

**COMPARISON OF DIAGNOSTIC PERFORMANCE OF MINI-FLOTAC WITH
FORMALIN-ETHER CONCENTRATION AND KATO-KATZ FOR DETECTION OF
INTESTINAL HELMINTHS AND PROTOZOA IN NURSERY SCHOOL CHILDREN IN
MBITA, WESTERN KENYA**

BY

OMUNDI NYABOKE JACKLINE

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FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL PARASITOLOGY**

DEPARTMENT OF BIOMEDICAL SCIENCES AND TECHNOLOGY

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DECLARATION

I declare that this thesis is my own original work and has not been presented to any institution of higher learning for the award of a degree certificate. All referenced sources are duly quoted and any semblance observed is coincidental.

OMUNDI NYABOKE JACKLINE

MSC/PH/00059/2013

Signature..... Date.....

This thesis has been submitted for examination with our approval as University supervisors:

Dr. Lilian Ogonda, PhD.

Department of Biomedical Science and Technology

Maseno University

Signature.....Date.....

Prof. Shinjiro Hamano, Ph.D.

Institute of Tropical Medicine, Nagasaki University

Signature..... Date.....

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DEDICATION

To my family

ABSTRACT

Schistosoma mansoni (*S. mansoni*), soil-transmitted helminths (STH) and protozoa affect approximately 2 billion people world-wide. Most of these infections occur in developing countries and children below 14 years of age are the most affected. Mbita in western Kenya is one of the regions in which these infections are widely spread. Kato-Katz and formalin-ether concentration (FEC) techniques are widely used for the diagnosis of *S. mansoni*, STH, and intestinal protozoa infections respectively, but these methods are labour intensive and have low sensitivity especially for low-intensity infections. Moreover, Kato-Katz does not allow for detection of intestinal protozoa while FEC is a qualitative technique. The newly developed Mini-FLOTAC technique has been shown to be more sensitive, hence the need to validate it in a population with low infection intensities especially in children below six years (nursery school children). Therefore as part of a search for a gold standard for diagnosis of intestinal helminths and protozoa, this study sought to compare the diagnostic performance of Mini-FLOTAC with FEC and Kato-Katz for the detection of *S. mansoni*, STH, and intestinal protozoa in nursery school children in Mbita western Kenya. The specific objectives were to compare the sensitivity and specificity of Mini-FLOTAC with FEC and Kato-Katz technique and also to determine the degree of agreement among the techniques in the detection and determination of prevalence of *S. mansoni*, STH and intestinal protozoa in nursery school in Mbita, western Kenya. Stool samples were collected from 435 children in 22 schools who were randomly sampled using Lot quality Assurance method. Stool samples were analyzed for intestinal parasites using Mini-FLOTAC, FEC, and Kato-Katz techniques. Using Kato-Katz as the reference standard for helminthes and FEC for protozoa, Mini-FLOTAC demonstrated a sensitivity of 78.4% to 100% and a specificity of 95.3% to 100% for the detection of helminthes, and a sensitivity of 68.7% and a specificity of 75.8% for the detection of protozoa. Therefore, Mini-FLOTAC is sensitive and specific for the qualitative diagnosis of *S. mansoni* and STH; but its sensitivity is low for protozoa. Moreover, there was significant moderate agreement $\kappa = 0.410$ (95% CI, 0.41 to 0.60), $p < 0.05$ between Mini-FLOTAC and Kato-Katz for detection of *S. mansoni*, a slight agreement for hookworm $\kappa = 0.121$ and *Ascaris lumbricoides* $\kappa = 0.063$ (95% CI, 0.01 to 0.20), $p < 0.05$. However, there was fair agreement $\kappa = 0.373$ (95% CI, 0.21 to 0.40), $p < 0.05$ between Mini-FLOTAC and FEC for the detection of protozoa. Hence, Mini-FLOTAC can be used as an alternative to Kato-Katz in *S. mansoni* and STH diagnosis. The sensitivity and specificity of Mini-FLOTAC can be improved by inclusion of a staining step e.g. with iodine to enhance visibility of the cysts and eggs for the diagnosis of intestinal protozoa and helminths.

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LIST OF ABBREVIATIONS AND ACRONYMS

DALY's- Disability Adjusted Life Years

ECD- Early Childhood Development

EPG- Egg Per Gram

FEC- Formalin-ether Concentration

HDSS- Health Demographic Surveillance System

KEMRI SSC- Kenya Medical Research Institute- Scientific Steering Committee

MDA- Mass Drug Administration

NPV-Negative Predictive Value

PEs- Parasite Elements

PPV- Positive Predictive Value

SOP- Standard Operating Procedure

STH- Soil-Transmitted Helminths

WHO- World Health Organization

DEFINITION OF TERMS

Diagnostic performance: Diagnostic performance evaluates the ability of a qualitative or quantitative test to accurately discriminate between two subclasses of subjects i.e. those with disease and without disease. The variables include sensitivity, specificity, negative and positive predictive value and degree of agreement.

Sensitivity: Sensitivity is the ability of a test to identify correctly all positive samples. It is calculated as the number of true positive samples detected by the test being evaluated, divided by the number of samples identified by the reference method as positive, expressed as a percentage.

Specificity: Specificity is the ability of a test to detect correctly all negative samples. It is calculated as the number of true negative samples recognized by the test being evaluated, divided by the number of samples identified by the reference test as negative, expressed as a percentage.

Predictive values: Positive and Negative predictive values describe a patient's probability of having disease once the results of his or her tests are known. Positive predictive value (PPV) expresses the proportion of persons with a positive test that has the disease condition. Negative predictive value (NPV) expresses the proportion of persons with a negative test that does not have the disease condition.

Intestinal protozoa: These are single-celled microorganisms, some of which are of medical important and they include; *Entamoeba histolytica*, *Entamoeba coli* and *Giardia lamblia*.

Intestinal helminths: These are worms that parasitize the gastrointestinal tract. They include; *A. lumbricoides*, hookworm, *Trichuris trichiura* and *Schistosoma mansoni*.

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Intestinal helminths such as *Schistosoma mansoni* (*S. mansoni*), Soil-transmitted helminths- STH (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworms, and *Strongyloides stercoralis*) and intestinal protozoa (*Giardia lamblia*, *Entamoeba coli* and *Entamoeba histolytica*) affects approximately 2 billion people worldwide (WHO, 2010). These infections are a major public health problem in developing countries especially in sub-Saharan Africa (Hotez *et al.*, 2009). *Schistosoma mansoni* causes an estimated global burden of 1.5 million disability adjusted life years (DALY's) lost (Steinmann *et al.*, 2006), while the global burden caused by STH is approximately 5 million DALY's lost (Bethony *et al.*, 2006). Infections with *G. lamblia* and *E. histolytica* lead to 140,000-500,000 deaths per year (WHO, 2010). *Schistosoma mansoni* and STH infections are highly endemic in western region of Kenya, with a prevalence of up to 80 % and 68% respectively in school going children (Handzel *et al.*, 2003; Mwinzi *et al.*, 2012). Mbita in western Kenya is one of the regions in which these infections are widely spread and studies have reported high prevalence of 76.8% and 13.3% for *S. mansoni* and STH respectively (Nagi *et al.*, 2014; Odiere *et al.*, 2012), however, the prevalence of intestinal protozoan infections remains unknown.

A variety of laboratory methods can be employed in the diagnosis of intestinal parasites (Ahmadi *et al.*, 2007). The choice of a particular technique for routine use is influenced by its affordability, simplicity, sensitivity and level of technical skill involved (WHO, 1994). Mass Drug Administration (MDA) programs have indicated the need for a low-cost, sensitive, specific,

and easy-to-perform quantitative test that can be used in developing countries where intestinal helminths and protozoan infections are endemic (Levecke *et al.*, 2011). However, such an ideal method does not exist. Additionally, polyparasitism has become a common phenomenon especially in developing countries, yet only a few studies have been carried to assess its extent in all age groups in endemic areas (Hotez *et al.*, 2009). This is due to lack of diagnostic tools that are able to detect multiple infections in stool samples. Kato-Katz has been the recommended method for a long time, but lately studies on comparison among techniques have proved that it is not the most sensitive and accurate method for egg counts (Albonico *et al.*, 2012). Thus, the use of a single diagnostic method such as Kato-Katz limits the diagnostic accuracy of intestinal parasites (Levecke *et al.*, 2011). Hence, there is the need to identify a method that can be used to concurrently detect different intestinal helminthes and protozoan parasites in the same stool sample.

Concentration techniques increase the chances of detecting parasitic elements (PEs) such as; eggs, larvae, oocysts and cysts, (Parameshwarappa *et al.*, 2012). Formalin-ether concentration technique (FEC) as described by Allen and Ridley (1970) is widely used particularly in reference laboratories and hospitals; it has an advantage over other diagnostic methods for detecting both intestinal helminthes and protozoa and stool samples are preserved and can therefore be analysed later after collection (Bogoch *et al.*, 2006; Cheesbrough, 2005). It also provides an economical and rapid diagnosis for intestinal parasites when they are present in sufficient density in faecal samples (Oguama and Ekwunife, 2007). Its centrifugation step further increases the sensitivity and specificity to allow the detection of low numbers of parasites, recover most PEs and retain their morphology (Ahmadi *et al.*, 2007). There is also less risk of infection from bacteria and viruses because they may not be able to survive the concentration process involved

(Cheesbrough, 2005). Akujobi *et al.* (2005) also indicated that this technique has an additional advantage by allowing for transportation and storage of faeces because they are preserved in formalin. However, the technique has some drawbacks; it is qualitative rather than quantitative that is, it cannot determine infection intensities, some PEs can be destroyed and hence under diagnosed, and it has a low sensitivity particularly in low infection intensity settings (Bergquist *et al.*, 2009; Utzinger *et al.*, 2010).

Currently, Kato-Katz technique is the only quantitative method used for diagnosis of STH, and *S. mansoni* infections (Kato and Miura, 1954; Katz *et al.*, 1972; WHO, 1998). Thus, it can be used to determine population prevalence and infection intensities. Owing to its simplicity and relatively low cost, it is recommended by the World Health Organization (WHO) for surveillance and epidemiological field surveys pertaining to human intestinal Schistosomiasis and STH control programs (WHO, 1998). It is also used to facilitate anti-helminthic drug efficacy assessment in clinical trials and monitoring and evaluation of community-based control programs (WHO, 1994). However, it cannot be used for the detection of intestinal protozoan infections (Cheesbrough, 2005). In addition, stool samples that are either loose or watery in nature cannot be processed by Kato-Katz method because of technical difficulties associated with analysing diarrhoeal specimens, and counting of eggs in Kato-Katz smears has also been found to be tedious and time-consuming (Cheesbrough, 2005). Moreover, it has low sensitivity for the detection of light-intensity of *S. mansoni* infections especially after deworming and in young children (Bergquist *et al.*, 2009; Berhe *et al.*, 2004). Studies on human infection with *S. mansoni* have suggested the role of immune response in efficient egg excretion; hence young children especially below six years have not developed an appropriate immune response to excrete eggs leading to false negative results when stool examinations are used for diagnosis

(Karanja *et al.*, 1997). The sensitivity of this method is further compromised by the small amount of stool used i.e. 41.7 mg (Enk *et al.*, 2008), the non-random distribution of eggs in stool (Ye *et al.*, 1997), the day-to-day and intraspecimen variation of helminth egg output (Booth *et al.*, 2003) and time delays from sample collection in the field and processing in the laboratory leading to rapid clearing of hookworm eggs (Dacombe *et al.*, 2007). Although the sensitivity of the method can be improved (Booth *et al.*, 2003) by examining multiple Kato-Katz thick smears produced from a single stool sample or by examining multiple stool samples collected over consecutive days, this has proved to be labour intensive and costly (Speich *et al.*, 2010).

A new and simplified diagnostic device called Mini-FLOTAC has been developed from FLOTAC technique (Cringoli, 2006). It is based on the principle of flotation and has been shown to have a high sensitivity for the diagnosis of intestinal parasites (Barda *et al.*, 2013a; Cringoli *et al.*, 2010; Knopp *et al.*, 2009a; Utzinger *et al.*, 2008). The technique was initially developed for veterinary parasitology (Cringoli, 2006; Gaglio *et al.*, 2008; Rinaldi *et al.*, 2007). But studies suggest that the method holds promise for the diagnosis of *S. mansoni*, STH infections and intestinal protozoans in humans (Barda *et al.*, 2013b). It is also a low cost method, which does not require any expensive equipment and most devices are reusable, therefore, it can be comfortably used in developing countries where resources are limited (Assefa *et al.*, 2014; Barda *et al.*, 2013a; Cringoli *et al.*, 2010). Due to its advantages, it is important to compare its sensitivity and specificity with standard tools such as Kato-Katz and FEC in the diagnosis of intestinal helminthic and protozoan infections especially in young children aged below six years (nursery school children) where there are likely to be low infection intensities.

Hence, this study compared the diagnostic performance of Mini-FLOTAC with Kato-Katz for the detection of STH and *S. mansoni* as well as the performance of Mini-FLOTAC with FEC for

the detection of intestinal protozoa in nursery school children in Mbita, western Kenya. The degree of agreement between the three techniques was also compared. Kato-Katz was used as the reference standard for intestinal helminthes while FEC was used as the reference standard for intestinal protozoa.

1.2 Statement of the Problem

The method used currently i.e. Kato-Katz for surveillance and epidemiological field surveys for *S. mansoni* and STH has a low sensitivity especially in low infection intensities and cannot be used to diagnose for intestinal protozoa. Similarly, FEC is mostly used in hospitals and reference laboratories for the detection of both intestinal helminths and protozoa, but it is a qualitative test and hence cannot be able to give infection intensities. Additionally, Polyparasitism (multiple infections) has become a common phenomenon particularly in developing countries, yet only a few studies have been carried out to assess the extent of polyparasitism in all age groups of communities living in endemic areas. The lack of diagnostic tools that are able to detect multiple parasitic infections with a high level of accuracy is an important underlying reason why so little is known about the extent of polyparasitism. Hence, development and validation of new diagnostic tools is necessary to assess the true extent of polyparasitism.

Therefore, my study compared the sensitivity and specificity of the new Mini-FLOTAC technique with the ones currently used so as to identify the most appropriate technique to be used to diagnose multiple infections.

1.3 Objectives of the Study

1.3.1 General objective

To compare the diagnostic performance of Mini-FLOTAC with formalin-ether concentration and Kato-Katz techniques in the detection of *S. mansoni*, soil-transmitted helminths and intestinal protozoa in nursery school children in Mbita, western Kenya.

1.3.2 Specific objectives

1. To compare the sensitivity and specificity of Mini-FLOTAC with Kato-Katz method in the detection of *S. mansoni* and soil-transmitted helminths such as *A. lumbricoides*, *T. trichiura* and Hookworms in nursery school children in Mbita, western Kenya.
2. To compare the sensitivity and specificity of Mini-FLOTAC with formalin-ether concentration method in the detection of intestinal protozoa such as *Entamoeba histoytica*, *Entamoeba coli* and *G. lamblia* in nursery school children in Mbita, western Kenya.
3. To determine the degree of agreement between Mini-FLOTAC, formalin-ether concentration and Kato-Katz in the detection and determination of prevalence of *S. mansoni*, soil-transmitted helminths, and intestinal protozoa in nursery school children in Mbita, western Kenya.

1.3.3 Null Hypothesis

1. There is no difference in sensitivity and specificity of Mini-FLOTAC and Kato-Katz method in the detection of *S. mansoni* and soil-transmitted helminths such as *A. lumbricoides*, *T. trichiura* and Hookworms in nursery school children in Mbita, western Kenya.

2. There is no difference in sensitivity and specificity of Mini-FLOTAC and Formalin-ether concentration method in the detection of intestinal protozoa such as *Entamoeba histolytica*, *Entamoeba coli* and *G. lamblia* in nursery school children in Mbita, western Kenya.
3. There is no difference in degree of agreement between Mini-FLOTAC, Formalin-ether concentration, and Kato-Katz in the detection and determination of prevalence of *S. mansoni*, soil-transmitted helminths, and intestinal protozoa in nursery school children in Mbita, western Kenya.

1.4 Significance of the Study

The findings of the study have shown that Mini-FLOTAC is sensitive compared to the other methods in the detection of all the parasites. Therefore, this study demonstrates that Mini-FLOTAC is a sensitive and relatively simple technique for the diagnosis of intestinal parasites and could be a good alternative to the standard Kato-Katz technique in disease surveillance and epidemiological studies. However, it had a low sensitivity for detection of intestinal protozoa. Hence, the results of the study have provided useful information that can inform identification of Mini-FLOTAC as the most appropriate diagnostic tool to be used for accurate diagnosis of multiple intestinal parasitic infections especially in low intensity settings. In addition, the technique can be used to identify the group at risk of infection and for accurate estimation of infection prevalence especially in children below six years.

1.5 Study Limitations

The limitation of this study was that the results can only be applied to a population of children below six years, thus the results cannot be generalized in the general population. Further research

can be done in a different setting e.g. in adults with low infection intensities to determine whether the results can be comparable.

CHAPTER TWO

LITERATURE REVIEW

2.1 Burden of Intestinal Parasites

Helminthic infections are important causes of morbidity and mortality throughout the world (Hotez *et al.*, 2009). There are about 20 major helminthic infections of humans, affecting more than one-third of the world's population (WHO, 2010). The major soil-transmitted helminths (STH) include; *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Necator americanus* and *Ancylostoma duodenale*) (Bethony *et al.*, 2006). Infections caused by *Schistosoma mansoni* accounts for most of the global helminth disease burden (Steinmann *et al.*, 2006). According to WHO, about 2 billion people are affected, and 300 million are ill as a result of these infections, the majority being children (Tekeste *et al.*, 2013). This occurs despite the worldwide efforts at controlling these diseases and the number of infected persons is growing, as well as the number of deaths (Tekeste *et al.*, 2013).

The burden of intestinal infections in human populations occurs in marginalized, low-income and resource-constrained regions of the world, with many people in developing areas of sub-Saharan Africa, Asia and the Americas affected (Lustigman *et al.*, 2012). In Kenya, communities along the shores of Lake Victoria in western Kenya suffer significant morbidities associated with *S. mansoni* and STH infections that contribute to poor health outcomes in infected persons, particularly children (Mwinzi *et al.*, 2012; Odiere *et al.*, 2011). *Schistosoma mansoni* and STH infections in this region are associated with contact with contaminated lake water and the surrounding environment (Handzel *et al.*, 2003; Nagi *et al.*, 2014). Other studies in this region have also shown that school children and members of communities that border Lake Victoria are

infected with schistosomiasis and STHs (Brooker *et al.*, 2000; Handzel *et al.*, 2003; Standley *et al.*, 2010; Thiong'o *et al.*, 2001).

Schistosomiasis remains the most important water-borne disease and common in many parts of the world, with about 200 million people infected globally (Terefe *et al.*, 2011). Of the global burden of schistosomiasis an estimated 85% of cases is found in sub-Saharan Africa (Terefe *et al.*, 2011). In Kenya, the two major endemic areas for *S. mansoni* are in the Lake Victoria Basin and Lower Eastern regions (Brooker *et al.*, 2009), and over 6 million people are estimated to be infected (Chitsulo *et al.*, 2000), and many more are at risk. The highest infection rates are found in adolescents aged 10-19 years, but adult workers in rural areas who are employed in activities associated with water contact are also affected (Mwinzi *et al.*, 2012). Overall, the prevalence of schistosomiasis is over 65% in endemic communities in Kenya and contributes to significant morbidity (Ouma *et al.*, 2001). In Mbita, schistosomiasis is largely associated with Lake Victoria (Nagi *et al.*, 2014), with a prevalence level among school children along the lake shores ranging between 29-94%.

For many years, the burden of schistosomiasis has been thought to be significant primarily in older children and adults (Odogwu *et al.*, 2006). Hence, lots of attention has been given to school going children while the pre-school children have been neglected. Reason being children in this tender age are often thought to have little contact with the contaminated waters to place them at risk of any infections (Odogwu *et al.*, 2006), compared to their active school-age counterparts. But studies have demonstrated that pre-school-age children can have high rates of *S. mansoni* and STH infections (Verani *et al.*, 2011). According to WHO, pre-school-age children comprise between 10%-20% of the 3.5 billion people living in Schistosomiasis and STH-endemic areas

(WHO, 2006). Since the parasitological method used in epidemiologic studies (examination of a single stool sample by Kato-Katz technique) lacks sufficient sensitivity especially in detecting light intensity infections, the schistosome and STH infections in most children may go undetected.

Among the many species of intestinal protozoa, *E. histolytica* and *G. Lamblia* are potentially pathogenic and constitute a public health problem in most parts of the world (WHO, 2010). The prevalence of Giardiasis is 2-7% in developed countries, whereas it is 20–30% in developing countries due to water and food contamination (Savioli *et al.*, 2006). Around 200 million people are infected around the world with 50,000 new cases occurring every year. *Entamoeba histolytica* infects hundreds of millions of people per year; while most individuals are asymptomatic, perpetuating the natural cycle of the organism through faecal excretion of infective cysts (Pritt and Clark, 2008). Minority suffers from the severe morbidity associated with invasive disease (approximately 50 million) with an estimated 100,000 dying every year from severe and invasive amebiasis (Xime'nez *et al.*, 2009). In Kenya, these infections are a public health concern and occur in all age groups, but the problem is predominant among school age children (Nguhiu *et al.*, 2009). This contributes to childhood growth retardation, malnutrition, and anaemia (Hotez *et al.*, 2009). The infections occur in both rural and public populations necessitating regular deworming especially in school going children who are more vulnerable (Nguhiu *et al.*, 2009).

Current estimates of the total number of people infected with STHs stand at 807-987 million for *A. lumbricoides* (Cheesbrough, 2005), 604-795 million for *T. trichiura*, and 576-740 million for hookworms (DeSilva *et al.*, 2003). It is estimated that approximately 10 million Kenyans are

infected with STHs and over 12 million people living in rural endemic areas in the country are at risk of infection with these parasites (Mwinzi *et al.*, 2012). Studies among school children in western Kenya have reported STH prevalence ranging from 16% in an urban setting (Odiere *et al.*, 2011), to 63% (Handzel *et al.*, 2003) and 68% (Riesel *et al.*, 2010) in rural areas. The morbidity caused by these worms is commonly associated with heavy infection intensities (Riesel *et al.*, 2010). Light and moderate STH infections are often associated with little or no acute disease, whereas heavy infections can lead to life-threatening conditions like intestinal obstruction (*A. lumbricoides*), acute dysentery (*T. trichiura*) and severe blood loss and anaemia (hookworms) (Bethony *et al.*, 2006). Compared with any other age group, school-age children, and pre-school children are the most vulnerable group, and they harbour the greatest numbers of intestinal worms (Drake *et al.*, 2000; Tchuente', 2012).

Studies have revealed that most effects resulting from infections with *A. lumbricoides* and *T. Trichiura* are seen among children, while hookworm-related morbidity is also found in adults, particularly women of child-bearing age (Bethony *et al.*, 2006). Chronic ascariasis leads to reduced vitamin A absorption and lactose intolerance and the constant blood loss resulting from hookworm infection gives rise to iron-deficiency anemia and protein malnutrition (Bethony *et al.*, 2006). Together, these symptoms lead to nutritional deficits which manifest themselves as impaired physical growth and fitness including poor worker productivity and impact on cognition, school attendance and performance (Drake *et al.*, 2000). Pregnant women with hookworms are at high risk of giving birth to low birth-weight babies, poor breast milk production, and babies who fail to thrive (Brooker *et al.*, 2008). Sufficient evidence demonstrates that the morbidity caused by these infections impose a substantial burden of disease, decrease

productivity and inadequate socio-economic development (Lustigman *et al.*, 2012). However, accurate identification of at-risk groups targeted for individual treatment, assessment of therapy efficacy, and evaluation of control strategies remains a challenge due to lack of “ideal” diagnostic tools that have constant high sensitivity and specificity.

While several factors may make estimating the number and burden of intestinal parasitic infections difficult, lack of accurate diagnostic tools with good sensitivity and specificity is a major one (Endris *et al.*, 2013). Efforts to identify an ‘ideal’ or alternative diagnostic technique based on fecal egg count need to be strengthened, especially considering the many MDA programs that are currently being rolled out. This will not only help in accurate identification of high-risk groups and infection rates in a population but also develop sound and targeted control measures to reduce the adverse health, social and economic outcomes caused by these diseases to individuals and the community at large.

2.2 Epidemiology and Clinical Indications of Common Intestinal Infections

2.2.1 *Ascaris lumbricoides*

Ascaris lumbricoides is the most common and important STH (Cheesbrough, 2005). Current estimates indicate that more than 807 people are infected worldwide, and over 250 million suffer from associated morbidity (WHO, 2010). Important factors associated with an increased prevalence of disease include socio-economic status, defecation practices and cultural differences relating to personal and food hygiene as well as housing and sewage systems (Bethony *et al.*, 2006). Most infections are subclinical; more severe complications occur in children who tend to suffer from the highest worm burdens (Drake *et al.*, 2000). The parasite is one of the major

public health problems in communities where the prevailing social environment is characterized by poverty, poor housing, inadequate sanitary practices and overcrowding (Bethony *et al.*, 2006).

Ascaris lumbricoides has been shown to play a significant role in childhood malnutrition, which leads to growth retardation, cognitive impairment, and poor academic performance, resulting in a poorer quality of life and less ability to contribute to society (Drake *et al.*, 2000). Studies on the health of Kenyan school going children indicated that ascariasis is most common and intensive in school children aged 6-15 years (Ngonjo *et al.*, 2012; Nguhiu *et al.*, 2009).

2.2.2 *Trichuris trichiura*

Trichuris trichiura (trichuriasis) is an intestinal nematode and is the third most common helminth infection of humans (Peters and Pasvol, 2005). Trichuriasis affects about 1 billion people throughout the world (WHO, 2010). It is more common in areas with tropical weather such as Asia, sub-Saharan Africa, and the Americas, particularly in impoverished regions of the Caribbean. It is spread via faecal-oral transmission and is more common in poor rural communities and areas that lack proper sanitary facilities with easily contaminated food and water (Despommier *et al.*, 2005). A large number of individuals who are infected actually harbour fewer than 20 worms and are asymptomatic; those with a larger burden of infection (greater than 200 worms) are most likely to develop clinical disease. School-aged children tend to be most heavily infected because of their high exposure risk (Bethony *et al.*, 2006). In heavy trichuriasis, infected people may show mild anaemia, eosinophilia, bloody diarrhoea (classic *Trichuris* dysentery syndrome, or chronic *Trichuris* colitis), prolapsed rectum (especially in children), and impaired physical and mental growth (Drake *et al.*, 2000). A study conducted by

Ngonjo *et al.* (2012) found over 10% prevalence of trichuriasis among school going children in Kenya.

2.2.3 Hookworms

Hookworms rank amongst the most widespread of STHs (Brooker *et al.*, 2004). The two principal species of hookworm infecting humans are *N. americanus* and *A. duodenale* (Hotez *et al.*, 2004). *Necator americanus* is the most widespread hookworm globally, whereas *A. Duodenale* is more geographically restricted in distribution (Bethony *et al.*, 2006). An estimated 576 million people are chronically infected with hookworm, and another 3.2 billion are at risk, with the largest number of afflicted individuals living in impoverished rural areas of sub-Saharan Africa, Southeast Asia and tropical regions of the Americas (Hotez *et al.*, 2004). Although hookworm infection does not directly account for substantial mortality, its greater health impact is in the form of chronic anaemia and protein malnutrition as well as impaired physical and intellectual development in children (Drake *et al.*, 2000). Reports have also indicated high prevalence of hookworm infections in Côte d'Ivoire (Booth *et al.*, 2003), Ghana (de Gruijter *et al.*, 2005), Zanzibar (Barda *et al.*, 2013a), and in Kenya (Nguhiu *et al.*, 2009).

2.2.4 *Schistosoma mansoni*

Schistosoma mansoni is the most prevalent of the schistosome species that affect the intestines and liver with an estimated 62 million persons infected worldwide (WHO, 2012). The global burden of the disease is mostly in sub-Saharan Africa though some infections occur in parts of South America, the Caribbean, and the Middle East (Steinmann *et al.*, 2006). Schistosomes have a snail intermediate host, and human contact with water is necessary for infection (Nagi *et al.*,

2014; Steinmann *et al.*, 2006). The adult worms of *S. mansoni* live in the small blood vessels of the liver and intestines, where they cause serious pathology, morbidity and even death in individuals with heavy and chronic infections (Cheesbrough, 2005). They produce eggs that typically have a lateral spine, non-operculated and contain a larva called miracidium, which are discharged in the faeces (Cheesbrough, 2005). Studies conducted in Kenya have shown that *S. mansoni* has a prevalence of up to 80 % in endemic areas (Nagi *et al.*, 2014; Odiere *et al.*, 2012; Verani *et al.*, 2011).

2.2.5 *Entamoeba histolytica* and *Giardia lamblia*

Among the many species of intestinal protozoa, *E. histolytica* and *G. lamblia* are potentially pathogenic and constitute a public health problem in most parts of the world (WHO, 2010). The highest prevalence of amebiasis is in developing countries where barriers between human feces and food and water supplies are inadequate (Pritt and Clark, 2008). The parasite is endemic in most tropical and subtropical areas of the world, where it causes millions of cases of dysentery and liver abscess each year. Around 200 million people are infected around the world with 50,000 new cases occurring every year and an estimated 100,000 dying every year from severe and invasive amebiasis (WHO, 2010).

The infection is transmitted orally by drinking infected water containing infective cysts (Xime'nez *et al.*, 2009). In Kenya, these infections are a public health concern (Ngonjo *et al.*, 2012). Although these infections occur in all age groups, the problem is predominant among school aged children. This contributes to childhood growth retardation and absenteeism (Nguhiu *et al.*, 2009). The infections occur in both rural and public populations necessitating regular deworming especially in school going children who are more vulnerable (Ngonjo *et al.*, 2012).

Giardiasis affects an estimated 200 million people and is most common in children aged one to five years old (Savioli *et al.*, 2006). In severe cases, it may be associated with acute and persistent diarrhoea, malabsorption of nutrients and impairment of children's growth and development. The prevalence of Giardiasis is 2– 7% in developed countries, whereas it is 20– 30% in developing countries (Savioli *et al.*, 2006). Most parasite transmission follows the oral-faecal route in which cysts are ingested. *Entamoeba histolytica* and *G. lamblia* are of public health importance in Kenya and have a prevalence of 12.6% and 4.2% respectively in school children (Ngonjo *et al.*, 2012).

2.3 Laboratory Procedures for the Diagnosis of Intestinal Helminths and Protozoans

Definitive diagnosis of helminths and protozoan infections depends on the demonstration of a stage of the parasite's life cycle in the human host (Garcia, 2001). The adult worms and protozoa that inhabit the intestine discharge their eggs, cysts or larvae in faeces (Cheesbrough, 2005). Therefore, laboratory diagnosis of intestinal parasites is based on detection and identification of characteristic eggs, cysts or larvae in stool samples (Cheesbrough, 2005). A wide variety of laboratory methods, including parasitological, molecular, serological and cultural approaches, have been developed over the years for the diagnosis of intestinal parasites (Garcia, 2001). However, these techniques come with significant difference in the cost, sensitivity, simplicity, and field applicability.

2.3.1 Parasitological methods

2.3.1.1 Stool microscopy

Microscopic diagnosis is generally sensitive, simple, and economical (Cheesbrough, 2005). If performed correctly, stool microscopy offers many advantages over other diagnostic methods for

detecting intestinal parasites (Bogoch *et al.*, 2006). Diagnostic tests involving microscopy include direct wet preparations, concentration methods and the Kato-Katz technique (Cheesbrough, 2005).

Concentration techniques increase the sensitivity of stool microscopy to allow the detection of small numbers of parasites that may be missed by using only a direct wet smear (Allen and Ridley, 1970). Basically, concentration techniques operate in two ways, either by sedimentation (Ritchie, 1948) in which the PEs sink to the bottom of the liquid suspension, or by flotation (Truant *et al.*, 1981) in which the PEs are suspended in a liquid of high specific density to make them buoyant and float to the surface where they are collected for examination (WHO, 1994). The floating medium generally employed include saturated aqueous solution of sodium chloride and zinc sulphate with specific gravities of approximately 1.20 and 1.18 respectively (Cheesbrough, 2005).

In general, flotation gives a “cleaner” preparation than sedimentation, yet each has a preference over another in certain aspects (Cheesbrough, 2005). The procedure is simple and more sensitive in detecting protozoan cysts and helminthic eggs or larvae (Cheesbrough, 2005). However, eggs of common intestinal helminths and protozoan cysts become shrunken. Hence they can be missed (Utzinger *et al.*, 2010). Therefore, no ideal method of concentration is capable of detecting all forms of parasites that may be present in stool specimens.

2.3.1.2 Kato-Katz technique

Kato-Katz technique (Kato and Miura, 1954; Katz *et al.*, 1972) is the most widely used method and diagnostic tool in epidemiological field surveys and surveillance involving human intestinal

helminthic infections and continues to be the standard method for assessing prevalence and intensity. It is preferred because of its simplicity, low cost, and the established system to stratify infection intensity into different classes based on cut-offs of egg-counts (WHO, 1998).

According to Cheesbrough (2005), the technique entails the examination of a standard sample (determined by the size of the template used) of fresh faeces pressed between a microscope slide and a strip of cellophane that has been pre-soaked in glycerin- Malachite green (Katz *et al.*, 1972). The cellophane coverslips, 22 x 30 mm, are pre-soaked for at least 24 hours in a glycerin-malachite green solution of 100 ml pure glycerin, 100 ml distilled water and 1 ml of 3% malachite green (Garcia, 2001). The Kato-Katz template may be made of stainless steel, plastic, or cardboard, and different sizes have been produced in different countries: a 50 mg template that has a hole of 9 mm on a 1 mm thick template; a 41.7 mg template that has a hole of 6 mm on a 1.5 mm thick template, and a 20 mg template with a hole of 6.5 mm on a 0.5 mm thick template (Ebrahim *et al.*, 1997). After the faecal film has cleared, eggs in the entire film are examined and counted, and the number of eggs of each species reported is multiplied by the appropriate multiplication factor to give the number of eggs per gram (epg) of faeces (Cheesbrough, 2005). When using a 50 mg template, a multiplication factor of 20 is used and for a 20 mg template, the multiplication factor of 50 is used (Katz *et al.*, 1972). But WHO (1998) recommended the use of a 41.7 mg template with a multiplication factor of 24.

This technique has some drawbacks, such as low sensitivity for the detection of light-intensity infections especially in young children and after deworming (Bergquist *et al.*, 2009; Berhe *et al.*, 2004), the sensitivity is further compromised by the small amount of stool used (Enk *et al.*, 2008). Though the sensitivity can be increased by examining multiple Kato-Katz thick smears

prepared from the same stool sample or, from multiple stool samples (Booth *et al.*, 2003), this has proved to be tedious and time-consuming. Furthermore, a study conducted by Siegel *et al.* (1990) demonstrated difficulty in processing diarrhoeal stools. Other drawbacks of the method include; high risk of infection when handling fresh stool, and rapid clearing of hookworm eggs (Cheesbrough, 2005). Further, counting of eggs in Kato-Katz smears can be tedious and time-consuming, and can lead to technical errors (Ebrahim *et al.*, 1997). The technique is also known to be unsuitable for detection of protozoan cysts, larvae, small fluke eggs or thin-shelled eggs such as *Hymenolepis* species because eggs clear during the clearing process in a short time of 30 minutes (Cheesbrough, 2005).

2.3.1.3 Formalin-ether concentration technique

The FEC procedure as described by Ritchie (1948), and Allen and Ridley (1970) provide the best diagnostic outcome in epidemiological studies. The technique requires the use of formalin as a fixative and ether (Allen and Ridley, 1970) or ethyl acetate (Young *et al.*, 1979) as a lipid removing agent. The formalin fixes and preserves the faecal specimen and ether or ethyl acetate extracts debris and fat from the faeces, leaving the parasites at the bottom of the suspension (Akujobi *et al.*, 2005). Several authors consider FEC technique as the most effective technique that recovers the broadest range of parasites, and hence, the “gold standard” method of all parasitological techniques (Cheesbrough, 2005; Oguama and Ekwunife, 2007).

The advantages of this method are that it will recover most ova, cysts, and larvae and retain their morphology, thereby facilitating identification (Cheesbrough, 2005). There is less risk of infection from bacteria and viruses because they may not be able to survive the concentration process involved (Cheesbrough, 2005). The concentration technique has an additional advantage

by allowing for transportation and storage after faeces are preserved in formalin (Akujobi *et al.*, 2005). Conversely, it has the disadvantage of destroying trophozoite stages and distorting cellular exudates, and liquid stools do not concentrate well (Cheesbrough, 2005). Because concentration procedures require a laboratory with well-trained personnel, centrifuge to separate parasites, electricity to run centrifuges, a well-ventilated work space, adequate water supply, a standard light microscope, and consistent availability of regular supply of reagents, it tends to be expensive running the test (Allen and Ridley, 1970).

2.3.1.4 Mini-FLOTAC technique

Mini-FLOTAC is a simplified diagnostic device recently developed from FLOTAC techniques to improve the quality of copromicroscopic diagnosis of intestinal parasitic infections (Cringoli, 2006). It is a multivalent technique based on the principle of floatation and subsequent translation of the apical portion of the floating suspension (Cringoli *et al.*, 2010). The technique was initially developed for veterinary parasitology (Cringoli, 2006; Rinaldi *et al.*, 2007), but has more recently been extended to human parasitology and validation is still underway for diagnosis of major intestinal protozoa, nematodes and trematodes parasitizing humans (Knopp *et al.*, 2009a; Utzinger *et al.*, 2008).

The Mini-FLOTAC kit uses two different floatation solutions for helminthic eggs and protozoan cysts. F2 (saturated sodium chloride; specific gravity 1.20) is recommended for the diagnosis of STHs and FS7 (zinc sulphate solution; specific gravity 1.35) is recommended for the diagnosis of *S. mansoni* and intestinal protozoa (Cringoli *et al.*, 2010). The Mini-FLOTAC kit has three components; a key, the base, and a reading disc. The base includes two 1-mL floatation chambers labelled 1 and 2 which are designed for optimal examination of faecal sample

suspensions and also permits maximum magnification. The Fill-FLOTAC is a reusable sampling device that is part of the Mini-FLOTAC kit. It consists of a container, a collector, and a filter. It facilitates the performance of homogenization, filtration and filling processes of the Mini-FLOTAC technique (Cringoli, 2006).

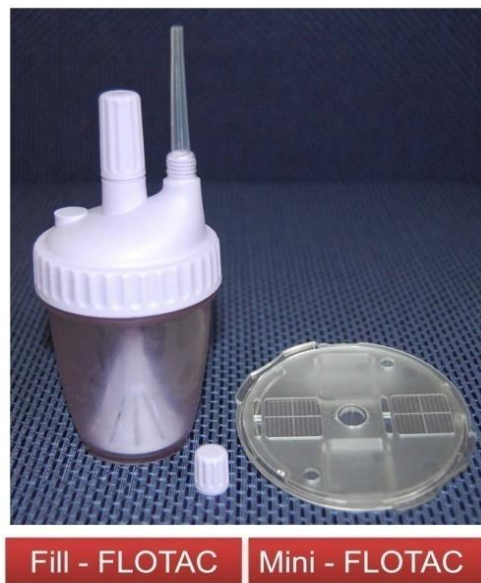


Plate 1: The Fill-FLOTAC and Mini-FLOTAC kit (Barda *et al.*, 2013b)

The Mini-FLOTAC has a high sensitivity for diagnosing intestinal parasites (Knopp *et al.*, 2009a; Utzinger *et al.*, 2008). It is also cheap and does not require expensive equipment and therefore, it can be comfortably used in developing countries (Barda *et al.*, 2013a; Knopp *et al.*, 2011). Other studies have also shown that Mini-FLOTAC has a higher sensitivity than multiple Kato-Katz thick smears for the diagnosis of STH and *S. Mansoni* (Glinz *et al.*, 2010; Knopp *et al.*, 2009b).

A study conducted in Tanzania and India also showed that Mini-FLOTAC is more sensitive (90 %) method for diagnosing helminth infections compared to FEC and direct method and further suggested that if the method can be improved, it can be used for the diagnosis of intestinal protozoa (Barda *et al.*, 2013b). Furthermore, a study conducted in Ethiopia also confirmed that a single FLOTAC is more sensitive than a single Kato-Katz for the diagnosis of STH infections, and suggested further standardization and validation of the method in different epidemiological settings with varying levels of infection intensities (Habtamu *et al.*, 2011). However, Assefa *et al* (2014) in their study in western Kenya reported that the sensitivity of Mini-FLOTAC and Kato-Katz was comparable, but Kato-Katz afforded greater cost-effectiveness and recommended future work to evaluate the cost-effectiveness of STH surveillance in different settings.

Further, it has been shown that Mini-FLOTAC can be carried out by local laboratory technicians after a short period of training. Moreover, it takes lesser time as compared to Kato-Katz to perform i.e. 3 minutes for sample preparation, 10 minutes for the eggs to float and approximately 5 minutes for reading, whereas Kato-Katz takes 1-2 minutes for sample preparation, 30 minutes for the glycerol to clarify the eggs and 3-5 minutes for reading (Barda *et al.*, 2013a). In terms of cost, Kato-Katz kits are purchased at a low price for developing countries, and Mini-FLOTAC is under negotiation with WHO to be donated free of charge, and most devices are reusable, and no further equipment is needed (Cringoli, 2006). An additional advantage of the fill-FLOTAC system is that it is a closed method, whereby the stool and the solutions are mixed in closed system and is thoroughly safe with no risk of contamination of the operator. Another recognized advantage of the Mini-FLOTAC method is that it can be performed on fixed stool, enabling processing at a later date and the formalin that is added into the mixture can help to preserve the samples and stored for future testing (Imali, 2015). This can help to increase the quality control

process and overcome some of the logistical difficulties in examining fresh stool samples in the field on the day of collection (Barda *et al.*, 2013a). Moreover, the technique can be used to test for watery or diarrhoeal stool.

With the limitations of the standard methods used, the current study therefore sort to compare the sensitivity and specificity of Mini-FLOTAC with the standard methods so as to identify the most sensitive technique that can be used for concurrent diagnosis of multiple infections especially in children below six years where infection intensities are likely to be low. The results of my study will help in the identification of a technique that can be used in accurate identification of high risk groups and also determine infection prevalence in these populations.

CHAPTER THREE

METHODOLOGY

3.1 Study Area

The study was conducted in eight villages (Kamasengre, Wakondo, Ngodhe Island, Wasaria, Kombe, Wanga, Nyawiya and Usungu) all of which are located 5 Km radius from Lake Victoria in Mbita sub County, in Homa-Bay County. The study was nested within the Health Demographic Surveillance System (HDSS) in Mbita, which covers an area of 163.28Km². It is between latitudes 0°21' and 0°32' South, and longitudes 34°04' and 34°24' East as shown in Figure 1. The HDSS follows the study population of my study of 102 primary schools (private and public) with a population of 5580 nursery school children (Kaneko *et al.*, 2012). The ethnic group in this area is predominantly Luo (98%). The main economic activities in the area are fishing and subsistence farming. The annual rainfall in Mbita district is between 800-1900 mm. However, the rains are slightly lower in Rusinga Island with an annual range of 800–1200 mm. The long rains start from March to May while the onset of short rains is from September to December. The temperatures in this region range from 15°C to 30°C (Kaneko *et al.*, 2012).

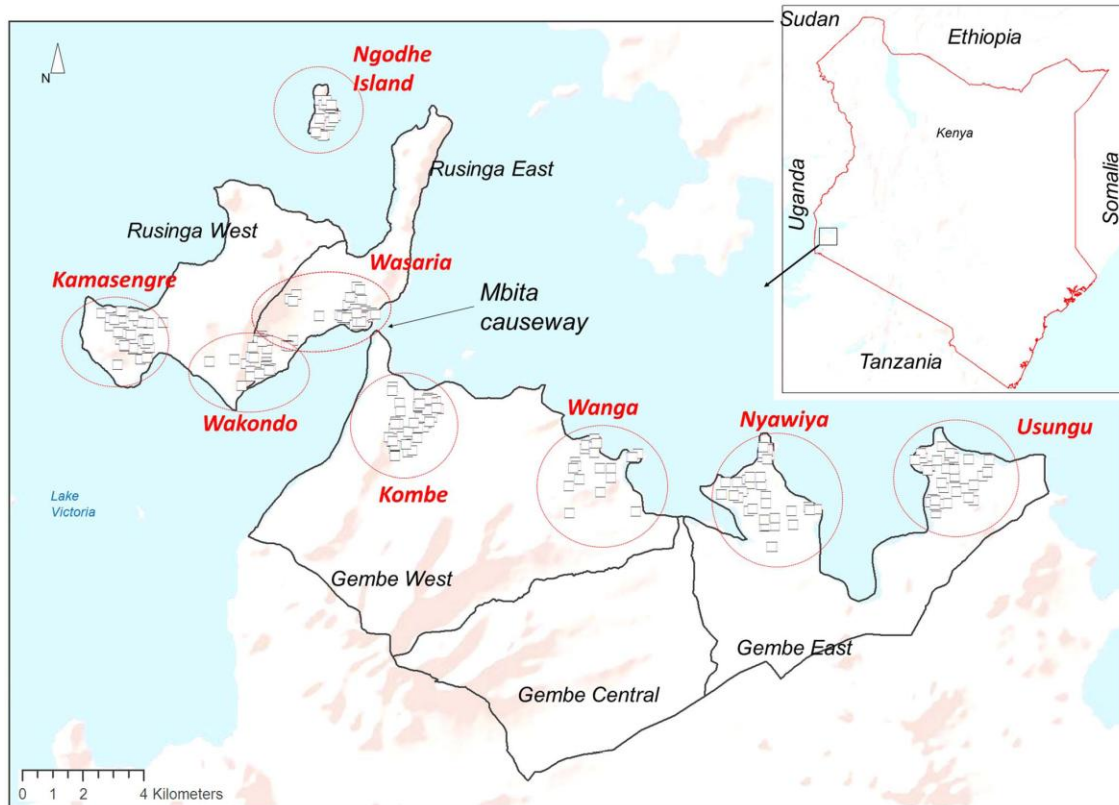


Figure 1: A map of the study area showing the areas under study

3.2 Study Design

This was a methodological study. It is another class of studies that have been designed due to advanced technology in diagnosis where new techniques and devices have been developed. It is designed to measure the repeatability/validity of an instrument or the agreement between two methods (Kothari, 2002). In this study design the sensitivity and specificity as well as degree of agreement of Mini-FLOTAC, formalin-ether concentration, and Kato-Katz techniques was compared. Laboratory experiments using standard procedures were used to detect the parasites.

3.3 Study Population

The target population was pre-school children aged below six years attending public and private nursery schools (Early Child Development- ECD) in Mbita. There are 102 primary schools (51 public and 51 private) in Mbita with a total population of 5580 children of which 2683 are boys, and 2897 are girls (Kaneko *et al.*, 2012).

3.4 Sampling Criteria

1. Schools from villages located within 5 km from the lakeshore.
2. School children registered in public and private primary schools and associated ECD centres.
3. In HDSS area in Mbita.

3.5 Inclusion Criteria

Children aged below six years old in ECD from both private and public schools.

3.6 Exclusion Criteria

1. Children who will not assent to participate to the study.
2. Children whose parents/guardians do not give consent to participate.
3. Children in special schools.
4. Children currently enrolled in different but related studies.
5. Children from schools which were given mass chemotherapy in the last 1 year.

3.7 Sampling Procedure

The method that was used for sampling was Lot Quality Assurance Sampling. It is a random sampling method that was originally used for quality control in industrial production. It is similar to cluster or stratified sampling where a sampling unit is drawn from the lot. It is cheap and quick to perform and it has been recommended by WHO to be used in modern research in the field of public health in health surveys. It has an advantage of taking a small fixed random sample from each set of items in the population (Kothari, 2002).

Sampling was carried out from 22 schools (lots) that were legible for the study. A sampling frame with a list of all the sampling units (children) was prepared for each school and sampling units were randomly drawn from each lot. In each school, a fixed sample of twenty children (10 boys and 10 girls) was randomly selected, but one school had 15 children; hence, a total of 435 children were sampled. All children whose parents or guardian consented were asked to provide stool samples for the study

3.8 Sample Size Calculation

The sample size was determined from the target population of all nursery school children. The total nursery school population in these schools is 5580 children (Kaneko *et al.*, 2012). The formula as used by Kothari, (1990) was used in sample size determination as shown below. A confidence level of 95% will be assumed.

$$n = \frac{Z^2 pqN}{e^2(N - 1) + Z^2 pq}$$

n = minimum sample size

N= Target population

Z= Standard normal deviate at the required confidence level (error 5% Z = 1.96)

p= Proportion of subjects in the sample population estimated to be infected by intestinal parasitic infections in Kenya is 53% (Nguhiu *et al.*, 2009).

q=1-p

q=1-0.53

e= Absolute precision expressed as a fraction of 100 (performance level of 5 % allowed = 0.05).

$$n = \frac{1.96^2 \times 0.53 \times 0.47 \times 5580}{0.05^2 (5579) + 1.96^2 \times 0.53 \times 0.47}$$

Therefore, the sample size is; 370 children, but this was adjusted to 435 children so as to include 10% of the sample to take care of any dropouts and also for insufficient stool sample.

3.9 Field Procedures

The day before sample collection, the children were instructed on the procedure for specimen collection after which they were given labeled specimen cups to bring overnight stool first thing the following morning. One stool sample was obtained from each participant. The stool specimens were collected by field staff before the start of classes. All collected samples were packed in cooler boxes and transported to Nagasaki University laboratory in Mbita for processing.

3.10 Laboratory Procedures

3.10.1 Mini-FLOTAC technique

The stool samples were processed as follows for the Mini-FLOTAC basic technique; Two fill-FLOTAC were performed for each sample, one using F2 solution and the other F7 solution. Eighteen ml of the F2 and FS7 solution were put in two different fill-FLOTACs and then 2 grams of stool was added. The fill-FLOTAC was closed, and the stool homogenized. After putting the tip on the fill-FLOTAC, the samples were homogenized again, and the two chambers filled until the meniscus was formed. Before reading the slide and translating the reading disc, the disc was allowed to stand for 10 minutes to allow the eggs and cysts to float. After 10 minutes the eggs and cysts within the grid were examined under the microscope at x10 magnification and recorded as positive or negative (present or absent) for *S mansoni*, *A. lumbricoides*, *T. trichiura*, Hookworm, *G. lamblia*, *E. histolytica* and *E. coli*. The Mini-FLOTAC procedure is illustrated in plate3 below.

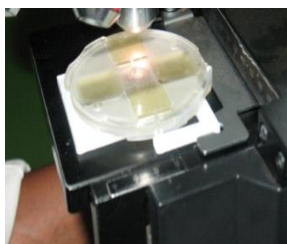
STEP 1 Sample preparation

- Add 18ml of floatation solution to the fill-FLOTAC
- Add 2g stool specimen



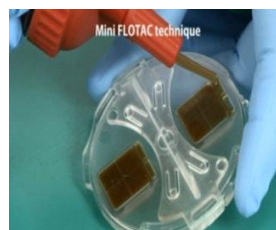
STEP 2 Homogenization

- Close the fill-FLOTAC tightly.
- Homogenize.



STEP 4 Microscopy

- Turn the reading disc clockwise
- Remove the key
- Examine under the microscope



STEP 3 Loading

- Tilt the Mini-FLOTAC kit and fill the chambers till a meniscus forms
- Leave undisturbed for 10 minutes.

Plate 2: Mini-FLOTAC procedure

3.10.2 Kato-Katz technique

Schistosoma mansoni and STHs were examined by Kato-Katz thick smear technique and it was used as a reference standard for the detection of helminthes (Kato and Miura, 1954; Katz *et al.*, 1972). Two slides of Kato-Katz thick smear were prepared from each sample using a standard 41.7mg template (Katz *et al.*, 1972). The slides were labelled with the sample ID plus letter A or B. A small amount of stool was placed on a newspaper and a piece of nylon sieve pressed on top so that some of the stool sieve through and accumulate on the top. Using a wooden spatula, the sieved stool was collected and added to fill the hole of the template placed on a slide. The spatula was passed over the filled template to remove excess stool from the edge of the hole. The template was then removed carefully so as to leave the stool on the slide. The stool was covered with Cellophane strip (pre-soaked in glycerine-malachite green) after which the slide was inverted and pressed firmly to spread evenly. The slide was then placed on the bench with the cellophane upwards to enable evaporation of water while glycerol clears the stool. After 30 minutes of clearing, the smears were examined microscopically by use of x10 magnification for hookworms, *S mansoni*, *A. lumbricoides*, *T. trichiura*, *G. lamblia*, *E. histolytica* and *E. coli* and recorded as present or absent (positive or negative). The slides were read by two qualified laboratory technologists and each adhered to either A or B series to avoid duplicate reading of the same stool sample by the same technologist. The procedure for Kato-Katz is illustrated in the plate below.

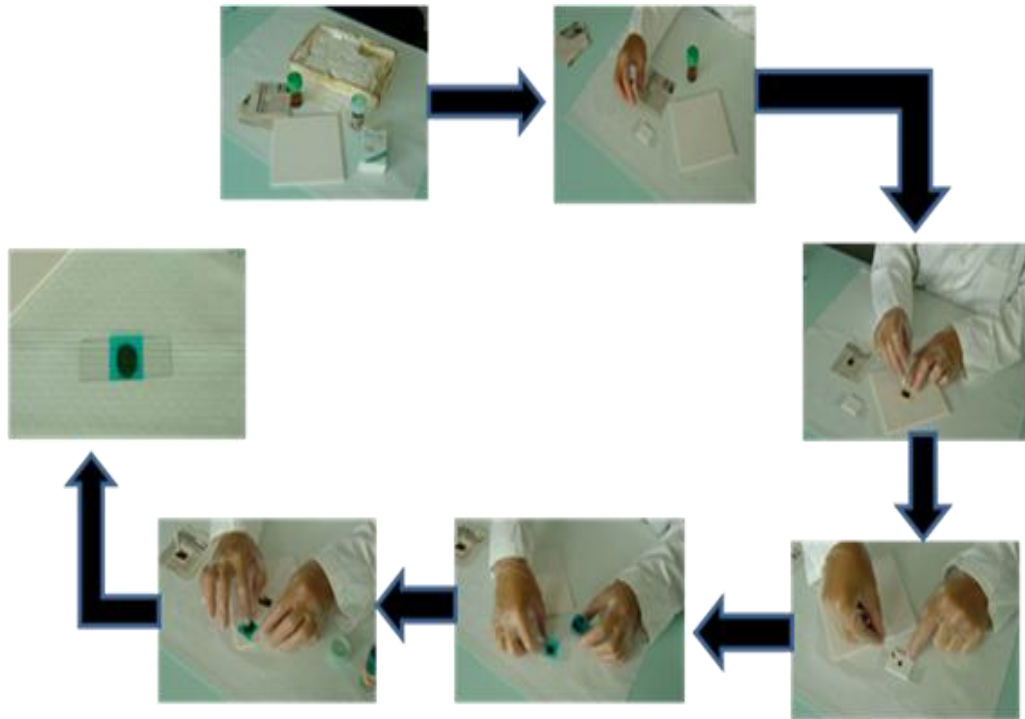


Plate 3: Kato-Katz procedure

3.10.3 Formalin-ether concentration technique

The technique was used as the reference standard for protozoans. The protocol was based on the work of Allen and Ridley as described by Cheesbrough (Allen and Ridley, 1970; Cheesbrough, 2005). One gram of stool was put in a polypot and 7ml of 10% formal saline added and then emulsified using applicator stick. The stool was then strained into a centrifuge tube by use of dump cotton gauze to remove the debris. Three ml of diethyl ether was added into the mixture then mixed thoroughly. The tube was centrifuged at 2500 rpm for 2 minutes. After centrifugation, the faecal material was dislodged from the walls of the tube and the supernatant discarded leaving behind the sediment. The tube was returned to its upright position to allow the fluid from the side of the tube to drain to the bottom. The bottom of the tube was tapped to re-

suspend and mix the sediment. One drop of Lugol's iodine added and mixed. The mixture was then picked by use of a Pasteur pipette and put on a microscope slide then cover-slipped and examined microscopically by use of x10 objective. The protozoan cysts were identified based on the morphology and recorded as present or absent (positive or negative). The comparison between the three techniques was then made on qualitative diagnosis as FEC is not a quantitative method. The procedure is summarized in figure 2 below.

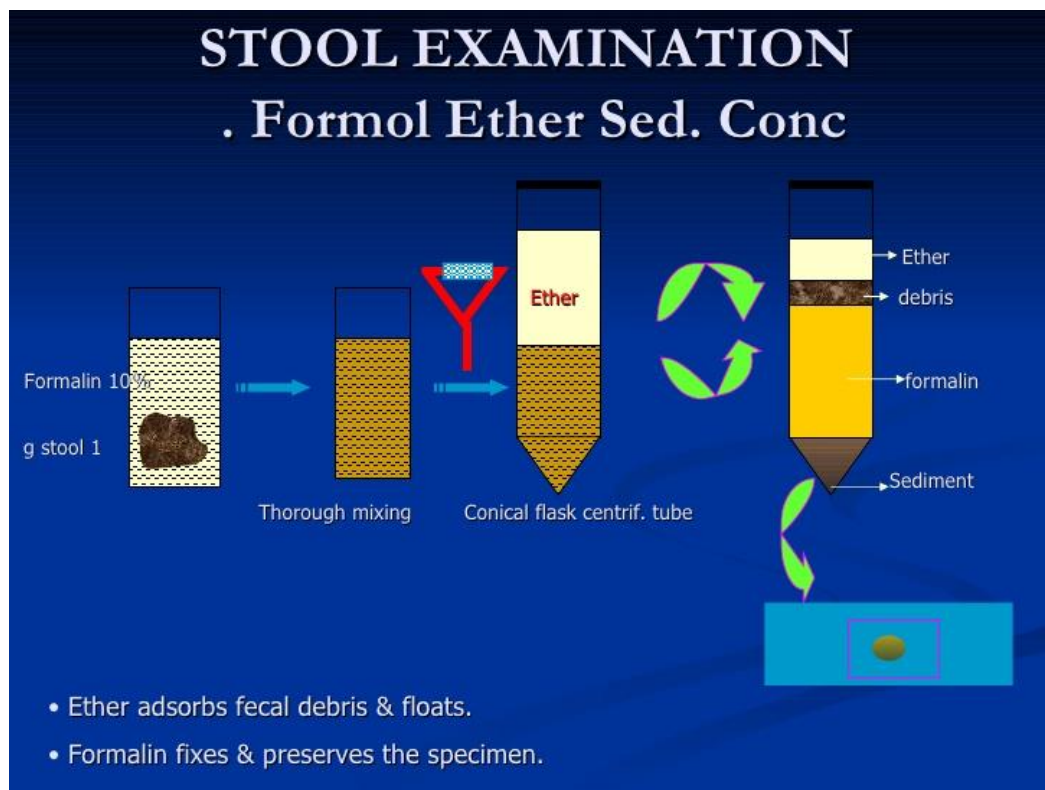


Figure 2: Procedure for Formalin-Ether Concentration method.

3.11 Data Analysis

All analyses were performed using SPSS version 21. Sensitivity and specificity of Mini-FLOTAC was estimated using 2x2 contingency tables where Kato-Katz and FEC were used as reference standards for helminths and protozoa respectively. Cohen's Kappa (κ) statistic was calculated to assess degree of agreement among all the three diagnostic techniques, with the strength of agreement determined using the following criteria: ≤ 0 = poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial and 0.81-1 = almost perfect. A probability (p-value) value of less than 0.05 was considered statistically significant. Prevalence for each parasite was determined using all the techniques.

3.12 Ethical Consideration

The study protocol was reviewed and approved by the KEMRI SSC (Kenya Medical Research Institute-Scientific Steering Committee). Written consent signature was sought from the parent/guardian for all the children and children themselves involved in the study, and only those who consented to participate were included. The consent form information was written in English language and translated in written form in the local languages (Kiswahili and Dholuo) in order to facilitate the understanding of the study (Appendix I). Children who were found to be infected with *S. mansoni*, STH and protozoa were treated with Praziquantel, albendazole and metronidazole respectively. Each study participant was assigned a unique study identification number and confidentiality was maintained during all phases of the study.

CHAPTER FOUR

RESULTS

4.1 Sensitivity and Specificity of Mini-FLOTAC Compared to Kato-Katz for the Detection of *S. mansoni* and STH.

4.1.1 Sensitivity of Mini-FLOTAC compared to Kato-Katz for the detection of *S. mansoni* and STH

With Kato-Katz as the reference standard, it was established that Mini-FLOTAC had a sensitivity of between 75.4% and 100% for the detection of all the helminths as shown in Table 4.1 below. The highest sensitivity was observed for hookworm at 100%, followed by *S. mansoni*, and *A. lumbricoides* with sensitivities of 87.9% and 80% respectively, while the least sensitivity was 78.4% for *T. trichiura*.

Table 4.1: Sensitivity and specificity of Mini-FLOTAC for the detection of helminthes and protozoa using Kato-Katz and FEC as reference standards respectively

TEST	SENSITIVITY				
	Protozoa	Hookworm	<i>A. lumbricoides</i>	<i>T. trichuria</i>	<i>S. mansoni</i>
Mini-FLOTAC – FS7	68.7%	N/A	N/A	N/A	87.9%
Mini-FLOTAC – FS2	N/A	100%	80%	78.4%	75.4%
	SPECIFICITY				
Mini-FLOTAC – FS7	75.8%	N/A	N/A	N/A	95%
Mini-FLOTAC- FS2	N/A	96.7%	95.3%	96%	72.7%

Table 4.1: Mini-FLOTAC demonstrated a sensitivity of 78.4% to 100% and a specificity of 95.3% to 100% for the detection of helminthes, and a sensitivity of 68.7% and a specificity of 75.8% for the detection of protozoa.

4.1.2 Specificity of Mini-FLOTAC compared to Kato- Katz for the detection of *S. mansoni* and STH

With Kato-Katz as the reference standard, Mini-FLOTAC demonstrated a specificity of 72.7% to 96.7% for all the helminthes as shown in Table 4.1 above. The highest specificity was observed for hookworm at 96.7%, followed by *T. trichiura* and *A. lumbricoides* with specificities of 96% and 95.3% respectively, while the least specificity was 72.7% for *S. mansoni*.

4.2 Sensitivity and Specificity of Mini-FLOTAC Compared to FEC for the Detection of Intestinal Protozoa

4.2.1 Sensitivity of Mini-FLOTAC compared to FEC for the detection of intestinal protozoa

With FEC as the reference standard, it was established that Mini-FLOTAC had a sensitivity of 68.7% for the detection of protozoa as shown in Table 4.1 above.

4.2.2 Specificity of Mini-FLOTAC compared to FEC for the detection of protozoa

Mini-FLOTAC demonstrated a specificity of 75.8% for the diagnosis of protozoans as shown in Table 4.1 above.

4.3 Kappa Test Agreement among Techniques

Kappa statistics was used to determine the degree of agreement among the techniques as shown in table 4.3 below. Mini-FLOTAC was compared with Kato-Katz for the detection of *S. mansoni*

and STH, whereas FEC was compared with Mini-FLOTAC for the detection of intestinal protozoa. There was moderate agreement between Mini-FLOTAC and Kato-Katz in the detection of *S. mansoni*, whereas there was slight agreement for *A. lumbricoides* and hookworm. However, there was fair agreement for the detection of *T. trichura*. For the detection of protozoa, there was fair agreement between FEC and Mini-FLOTAC.

Table 4.3: Kappa Test Agreement among techniques

	Hookworm		<i>A. lumbricoides</i>		<i>T. trichura</i>		<i>S. mansoni</i>		Protozoa	
	Kappa	P-value	Kappa	P-value	Kappa	P-value	Kappa	P-value	Kappa	P-value
MF vs FEC	-	-	-	-	-	-	-	-	0.373	<0.001
MF vs KK	0.121	<0.001	0.063	0.034	0.262	<0.001	0.410	<0.001	-	-

Table 4.3: Significant moderate agreement $\kappa = 0.410$ (95% CI, 0.41 to 0.60), $p < 0.05$ was found between Mini-FLOTAC and Kato-Katz for detection of *S. mansoni*, a slight agreement for hookworm $\kappa = 0.121$ and *Ascaris lumbricoides* $\kappa = 0.063$ (95% CI, 0.01 to 0.20), $p < 0.05$. Kappa agreement between Mini-FLOTAC and FEC for protozoa was fair, $\kappa = 0.373$ (95% CI, 0.21 to 0.40), $p < 0.05$.

Key: ≤ 0 = poor, 0.01-0.20 = slight, 0.21-0.40 = fair, 0.41-0.60 = moderate, 0.61-0.80 = substantial and 0.81-1 = almost perfect. FEC-Formalin-ether Concentration, KK- Kato-Katz, MF-Mini-FLOTAC

4.4 Prevalence of Intestinal Helminths and Protozoa in Nursery School Children in Mbita

Single stool samples were randomly collected from a total of 435 children of which there were 218 boys and 217 girls aged below six years. The stool samples were analysed for intestinal parasites using Mini-FLOTAC, Formalin-ether Concentration, and Kato-Katz techniques. From the results, it was established that the prevalence of Hookworms was 3.4%, 5.5% for *A.*

lumbricoides, 5.5% for *T. trichiura*, 60% for *S. mansoni*, and 34% for protozoa as indicated by Mini-FLOTAC. The study also showed that there was a high prevalence of *S. mansoni* of 60% and protozoa of 34% compared to the soil transmitted helminths (13.3%) in nursery school children in Mbita as shown in Table 4.4 below.

Table 4.4: Prevalence of intestinal helminthes and protozoa in nursery school children in Mbita

Test	Total examined	Number positive for hook worm	Number positive for <i>A. lumbricoides</i>	Number positive for <i>T. trichiura</i>	Number positive for <i>S. mansoni</i>	Number positive for Protozoa
Mini-FLOTAC	435	15(3.4%)	24(5.5%)	24(5.5%)	261(60.0%)	148(34%)
Formalin-ether Concentration	435	2(0.5%)	5(1.1%)	13(3.0%)	116(26.7%)	96(22.1%)
Kato-Katz	435	1(0.2%)	3(0.7%)	5(1.1%)	178(40.9%)	NA

Table 4.1: Prevalence of Hookworm was 3.4%, 5.5% for *A. lumbricoides*, 5.5% for *T. trichiura*, 60% for *S. mansoni*, and 34% for protozoa as indicated by Mini-FLOTAC.

4.5 Negative Predictive Value (NPV) and Positive Predictive Value (PPV) for Mini-FLOTAC

From the results in table 4.5 below it was established that the NPVs were between 91.9% to 100 % for the helminths and 89.5 % for the intestinal protozoa. For instance, the NPV for hookworm was 100% meaning that 100 % of the children whose test was negative for Mini-FLOTAC did not have the disease, whereas 89.5 % of the children did not have protozoa.

Table 4.5: Negative Predictive Value (NPV) and Positive Predictive Value (PPV) for Mini-FLOTAC

Test	Hookworms		<i>A. lumbricoides</i>		<i>T. trichiura</i>		<i>S. mansoni</i>		Protozoa	
	NPV	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV	PPV
Mini-FLOTAC	100%	13.3%	99.8%	16.7%	98.0%	29.2%	91.9%	39.1%	89.5%	44.7%

Table 4.3: The PPV for Mini-FLOTAC was between 13.3% and 44.7% while the NPV was between 89.5% and 100%.

CHAPTER FIVE

DISCUSSION

This study sought to compare the diagnostic performance of Mini-FLOTAC with FEC and Kato-Katz for detection of intestinal helminths and protozoa. Findings from this study have shown a significant difference in performance among the three techniques in estimating the prevalence of *S. mansoni*, STH infections, and intestinal protozoa.

From the results, Mini-FLOTAC demonstrated a sensitivity of 75.4% to 100% for all the helminths. This is in agreement with a study that was conducted in Ethiopia that reported a sensitivity of 100% for Mini-FLOTAC (Habtamu *et al.*, 2011). The study further demonstrated that Mini-FLOTAC had a higher sensitivity than multiple Kato-Katz thick smears of 2 slides of one stool sample for the diagnosis of STH. The results are also similar to a study that was conducted in Tanzania and India which also showed that Mini-FLOTAC was the most sensitive (90%) method for diagnosing helminthic infections compared to FEC and direct method and further suggested that if the method can be improved, it can be used for the diagnosis of intestinal protozoa (Barda *et al.*, 2013a). Similarly, a study conducted by Glinz *et al.*, (2010) demonstrated Mini-FLOTAC to be the most sensitive technique for the detection of *S. mansoni* and STH (Glinz *et al.*, 2010). However, a study conducted in Western Kenya by Assefa *et al.* (2013) reported that the sensitivity of Kato-Katz and Mini-FLOTAC was comparable for the detection of any STH species over single day (Kato-Katz: 52.0%, Mini-FLOTAC: 49.1%) and consecutive days (Kato-Katz: 76.9%, Mini-FLOTAC: 74.1%).

The high sensitivity reported here could be attributed to two reasons; one is the amount of stool sample used in Mini-FLOTAC compared to Kato-Katz technique, the small amount of stool used

of 41.7mg in Kato-Katz technique, is likely to increase the chances of missing parasites, as compared to 2g of stool used in Mini-FLOTAC procedure and two is the low intensity settings of where the study done. The study was conducted in a low intensity population of children below six years and it is well acknowledged that sensitivity of coprological techniques such as Kato-Katz can be poor in low intensity settings.

For intestinal protozoa, the results are similar to a study that was conducted in Tanzania that showed Mini-FLOTAC to have a sensitivity of 68%. However, the most sensitive method for intestinal protozoa diagnosis was FEC (88%) followed by direct method (70%) and Mini-FLOTAC (68%) (Barda *et al.*, 2013b). The slight difference could be attributed to the poor visibility of the cysts by faecal debris when using Mini-FLOTAC, leading to many of the cysts being undetected. Conversely, a study by Becker *et al.* (2011) found Mini-FLOTAC to be more sensitive for *E. coli*, and *G. lamblia* whilst FEC was more sensitive for *E. histolytica* and *E. dispar* (Becker *et al.*, 2011).

In terms of specificity, the results of this study differed from those in a study that was done in western Kenya whereby estimates of specificity for Mini-FLOTAC were generally between 93.5% and 99.6% (Assefa *et al.*, 2014). The likely reason for this is that the current study was conducted in a low intensity setting especially for STH infections whilst Assefa *et al.* (2014) conducted their study in a treatment naïve high-intensity setting. Hence, Kato-Katz would fail to positively identify most of these parasites since its sensitivity is low in low infection intensities.

The degree of agreement observed in the study differs from the results of similar studies done in India and Tanzania whereby the agreement among the three techniques was only moderate ($k = 0.40$; $p < 0.001$) and the best match was between FEC and Mini-FLOTAC ($k = 0.49$ – 0.81 ;

p<0.001) (Barda *et al.*, 2013a). The difference can be attributed to the difference in epidemiological settings and the low sensitivity of Kato-Katz especially in low infection intensities.

This study therefore, demonstrates that Mini-FLOTAC is a sensitive and simple technique for qualitative diagnosis of intestinal helminthes, though it can still be improved for the diagnosis of intestinal protozoa. Thus, the qualitative results of the Mini-FLOTAC from this study should foster for further trials that would compare mini-FLOTAC with other quantitative techniques for the diagnosis of helminthic and protozoan infections.

The study has shown that there is still a high prevalence of *S. mansoni* (60%), as indicated by Mini-FLOTAC, compared to STH which had prevalence of 3.4% and 5.5% among the study participants, despite the Schistosomiasis control programs being rolled out in the area. The results are consistent with the data obtained from a study that was done in the same area which had shown *S. mansoni* to be the most prevalent helminthic parasite among nursery school children (Nagi *et al.*, 2014). The findings also support other surveys conducted in Kenya which reported a prevalence of *S. mansoni* of up to 80 % in endemic areas (Odiere *et al.*, 2012; Verani *et al.*, 2011). This observation further confirms a report by Hotez and Kamath (2009) that Schistosomiasis infection still remains a significant public health problem in sub-Saharan Africa (Hotez and Kamath, 2009). Therefore, the high prevalence reported in the study is associated with the close proximity to the lake leading to continuous contact with contaminated lake water which is the sole source of infection.

Furthermore, the study also reported a high prevalence of protozoa (34%), though no study has been done in the same area to include the protozoa. However, similar studies have been carried

out in Kenya as was observed by Ngonjo *et al.* (2012) in Thika District where there was a high prevalence (37.8%) of protozoan infections in school children. The high prevalence of the protozoans can be attributed to the lack of any control programs for protozoa in the area as well as poor rural settings, together with the lack of access to clean piped water.

However, the study showed an overall low prevalence of STH of between 3.4% and 5.5%, the results agree with a similar study done in the same area which reported a prevalence of STHs of between 2.3% and 6.1% (Nagi *et al.*, 2014). The results agree with the global prevalence of STH which is estimated to be less than 10% and these infections form part of the Neglected Tropical Diseases (NTDs). The low prevalence may be due to on-going MDA programs for *S. mansoni* and STH infections that have been carried out in the area for school going children.

The limitation of this study was that the results can only be applied to a population of children below six years, thus the results cannot be generalized in the general population. Further research can be done in a different setting e.g. in adults with low infection intensities to determine whether the results can be comparable.

CHAPTER SIX

SUMMARY OF FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Findings

This study has shown that there is a significant difference in performance between Mini-FLOTAC, Formalin-ether Concentration and Kato-Katz techniques in the detection and estimation of prevalence of *S. mansoni*, STH, and intestinal protozoa. With Kato Katz as reference standard, Mini-FLOTAC demonstrated that it is a sensitive and specific technique for detection of *S. mansoni*, and STH. However, there was low sensitivity and specificity of Mini-FLOTAC compared to FEC in the detection of intestinal protozoa. In terms of degree of agreement, there was moderate agreement between Mini-FLOTAC and Kato-Katz in the detection of *S. mansoni*. However, there was generally fair agreement among the three techniques for the detection of STH and intestinal protozoa. The results also showed that *Schistosoma mansoni* and intestinal protozoa were the most prevalent parasites in the area. The prevalence of *S. mansoni* was 60% while that of intestinal protozoa was 34% as indicated by Mini-FLOTAC. However, the prevalence of STH infections as indicated by the three techniques was low.

6.2 Conclusions

1. With Kato-Katz as the reference standard for helminthes, the study demonstrated that Mini-FLOTAC is a more sensitive and simple technique for the qualitative diagnosis of helminthic infections.
2. With FEC as the reference standard for intestinal protozoa, the study demonstrated that Mini-FLOTAC had a lower sensitivity for the detection of intestinal protozoa; however it can be improved for the diagnosis of intestinal protozoa.

3. The Mini-FLOTAC technique can be a better alternative to the Kato-Katz technique for diagnosis of intestinal helminths and estimation of prevalence.

6.3 Recommendations from this Study

- i. The study recommends the use of Mini-FLOTAC in Schistosomiasis and STH control programs and drug efficacy evaluation programs as an alternative to the Kato-Katz technique in endemic areas.
- ii. The study recommends the improvement of Mini-FLOTAC for the diagnosis of intestinal protozoa i.e. by inclusion of a staining step using iodine.
- iii. The study also recommends the use of Mini-FLOTAC to help in the identification of people at risk of intestinal helminths.

6.4 Recommendations for Further Research

- i. The study recommends further research to include staining using iodine in Mini-FLOTAC so as to improve its sensitivity in the diagnosis of intestinal protozoa.
- ii. The study recommends further research to include centrifugation step with Diethyl ether in order to eliminate the faecal debris to improve visibility.
- iii. The study also recommends a further comparison of Mini-FLOTAC with other quantitative techniques in the detection of intestinal protozoa and helminths.

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APPENDIX

APPENDIX I: Informed Consent form

Consent agreement declaration by parent/guardian

Title of study: Comparing diagnostic performance of Mini-FLOTAC with formalin-ether concentration and Kato-Katz for detection of intestinal helminths and protozoa in nursery school children in Mbita, Western Kenya

Institutions

1. Institute of Tropical Medicine, Nagasaki University
2. Maseno University

Principal Investigator

Omundi Nyaboke Jackline, Maseno University

Co-Investigators

Prof. Shinjiro Hamano, MD, PhD

Nagi Sachiyo

Evans Chadeka

Introduction:

Your child is requested to participate in a medical research study to investigate the most appropriate diagnostic method that can be used to diagnose infectious diseases such as schistosomiasis (*S. mansoni*), Soil-transmitted helminths (STH) and intestinal protozoa. These parasitic diseases are common in Mbita area and are affecting many inhabitants of rural settings, although mass chemotherapy under the parasitic diseases control program has been operated by the government of Kenya. Re-infection of parasites is very common.

These diseases cause many health problems in the infected persons, such as abdominal pain, diarrhoea, liver enlargement and may cause death. In children, they interfere with school performance and affect growth and development. There are many ways to control these infections but these methods have not been very effective in African countries mainly because of some undesirable social-economic, cultural and geographical factors found in many communities. Such factors prevent the communities from understanding and appreciating the parasitic infections, their causes and the role each person must play to prevent and control the disease.

Reduction of the diseases in the community depends on the ability to correctly diagnose the disease and involvement of the community in the control measures. It is therefore very important to use a screening method that has a high sensitivity and specificity and also for the community to be enlightened about the diseases as a major health problem that can be eliminated, and the role the community can play towards reduction of transmission.

To be able to educate the communities about the disease, it is important for the researcher to first identify an appropriate diagnostic method that can be used to study the pattern of the current infection of parasites and their impact on health in the region. Infected person with parasites shows the eggs in stool. To be able to get the required results in this study, it will be necessary to get stool from the children.

Schistosomiasis, STH and intestinal protozoa are chronic infections and therefore are considered to be underlying condition of your child's health. We would therefore like to get more information about the diseases from the nursery school children by examining their stool. We will therefore visit your child's school to collect stool.

Purpose of Consent form:

The purpose of this consent form, therefore, is to give you information that might help you to decide whether your child will participate in the study or not. You are allowed to ask questions related to the study and implications on your part.

Persons to be contacted in case of any questions:

1. Principal Investigator: Omundi Nyaboke Jackline. Tel. 0720661161.
2. Co- Investigator: Prof. Shinjiro Hamano, MD, PhD (Chief Representative, NUITM-KEMRI), P.O. Box 54840, Nairobi. Tel. 020-272-5120.

Purpose of study:

This study will help to identify an appropriate screening technique with a good diagnostic performance that will allow simultaneous examination of *S. mansoni*, STH and intestinal protozoa.

Procedures to be followed:

Your child will be requested to provide overnight stool specimen early in the morning, which we will collect at your child's school. The sample will be processed using Mini-FLOTAC, Formalin-ether concentration and Kato-Katz techniques. The results from each method will be recorded and analysed so as to determine which method is sensitive.

Risks:

There are minimal risks involved in this study. The deworming drugs used are known to be safe in most people, and are in common use in Kenya. Your child will not be involved in any other part of experiment in this study other than physical examination, interview, and collection of stool.

Benefits:

Your child will be treated with praziquantel, albendazole or metronidazole (free of charge), after examination of stool specimen if found infected. You will receive the results of all of the examination which will be useful for your child's health care in future.

Assurance of confidentiality:

No name of the child, parent or guardian will appear in our records, but instead a coding system will be used. This is to ensure the confidentiality of all information related to you/your child. The records will remain confidential and will not appear when we present this study or publish the results. You as a parent or guardian of the child will receive a copy of the consent form.

Storage of specimens:

Immediately after the completion of the examination, all specimens will be destroyed.

Right to refuse or withdraw:

It is important that you understand the following general principles that will apply to all participants in the study:

1. Participation is entirely voluntary.
2. You/your child may withdraw from this study at any time without penalty or loss of benefits.
3. Please feel free to ask any questions that you may have.

Do you agree your child to participate?

I acknowledge that this consent form has been fully explained to me in a language that I understand and I do agree that my child can participate in the study.

Participant's name (child)

Parent's/guardian's signature or thumb printDate

Name of parent/guardian:

Investigator's signature: Date

This part will be used if the parent/guardian cannot read:

I *attest that the information concerning this research was accurately explained to and apparently understood by the parent/guardian and that informed consent was freely given by the subject/parent/guardian.

Witness' signature: **Date**

Witness' name:

***A witness is a person who is independent from the trial or a member of staff who was not involved in gaining the consent.**

Thumbprint of the parent as named above if they cannot write:

APPENDIX II: ETHICAL APPROVAL LETTER



KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org Info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1

April 16, 2015

**TO: PROF. SHINJIRO HAMANO
PRINCIPAL INVESTIGATOR**

**THROUGH: PROF. SAMMY NJENGA,
THE DIRECTOR, ESACIPAC
NAIROBI**

*Forwarded 20/04/15
S. Njenga*

Dear Sir,

**RE: SSC PROTOCOL No. 2084 (REQUEST FOR ANNUAL RENEWAL):
POLYPARASITISM AND OTHER MAJOR INFECTIOUS DISEASES IN RURAL
SETTINGS: PREVALENCE SURVEY IN SCHOOL CHILDREN IN KWALE AND
MBITA, KENYA**

Thank you for the continuing review report for the period **22nd April 2014 to 6th March 2015**.

This is to inform that during the 238th A meeting of the KEMRI/Scientific and Ethics Review Unit (SERU) held on **14th of April 2015**, the Committee **conducted the annual review and approved** the above referenced application for another year.

This approval is valid from today **16th April 2015** through to **15th April 2016**. Please note that authorization to conduct this study will automatically expire on **April 15, 2016**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to SERU by **4th March 2016**.

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to SERU for review prior to initiation.

Yours faithfully,

EAB

**PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI / SCIENTIFIC AND ETHICS REVIEW UNIT**

In Search of Better Health