosomiasis because they likely to spend time swimming or bathing in water containing infecting stage of parasite cercariae, hygiene and play habits make children especially vulnerable to infection (WHO; 2006, 2011)**.**

Schistosomiasis is the prototype of NTD whose control is advocated by the WHO and other international agencies with the strategy based on preventive chemotherapy (WHO, 2010, 2011).

In the current era of preventive chemotherapy, that is the mass drugs administration of praziquantel to SAC and other populations at risk of morbidity, accompanied with access to safe water, improved sanitation, hygiene education and snail control (WHO, 2006, 2011).

The WHO has formulated a roadmap to guide implementation of the policies and strategies set out in a global plan to combat NTDs (period 2008–2015), and more than 70 pharmaceutical companies, governments, and global health organizations committed to supporting this roadmap (WHO, 2011) in the London Declaration on NTDs in January 2012 by sustaining or expanding drug donation programs (NTD Partner Website, 2012). These pledges of drug donations are now being stepped up. However, there are different factors that might hinder the success of these programs, of which rapid assessment of the prevalence and intensity of infections take the frontline, because most studies and survey relied on standard quantitative techniques.

So far rapid and cost effective techniques to undertake epidemiological survey, estimate intensity (burden) of infection and monitoring the impact of control intervention are lacking.

Therefore development of new, rapid and inexpensive (cost effective), simple, and yet accurate, diagnostic approaches for the prevalence and intensity of schistosomiasis constitute an important part of the strategy.

The assessment of schistosomiasis infection intensity is traditionally achieved through the examination of individual stool samples. However, this strategy hampers the improvement of epidemiological surveys, burden of disease estimation and monitoring of the impact of control measures that is required to support evidence based health care decision makers, in terms of labor cost, time and material costs.

Thus, pooling of stool sample and examination might serve as the new possible alternative to assess/measure the intensity of schistosomiasis infection in resource limited countries in comparison to individual examination (Mitchell & Pagano, 2012; Mekonnen *et al.*, 2013) **.**

In animal health it has been shown that pooling stool samples allows for a rapid assessment of infection intensity and drug efficacy. Pools of up to 5- 10 animals provided estimates of intensity of helminth infections by means of fecal egg counts (FECs) comparable to those obtained by examination of individual fecal samples (Eysker *et al*., 2008) .

In human pooling stool samples of the different individuals has been validated and found valuable for the diagnosis of STHs using McMaster technique (Mekonnen *et al.*, 2013)**.**

The definite diagnosis of *S. mansoni* infection requires the demonstration of eggs in stool samples and an ideal diagnostic technique to be applied in routine and field laboratories of intestinal schistosomiasis endemic countries should be able to combine robustness, simplicity, low cost and good sensitivity (WHO, 1994).

The Kato-Katz technique (Katz *et al*., 1972) originally developed for detection and quantification of *S. mansoni* eggs in human stool samples , is the only available quantitative technique currently most widely used and found to be the recommended diagnostic method of choice by WHO for *S. mansoni***.** In fact, the method is relatively direct, requires minimal equipment which is mostly reusable, and hence the method is thought to be inexpensive. Moreover, the Kato Katz method is simple to apply and laboratory workers can be familiar easily (WHO, 1991, 1994).

However, studies evaluating and validating a pooling strategy for human schistosomiasis for settings with low to moderate and high prevalence and cost effectiveness related to time required for the preparation and examination of both pooled and individual stool samples are lacking.

Therefore, the ultimate aim of this study was to evaluate pooling of stool samples as a rapid and cost-effective alternative to individual stool examination for the assessment of *S. mansoni* infection intensity and as well STHs in Jimma Zone of South West, Ethiopia.

CHAPTER TWO

2. LITERATURE REVIEW

Schistosomiasis is distributed throughout the tropics and subtropics, which is next to malaria in terms of socioeconomic and public health importance. The prevalence of the disease is higher in sub-Saharan countries including Ethiopia. Children whose age ranged 10-14 are the most affected groups (WHO, 2002).

According to a study conducted to determine the factors associated with *S. mansoni* infection in a population of Minas Gerais, Brazil, the prevalence of *S. mansoni* infection among school children was 8.6% (Massara *et al*., 2004).

In study conducted to determine the emergence of *S. mansoni* infection in Upper Egypt, Giza governate the prevalence of *S. mansoni* among school children was 57.7% by the Kato Katz technique. (Talaat *et al*., 1999).

A study conducted near Lake Victoria in Kenya among children of 10-12 years has shown 16% prevalence of *S. mansoni*. According to this study the prevalence increased with each year of age, which was consistent with typical age prevalence curve that peak in early adolescence (Handzel *et al*., 2003). In another study conducted in Busia, District , Kenya , to describe the pattern of single and multiple helminth infection in school children , the overall prevalence of *S. mansoni* was 22% (Brooker *et al*., 2000).

According to a parasitological survey conducted in southern Sudan, in Lui and Nayal (upper Nile region) on *S. hematobium* and *S. mansoni* infection among school children, the prevalence of *S. mansoni* infection was 70% (Deganello *et al*., 2007).

In Ethiopia the endemicity of schistosomiasis has long been established, and new foci have also been continuously discovered. *S. mansoni* is recorded from 50% of the communities studied with prevalence rates ranged from less than 1% up to more than 90% (Tedla and Jemaneh, 1998). From the result of 219 communities surveyed the prevalence of *S. mansoni* was 15%. In general the overall prevalence of *S. mansoni* in Ethiopia ranged between 15-20% (Kloos and Tesfamichael, 1998).

Many studies had been conducted to determine the prevalence and intensity of *S. mansoni* infection in different parts of Ethiopia. According to a study conducted among students of Gorgora town, North West Ethiopia, the prevalence of *S. mansoni* was 20.6% with moderate mean of intensity infection (125 epg). And almost a similar prevalence was observed in the age group 5-9 and (15%), 10-14 (20.9%) and 15-19 (22%) respectively (Essa, Birhane, Endris, Moges, & Moges, 2013) .

Another study conducted among students attending elementary school of Amibera District, Ethiopia, the over prevalence of *S. mansoni* was 0.8% (Awoke, Bedimo, & Tarekegn, 2013). A similar study conducted among primary school students in Adwa, North Ethiopia, the prevalence of *S. mansoni* was found to be 58.7% with mean intensity of infection (95.8 epg) and the highest prevalence was found in male (70.7%) and female (57%) (Legesse *et al.*, 2010).

Another study also conducted among school children attending Hayk number 1 and Hayk number 2 primary school of Hayk area, North East, Ethiopia, the prevalence of *S. mansoni* was found to be 45% with mean intensity of infection (161 epg) and children in the age groups 15-19 had the highest infection rate, followed by 10-14 and 5-9 years age groups (Amsalu & Erko, 2010).

A study conducted among school children and residents of Bushulo Village near Lake Hawassa, Southern Ethiopia, the overall infection rates of *S. mansoni* was found to be 73.7% (Terefe *et al*., 2011). A similar study was conducted in school children in Tikur Wuha area, Southern Ethiopia, the prevalence of *S. mansoni* was 12% with mean intensity of infection (69 epg) by Kato Katz technique (Mitiku, Legesse, Teklemariam, & Erko, 2010).

Jimma is one of the areas where *S. mansoni* infection is prevalent and documented by different researchers. According to the study conducted among Jiren elementary and junior secondary School students 0.3% of *S. mansoni* was observed (Haile,1994). Another study conducted among individual living nearby the three river of Jimma Town, South West Ethiopia , the mean prevalence of *S.mansoni* was 26.3% with mean intensity of infection (108 epg) and the highest prevalence was found in age group 10-14 and 15-19 was 42.9% and 40.9% respectively (Mengistu *et al*., 2011).

Limited studies were conducted to reduce the labor cost, time, and material costs of processing and examining individual stool specimens. A possible alternative to individual stool examination is the examination of pooled stool samples. Pooling of the same individual has been found efficient,

valuable, practical and cost effective for diagnosis of various pathogens, including Giardia (Wahlquist *et al*., 1991), Chlamydia (Shipitsyna *et al*., 2007), Salmonella (Singer *et al*., 2006), and HIV (Verstraeten *et al*., 1998).

According to a study conducted in a children attending 14 primary school in Jimma, South west Ethiopia, individual and pooled samples were examined with the McMaster egg counting method, for each of the three STHs (*A. lumbricoides, T. trichiura* and hookworms) they found a significant positive correlation between mean fecal egg counts (FECs) of individual stool samples and FEC of pooled stool samples, ranging from 0.62 to 0.98 (Mekonnen *et al*., 2013).

However, studies validating pooling strategy for the detection and quantification of *S. mansoni* and human STHs infection intensity as a rapid cost effective assessment, by Kato Katz technique are lacking.

Thus, the present study facilitated a rapid and cost effective assessment of *S. mansoni* and human STHs infection intensity in Jimma Zone of South west Ethiopia.

Significance of the study

Recently, there has been increased interest in the development and assessment of control and elimination programs for schistosomiasis. For design of effective control programs, it is important to determine an accurate estimate of infection intensity and prevalence in the program area.

To reduce screening costs by reducing the number of slides examined, without sacrificing any accuracy, pooling may be a new possible alternative way to measure intensity of *S. mansoni* infection in comparison to individual examination.

Therefore, the significance of this study was to substantiate pooling may be a cost effective strategy for rapid assessment of *S. mansoni* infection intensity for epidemiological survey and thereby facilitating control measure through MDA compared to individual stool samples.

Finally, the outcome of this study may help to guide evidence based healthcare decision makers and researchers in budget planning and funding for epidemiological surveys, undertaking the control measure through MDA and monitoring of control interventions in case of limited resources.

CHAPTER THREE

3. OBJECTIVES

3.1. General Objective

To compare individual and pooled stool samples for the assessment of *S. mansoni* and STHs infection intensity as a cost effective strategy in Jimma Zone, South West, Ethiopia from February – May, 2014.

3.2. Specific Objectives

1. To compare infection intensity of *S. mansoni* by individual stool examination with those by pooling stool samples using Kato Katz thick smear techniques
2. To compare infection intensity of STHs by individual stool examination with those by pooling stool samples using Kato Katz thick smear techniques
3. To compare the time required for assessing infection intensity obtained by individual stool examination with those by pooling stool samples
4. To determine the prevalence and infection intensity of *S. mansoni* and STHs

CHAPTER FOUR

4. MATERIALS AND METHODS

4.1. Study area and period

The study was carried out in Jimma Town and Kore village (Mana district), Jimma Zone South West Ethiopia, From February – May, 2014.

Jimma is located 352 kilo meters southwest of Addis Ababa having a latitude and longitude of $7^0$40'N$36^0$50'E, with an area of 50.52 square km. It is characterized by a semi-arid type climate with an average annual rainfall of 800–2,500 mm. The mean daily temperature is 19 °C, and ranges from 12 to 30°C. It is located 1,720–2,010 m above sea level. The town has a population density of 174,000, according to the data from the towns' municipality 2011. Public health facilities in Jimma Town include Jimma University Specialized Hospital (JUSH), Shenen Gibe Hospital, Jimma health center, higher two health center and Mendera Kochi health center, and a couple of private clinics.

Kore village is found in Mana district of Jimma zone and located 32 Km to the west of Jimma Town situated 384 km from the capital Addis Ababa. The district is found at an average altitude of about 1,450 m above sea level. The district is generally characterized by warm climate with a mean annual maximum temperature of 25°C and a mean annual minimum temperature of 18°C. The annual rainfall ranges from 1138-1690 mm. Kore health center is the only health service providing facility.

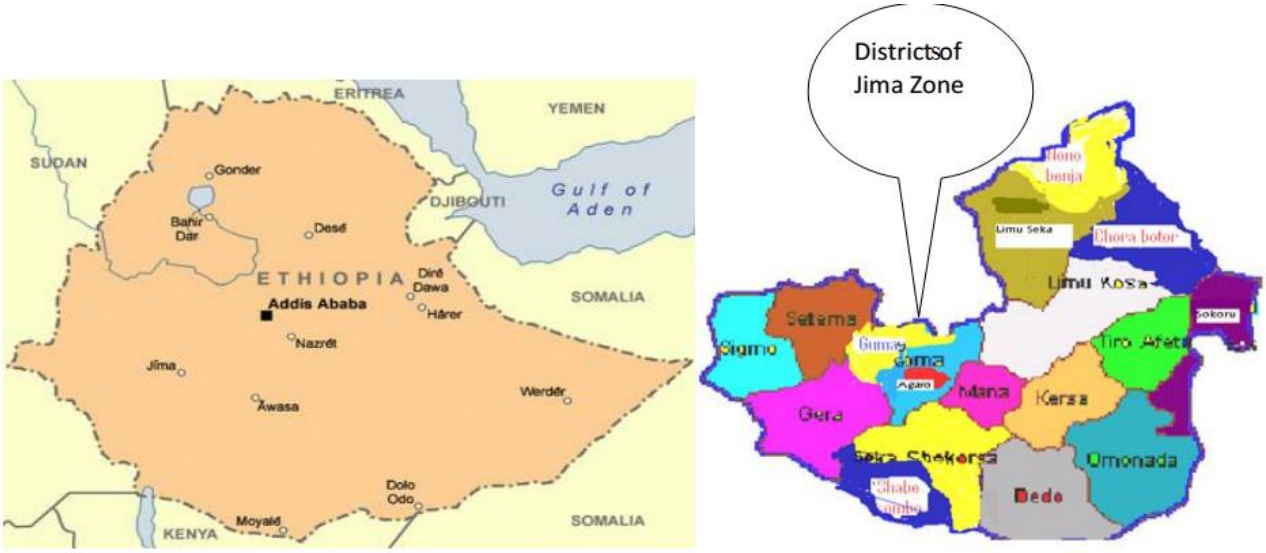

Figure 4: Map of Jimma zone with in Ethiopia (Source: Jimma Zone administration)

4.2. Study design :

School based cross-sectional study design was employed

4.3. Population :

4.3.1. Source population

All school age children (5-18) in the respective study areas (Jimma Town and Kore village) were used as a source population.

4.3.2. Study population

School age children, who were actively involving in learning process and voluntarily selected for this study were used as study population.

4.3.3. Study unit

Individuals (students), who fulfills the eligibility criteria

4.4. Eligibility Criteria

4.4.1. Inclusion Criteria

(i) School children whose parents or guardians signed the written informed consent , (ii) School children expressed assent verbally in responding providing stool samples , (iii) Schools hosting grade 1-8 students and provided at least 60 samples (iv) Schools children from age 5-18 years and (v) Schools children that can offer sufficient amount of stool samples were incorporated in this study.

4.4.2. Exclusion Criteria

(i) Subjects who were unable to provide sufficient amount of stool sample (ii) Subjects who did not give written informed consent and assent (iii) Subjects who treated with praziquantel and albendazole for the last two months.

4.5. Sample size estimation

Sample size was estimated by using sample size determination technique for correlation (Assumption: *Power* = 80%, *a*= 5% and Rs= 0.60 (from previous study result on pooling (Mekonnen *et al.,* 2013)) using **StatsToDo** (Sample size for correlation program) free online Software (available at: http://www.statstodo.com/SSizCorr_Pgm.php ,accessed on December 12/2013, at 11: 32: 13) n = 16 (pool size) since we intended to pool, pools of 5, 10, and 20. By taking the maximum pool size which is 20, therefore **n** (individual sample size) = 20x16 = 320 if we incorporate pool of 60 for simplicity and uniformity and the recommended sample size per school for pooling (Mekonnen *et al.,* 2013) we need to add 40 samples which is n= 320+40= 360 =6x60, therefore a total of 360 study participant were recruited.

4.6. Sampling technique/ sampling procedures

The study was conducted in three primary schools in Jimma Town and three primary school in Kore village, a total of six public primary schools hosting 1-8 grades of students were selected purposively (Figure 5), based on their endemicity level as follow. Among a total of 11 government primary schools found in Jimma Town, only three schools namely Mendera, Kito and Seto Yido were selected based on the prevalence and their low-moderate endemicity (Bajiro *et al*., unpublished data). And three primary schools namely Saye Oddo, Wollo Sefer and Kore Konjo among 4 primary schools found in Kore village were recruited. Pre –visit to this schools indicated substantial *S. mansoni* prevalent area.

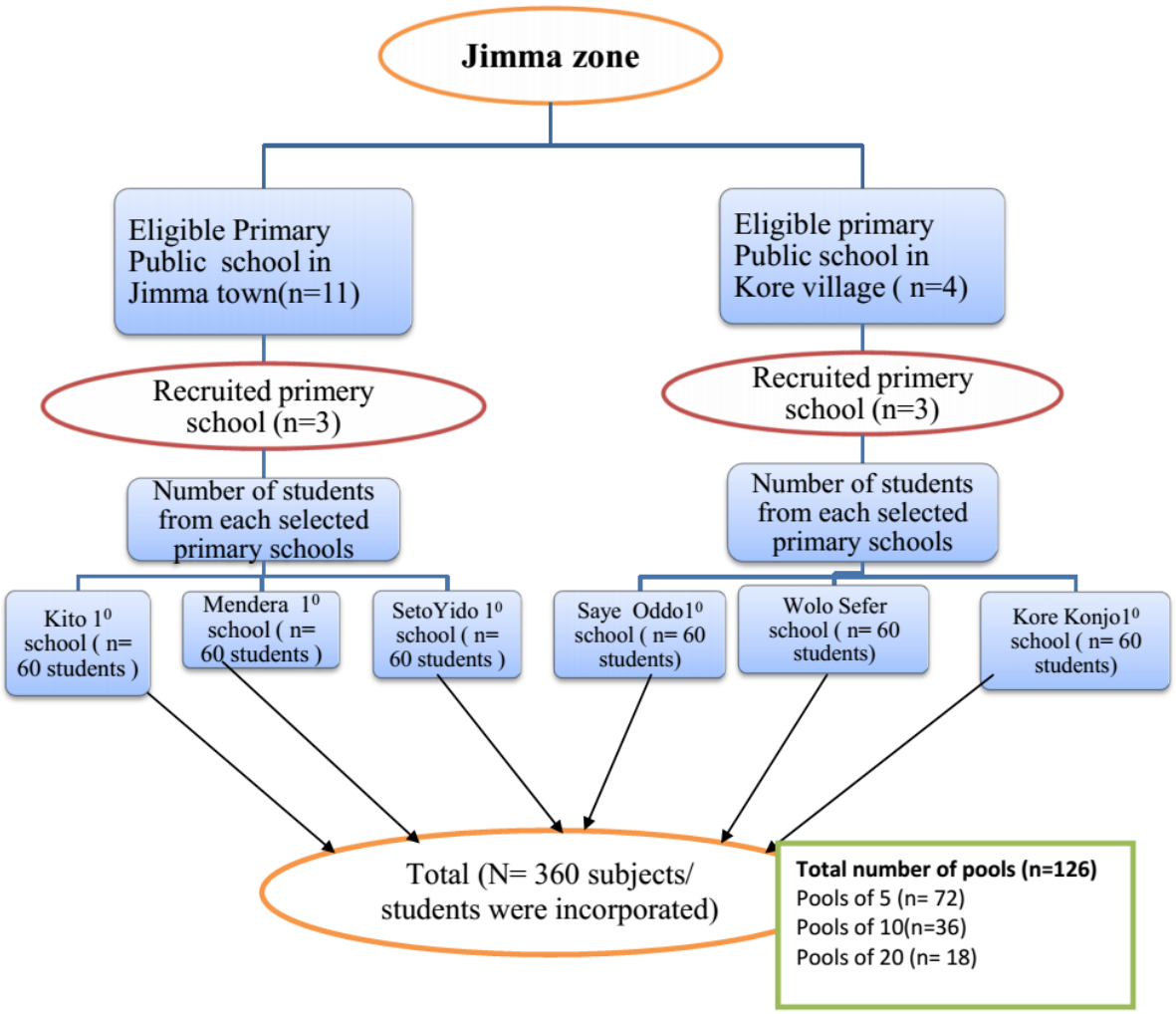

Figure 5 : Number of schools and stool samples for assessing infection intensity of *S. mansoni* and STHs in Jimma Zone, South West, Ethiopia, from February – May, 2014.

In each school subjects were stratified, according to three age classes (age class A: age 5–9 years, B: age 10–14years, and C: age 15–18 years). For each age class at least 20 subjects were selected by simple random sampling technique, on a voluntary basis, resulting in a total of at least 60 subjects per school.

4.7. Ethical Consideration

The study was approved by the Research Ethics Review Board of Jimma University. Official letter was written form Jimma University, College of Public Health and Medical Science, Department of Medical Laboratory Science and Pathology to the respective schools. Prior to the survey the school directors, teachers, and the children were informed about the purpose and procedures of the study and permission also obtained. The written informed consent form was prepared in English and translated in to the two commonly used local languages (Afaan Oromo and Amharic) and read and handed over to the children's parents/caretakers. Only those children (i) who were willing to participate and (ii) whose parents or caretakers signed the written informed consent form were included in the study. Moreover, an additional separate written informed consent form for children older than 12 years were prepared, read, and handed over to them and their additional written informed consent were obtained. Any information obtained from participants during the study was kept confidential. Children who were found positive for helminthes (*S. mansoni* and STHs) infection were treated free of charge with praziquantel (single 40 mg/kg oral dose) and albendazole (single 400 mg oral dose) according to WHO recommendations (WHO, 2006), by clinicians. In addition, the clinicians and the PI gave them health education relevant to helminthic infection prevention and control.

4.8. Stool collection and parasitological examination

Principal investigator (PI) interviewed the study subjects about their voluntariness and socio-demographic status (Name, Age, Sex, Grade level and Address) using semi-structured questionnaire/tool.

After getting their written consent and assent, each child was registered and identification number was assigned and stool samples was individually collected from all participants. The study participants were provided with labeled screw caped stool cap and informed on how to collect at least 5 gm stool sample of their own. This quantity of stool was required to examine the samples individually and to pool individual stool samples for Kato Katz. The collected stool samples were soon transported to Jimma University NTDs laboratory where it was processed and analyzed within 24 hours (figure 6) following the standard operating procedures of Kato-Katz technique, as described elsewhere (WHO, 1994).

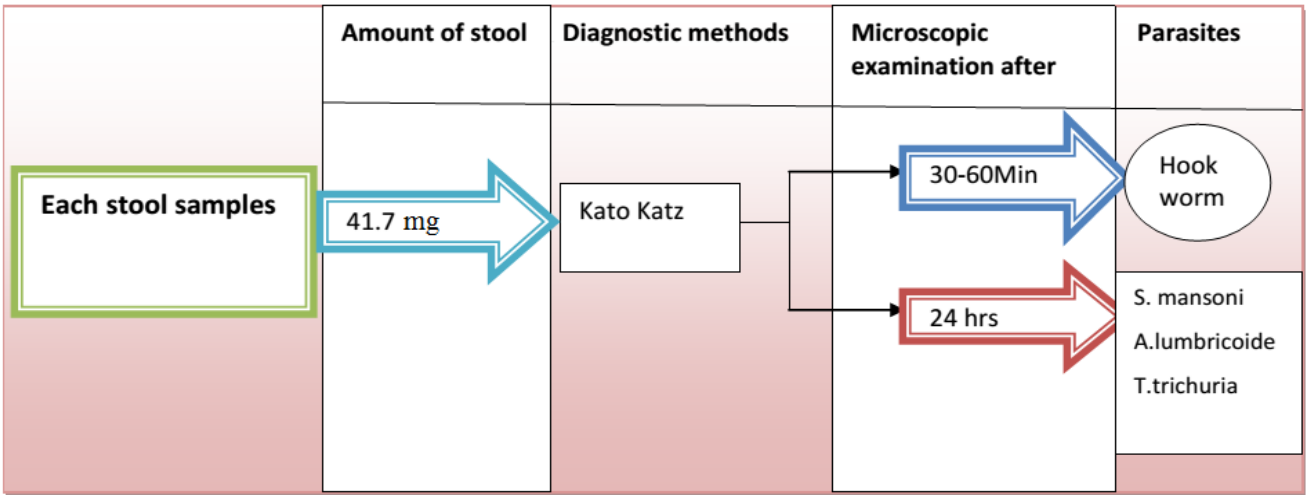

Figure 6: Diagnostic method used to detect *S. mansoni* and soil-transmitted helminth infections. The flowchart details the diagnostic approach and its temporal sequence, as well as the amount of stool examined for the detection of helminth eggs, applied to 360 stool samples from school children in Jimma zone, South West, Ethiopia, in February-May 2014.

The Kato-Katz was performed using the 41.7 mg template (prepared from 1 gm of stool), according to the WHO recommendation (analytic sensitivity = 24 eggs/ per gram of faeces) (WHO, 1994). Before reading the smear, an average time of 30-60 minutes were needed for STHs and 24 hr for *S. mansoni* clearance. Eggs of *S. mansoni* and STHs were detected and counted systematically under light microscope using 100X magnification and the FEC, expressed as epg for each helminth species, obtained by multiplying the total number of eggs counted under the microscope by a factor 24.

In addition a subset (1 gm) from each stool samples were pooled in pools of 5, 10 and 20 individual samples, considering pooling may be a rapid alternative to individual stool examination (Figure 7).

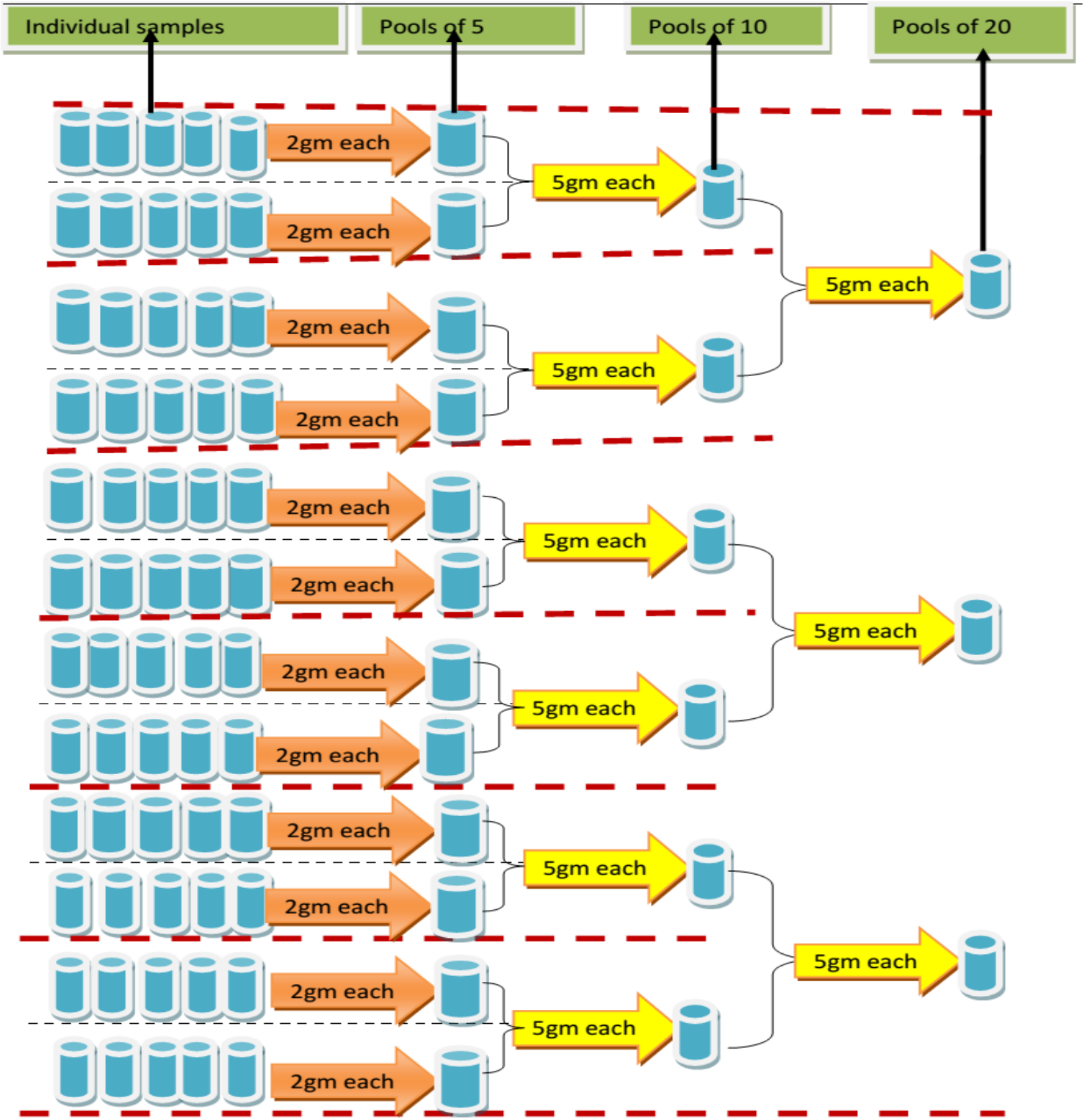

Figure 7: Procedure to obtain pools of 5, 10 and 20 individual stool samples. Sixty individual samples were arranged in 12 rows with each row consisting of 5 individual samples, subsequently 12 pools of 5, 6 pools of 10 and 3 pools of 20 individual samples, resulting in total of 21 pooled samples per school. NTD and STHs Lab. Jimma University, February-May 2014.

For uniformity at first, 60 individual samples were randomly organized in 12 rows of 5 individual stool samples. From each row 2 gm of each of the 5 individual stool samples were transferred into a new pre-labeled plastic beaker (resulting in a total of 12 pools of 5 individual stool samples). Then homogenization was followed, it was standardized by means of stirring the stool samples until homogenized. Stools from different subject have different colors. We stopped stirring the pooled stools sample when the pool had one homogenous color. Then 5 gm from each 2 plastic beakers representing pools of 5 individual samples were transferred into another new pre-labeled plastic beaker, resulting in a total of 6 pools of 10 individual samples. Again after homogenization 5 gm from each 2 plastic beakers representing pools of 10 individual samples were transferred into another new pre-labeled plastic beaker, resulting in a total of 3 pools of 20 individual samples.

Finally, each of the pools was processed by the Kato Katz thick smear only as done for individual samples. This pooling procedure may have two important advantages. First, the cascade procedure applied (e.g. we pooled pools of 5 to make pools of 10 etc…) allow for pooling samples into different pool sizes with only 1 gm per individual sample. Second, it avoids the homogenization of too large quantities of stool.

For the assessment of infection intensity, samples were randomized according to age class (4 rows of 5 samples per age class). And subjects were classified as light, moderate and heavy infection according to the WHO criteria (for *S. mansoni*: light infection (1-99 epg), moderate (100-399 epg) and heavy (greater than 400 epg) (WHO, 2002) .

Finally, the total time required for each distinct step in the laboratory needed to perform both individual and pooled stool samples, preparation and examination by the Kato Katz technique was measured and recorded in the prepared laboratory format after preparing all the necessary materials and labeling. Then the total time was summed up and the cost effectiveness of pooling stool samples in terms of time was assessed.

4.9. Quality Control

In order to ensure quality of the data, **e**ach of the questionnaires was checked whether the necessary information's were properly filled (name, age, sex, and grade level of the participants)**.** All the necessary reagents and equipments were checked by known positive and negative samples before sample preparation and examination using already preserved stool samples. The smears were examined independently by two experienced trained and certified laboratory technologists for Kato

Katz techniques blindly to avoid observer bias and finally the results were checked by the principal investigator in case where the results were discordant , the results of the third expert reader were considered as the final results **.** The samples were processed within 4 hours on average. Verification of the sensitivity of the scale weighing the stool samples was ensured. Supervision of the Kato-Katz technique procedure was done regularly. Finally re-examination of 10% of the Kato-Katz slides was examined by a senior medical lab. technologist and principal investigator were checked.

4.10. Data processing and analysis

Data were coded, entered and cleaned before and during data processing using statistical software Epidata programme (version 3.1; Area of Health Analysis and Information Systems Pan American Health Organization, January 2006) and were exported to SPSS for windows version 20 statistical software package for analysis of variables (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.). Simple frequency, percentages and Pearson chi-square were used as the statistical parameter in the descriptive analysis to determine prevalence and distribution of *S. mansoni* and STHs.

The infection intensity was determined for *S. mansoni* and STHs (*A. lumbricoides, T. trichiura* and hookworm), and expressed as arithmetic mean epg of stool for each individual and each pooled sample. A total of 126 pools (72 pools of 5, 36 pools of 10, and 18 pools of 20) consisting a total of 360 individual samples were pooled.

Then, the agreement between mean FEC based on the examination of individual samples and the pooled sample was evaluated by the Spearman rank correlation coefficient. In addition Wilcoxon rank sum test was applied to test for differences in mean FEC between examination of individual and pooled samples.

Furthermore we summed up the total time required to prepare and examine both individual and pooled samples to determine the cost effectiveness of pooling stool samples in terms of time.

A p-value of < 0.05 was considered to be statistically significant.

Finally, the results were displayed using tables, and figures.

Operational definition

Eggs per Gram (epg): The number of parasite eggs per gram of stool samples, which provides an indirect measure of the intensity of helminth infection.

Intensity of infection: The number of helminths infecting an individual.

Prevalence of infection: Percentage of individuals in a population who are infected.

Schistosomiasis: Parasitic disease caused by schistosomes.

School-age children: Usually defined as children between 5 and 14 years of age who may or may not be enrolled in school.

Soil-transmitted helminths: Four species of nematodes are collectively referred to as "soil-transmitted helminths": the roundworm, *Ascaris lumbricoides*; the whipworm, *Trichuris trichiura*; and the hookworms *Necator americanus and Ancylostoma duodenale*.

4.11. Dissemination Plan

The finding or the result of this study will be submitted to Jimma University College of Public Health and Medical Sciences, Department of medical laboratory sciences and pathology. It will also be presented to Jimma university scientific community through thesis defense. Then the final report will be disseminated to the concerned bodies so that the outcomes of this study will be used by healthcare policy makers, budget planners , researchers and other health professionals for epidemiological survey, undertaking the control measure through mass drug administration and monitoring of control measures in prevention and control of *S. mansoni* and soil transmitted helminthiasis. Furthermore, great endeavors will be made to publish on peer-reviewed journal nationally or internationally and present in meeting /conferences.

CHAPTER FIVE

5. RESULTS

5.1. Description of socio-demographic characteristics of the study participants

A total of three hundred and sixty school children from grade one to eight from six different public primary schools were recruited in the study. The age composition of the study participants ranged from 6-18 years. The mean and standard deviation of age of the study participant was 11.65 (SD± 3.198).

Out of the total study participants 161 (44.7%) were females and 199 (55.3%) were males. Most of the study participants 215 (59.7%) had educational level of grade 4-6, followed by 110 (30.6%) with education level of grade 1-3 and 35 (9.7%) grade 7-8 (Table 1).

Table 1: Socio-demographic characteristics of the study participants among primary school children in Jimma Zone , South West , Ethiopia, February- May, 2014 (n=360).

Socio-demographics	**Frequency (n)**	**Percent (%)**
Age in years		
5-9	120	33.3
10-14	120	33.3
15-18	120	33.3
Sex		
Female	161	44.7
Male	199	55.3
Educational level		
Grade 1-3	110	30.6
Grade 4-6	215	59.7
Grade 7-8	35	9.7
Total	360	100

n - Number of study participants

5.2. Prevalence and infection intensity of *S. mansoni* and STHs infections

Out of 360 study participants in the six public primary schools, 218 (60.5%) of them were found positive for any helminthic (*S. mansoni* and the three STHs) infection.

The prevalence rate of *S. mansoni* and STHs infection were 25.3 % (11.7% for females and 13.6% for males) and 48.3% (22.8% for females and 25.5% for males) respectively. *T. trichiura* was the predominant species (30.6 %), followed by hookworms (21.4%) and *A. lumbricoides* (18.1%), among STHs infection respectively. Among the study participants 14 (3.9%) triple infection, 50 (13.9%) double infection and 110 (30.5%) had single infections with STHs (Figure 8).

The arithmetic mean FEC was 2,596.3 EPG, 126.0 EPG, 47.3 EPG and 40.7 EPG for *A. lumbricoides, T. trichiura, S. mansoni* and hookworms, respectively (Table 2). Moreover the intensity of *S. mansoni* infections ranges from 24 -1536 epg, among those infected subjects, the rates of light, moderate and heavy infections were 56 (15.6%), 21 (5.8%) and 14 (3.9%) respectively.

The prevalence of *S.mansoni* in Jimma Town was 40 (22.2%) of which in Kito 14 (23.3%), Mendera 8 (13.3%) and Seto Yido schools 18 (30%) respectively. Similarly the prevalence of *S.mansoni* in Kore village, Mana District was 51 (28.3%) of which in Kore Konjo 19 (31.7%), Saye Oddo 28 (46.7%) and Wolo Sefer schools 4 (6.7%) respectively (Table 2).

Out of 91 study participants who were infected with *S. mansoni* 36 (39.6%) of the students found in age group of 10-14 years and the prevalence was more in males 49 (13.6%) than females 42 (11.7 %) and grade 4-6 study participants were more infected 61 (16.9%) than the rest (Table 3).

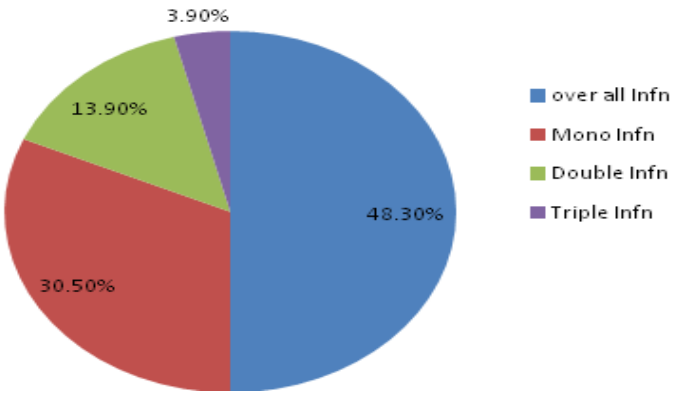

Figure 8: Prevalence of STHs mono parasitism and poly parasitism among primary school children in Jimma Zone South West Ethiopia, February - May, 2014.

Table 2: Prevalence and intensity of *S. mansoni* and STHs infections in Jimma Town and Kore village, Mana District, South West, Ethiopia, February–May , 2014 (N=360).

School Address (Name)		Group total	*S. mansoni*		*A. lumbricoides*		*T. trichiura*		hookworm	
			Prevalence No (%)	**Mean FEC (epg)**	**Prevalence No (%)**	**Mean FEC (epg)**	**Prevalence No (%)**	**Mean FEC (epg)**	**Prevalence No (%)**	**Mean FEC (epg)**
Jimma town (n=60 for each)	Kito	60	14 (23.3)	54.8	16 (26.7)	4,661.6	27 (45)	289.6	5 (8.3)	13.2
	Mendera	60	8 (13.3)	59.6	12 (20)	25.2	23 (38.3)	26.2	6 (10)	42.0
	SetoYido	60	18 (30)	31.2	26 (43.3)	3,279.2	24 (40)	284.8	11 (18.3)	12.0
	Sub total	180	40 (22.2)	40.67	54 (30)	5053.2	74 (41.1)	233.47	22 (12.2)	18.8
Kore Village (n=60 for each)	Kore Konjo	60	19 (31.7)	98.0	1 (1.7)	83.2	19 (31.7)	7.2	19 (31.7)	113.6
	Saye Oddo	60	28 (46.7)	36.0	5 (8.3)	7,218.8	9 (15)	126.0	17 (28.3)	31.2
	Wolo Sefer	60	4 (6.7)	4.4	5 (8.3)	310.0	8 (13.3)	18.4	19 (31.7)	35.6
	Sub total	180	51 (28.3)	54	11 (6.1)	139.47	36 (20)	18.53	55 (30.5)	62.53
Total		**360**	**91 (25.3)**	**47.3**	**65 (18.1)**	**2,596.3**	**110 (30.6)**	**125.4**	**77 (21.4)**	**41.3**
Age groups in years (n=360)	5-9	120	21 (5.8)	21.2	28 (7.8)	2666.6	40 (11.1)	145.2	24 (6.7)	65.2
	10-14	120	36 (10)	44.6	21 (5.8)	2594.8	36 (10)	47	27 (7.5)	25.4
	15-18	120	34 (9.4)	76.2	16 (4.4)	2627.6	35 (9.7)	185.8	25 (6.9)	31.4
Sex (n=360)	Female	161	42 (11.7)	53.4	30 (8.3)	2131.4	50 (13.9)	186.9	37 (10.3)	39.9
	Male	199	49 (13.6)	42.4	35 (9.7)	2972.5	61 (16.9)	76.7	39 (10.8)	41.2
Educational level **(n=360)**	Grade 1-3	110	20 (5.5)	13.7	35 (9.7)	5279.8	45 (12.5)	200.7	23 (6.4)	30.8
	Grade 4-6	215	61 (16.9)	67.6	28 (7.8)	1624.7	61 (16.9)	105.1	47 (13)	48.3
	Grade 7-8	35	10 (2.8)	28.1	2 (0.5)	131	5 (1.4)	19.2	6 (1.7)	24.7

Table 3: The association between the prevalence of *S. mansoni* infection and socio-demographic variables among school children in Jimma Zone , South West , Ethiopia, February - May, 2014 (n=91).

Variables	***S.mansoni* (n (%))**	χ^2	***P*-value**
Age in years		5.8	0.054
5- 9	21 (23.1)		
10-14	36 (39.6)		
15-18	34 (37.4)		
Sex		0.101	0.751
Female	42 (11.7)		
Male	49 (13.6)		
Educational level		4.2	0.121
Grade 1-3	20 (22)		
Grade 4-6	61 (67)		
Grade 7-8	10 (11)		
Address		1.8	0.182
Jimma town	40 (43.9)		
Kore Village	51 (56)		

n - Number of *S. mansoni* infected individual among study participants; x^2- Pearson chi square

As indicated in Table 3, variables such as age, sex, educational level, and address did not show any association with *S .mansoni* ($P > 0.05$).

5.3. Correlation in infection intensity of *S. mansoni* and STHs

Overall, there was a significant positive correlation between mean FEC of individual samples and the FEC of the pooled samples for *S. mansoni* (Rs $_{S.\ mansoni}$ = 0.66-0.75, p <0.01), *A. lumbricoides* ($R_{A.\ lumbricoides}$ = 0.90-0.95, p <0.01); Rs $_{\text{hookworm}}$ = 0.68, p <0.01) and *T. trichiura* (Rs $_{T.\ trichiura}$ = 0.52-0.72, p <0.01).

For hookworm, a positive correlation was found for each pool size, but only for a pool size the correlation was significant different from zero ($R_{\text{pool of 5}}$ = 0.34, p <0.01; $R_{\text{pool of 10}}$ = 0.19, p = 0.26; $R_{\text{pool of 20}}$ = 0.45, p = 0.06) (Figures 9 and 10).

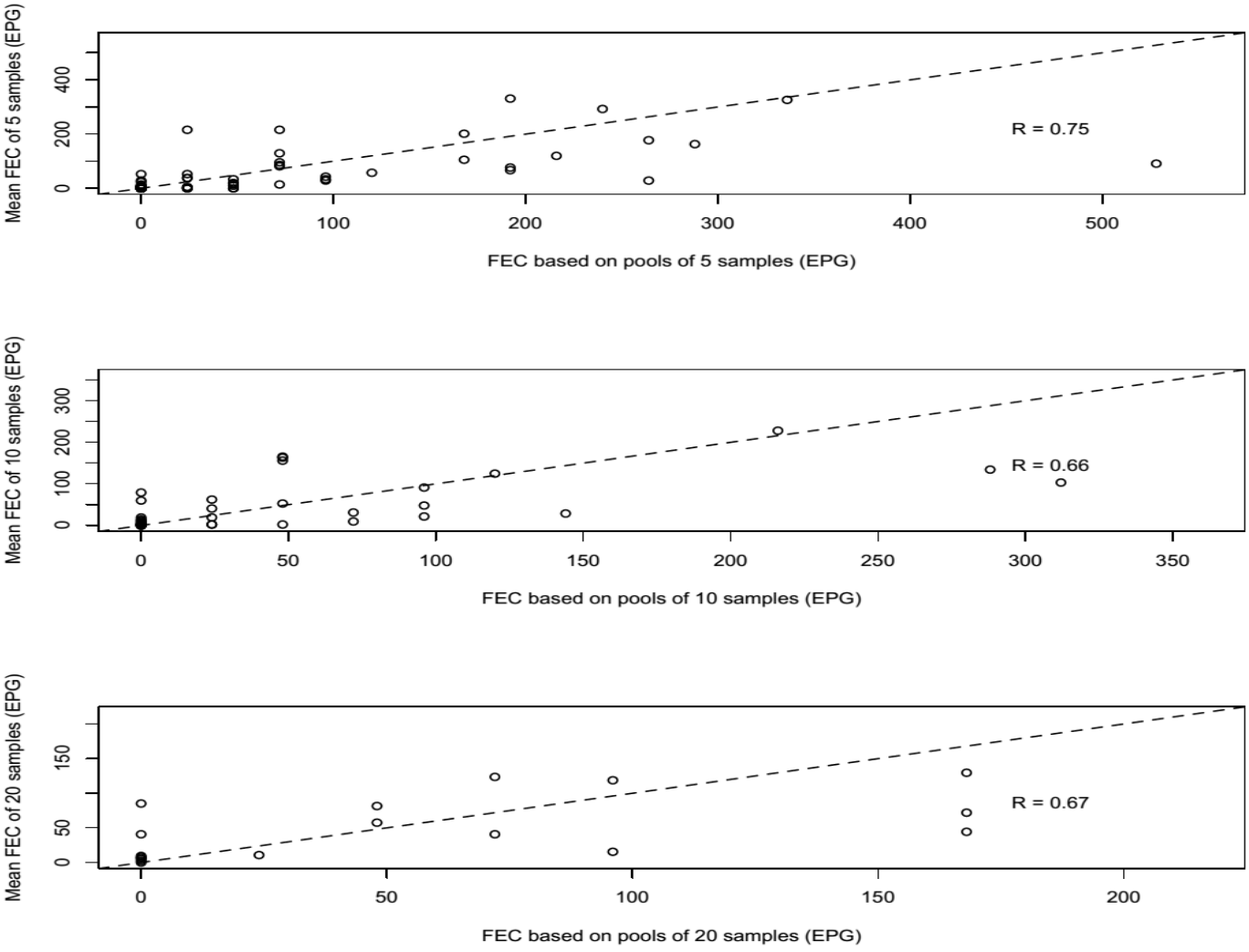

Figure 9: Agreement in FEC of *S. mansoni* between individual and pooled samples. Each of the 3 scatter plots represents the agreement in mean individual FEC and pooled FEC of stool samples. The plots in top, middle and bottom row represent pool sizes of 5, 10 and 20, respectively. The magnitude of correlation for each plot is based on the Spearman correlation coefficient (Rs), Jimma Zone, South West Ethiopia, February- May, 2014.

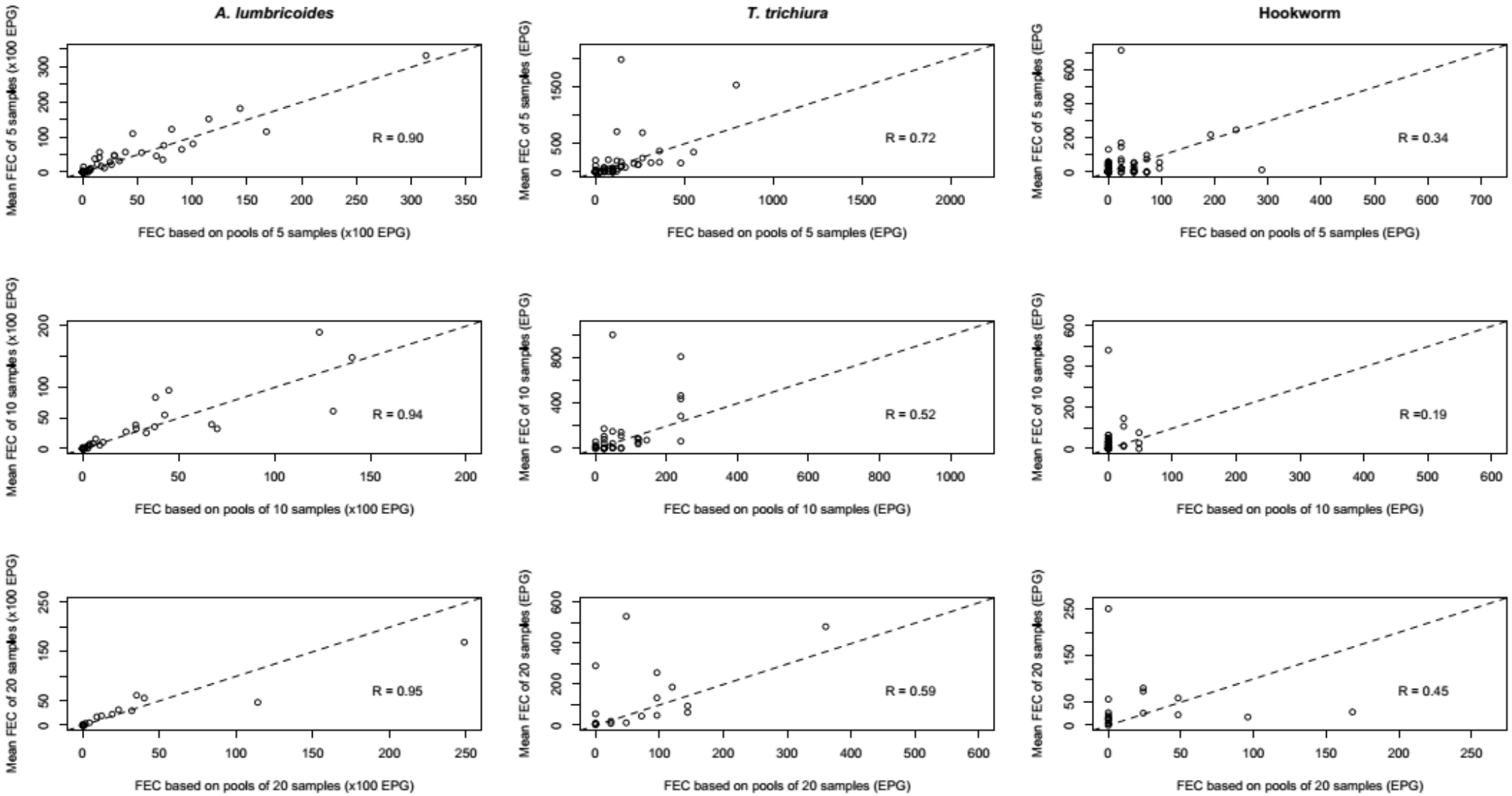

Figure 10: Agreement in FEC of STHs between individual and pooled samples. Each of the 9 scatter plots represents the agreement in mean individual FEC and pooled FEC of stool samples. The plots in column A, B and C represent *A. lumbricoides*, *T. trichiura*, and hookworm, respectively. The plots in top, middle and bottom row represent

pool sizes of 5, 10 and 20, respectively. The magnitude of correlation for each plot is based on the Spearman correlation coefficient (Rs), Jimma Zone, South West Ethiopia, February- May, 2014**.**

5.4. Difference in infection intensity *S. mansoni* and STHs

Table 4 summarizes the mean FEC for both individual and pooled samples. Overall, there were no significant differences in FEC between individual and pooled samples for S. *mansoni* and the three STHs (*A. lumbricoides, T. trichiura* and hookworms). Only for hookworm was a significant difference in FEC observed for a pool size of 10, examination of pools resulting in lower FECs ($FEC_{pools\ of\ 20}$ = 6.7 EPG *vs.* $FEC_{individual\ =}$ 40.7 EPG, p <0.01).

Table 4 : Mean fecal egg counts for *S. mansoni* and STHs based on individual and pooled samples, in Jimma Zone, South West, Ethiopia, from February -May, 2014.

Pool size	Sample size	*S. mansoni*		*A. lumbricoides*		*T. trichiura*		hookworms	
		Mean FEC (epg) *(95% CI)*	P - value	Mean FEC (epg) *(95% CI)*	P - value	Mean FEC (epg) *(95% CI)*	P - value	Mean FEC (epg) *(95% CI)*	P - value
1	360	47.3 (31.2; 66.3)	_	2,596.3 (1,688.4; 3,588.5)	_	126.0 (66.9; 206.1)	_	40.7 (23.9; 64.7)	-
5	72	61.7 (40.7; 86.3)	0.12	2,281.7 (1,271.6; 3,521.0)	0.11	95.3 (65.0; 131.0)	0.39	26.3 (15.7; 39.7)	0.23
10	36	52.0 (29.3; 79.3)	0.68	2,367.3 (1,246.0; 3,688.7)	0.53	73.3 (49.3; 100.0)	0.12	6.7 (2.7; 12.0)	<0.01
20	18	53.3 (26.7; 82.7)	0.64	3,008.0 (853.3; 6,161.5)	0.51	70.7 (36.0; 116.0)	0.11	24.0 (6.7; 46.7)	0.40

*epg: Eggs per Gram of Stool (Arithmetic mean); FEC, fecal egg count; CI, confidence interval

5.5. Assessment of the time required to screen individual and pooled samples

The tables 5 below summarized, the total time required to prepare pooled stool samples and the total time required to prepare and read Kato Katz slides for both individual and pooled stool samples. Thus the total time required to determine individual FECs for 360 samples was 65 hours and 5 minutes; for pooled FECs, this was 19 hours and 12 minutes for pools of 5, 14 hours 39 minutes for pools of 10 and 12 hours 42 minutes for pools of 20.

Table 5: Total times required to prepare and read based on individual and pooled stool samples (Individual stool samples =360, Pooled stool samples=126 (Pools of 5=72, Pools of 10 =36, Pools 20=18)), Jimma Zone, South West, Ethiopia, February - May, 2.

Pool size	Sample size (N)	Total time required to prepare pools stool samples	Average time required to prepare one batch of 10	Total time required to prepare KK slides (Batch of 10)	Total time required to read (examine) KK slides	Total time
1	360	----------	**25.8 min**	15 hr 29 min	49 hr 36 min	65 hr 5 min
5	72	6 hr 35 min		3 hr 6 min	9 hr 30 min	19 hr 12 min
10	36	8 hr 29 min		1 hr 33 min	4 hr 37 min	14 hr 39 min
20	18	9 hr 20 min		46 min	2 hr 36 min	12 hr 42 min

CHAPTER SIX

6. DISCUSSION

S. mansoni and STHs infections are continuous to become a major public health burden in the tropics and subtropics, including Ethiopia which is next to malaria in terms of socioeconomic and public health importance. SAC in the developing world are at highest risk of morbidity due to intestinal schistosomiasis because they tend to spend time swimming or bathing in water containing infecting stage of parasite cercariae, hygiene and play habits (WHO, 2011, 2006, 2002). Health strategy for attainment of effective parasitic disease control programs demand knowledge of magnitude of the disease.

Hence, the present study also provided current information on prevalence and intensity of infections in the study areas. In this study the overall prevalence of *S. mansoni* was 25.3% which is considered a moderate prevalence according to WHO classification of prevalence of *S.mansoni* with low arithmetic mean of intensity (47.3 epg) (WHO, 2002) . This result is lower when compared to similar studies conducted in different areas in developing countries including Ethiopia (Talaat *et al*., 1999 ; Deganello *et al*., 2007 ; Legesse *et al*., 2010 ; Amsalu and Erko, 2010; Terefe *et al*., 2011) . However, this finding is higher when compared to similar studies conducted in different parts of the world including Ethiopia (Handzel *et al*., 2003 ; Massara *et al*., 2004 ; Mitiku *et al*., 2008 ; Awoke *et al*., 2013 ; Haile, 1994).

The differences in findings among the studies might be explained by variations in geography, socio-economic conditions, hygienic conditions of the population under consideration, the category of the study population, the methods employed for stool examination and the time of study. Also this finding is some what comparable with the previous report from different parts of the world including Ethiopia (Brooker *et al*., 2000; Mengistu *et al*., 2011; Essa *et al.*, 2013).

In this studies males than females and children with age group 10-14 years were more infected. These finding is in line with the previous survey conducted by WHO on schistosomiasis and in Kenya and Ethiopia on *S. mansoni* (WHO, 2002; Handzel *et al*., 2003; Essa *et al*., 2013) .This could partly be explained by the fact that males are usually more likely to play in the stream as compared to females and outdoor activities also increase as the age increases. This also holds true for STHs.

Provided that the recent pledges of continuing donations of anthelminthic drugs NTD Partner Website (2012) , and thus prospects of increasing drug pressure on parasite populations, cost-effective tools to guide evidence based healthcare decision makers on how to estimate the burden, on how to optimize treatment strategies and on how to monitor the control of *S. mansoni* and STHs are critically needed.

In comparison with studies conducted in animal health and public health, our results show that pooling stool samples also holds promise as a rapid cost effective strategy in public health as well in assessing infection intensity through epidemiological surveys, undertaking the control measure through MDA and monitoring of control interventions compared to individual stool samples.

In the present study we evaluated our pooling strategy for the assessment of intensity of *S. mansoni* and STHs infections. And we found a significant correlation (coefficient = 0.53-0.95) between the mean of individual FECs and the FECs of pooled samples for *S. mansoni, A. lumbricoides* and *T. trichiura*, regardless of the pool size. The arithmetic mean FEC was 2,596.3 EPG, 126.0 EPG, 47.3 EPG and 40.7 EPG for *A. lumbricoides*, *T. trichiura*, *S. mansoni* and hookworms, respectively. Except hookworm, there was no significant difference in FECs between the examination of individual and pooled stool samples. The finding of this study is partly comparable with a similar study conducted in Jimma Town , South west , Ethiopia (Mekonnen *et al*., 2013), in that they found a significant positive correlation between mean fecal egg counts (FECs) of individual stool samples and FEC of pooled stool samples, ranging from 0.62 - 0.98 for STHs . The minor difference might be attributed by difference in sample size, pools size, and the laboratory method employed for stool examination.

Furthermore, since pooling samples does not provide prevalence data, various models have been developed for other pathogens to estimate prevalence based on pooled samples both in animal health and public health (Wahlquist *et al*., 1991 ; Verstraeten *et al*., 1998 ; Singer *et al*., 2006 **;** Shipitsyna *et al*., 2007).

In addition the present study was also designed to verify/substantiate the cost effectiveness of this pooling strategy in terms of time, hence we assessed the time to screen individual and pooled samples(pools of 5 , pools 10 and pools of 20) , therefore the total time to obtain individual FECs was 65 hr 5 min. For pooled FECs; this was 19 hr 12 min for pools of 5, 14 hr 39 min for pools of 10 and 12 hr 42 min for pools of 20. This implies that in our setting, time in the laboratory can be reduced with 76%, 84%, and 87% when pools of 5, 10 and 20 instead of individual stool samples, are screened respectively.

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATIONs

7.1. CONCLUSION

In the present study we highlighted that pooling stool samples holds promise as a means of rapidly assessing of the infection intensity of *S. mansoni* and STHs on a population level as a cost effective approach in terms of time.

In this study, pooling samples does not provide prevalence data, however various models have been developed for other pathogens to estimate prevalence based on pooled samples both in animal health and public health.

In addition, the present study also revealed the prevalence and infection intensity of *S. mansoni* and STHs in the study areas with moderate level.

7.2. RECOMMENDATIONs

Further insight is required to validate when and how pooling stool samples are applied for prevalence study of *S. mansoni* and STHs.

Research is still paramount important to determine when and how pooling of stool samples applicable with different sample size, diagnostics techniques and areas for a control programs for this *S .mansoni* and STHs.

Moreover further research is demanded, to verify the cost effectiveness of this pooling strategy in terms of labor and material cost for this pooling strategy.

Since the prevalence of *S. mansoni* and STHs in the study areas was considerable, immediate treatment of all school children in the study areas are recommended (WHO, 2006). Also other measures such as health education and improved access clean water should be in line with chemotherapy (PZQ) to maintain the low level of morbidity and transmission achieved by drug treatment until *S. mansoni* and STHs becomes eliminated from the study areas.

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ANNEXES

Annex-I: Laboratory procedure for Kato-Katz technique

In the Kato-Katz technique faeces are pressed through a mesh screen to remove large particles. A portion of the sieved sample is then transferred to the hole of a template on a slide. After filling the hole, the template is removed and the remaining sample (approx. 41.7 mg depending on size of template) is covered with a piece of cellophane soaked in glycerol (glycerin). The glycerol 'clears' the faecal material from around the eggs. The eggs are then counted and the number calculated per gram (g) of faeces.

A. Materials

- Kato-set (Template with hole, screen, nylon or plastic, plastic spatula)
- Newspaper or glazed tile
- Microscope slides
- Cellophane as cover slip, soaked in Glycerol-malachite green solution
- Fresh stool
- Gloves

B. Procedure

1. Place a small amount of faecal material on the newspaper or plastic.
2. Press the screen on top so that some of the faces filter through and scrape with the flat spatula across the upper surface to collect the filtered faeces.
3. Add the collected faeces in the hole of the template so that it is completely filled.
4. Remove the template carefully so that the cylinder of faeces is left on the slide.
5. Cover the faecal material with the pre-soaked cellophane strip.
6. Invert the microscope slide and firmly press the faecal sample against the cellophane strip on a smooth hard surface such as a tile. The material will be spread evenly.
7. Carefully remove the slide by gently sliding it sideways to avoid separating the
8. Cellophane strip. Place the slide with the cellophane upwards.

- The smear should be examined in a systematic manner and the eggs of each species reported based on cut-off values for classification of intensity of infection.
- Having used the WHO recommended 41.7 mg template, the number of eggs per gram (epg) of faeces is obtained by multiplying the number of eggs by a factor of 24.

Table 6: Classification of infection intensities by the Kato Katz (WHO, 2002).

	Light intensity infection	Moderate intensity infection	High intensity infection
S. mansoni	1-99 epg	100-399 epg	≥400 epg
A. lumbricoides	1-4999 epg	5000-49,999 epg	≥50,000 epg
T. trichiura	1-999 epg	1000-9999 epg	≥10,000 epg
Hook worm species	1-1,999 epg	2000-3999 epg	≥4,000 epg

epg = eggs per gram of faeces.

Annex-II: Preparation of glycerol-malachite green solution

1. A stock solution of malachite green, 1% solution is prepared as follows:
   Malachite green crystals…. ……..…1gm
   Distilled water …….. 100ml
2. A working solution of glycerol-malachite green solution is prepared as follows:
   Glycerol ……..……..100ml
   Malachite green, 1% stock solution……1ml
   Distilled water ……..……100ml

The glycerol, malachite green stock solution and distilled water are mixed and poured into a 250ml glass stoppered bottle, and then labeled as "GLYCEROLMALACHITE GREEN SOLUTION". It is mixed gently before use.

Annex –III: Information Sheet (English Version) Form- For Parents/Guardians

FOR PARENTS/GUARDIANS OF CHILDREN PARTICIPATING IN A STUDY IN MENDERA, KITO , AND SETO YIDO (JIMMA TOWN) AND SAYE ODDO, WOLO SEFER AND KORE KONJO (KORE VILLAGE , MANA DISTRICT) ELEMENTARY SCHOOL IN JIMMA ZONE .

Investigators: Ashenafi Kure (PI)

Daniel Dana

Zeleke Mekonnen

Bruno Levecke

Organization: Department of Medical Laboratory Science and Pathology, College of Public Health and Medical Sciences, Jimma University, Ethiopia.

Project title: Comparison of individual and pooled stool samples for the assessment of *schistosoma mansoni* infection intensity by Kato Katz technique in Jimma zone, south-west, Ethiopia

This informed consent has two parts:

Part I- Information sheet, and

Part II- Certificate of Consent

Read and give a copy of the full informed consent form to the participants.

Part I- Informed sheet

Introduction:

My name is Ashenafi Kure (Medical Laboratory Technologist & principal investigator) from Jimma University**.** This information sheet is prepared by group of researchers whose main aim is to compare individual and pooled stool samples for the assessment of *S. mansoni* infection intensity in Jimma zone ,South West Ethiopia from February – June; 2014. We are planning to conduct a research among school children in Jimma town and Kore village (Mana district) . This study will be done by a research group which includes one principal investigator, laboratory professionals from Jimma University NTD and STHSs laboratory and advisors from Jimma University, College of public health and Medical sciences, Department of medical laboratory sciences and pathology. In this study comparison of individual and pooled stool samples for the assessment of *S. mansoni* infection intensity will be done. Also, the time (duration) required assessing infection intensity obtained by individual stool examination with those by pooling stool samples will be compared.

Purpose:

The purpose of this research is to compare individual and pooled stool samples for the assessment of *S. mansoni* infection intensity as a cost effective strategy among school children in South West Ethiopia from February – May; 2014.

Schistosomiasis (bilharziasis) remains one of the most pervasive parasitic diseases in the world. In terms of impact this disease is ranking second only to malaria as the most devastating parasitic disease. In children the disease contributes to stunted (undermined) growth, impaired cognitive development, malnutrition, and anemia and disrupts school attendance and even sometimes death, because of their hygiene and play habit. Depending on the species of parasite, long-term infection can cause serious damage either the bladder and kidneys, or the liver and gastrointestinal tract, and can hinder people's ability to work and contribute to society. The study and development of new diagnostic methods for schistosomiasis are vital for epidemiological survey, estimating the burden (intensity) infection and monitoring of large scale community based control programs. Therefore in this study, a rapid cost-effective alternative to individual stool examination for the assessment of *S. mansoni* infection intensity will be highlighted.

Procedure:

Your child is kindly invited to take part in this research which aimed to compare individual and pooled stool samples for the assessment of *S. mansoni* infection as a cost effective strategy in South West Ethiopia from February – May; 2014. If your child is participating in this study, you need to understand and sign the agreement form. Your child should ask some information about him/herself (Name, age, sex and grade level). For laboratory examination your child will provide 5 gram stool sample. Collected following standard laboratory procedure. The results of the laboratory investigation would be kept confidential. The information that he/she provides during the interview and the laboratory findings would be used to initiate appropriate treatment for the said infections of your child. The study findings would also be used to design and implement control strategies in the study area in the future.

Risk and discomfort:

There are no expected risks to your child in this study. Your child may feel a little embarrassed of providing a stool samples but we would like to say that we are very familiar with this kind of samples and we only are interested in finding parasites and comparing the said methods using their stool samples.

Benefits:

If your child participates in this research, he/she will get appropriate interventions and we will provide drug prescription request for drug seeking individuals. The laboratory findings would be used to initiate appropriate treatment for the said infections. The study findings would also be used to design and implement control strategies in the study area in the future.

Incentives:

Your child will not be provided any incentives to take part in this research.

Confidentiality:

The information we collect from your child in this research project will be kept confidential. Your name and the name of your child will not be given to anyone outside of the research team.

Voluntary participation and the Right to refuse or withdraw:

The participation of your child in this study is strictly voluntary. You may refuse your child's participation and you may discontinue his/her participation at any time without explanation, and without penalty or lose of benefits to which you are otherwise entitled.

Whom to contact:

If you have any questions regarding to this study, you can contact any of the following individuals and you may ask at any time you want:

Ashenafi Kure: SNNP Regional Health Bureau, Public Health Lab., Hawassa (Tel: 0910011182).

Daniel Dana: Jimma University, College of Public Health and Medical Sciences, Department of Medical Laboratory Sciences and Pathology, Jimma. (Tel: 0917800188)

Zeleke Mekonnen : Jimma University, College of Public Health and Medical Sciences, Department of Medical Laboratory Sciences and Pathology, Jimma (Tel: 0917765427)

Dr. Bruno Levecke: Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, and Merelbeke, Belgium (E-mail: bruno.levecke@ugent.be)

Part II – Certificate of consent for parents/guardians of children participating in the research study

I, the undersigned, confirm that the objective of the study has been explained to me in the language I am convinced and that I have given my consent to participate my child in this study.

Name of parent (or guardian) _____

Signature _____

Date _____

Annex-IV: Informed Consent (Amharic) for parents/guardians

የዋናው ተመራማሪ ስም፡- አሸናፊ ኩራ

የአማካሪዎች ስም፡- ዳንኤል ዳና

ዘለቀ መኮንን

ቡሩኖ ሌቪኬ

ድርጅት : ጅማ ዩኒቨርሲቲ የህብረተሰብ ጤናና ህክምና ሳይንስ ኮሌጅ የሜዲካል ላቦራቶሪ ሳይንስና ፓቶሎጂ ትምህርት ክፍል፡፡

የጥናቱ ርዕስ፡ በጅማ ከተማና በቆሬ አካባቢ (ማና ወረዳ) አንደኛ ደረጃ ት/ቤት ተማሪዎች ላይ የብልሃርዚያ በሽታ ስርጭት ማወቅ እና ጠቃሚ የመመርመሪያ ዘዴ ለማወቅ ነው፡፡

ማብራሪያ

ጤና ይስጥልኝአሸናፊ ኩራ እባላለሁ እኔ በጂማ ዩኒቨርስቲ የ ህክምና ፓራሳይቶሎጂ የማስተር ተማሪ ነኝ፡፡ ይህ ስለ ጉዳዩ መረጃ የሚሰጥ ክፍል የተዘጋጀው በተመራማሪዎች ቡድን ሲሆን ዋናው ዓላማ የብልሃርዚያ በሽታ ስርጭት ማወቅ እና ጠቃሚ የመመርመሪያ ዘዴ በጅማ ከተማና በቆሬ አካባቢ (ማና ወረዳ) አንደኛ ደረጃ ት/ቤቶች በሚገኙት ተማሪዎች ላይ እጥነቶ ለመለየት ነው፡፡

እንደሚታወቀው የብልሃርዚያ በሽታ ከሆድ ትላትሎች አንዱ ሲሆን በሐገራችን ዋንኛ የጤና ችግር ነው፡፡ በዚህ ጥናቱ በሚደርግባቸው አካባቢ በተለይም በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛል፡፡ ይህ ምርምር የሚካሄደው በተመራማሪዎች ቡድን ሲሆን አንድ ዋና ተመራማሪ፣ የላቦራቶሪ ባለሙያዎችና አማካሪዎች ከጅማ ዩኒቨርሲቲ የህብረተሰብ ጤናና ህክምና ሳይንስ ኮሌጅ ሜዲካል ላቦራቶሪና ፓቶሎጂ ትምህርት ክፍል ናቸው፡፡ በዚህ ጥናት ዋነኛው የብልሃርዚያ በሽታ ስርጭቱ ጥናቱ በሚደረግበት አካባቢ የሚለይ ሲሆን እንዲሁም ጠቃሚ የመመርመሪያ ዘዴም ይለይበታል፡፡

ዓላማ

የዚህ ጥናት ዋና ዓላማ የብልሃርዚያ በሽታ ስርጭት ማወቅ እና ጠቃሚ የመመርመሪያ ዘዴ በጅማ ከተማና በቆሬ አካባቢ (ማና ወረዳ) አንደኛ ደረጃ ት/ቤቶች በሚገኙት ተማሪዎች ላይ ለማወቅ ነው፡፡

እንደሚታወቀው የብልሃርዚያ በሽታ የእድገት መቀጨጭን ትምህርት የመቀበል ችግር ፣ የምግብ ማነስ እና የደም ማነስና በታዳጊ ልጆች ላይ ያስከትላል፡፡ ስለዚህ በዚህ ጥናት ላይ የብልሃርዚያ በሽታ ስርጭት የሚታውቅ ይሆናል እንዲሁም ጠቃሚ የመመርመሪያ ዘዴ ይለይበታል፡፡

የአካሄድ ቅድመ ተከተል

የእርሶ ልጅ በዚህ ጥናት የሚጋበዝ ሲሆን ዓላማው የብልሃርዚያ በሽታ ስርጭት ማወቅ እና ጠቃሚ የመመርመሪያ ዘዴ በጅማ ከተማና በቆሬ አካባቢ (ማና ወረዳ) አንደኛ ደረጃ ት/ቤቶች በሚገኙት ተማሪዎች ላይ ለማወቅ ሲሆን፡፡ በጥናቱ ላይ ልጅዎ እንድትሳተፍ/እንዲሳተፍ ከተስማሙ ስለጥናቱ ማወቅና የስምምነት ሰነድ መፈረም ይኖርቦታል፡፡ በጥናቱ ልጅዎ ለላቦራቶሪ ምርመራ የሚሆን የሰገራ ናሙና ይሰጣል፡፡

ስጋትና ጉዳት

በዚህ ጥናት ከልጅዎ የሰገራ ናሙና እንዲሰጥ ይጠየቃል፡፡ በዚህ ጥናት ልጅዎ ቢሳተፍ ከሆድ ትላትል ነፃ እንዲሆን ይደረጋል፡፡

ጥቅም

በዚህ ጥናት ልጅዎ ቢሳተፍ ተገቢውን መድሀኒት ለሆድ ትላትል የሚያገኝ ሲሆን በሀገራችን በሽታውን ለመከላከል በሚደረገው ጥረት ላይ ከፍተኛ አስተዋፅኦ ያደርጋሉ፡፡

ሚስጥራዊነት

ከምርምሩ የምወሰደው የልጅዎ መረጃ በሚስጥር ይያዛል፡፡ የላቦራቶሪ ምርመራ ውጤት ከእርሶ ፍቃድ ውጪ ለማንም አይሰጥም፡፡

ያለመሳተፍና ከተሳታፊነት የማቋረጥ መብቶች

በዚህ ጥናት ልጅዎ ላይ ምንም አይነት ጉዳት እንደማይደርስ ለማረጋገጥ እንወዳለን፡፡ በጥናቱ ልጅዎን ለማሳተፍ ፍቃደኛ ባይሆኑ የሚደርስቦት ምንም አይነት ተፅእኖ የለም፡፡ ልጅዎት መሳተፍ ከጀመርም በኃላ በማናቸውም ሰዓት ከጥናቱ ሊያስወጡ ይችላሉ፡፡

ማንን ማነጋገር እንዳለቦት

ማንኛውም አይነት ጥያቄ ካልዎት አሁን ወይም ሌላ ጊዜ ሊጠይቁ ይችላሉ፡፡ሌላ ጊዜ ሊጠይቁ ቢፈልጉ ከዚህ በታች ያሉትን ግልሰቦች ማነጋገር ይችላሉ፡፡

1. አሸናፊ ኩሬ ፡ ስልክ ቁጥር 09 10 01 11 82
2. ዳንኤል ዳና ፡ ስልክ ቁጥር 09 17 80 01 88
3. ዘለቀ መኮንን ፡ ስልክ ቁጥር 09 17 76 54 27
4. ቡሩኖ ሌቨኬ ፡ ስልክ ቁጥር E-mail: bruno.levecke@ugent.be

Part II – Certificate of consent for parents/guardians of children participating in the research study

በጥናትና ምርምሩ ልጄ እንዲሳተፍ/እንድትሳተፍ በተጠየቅኩት መሰረት አላማው

በደንብ አንብቤ (ተነቦልኝ) እንዲሁም ያልገባኝን ጥያቄ ስለተገነዘብኩ የተሳትፎ

ፍቃዴን ልጄ በዚህ ጥናት እንዲሳተፍ/እንድትሳተፍ በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊው ወላጅ/ አሳዳጊ ስም ----------

ፊርማ :----------

ቀን :----------

Annex-V: Information sheet (Afan Oromo) for parents/guardians

Odeefannoo

Maqaa Qowaataa isa jalqabaa: Ashanafii Kuree

Maqaa Gorsitoota: Daniiel Daanaa

Zeleqee Mekoonin

Bruunoo Leveekee

Dhaabata: Yuniversiitii Jimmaatti Kollejjii Saayinisii fayyaa Hawaasafi Meedikaala Dipartmeenti Labooratoory fi Patoologii

Matadureen Qowaanichaa: Magaala Jimmatiif nannoo qoree anaa Maanaa baratoota sadarkaa tokkoffa iratti babal'ina dhukuba Bilhaarziyaa beekuuf toofta ittiin Sakata'an baruu nita'a.

Hubachiisa:

Wa'ee dhimichaa kan odeefannoo kennu kuni kan qopha'ee garee baratootaan yammuu ta'u kayyoon isaa inni jalqabaas babali'inni dhukuba bilharziyaa beekuufi tooftaa ittin sakata'amu magaala Jimmaa fi naannoo qoree aanaa maannaa barattoota mana barumsa sadarkaa tokoffaa jiran irratti beekuu ni ta'a. A kuma beekamu dhukubin bilharziyaa ramoolee garaa kessaa tokko yammuu ta'u biyya keenya keessatti rakina fayyaa isa cimmaadha. Qowannichi bakka itti gageefamu kessumattu ijoollee dargagoota irratti bal'inaan argama. Qowanaan kunis kan gageefamu garee qowatootaan yammuu ta'u Qowataa isa jalqabaa; ogeesota laboratorii fi gorsitoota universitii Jimmaa Kolleejii saayinsii fayyaa Hawasaaf medikaalaatti kutaa barnootaa medical laboratoriiti Qowanna kana irratti fayyida kan qabu gosni ittiin sakata'amu ittiin beekama.

Kaayyoo:

Kaayoon Qowaannnaa kanaa babal'ina dukuba bilharziyaa hubachuufi akkasumas gosa ittiin sakata'amu magaalaa Jimmattii fi aanaa mannaatti naanoo Qoreetti barattoota mana barumsa sadarkaa tokkoffaa nita'aa. Akkuma beekamu dhukuban bilharziyaa hanqina gudina, rakina barumsa hubachuu, feedhii nyaata hirdhisuufi dhiga hirdhisu da'imani olgudachaa jirani irratti nigeesisa.

Kanaafuu Qowannoo kana irratti babal'ina dhukuba bilharyiyaa akasumas gosa ittiin sakata'amu ittiin beekama.

Adeemsa:

Mucaan keesaan Qowannaa kana iratti akka hirmaatu waan aferameef mucaan keessan akka irratti hirmaattu fi/ hirmaatuuf yoo fedhii keesan ta'e wa'ee qowanichaa baruufi akkasumas malattoo waligaltee malatteesuun isin iraa eegama Qowani chaaf. Kan ta'uus mucaan keessan naamunaa booliis ni kenna.

Sodaafi Midhaa:

Qowannaa kana irratti mucaan kessan bolii akka kennu ni gaafatama. Yoo qowannaa kana irrattii hirmaate mucaan keesan rakkina ramoolee garaakeessaa irra bilisa ni ba'a.

Faayidaa:

Qowannoo kana irratti yoo hirmaatee dawaa ramoolee garaa keesaat ta'a waan argatuuf biyya keenya kessatti dhukubicha ofirra ittisuuf tatafii godhamu irratti ga'ee gudaa hirmaata.

Icitii:

Qowanichaaf odeefannoon mucaa keesan irra fudhamu iciitii dhaan qabama. Firiin laboratoriis fedhii keesanin allatti enyummafu hinkennamu.

Itti hirmannaa isuufi mirga hirmanna irra kutuu:

Qowannoo kana irratti rakkin kamiyyuu mucaa keesan irra akka hingeenye. Isinii mirkaneesuu barbaanna. Qowanna kana irratti mucaan keesan akka hinhirmanne yoobarbaadan dhiibaan isin irra ga'u tokollee hinjiru. Mucaan keesan akka itti hirmaa tu, erga jalqabee booda illee yeroo kamiyuu qoqanna kana keesa baasuuf mirga qabdu.

Eenyuun akka dubisuu qabdan:

1. Ashanafi kuree Lak. Bil : 09 10 01 11 82
2. Daani'eel Daanaa ›› ›› : 09 17 80 01 88
3. Zeleqee Mekonin ›› ›› : 09 17 76 54 27
4. Bruno Leveekee ›› ›› : E-mail: bruno.levecke@ugent.be

Waliigaltee:

Qowannaa kana irratti mucaan koo akka hirmaatuuf gafatamee kaayyoo isaas sirritti dubbifadhee/ naaf dubbifamee akkasumas waan naaf hingalle naaf dhibsamee mucaan koo qowannoo kana irratti akka hirmaatu malattookootiin nan mirkaneessa.

Maqaa Maatii mucaa/ Guddisa: ----------

Mallattoo: ----------

Guyyaa: ----------

Translation made by Mr. Mio Ayana from Amharic to Afaan Oromo.

Annex -VI: Consent (English) form for children older than 12 years

Project title: Comparison of individual and pooled stool samples for the assessment of *schistosoma mansoni* infection intensity by Kato Katz technique in Jimma zone, south-west, Ethiopia

Principal investigators : Ashenafi Kure (PI)

Daniel Dana

Zeleke Mekonnen

Bruno Levecke

Part I- Informed sheet (statement)

Introduction:

My name is Ashenafi Kure (Medical Laboratory Technologist & principal investigator) and I and my colleagues are from Jimma University. This information sheet is prepared by group of researchers whose main aim is to compare individual and pooled stool samples for the assessment of *S. mansoni* infection intensity in Jimma zone ,South West Ethiopia from February – May; 2014.

Purpose:

The purpose of this research is to compare individual and pooled stool samples for the assessment of *S. mansoni* infection intensity as a cost effective strategy among school children in South West Ethiopia from February – May; 2014.

Schistosomiasis (bilharziasis) remains one of the most pervasive parasitic diseases in the world. In terms of impact this disease is ranking second only to malaria as the most devastating parasitic disease. In children the disease contributes to stunted (undermined) growth, impaired cognitive development, malnutrition, and anemia and disrupts school attendance and even sometimes death, because of their hygiene and play habit. Depending on the species of parasite, long-term infection can cause serious damage either the bladder and kidneys, or the liver and gastrointestinal tract, and can hinder people's ability to work and contribute to society. The study and development of new diagnostic methods for schistosomiasis are vital for epidemiological survey, estimating the burden (intensity) infection and monitoring of large scale community based control programs. Therefore in this study, a rapid cost-effective alternative to individual stool examination for the assessment of *S. mansoni* infection intensity will be highlighted.

Procedures:

If you agree to participate, we will visit you at the school and give you a small plastic container and ask you to collect your fresh stool sample (about 5 gm). Then we will check the stool to see if you have infection of intestinal worms.

Confidentiality:

The information obtained during the conduct of this study will remain confidential. The results of the research study may be published, but subjects' names or identities will not be revealed. Records will remain confidential. Only the researchers doing the study and principal investigator will use these forms. All will have a duty of confidentiality to you as a research participant.

Safety:

For this survey, you are inquired to provide your feaces only, which is a non invasive procedure. Therefore, we do not expect any harm to occur on you. If you are infected with any of the schistosomiasis or soil transmitted helminthes you will be treated with praziquantel or albendazole respectively. The drugs will only be administered by qualified health professional.

Benefits:

By participating in the survey you will directly benefit by being investigated for intestinal worm infections and receiving appropriate treatment free of charge.

Right to refuse or withdraw:

We assure you that our best care will be taken for you if you agree to take part in the study. You are free to withdraw from the study at any time and no one will force you to participate and you will not be discriminated in any form for education or health services. If you have questions, feel free to ask

Mr. Ashenafi Kure (Medical Laboratory Technologist & principal investigator, Tel. 0910011182) or Mr. Zeleke Mekonnen (Tel: 09 17 76 54 27)

Part II: Certificate of consent

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I will participate in this study by giving my stool for intestinal worm diagnosis and management and I understand that I have the right to withdraw from the study at any time.

Print name of subject, date and signature

_____ ____/____/____ (dd/mm/yy)

Annex –VII: Consent (Amharic) form for children older than 12 years

ቅ□ 7፡5 □እድሜያቸው ከ12 ዓመት በላይ የሆኑትና በጥናቱ ላይ በሚሳተፉት ተማሪዎች የሚሞላ ተጨማሪ የስምምነት ቅፅ

የጥናቱ ርዕስ፡ በጅማ ከተማና በቆሬ አካባቢ (ማና ወረዳ) አንደኛ ደረጃ ት/ቤቶች ተማሪዎች ላይ የብልሃርዚያ በሽታ ስርጭት ማወቅ እና ጠቃሚ የመመርመሪያ ዘዴ ለመለየት የሚደረግ ነው፡፡

የዋናው ተመራማሪ ስም፡ አሸናፊ ኩሬ

ዳንኤል ዳና

ዘለቀ መኮንን

ቡሩኖ ሌቪኬ

□□ል ሀ፡ ማብራሪ□

ጤና ይስጥልኝ፡፡ አሸናፊ ኩሬ እባላለሁ፡፡ እኔ በጅማ ዩኒቨርሲቲ የህክምና ፓራሳይቶሎጂ የማስተር ተማሪ ነኝ፡፡ እንደሚታወቀ□ ብልሃር□□ በሽታ የሀገራችን ዋነኛ የጤና ችግር ነዉ፡ በጅማ ከተማ □እና በቆሬ አካባቢ በተለይ በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛል፡፡ ስለ□ህ ጥናቱ በሚካሄድባቸው ት/ቤቶች ላይ ይህንን የጤና ችግር የሆነውን የብልሃርዚያ በሽታ ስርጭቱንና ጠቃሚ የመመርመሪያ ዘዴ ላይ ጥናት ማድረግ እንፈልጋለን፡፡

አላማ

በሀገራችን ኢትዮጵያ የብልሃርዚያ በሽታ በተለ□ በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛል፡፡ በዚህ ጥናቱ በሚካሄድበት አካባቢዎችም በብዛት ተስፋፍቶ ይገኛል፡፡ በልጆች ላይ የደም ማነሰ እና የእድገት መቀጨጭን ያስከትላል፡፡ ስለዚህ እኔ ከጅማ ዩኒቨርሲቲ ስለ ስርጭቱና ጠቃሚ የመመርመሪያ ዘዴ ለመለየት አቅጃለሁ፡፡ በመሆኑም ከዚህ ት/ቤት እድሜያቸው ከ 5 -18 አመት የሆኑትን በዘህ ጥናት እንድሳተፉ እ□ል□ለሁ፡፡ ስለ□ህ አንተ ወይም አንቺ በዚህ ጥናት ዉስጣ እንድትሳተፍ/ፊ □ቃ□ኛ እን□ትሆን/ኝ በትህትና እ□□ቃለሁ፡፡

የአካሄድ ቅድመ ተከተል

በጥናቱ ላይ ለመሳተፍ ከተስማማህ/ሽ/ በጥናቱ ቀን የሠገራ ናሙና እንድታመ□/□ □ስ□ራ ማም□ እቃ እንሰጥሃለን፡፡ የሰገራው ናሙናዉ ብልሃርዚያ ትላትል እንዲሁም ሌሎች የሆድ ውስጥ ትላትሎች እንዳለዉ ለማረጋገጥ ላቦራቶሪ ተወስዶ ምርመራ ይደረግበታል፡፡ በሽታ ከተገኘበት መድሐኒት እንሰጥሃለን/ሻለን ::

ሚስጥራዊነት

ከምርምሩ የምንሰበስበው መረጃ በሚስጥር ይያዛል። ከጥናቱ በሚገኘው ውጤት የግለሰብ ማንነት በማይታወቅ መልኩ ሊታተም ይችላል መዝገቦች በሚስጥርነት ይያዛሉ እና የተለየ የጥናቱን ስነምግባር የሚከታተል ኮሚቴ ስለ አንተ መረጃ ሊሰጣቸው ይችላል። ይህ ደግሞ ጥናቱ ደረጃወን □□በቀ እንዲሆን ለማድረግ ነው። እነዚህ አካላትም ምስጢርነቱን የመጠበቅ ኃላፊነት አለባቸው ።

ጥንቃቄ ስጋትና ጉዳት

ጉዳት፡ የሰገራ ናሙና መስጠት ምንም አይነት ጉዳት አይኖረውም

ጥቅሞች

በዚህ ጥናት የሚሳተፉ ልጆች ከሆድ ትላትል ነፃ እንዲሆኑ ይደረጋል። በተጨማሪ የሽታው ስርጭት እና ጠቃሚ የመመርመሪያ ዘዴ ለማወቅ ይረዳል። የመመርመሪያ ዘዴዎችን በማመላከት በሃገራችን በሽታዉን ለመከላከል በሚደረገው ጥረት ከፍተኛ አስተዋጽኦ ያደርጋሉ።

ያለመሳተፍና ከተሳታፊነት የማቋረጥ መብቶች

በዚህ ጥናት ላይ ብትሳተፍ /ብትሳተፊ/ ምንም አይነት ጉዳት እንደማይደርስ ለማረጋገጥ እንወዳለን።በ□ሮ□□ቱ ላለመሳተ□ □ቃ□ኛ ባትሆን/ኚ የሚደርስብህ/ሽ ምንም አይነት ተጽንኦ □ለም። በጥናቱ መሳተፍ ከጀመርክ/ሽ/ በኋላ በማናቸውም ሰዓት ከጥናቱ መወጣት ወይም ማቋረጥ ይቻላል። ከጥናቱ በመውጣትህ/ሽ/ በማቋረጥህ/ሽ/ የሚገባህን/ሽ/ የህክምና አገልግሎት አይነፈግህም/ሽም/ ። ጥያቄ ካለህ/ሽ አሸናፊ ኩሬ /ዋና ተመራማሪ 0910011182 ወይም ዘለቀ መኮነን፡ 09 17 76 54 27 መጠየቅ ይቻላል።

□□ል ለ፡ □ተሳታፊዎች የስምምነት ሰነድ

ከላይ የተገለፀትን ነገሮች በሙሉ አንብቤያለው ወይም ተነቦልኛል። ግልፅ ያልሆነልኝ ነገር ካለ ጥያቄዎች እንድጠይቅ እድል ተሰቶኛል ለጥያቄም በቂ ምላሽ አግኝቻለሁ። በሙሉ ፍቃደኝነት በዚህ ጥናት ላይ የሰገራ ናሙና በመስጠት በመሳተፍ በፍቃደኝነት ተስማምቼአለሁ።

□ተሳታፊ ስም፡ ቀን፡ ፊርማ/የጣት አሻራ

ቀን----------ወር----------□.ም

Annex- IX: Consent (Afan Oromo) form for children older than 12 years

Guca haayyama Hirmaannaa ijoollee waggaa 12 fi isaa oli

Qorattoonni: Ashanafi Kuree (Qorataa duraa)

Daniel Daanaa

Zeleqee Mekonnen

Bruno Leveke

Dhaabata: Yuniversiitii Jimmaatti Kollejjii Saayinisii fayyaa Hawaasafi Meedikaala Dipartmeenti Labooratoory fi Patoologii

Matadureen Qowaanichaa: Magaala Jimmatiif nannoo qoree anaa Maanaa baratoota sadarkaa tokkoffa iratti babal'ina dhukuba Bilhaarziyaa beekuuf toofta ittiin Sakata'an baruu nita'a.

- Gucinni hayyammi hiirmaanna tun kutaa lama qaba

  Kutaa 1. Fuula odeefannoo fi

  Kutaa 2. Warqa ragaa Eeyyamaa

Kutaa 1- Odeefannoo/ibsa

Seensa:

Maqaan Koo Ashanafi Kuree (Medikal Laboratory Tekenologistii fi qorataa isa duraati). Ani fi qorattoonni kannen yuunivarsiitii Jimmaa. Duraan dursa nagaan isin gaafadha ; akkam jirtu . Akkuma beekamu dhukkubni Bilhaarziyaa biyya keenya keessatti babal'atee akka jiru nihubatama. Keessumattuu naannoo Qoree fi magaalaa Jimmaa Keessatti bal'inaan Ijoollee irratti mul'ata. Kanaafuu mana barumsaa qorannoon kun keessatti adeemsifamu keessatti dhukkubni kun tamsa'inni isaa maal akka fakkaatu, dabalataanis mala ittiin dhukkuba kana adda baasamu irratti qorannoo geggeessina.

Kaayyoo qorannichaa

Kaayoon Qowaannnaa kanaa babal'ina dukuba bilharziyaa hubachuufi akkasumas gosa ittiin sakata'amu magaalaa Jimmattii fi aanaa mannaatti naanoo Qoreetti barattoota mana barumsa sadarkaa tokkoffaa nita'aa. Akkuma beekamu dhukuban bilharziyaa hanqina gudina, rakina barumsa hubachuu, feedhii nyaata hirdhisuufi dhiga hirdhisu da'imani olgudachaa jirani irratti nigeesisa.

Adeemsa

Yoo fedha qabaattan hojjetoota qorannoo keenya mana barumsa keessan dhaquun qabdu iddattoo pilaastikii irraa tolfamte sinii kennuun akka iddattoo bobbaa ho'aatilmaamaan gm 5 fiddan isin gaafatu. Firii qorannoo keessan irratti hundaa'uudhaan qorichi praziquaanteli fi albendaazoli isin tola kenname ni fayyitu.

Icitii

Odeeffannoon yommuu qorannoo kanaa argamu icitiidhaan eegama. Odeeffannoon argame kun qaama biraaf dabarfame hin kennamuu. Argannoo qo'annoo kanaa maxxansumuu ni danda'a haata'u malee maqaaf eenyummaan namootaa hin ibsamu. Galmeewwn icitiin eegamu. Iciitii kana eeguudhaaf, qorataan galmeewwan kana sanduqa furtuu qabu keessa Biiroo Yunivarsitti Jimmaatti argamu keessa kaa'a. Friin qorannoo taasifamuu kun koodiin itti kennamee akka eenyummaan namoota fedhiitiin hirmatamanii akka hin beekamnne ta'a. Namoota firii laaboraatorii ilaalani allatti akka ragaan kampiitera keessa taa'u ni taassifama. Kompuuterichis koodiitiin cufaa ta'a. odeeffannoon namoota dhuunfaa yoo fedhii saanii ta'e qofa ogeessota fayyaaf kennamuu danda'a. Bobbaa funaanamu haala protokolii keessaatti ibsameen alatti qo'annoo biraatiif osoo hin ooliin haala miidhaa hin finneen qo'annoon kun erga dhumee booda ni gatama.

Of-eegannoo

Kan isin irra dheegamu bobba furdaa qofaa kennuu dha. Yaaliin kun Praziquantalii fi albendaazolii yeroo ammaa kana gabaa irratti argaman waan laatuuf bu'aa malee miidhaa hin qabu.Qorichis kan kennamu ogeessa fayyaati qofaani dha.

Faayidaawwan

Isin ijoleen kana keessatti hirmaattan raamolee marri'imaaniif faalamuu fi hin faalamne qorannoon adda baasa fi wala'ansa barbaachisaa argachuun ni fayyadamtu. Kan dhuma irrattis raamootiin qabamtanis haala tokko tokko irratti hunda'uun yaalii atattamaa ni argattu.

Mirga diduu fi addan kutuu

Yoo qorannoo kana keesatti hirmaattan of eegannoo guddoo akka goonu isinii mirkaneesina. Akkasumas yeroo barbaadanii hirmanaa qoranichaa addan kutuuf mirga guutuu qabda. Kanaaf immoo miidhaan/loogiin karaa barnootaa fi waalansaa isin irraan ga'u hin jiru. Yoo gaaffi qabaatte battaluma Oboo Ashanafi Kuree (Bil. 0910011182) fi Zeleqee Mekonnen (Bil: 09 17 76 54 27) gaafachuu dandeessa.

Kutaa II – Guca raga Heeyyamaa

Odeefannoo armaan olii dubbiseera ykn naa dubbisameera carraan gaafii gaafachuu naa kennameera gaaffii kootifisi deebii gahaan argadheera. Fedhii kootiin qorannoo keessan keesatti hirmaachuu akkan barbaade isin mirkaneessa:

Maqaa hirmaata _____Mallato _____

Guyyaa _____ g/j/w

Maqaa qorataa guyyaa fi mallattoo qorataa _____guu/j/w_____

Annex- X: Questionnaire (English Version)

JIMMA UNIVERSITY COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY

A RESEARCH QUESTIONNAIRE, 2014

AIM: Dear participant, first I wonder your participation that you think your Participation will help us to provide a rapid and cost effective diagnostic method for the assessment of Schistosomiasis in the area. As I told you the objective of the study is to compare individual and pooled stool samples for the assessment of *S. mansoni* infection as a cost effective strategy among school children in Jimma town and Kore village (Mana district).

Data collectors code no _____

I. Subject code_____

1. Name of School _____
2. Name of the student _____
3. Grade /section of student_____
4. Age (in years) _____
5. Sex _____
6. Pregnancy for Female _____
7. Recent treatment status with PZQ and Albendazole and any adverse effect (allergy): ___

Annex-XI: Questionnaire (Amharic Version)

ጅማ ዩኒቨርሲቲ የህብረተሰብ ጤናና ሕክምና ሳይንስ ኮሌጅ የሜዲካል ላቦራቶሪ ሳይንስና ፓቶሎጂ ትምሕርት ክፍል መጠየቂያ፡ 2014

ዓላማ፡ ውድ ተሳታፊያችን በጥናቱ ላይ በመሳተፎ እጅግ እያመሰገንን የእርሶ መሳተፍ የችግሩን መጠን በአካባቢው እንድናውቅ ያስችለናል፡፡ በመሆኑም የጥናቱ አላማ እንደገለጽነው የብልሃርዚያ በሽታ ስርጭት ማወቅ እና ጠቃሚ የመመርመሪያ ዘዴ ለማወቅ የሚደረግ ጥናት ነው፡፡

የጠያቂው መለያ ቁጥር ----------

ክፍል አንድ፡ የተሳታፊው መለያ----------

1. **የትምህርት ቤት ስም** ----------
2. **የተማሪው ስም**----------
3. **ክፍል** ----------
4. **ዕድሜ** ----------
5. **ያታ** ----------
6. **እርግዝና ሁኔታ ለ ሴቶች**----------
7. **የቅርብ ጊዜ (በ** PZQ and AL)**የህክምና ሁኔታ እና ያጋጠሞት የጎንዮሽ ጉዳት ካለ(አለርጂ)፡** --------

Annex-XI: Questionnaire (Afan Oromo Version)

Yuniversiitii Jimmaatti Kollejjii Saayinisii fayyaa Hawaasafi Meedikaala Dipartmeenti Labooratoory fi Patoologii, Guca gaaffii: 2014

Hirmaannaa keessaniif guddaa isin galateeffanna, hirmaannan keessan kunis tamsaina dhukkuba Bilaarziyaa beekuuf nugargaara.

Koodi nama gaafatu: ----------

Kutaa 1: Koodii Barataa: ----------

1. Maqaa M/ barumsaa:----------
2. Maqaa barataa:----------
3. Kutaa :----------
4. Umurii:----------
5. Saala :----------

Annex XI: Formats for parasitological examination

1. Data collection format (Summary)

Laboratory method: ----------

Name of the school…… Date of data collection……..

S. no	Name of the student	Name of School	Grade	Age	Sex	Pregnancy for female	Recent Tx. status with PZQ and Al & any adverse effect(Allergy)	Name & signature of Data collector
1								
2								
3								
4								
5								

2. Stool Sample Processing Format for Pooling

Name of School: ---------- Date :----------(DD/MM/YY)

ID subject	ID Pool 5	ID Pool 10	ID Pool 20	ID Pool 60
	Time: Initial:			
	Time: Initial:	Time: Initial:		
	Time: Initial:			
	Time: Initial:			
	Time: Initial:	Time: Initial:	Time: Initial:	

3. Sample preparation format for Kato Katz Slides (In batch of 10)

Name of School: ----------Date :----------(DD/MM/YY)

Batch of 10	Time	Initial
1		
2		
3		
4		
5		
6		
7		
8		

4. Format for individual Kato Katz slides Reading

Name of School: ----------Date: ---------- (DD/MM/YY)

Subject ID	*S.mansoni*	*A. lumbricoides*	*T.trichiuria*	*H. worm*	Time (Min)	Time (Sec)	In

5. Format for Pooled Kato Katz Slides Reading

Name of School: ---------- Date :----------(DD/MM/YY)

Pool ID	*S.mansoni*	*A. lumbricoides*	*T. trichiura*	hookworms	No. Samples	Time (min)	Time (sec)	In
1P5					5			
2P5					5			
3P5					5			
4P5					5			
5P5					5			
6P5					5			
7P5					5			
8P5					5			
9P5					5			
10P5					5			
11P5					5			
12P5					5			
1P10					10			
2P10					10			
3P10					10			
4P10					10			
5P10					10			
6P10					10			
1P20					20			
2P20					20			
3P20					20			
1P60					60			

CERTIFICATE

This is to certify that the research entitled "Comparison of individual and pooled stool samples for the assessment of *schistosoma mansoni* soil-transmitted helminth infection intensity by Kato Katz technique in Jimma zone, south-west, Ethiopia, was carried out by Ashenafi Kure under direct supervision of the advisor(s) listed below. Further, the advisor(s) certify that this work has not been submitted in part or full in any University or Institution for any Degree or Diploma.

1. Name: Mr. Daniel Dana Signature: _____Date: July,14,2014
2. Name: Mr. Zeleke Mekonnen Signature: _____ Date: July,14,2014
3. Name: Dr. Bruno Levecke Signature: _____ Date: July,14,2014

Approval of the Examiners:

DECLARATION

I hereby declare that the work embodied in this thesis was carried out by me under direct supervision of the advisor(s), from Department of Medical Laboratory Science and pathology, College of Public Health and Medical Sciences, Jimma University. This work has not been submitted in part or full in any University or Institution for any Degree or Diploma. I further endorse that this work is the property of Jimma University and all rights in this regard are reserved with Jimma University.

Name of the PI: Ashenafi Kure Signature: _____ Date: July, 14, 2014

Head of the Department: Tesfaye Kassa Signature: _____ Date: July, 14, 2014