PERFORMANCE OF KATO-KATZ, MINI-PARASEP AND MINI-FLOTAC IN DETECTION OF INTESTINAL HELMINTHES IN MBITA, HOMABAY COUNTY, KENYA

BY

ANNETTE IMALI

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DEPARTMENT OF ZOOLOGY

MASENO UNIVERSITY

ABSTRACT

Schistosomiasis and soil-transmitted helminthiasis remain a serious public health problem that cause significant morbidity and mortality in developing countries. Fishing, car washing and use of fresh waters infested with cercariae for domestic purposes, in addition to soil contaminated by fecal matter, predispose humans to these infections. Kato-Katz has for decades been used as a reliable diagnostic method for most intestinal parasitic infections, but has major drawbacks that include low sensitivity, exposure to infectious agents in stool, and requirement to process and examine stool samples soon after collection. Thus, the combination of Kato-Katz with other diagnostic procedures that allow collection and preservation of stool samples to be processed at a later time will help in logistical organization of surveys, evaluation of effectiveness of interventions and accurate estimation of disease prevalence. This cross-sectional study sought to evaluate the performance of Kato-Katz, Mini-Parasep and Mini-FLOTAC for laboratory detection of *Schistosoma mansoni* and soil transmitted helminth ova. One stool sample was randomly collected from 282 mother-preschool child pairs and individuals ≥6 years from 4 villages along the shores of Lake Victoria, Mbita. Aliquots for Mini-Parasep and Mini-FLOTAC techniques were preserved in 10% and 5% formalin, respectively, before processing and microscopy, while for Kato-Katz, fresh stool was used. The recovery of intestinal helminth ova by Kato-Katz, Mini-Parasep and Mini-FLOTAC was comparable. Mini-Parasep and Mini-FLOTAC FS7 detected an additional *Enterobius vermicularis* and *Taenia* respectively. Using Kato-Katz as reference standard, Mini-Parasep showed a higher sensitivity for detecting *S. mansoni* (85.0%) and hookworm (33.3%) than Mini-FLOTAC FS7 (27.7% *S. mansoni*) and Mini-FLOTAC FS2 (8.5% *S. mansoni*). Kappa statistic for agreement showed a moderate agreement between Kato-Katz and Mini-Parasep (k=0.49), and a fair agreement between Kato-Katz and FS7 (k=0.28) in detecting *S. mansoni* ova. Using Fisher's exact test, Mini-Parasep detected more light intensity *S. mansoni* infections (70.2%), while Kato-Katz detected more heavy intensity *S. mansoni* infections (16.5%). Spearman correlation showed a significant positive association among the techniques in estimating *S. mansoni* egg counts such that Kato-Katz vs FS2 was (Υ_s 0.28, $p=0.0018$), Kato-Katz vs FS7 was (Υ_s 0.40, $p=<0.0001$), Kato-Katz vs Mini-Parasep was (Υ_s 0.68, $p=<0.0001$) and FS2 vs FS7 was (Υ_s 0.23, $p=0.0085$). Mini-Parasep is a promising technique with high sensitivity for *S. mansoni* and hookworm eggs and is recommended to be included into schistosomiasis and soil transmitted helminth control programs as an alternative to Kato-Katz. This study also recommends the combined use of Mini-Parasep and Kato-Katz in disease surveillance and epidemiological studies to increase diagnostic sensitivity for detecting intestinal schistosomiasis.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Schistosomiasis and soil-transmitted helminthiasis (STHs) are widespread in underdeveloped parts of the world, where they negatively impact on human health (make people sick for long periods of time) and wellbeing (cause long-lasting disabilities), thus, exacerbating poverty (Glinz *et al.*, 2010; Skolnik and Ahmed, 2010). In areas with insufficient sanitation, soil transmitted helminthes and schistosomes are transmitted by eggs excreted in human stool and/or urine that contaminate soil and water sources (Bethony *et al.*, 2006). Infections caused by soil transmitted helminthes include hookworms (*Necator americanus, Ancylostoma duodenale*), roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) (Bethony *et al.*, 2006), while schistosomiasis, *Schistosoma haematobium* (causal agent of urogenital schistosomiasis), *Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi,* and *Schistosoma intercalatum* which provoke the intestinal, hepatic or hepatosplenic forms of the disease, (Gryseels *et al.*, 2006). Co-infections between soil transmitted helminthes with S. mansoni have been reported in Kenyan school children (Brooker *et al.*, 2000). Periodic drug treatment of populations has been adopted as the global strategy to control these widespread infections. Yet, many mass drug administration (MDA) programs that are currently being rolled out, targeting STHs and schistosomiasis have very little focus on diagnosis.

Prevalence of intestinal parasitic infections varies from one region to another, and depends on the diagnostic methods used and the number of stool examinations done (Parameshwarappa *et al.*, 2012). In mass treatment programs, changes in infection prevalence is monitored primarily

using the Kato-Katz method probably due to its low material and infrastructure requirements (Cringoli *et al.*, 2010; Tchuente', 2012). However, the Kato-Katz technique has several drawbacks including low sensitivity, unnecessary high risk exposure to infectious agents in stool, and the requirement to process and examine stool samples soon after collection. Additionally, the sensitivity of Kato-Katz in single stool sample examination is limited by day-to-day variation in egg excretion, leading to errors in estimating the presence of infection, especially in settings with high proportions of light-intensity infections (Tchuente', 2012). Examination of several stool specimen collected over consecutive days has been suggested to improve the sensitivity of Kato-Katz (Booth *et al.*, 2003; Tarafder *et al.*, 2010), however, consistency in provision of stool samples by all study participants is rarely achieved (Tarafder *et al.*, 2010). Moreover, the small amount of stool analyzed (41.7mg) explains why the Kato-Katz technique has a low sensitivity to detect eggs whenever they are present in low frequencies or appear highly clustered (Glinz *et al.*, 2010). Since use of Kato-Katz as a confirmatory test may significantly increases misdiagnosis of intestinal helminth infections, identification of other diagnostic procedures that combine simplicity, high sensitivity and specificity is important. Accurate diagnosis helps reduce disease burden and the consequences caused to the community due to intestinal helminth infections (Endris *et al.*, 2013). In fact, accurate diagnosis is important for specific and timely patient management, drug efficacy evaluations, monitoring of large-scale community-based control programs, and disease control managers for all aspects of prevention, control, monitoring and surveillance (Handzel *et al.*, 2003). However, these alternatives will only be useful to national control programs if their diagnostic performance (sensitivity and specificity) for *Schistosoma mansoni* and soil transmitted helminth eggs is equal or better than that of Kato-Katz.

Although use of concentration techniques will potentially increase the ability to detect rare infections and protozoa, they are not a perfect option: some concentration techniques like the FLOTAC are more complicated and requires better equipped laboratories (Assafa *et al.*, 2014; Duthaler *et al.*, 2010; Speich *et al.*, 2010), and extensive training of laboratory workers. Never the less, the newly introduced Mini-FLOTAC technique overcomes this constraint. It includes a closed chamber for mixing and floatation, and a separate reading disc (Assafa *et al.*, 2014). The Mini-FLOTAC does not require any expensive equipment or energy source, and so can be comfortably used in developing countries for larger studies (Barda *et al.*, 2013a). A study in Tanzania demonstrated that Mini-FLOTAC was the most sensitive method than Formal-ether concentration and direct fecal smear for helminth infections (Barda *et al.*, 2013b). Other work has shown Mini-FLOTAC and Kato-Katz to have comparable sensitivity for detection of any soil transmitted helminth species over a single day and consecutive days (Assafa *et al.*, 2014). In terms of cost, Mini-FLOTAC is relatively expensive as it requires use of two different floatation solutions for soil transmitted helminthes and *Schistosoma mansoni* (Barda *et al.*, 2013a) In contrast, *S. mansoni* can be read alongside STHs in the Kato-Katz method.

The Mini-Parasep, on the other hand, is a single-use *in vitro* diagnostic device for fecal concentration of helminth ova and larvae/protozoan cysts and oocysts. It has a flat bottom tube that allows mixing of stool sample, formalin and the solvent (i.e., ethyl acetate), a sedimentation cone, where deposition of helminth ova and protozoan oocysts occur after centrifugation, and a built-in filter. Use of Mini-Parasep technique in epidemiological studies and control of intestinal parasitic infections have been reported elsewhere (Kurup, 2010; Nyakango *et al.*, 2015).

One key advantage of the Mini-FLOTAC and Mini-Parasep techniques is that they permit work with fixed/preserved fecal samples, and can also be performed on fresh stool samples. The

possibility of examining stool samples in different days is enhanced, and hence the quality control process (Barda *et al.*, 2013b). In addition, the combined use of the Fill-FLOTAC device prevents any hazard of contamination of the operator (Barda *et al.*, 2013b).

It is possible to overcome some of the limitations facing national programs if an alternative to Kato-Katz can be identified. Diagnostic procedures that provide the ability to collect and preserve stool to be processed at a later time will help in accurate estimation of disease prevalence in a population, logistical organization of surveys, and evaluation of effectiveness of interventions in a community, while also providing opportunities for integration with other health-related activities. The present study sort to evaluate the ability of three techniques to detect intestinal helminth eggs in preserved stool. This study compared the sensitivity and specificity Mini-Parasep (DiaSys England) and Mini-FLOTAC (University of Naples Italy) to Kato-Katz (WHO recommended quantitative technique for diagnosis of intestinal helminth infections), for laboratory detection of *S. mansoni* and STHs in Mbita, western Kenya. The Kato-Katz technique was used as the reference standard. Agreement among the techniques was also assessed.

1.2 Statement of the problem

In the current era of Preventive chemotherapy, intensification of mass administration of anthelminthic drugs (praziquantel/albendazole/mebendazole) and repeated deworming of school-age children and other at-risk groups will significantly reduce worm burden and intensity of infection due to schistosomiasis and soil transmitted helminthes. Hence, there will be an increase of light intensity infections which is often undetected if single stool samples are examined by Kato-Katz method only. As a consequence, there will be high rates of underestimation of the prevalence and burden of schistosomiasis and soil transmitted helminth infections. Therefore, the simultaneous use of Kato-Katz with other diagnostic procedures preferably concentration techniques, which are thought to increase the sensitivity for diagnosis of intestinal parasitism, holds the key to accurate estimation of disease burden and eventually achieve elimination of intestinal helminthiasis.

1.3 Justification of the study

A disproportionate burden of helminth infections in humans occur 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 infected with one or more helminth species (Lustigman *et al.*, 2012). Communities along the shores of Lake Victoria ,western Kenya are populated with individuals suffering significant morbidities associated with *S. mansoni* infections (Odiere *et al.*, 2012), attributable to water contact (Handzel *et al.*, 2003), and contaminated environment. Several studies in this region have shown high prevalence of schistosomiasis and STHs among school children bordering Lake Victoria (Brooker *et al.*, 2000; Handzel *et al.*, 2003; Standley *et al.*, 2010; Thiong'o *et al.*, 2001). In addition to causing severe disease, schistosomiasis and soil

transmitted helminth infections cause high morbidities that contribute to poor health outcomes in infected persons, particularly children (Mwinzi *et al.*, 2012). 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 (Skolnik and Ahmed, 2010).

While several factors may make estimating the number and burden of intestinal parasitic infections difficult, lack of accurate diagnostic tools is a major one (Endris *et al.*, 2013). Efforts to identify an 'ideal' or alternative diagnostic technique based on fecal egg detection have been strengthened including simultaneously using different diagnostic techniques to analyze stool. Currently, many Mass Drug Administration (MDA) programs are being rolled out, and are likely to create a shift from heavy to light intensity infections, which more often go undetected. Therefore, diagnostic procedures that accurately detect an infection will not only help in identification of risk groups to be targeted for treatment, but accurately estimate infection rates in a population, hence develop sound and targeted control measures.

1.4 Overall objective

To evaluate the performance of Kato-Katz, Mini-Parasep and Mini-FLOTAC in detection of intestinal helminth ova in Mbita, Homabay county, Kenya

1.4.1 Specific objectives

1. To compare the sensitivities and specificities of Mini-Parasep and Mini-FLOTAC techniques to Kato-Katz in detecting *Schistosoma mansoni* and soil-transmitted helminth ova.
2. To determine the degree of agreement between Kato-Katz, Mini-Parasep and Mini-FLOTAC in detecting *Schistosoma mansoni* and soil-transmitted helminth ova.

1.5 Null Hypothesis

1. There is no difference in sensitivity and specificity of Mini-FLOTAC and Mini-Parasep in detecting *Schistosoma mansoni* and soil-transmitted helminth ova.
2. There is no difference in strength of agreement between Kato-Katz, Mini-Parasep and Mini-FLOTAC in detecting *Schistosoma mansoni* and soil-transmitted helminth ova.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diagnosis of schistosomiasis and soil transmitted helminthiasis

A multitude of techniques for diagnosis of intestinal parasites exist. Some of the main parasitological methods used include Kato-Katz, concentration techniques, immunological and molecular techniques. However, these techniques come with significant difference in the cost, sensitivity, simplicity and field applicability.

2.2.1 Kato-Katz

Owing to its simplicity and relatively low cost, the Kato-Katz technique is recommended by the WHO for epidemiological surveys pertaining to intestinal schistosomiasis and STH control programs (Cringoli *et al.*, 2010). Moreover, the Kato-Katz equipments are mostly reusable and laboratory workers can be trained within half a day (Speich *et al.*, 2010). However, it is widely acknowledged that single Kato-Katz thick smear examinations underestimate the "true" prevalence of *S. mansoni* and *S. japonicum* (de Vlas and Gryseels, 1992; Utzinger *et al.*, 2011). The sensitivity of Kato-Katz in single stool sample examination is limited by day-to-day variation in egg excretion leading to errors in estimating the presence of infection, particularly in settings with high proportions of light intensity infections (Tchuente', 2010). Suggestions have been put foreward to examine several stool specimens collected over consecutive days to improve the sensitivity of the test (Booth *et al.*, 2003; Tarafder *et al.*, 2010). Examination of 3 insead of 1 stool specimen was shown to have increased the sensitivity of hookworm diagnosis (Steinmann *et al.*, 2008). However, collection of the desired and equal number of stool samples on consequtive days from all participants is rarely achieved (Tarafder *et al.*, 2010) and increases

costs. The small amount of stool analyzed (usually 41.7mg) explains why the Kato-Katz technique has a low sensitivity to detect eggs whenever they are present at low frequency or appear highly clustered (Glinz *et al.*, 2010). In order to boost diagnostic sensitivity, employment of multiple methods for the same stool sample, and analysis of several Kato-Katz thick smears has been recommended (Ebrahim *et al.*, 1997; el-Morshedy *et al.*, 2002; Utzinger *et al.*, 2011). This has been found to be more feasible at the individual level and in field studies (el-Morshedy *et al.*, 2002).

2.2.2 Concentration techniques

Direct microscopy, although useful for observation of motile protozoan trophozoites and cellular exudates, is not recommended solely for the routine examination of faeces with suspected parasitic infections. Therefore, in order to maximise the number of organisms detected, a concentration method is essential to increase the possibility of recovering ova, cysts and larvae which may be too scanty to detect by direct microscopy alone.

A wide variety of concentration techniques have been developed and evaluated (Peters *et al.*, 1980; Xu *et al.*, 2011). Examples of these tests are the Merthiolate-Iodine Concentration technique (MIFC), Merthiolate- Formaldehyde concentration techniques (MFCT) and Formaldehyde- Ether. These tests, however, are time consuming, laborious to perform, and also impractical for field-based screening (Olveda *et al.*, 2013). Despite these limitations, the ether-based concentration methods are still widely used. In a study in Ghana, the formol-ether concentration method gave the highest prevalence of 11.1% for helminth parasites (Tay *et al.*, 2011). Parameshwareppa et al showed that there was a significant increase in the number of parasites detected and sensitivity following application of concentration methods. The formol

ether concentration method detected 157 (56.9%) while the modified formol ether concentration method detected 179 (64.9%) positive cases (Parameshwarappa *et al.*, 2012). In the same study, it was observed that the inclusion of the modified formol ether concentration method and the simple salt floatation technique increased the sensitivity of parasite detection (Parameshwarappa *et al.*, 2012). In Tanzania and India, the formol ether concentration method was the most sensitive method in detection of intestinal protozoa infections (88% FECM, 70% direct smear, and 68% Mini-FLOTAC) (Barda *et al.*, 2013b). Stool samples in concentration techniques can be preserved and analyzed in the laboratory several days or weeks later, and allow for the diagnosis of intestinal helmithes and protozoa (Cringoli *et al.*, 2010). Additionaly, several preservatives including SAF (sodium-acetate acetic acid formalin) are known to remain stable for long periods of time when stored at ambient temperatures, and can allow for neutralization of infectious agents in faeces.

2.2.3 Floatation techniques

The methods most frequently used to recover parasite eggs and oocysts are floatation techniques that rely on the differences in specific gravity (SG) of the egg (s), fecal debris, and floatation solution (Dryden *et al.,* 2005). For parasite eggs to float, the SG of the floatation solution must be greater than that of the eggs. Additionally, helminth eggs and protozoan oocysts should float and still maintain their morphologic integrity, while fecal debris should sink in the chosen floatation solution. Common floatation solutions include saturated Sodium chloride (SG 1.18), sugar (sheather's solution SG 1.27-1.33), Sodium nitrate (SG 1.18-1.20), Magnesium sulphate (SG 1.20) and Zinc sulphate (SG 1.35) (Dryden *et al.*, 2005).

Floatation procedures vary from the simplest to the complex. The simplest procedure involves mixing a small amount of faeces with floatation solution in a cylinder (shell vial or centrifuge tube), centrifugation to spin down the debris and allowing eggs to float to the surface of the solution. A glass coverslip is placed on top of the solution and allowed to stand until the eggs have had time to float to the top. The coverslip is then removed to a microscope slide and examined (Dryden *et al.*, 2005).

New research has revealed that the recently developed FLOTAC technique (Cringoli *et al.*, 2010) shows a higher sensitivity than multiple Kato-Katz thick smears for the diagnosis of STH infections (Speich *et al.*, 2010). Upto 1g of stool can be examined by a single FLOTAC test, and hence the theoretical sensitivity is 1 egg per gram (EPG), which is 24 times greater than for a single Kato-Katz thick smear (Utzinger *et al.*, 2011). It is, therefore, expected that its sensitivity for detection of helminth ova to be higher, especially in situations of low infection intensities (Cringoli, 2006). In particular, the FLOTAC technique improves the ability to diagnose human hookworm infections accurately (Utzinger *et al.*, 2008), which is generaly underestimated when using Kato-Katz thick smears due to rapid disintegration of hookworm eggs and the constraint to read the slides shortly (within 30 minutes) after preparation (Tchuente', 2010).

The Mini-FLOTAC (a modification of the FLOTAC technique) is a new simplified device that has been developed to improve the quality of copromicroscopic diagnosis of intestinal parasitic infections (Barda *et al.*, 2013b; Cringoli *et al.*, 2013). In Mwanza, Tanzania, a study by Barda *et al* showed that Mini-FLOTAC detected a higher number of positive children for hookworm than Kato-Katz (73% vs 68%) as well as for *S. mansoni* (40% vs 33%) (Barda *et al.*, 2013a). In the same study, the two floatation solutions (FS), FS2 and FS7 detected different helminth eggs. FS2 was more sensitive than FS7 for hookworms, but did not detect *S. mansoni* whose eggs float only

with FS7 (Barda *et al.*, 2013a). This, therefore, shows that in settings where both STH and schistosomiasis are present, both floatation solutions are necessary.

2.2.4 Molecular and immunological techniques

Although immunodiagnosis usually requires better equiped laboratories than using microscopy, they may yield higher sensitivities especially for antibody detection. Since antibody detection is not quantitative, it is difficult to differentiate between light and heavy infection. Moreover, antibody levels remain high for prolonged periods of time following successful chemotherapy, which represents a diagnostic dilemma: failure to differentiate between active and cured infection (Utzinger *et al.*, 2011).

Various serodiagnostic methods have been developed to detect anti-schistosomal antibodies, such as indirect hemagglutination assay (IHA), and Enzyme-Linked Immunosorbent Assay (ELISA) using different types of antigens (Ibrahim and Ibrahim, 2014; Kinkel *et al.*, 2012). In a study in Sennar state, Sudan, the majority of positive cases were detected by ELISA (56%) followed by IHA (33%) and Kato-Katz (21%). The sensitivities of ELISA and IHA were much higher than the Kato-Katz, (93% and 84%), respectively (Ibrahim and Ibrahim, 2014). Detection of schistosome antigens, such as circulating anodic antigens (CAA) and circulating cathodic antigens (CCA) or *S. mansoni* soluble egg antigen (SEA) in blood or urine, using ELISAs hold several advantages over antibody detection (Utzinger *et al.*, 2011). Active infections can be readily demonstrated, and because of their high specificity, they are useful for drug efficacy trials. Classical ELISA procedures, however, are quite slow, require well-equiped laboratories and highly qualified technicians.

The detection of helminth DNA in stool samples using real-time PCR provides a specific and sensitive tool for detecting low-grade, patent helminth infections (Verweij *et al.*, 2007). For instance in a population study in Brazil, the prevalence of *S. mansoni* infection was found to be 31% when 3 fecal samples were examined using Kato-Katz, but the prevalence rose to 38% when PCR was applied using only one stool sample (Pontes *et al.*, 2003). However, PCR methods require expensive and extensive equipment and will thus not become a routine diagnostic tool in most endemic and poor countries for the near future (Verweij *et al.*, 2009).

It might not be possible to screen an entire population for all potentially endemic parasites, but concentrating on one kind of biological sample (stool, urine or blood) and subjecting it to a suite of diagnostic approaches could prove useful. Furthermore, the increasing multiparasitism in developing countries calls for sensitive diagnostic tools that can concurrently detect different intestinal parasite species in the same stool sample (Parameshwarappa *et al.*, 2012).

2.2 Control of schistosomiasis and soil-transmitted helminth infections

These diseases affect the poorest populations and infections are particularly abundant among people living in rural or deprived urban settings with low socio-economic status, lack of clean water and poor sanitation (Hotez *et al.*, 2006). As with other Neglected Tropical Diseases (NTDs), schistosomiasis and STH control efforts have been overshadowed by other health priorities, with more emphasis given to the ''big three'', that is, HIV/AIDS, tuberculosis and malaria (Tchuente', 2012). Probably, the chronic and focal nature of these neglected tropical dieases make them feature much less prominently on national strategic plans and global health initiatives, which still tend to focus on mortality outcomes (Hotez *et al.*, 2006). Consequently,

the level of resources allocated for research and their control have been insufficient (Hotez and Yamey, 2009).

Large-scale administration of anthelminthic drugs to at-risk groups has been celebrated for a long time as an effective tool to reduce worm burden and prevent morbidity due to schistosomiasis and STHs. All member states of WHO (over 200 countries) endorsed in May 2001, the World Health Assembly resolution (WHA), with as a major objective the regular treatment of at least 75% of all school-aged children and other high-risk groups regularly with praziquantel against schistosomiasis and albendazole or mebendazole against soil-transmitted helminthiases (Tchuente', 2012; WHO, 2002). Under the WHO guidelines, the decision to treat all persons (mass treatment) or only school children and other high-risk groups (selective treatment) depends on the prevalence of infection in a particular region (WHO, 1998). In the early stages of a control program when morbidity control is the main objective, infection prevalence and intensity are usually high, and hence direct methods show reasonable diagnostic accuracy (Utzinger *et al.*, 2011). However, prevalence and particularly intensity of infection are reduced after treatment, and hence direct methods become less sensitive. In order to surpass this diagnostic limitation, suggestions have been put forward to augmented or replaced direct methods with immunological techniques based on antigen or antibody detections (Bergquist *et al.*, 2009), or molecular tools such as polymerase chain reaction (PCR)-based approaches (Gomes *et al.*, 2010). The simultaneous use of different diagnostic methods, such as antibody detection followed by stool examination of seropositive individuals has been applied to identify the small number of infected people once morbidity control is achieved (Pontes *et al.*, 2003). For instance use of PCR-ELISA in a low parasite area of Brazil identified 30% infected persons as compared with only 18% found by microscopic examination of stool (Gomes et al., 2010). In

Sennar state, Sudan, higher positive results as detected by ELISA was 56.1%, 33.2% by IHA and 21% by Kato-Katz. In the same study, the combined use of the ELISA and IHA (indirect haemagglutination) yielded a good sensitivity of 93.3% (Ibrahim and Ibrahim, 2014). Sero-diagnostic tools help detect more positive cases, However, they require blood sample collection (invasive), and they are expensive, hence limiting their integration into large-scale national control programs.

Schistosomiasis and STH infections occur concurrently with many of the other NTDs. Therefore, an integration of schistosomiasis and STH control with other helminth control programs would be highly beneficial for their control (Tchuente', 2012). Other control strategies include health education, snail control using molluscicides or by lining and covering water conduits, improved sanitation and provision of safe water supplies (Tomblin *et al.*, 2007).

Despite the many control initiatives that have been put in place to control helminth infections, very little success has been achieved as these infections are still a public health concern in many parts of the world. Therefore, to facilitate progress towards their control and elimination, it is necessary to develop new diagnostic tools for determination of possible anthelminthic resistance, and treatment end points, also, quantification of infection prevalence and intensity that can aid more accurate mapping of helminth infections.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

This study was carried out in beach villages of Mbita, (Kisui, Wariga, Usare and Nyamanga) (figure 1), located at latitude (0^0 25'S) and longitude (34^0 12'E), between May and June 2013. Mbita district has a population estimated at 111,409, according to the Kenya National Bureau of Statistics, 2010. Occupational hazards associated with the lake such as sand harvesting, fishing and car washing predispose individuals living close to the lake shores to schistosome infections, while lack of clean water and poor sanitation within these beach villages promote STH infections among the population. Several studies in this region have shown high prevalence of schistosomiasis and STHs among school children bordering Lake Victoria (Brooker *et al.*, 2000; Handzel *et al.*, 2003; Standley *et al.*, 2010; Thiong'o *et al.*, 2001).

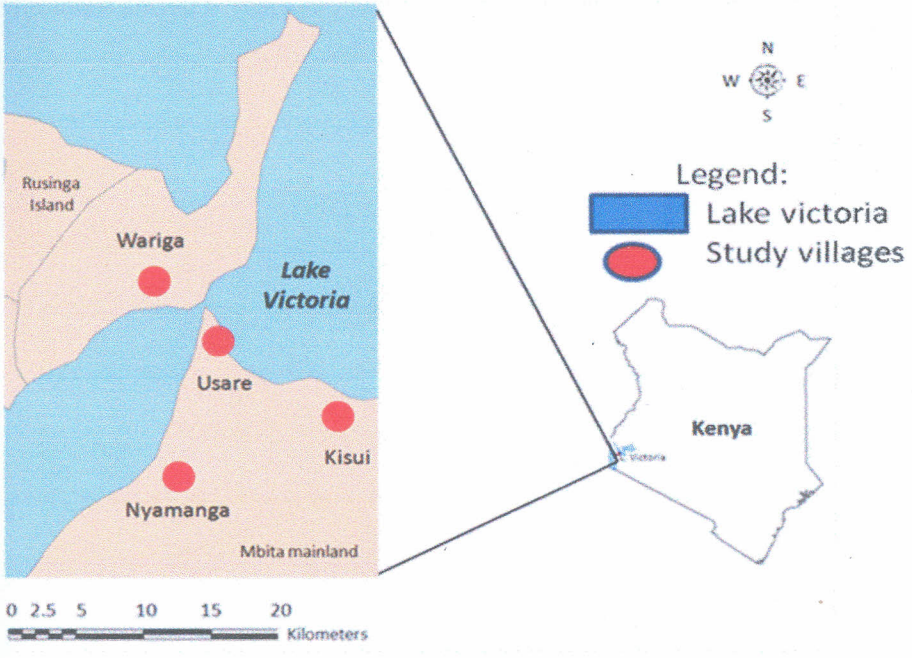


Figure 1: A map of the study area showing the four villages under study

3.2 Study design and recruitment

This was a cross-sectional study. Baseline prevalence of *S. mansoni* in the study area has been previously reported (Odiere *et al.*, 2012). Four villages (Kisui, Wariga, Usare and Nyamanga) located within 5 km from the lakeshore and with *S.mansoni* prevalence >25% were selected as shown in table 1. Mini-Parasep and Mini-FLOTAC were not applied in some villages considering that all the villages are fairly homogenous and data would be representative. The minimum sample size of 50 has been achieved from the four villages, and also the logistical feasibility in terms of work load was considered. Kato-Katz was used as the reference standard in this study since it has been recommended by the WHO for epidemiological studies pertaining to intestinal schistosomiasis and soil transmitted helminthiasis (Cringoli *et al.*, 2010). In each of these villages, the community health workers and the field team mobilized all resident mother-preschool child pairs and individuals above 6 years to participate in this study. Informed consent (and assent where necessary) were obtained from parents of preschool-age children and from participants who were above 6 years.

Table 1. Summary of study design and total number of stool samples per technique/village

Village	Base line *S. mansoni* prevalence %	Kato-Katz[1]	Mini-FLOTAC FS2	Mini-FLOTAC FS7	Mini-Parasep
Nyamanga	48.5	54	54	54	54
Kisui	71.7	73	73	73	-
Wariga	67.9	81	-[2]	-	81
Usare	62.1	74	-	-	74
TOTAL		**282**	127	127	209

[1] Kato-Katz, the reference standard was applied across all the four villages
[2] Mini-FLOTAC and Mini-Parasep were not applied in these villages

3.3 Field procedures

One stool sample was obtained from each participant as shown in plate 1. The stool was thoroughly mixed in the stool cup using a wooden spatula and 0.5g of the sample was weighed using a digital weighing scale (CS 200, Ohaus Corporation USA). The measured stool sample was transferred to the flat-bottom tube of the Mini-Parasep kit and 2.5mL of 10% formalin added for preservation (plate 1). One gram of the same stool sample was weighed in a 15ml centrifuge tube for Mini-FLOTAC, and 4 mL of 5% formalin added for preservation (plate 1). The remaining stool sample was stored unpreserved to be examined by the Kato-Katz method. All collected samples were packed in cooler boxes and transported to the Division of Vector-Borne and Neglected Tropical Diseases (DVBNTD) laboratory in Homa-bay for processing and microscopy (Kato-Katz) or temporary storage (Mini-Parasep and Mini-FLOTAC). Mini-Parasep

and Mini-FLOTAC samples were processed one month later at the NTD Parasitology laboratory, KEMRI's Centre for Global Health Research (CGHR), Kisumu.

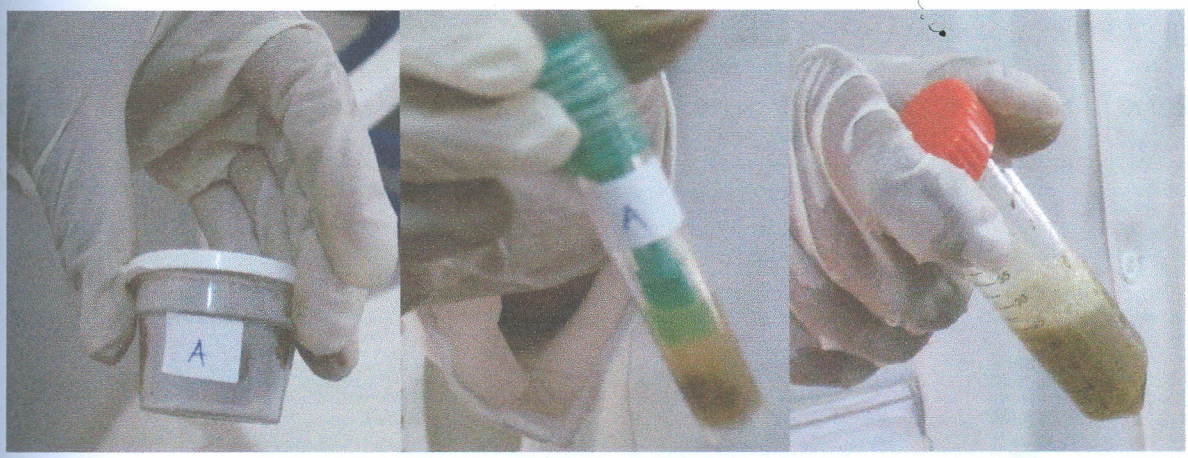


Plate 1 Photos showing a sample of stool in stool cup, and preserved stool for Mini-Parasep and Mini-FLOTAC respectively.

3.4 Laboratory procedures

3.4.1 Kato-Katz

The Kato-Katz is a quantitative technique that allows good visualization of helminth eggs/ova in faeces and has been recommended by the World Health Organization for diagnosis of intestinal helminthiasis (Cringoli *et al.*, 2010; WHO, 2002). All stool samples were analyzed in duplicate for eggs of *Schistosoma mansoni, Ascaris lumbricoides, Trichuris trichiura* and hookworms (WHO, 1994) (plate 3). A template was used that when filled contained approximately 41.7 mg of feces (plate 2). Slides were allowed to clear for 30 minutes prior to microscopic examination. Eggs were counted by two independent microscopists and any discrepancy in results of the two was reconciled by comparing to results of a third independent and more experienced microscopist. The concentration of eggs was expressed as eggs per gram (epg) of feces by a factor of 24. Intensity of infection for each helminth was categorized as light, moderate or heavy

as follows: *S. mansoni*, light, moderate and heavy infections as 1-99, 100-399 and ≥ 400 eggs grams^{-1}, respectively; hookworm, light, moderate and heavy infections as 1-1999, 2000-3999 and ≥4000 eggs grams^{-1}, respectively; *Ascaris lumbricoides*, light, moderate and heavy infections as 1-4999, 5000-49999 and ≥ 50000 eggs grams^{-1}, respectively; while *Trichuris trichiura*, light, moderate and heavy infections as 1-999, 1000-9999 and ≥ 10000 eggs grams^{-1}, respectively (WHO, 2002).

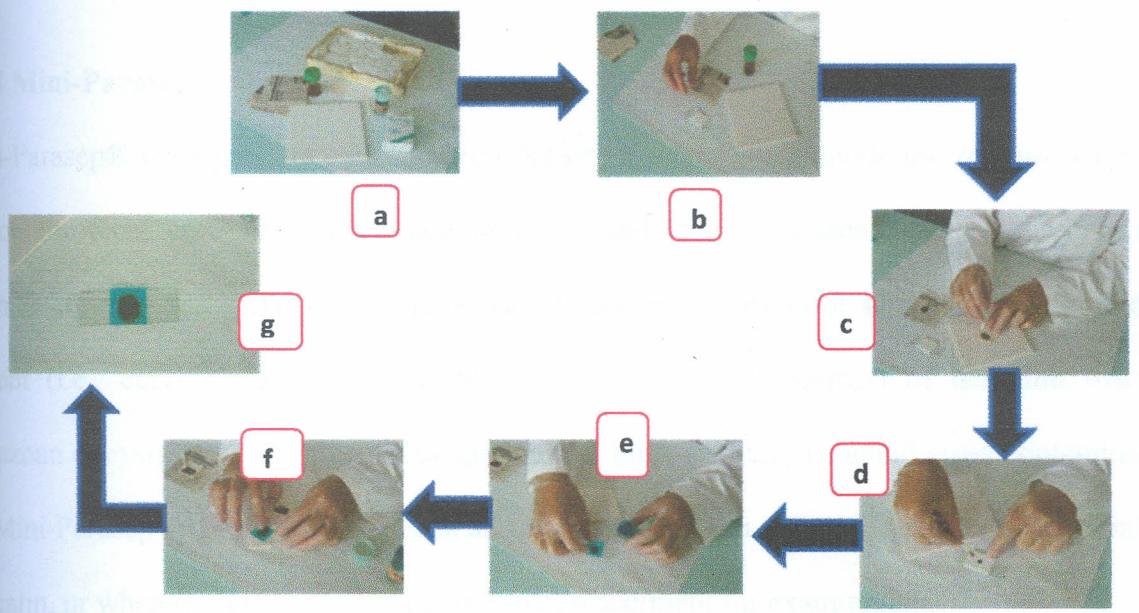


Plate 2: Kato-Katz procedure (photos adapted from a) assembly of Kato-Katz requirements, b) placing Kato-Katz template onto a microscope slide, c) sieving faeces using the Kato-Katz mesh, d) filling template with sieved faeces, e) placing cellophane soaked in malachite green solution onto fecal matter on microscope slide, f) inverting slide and pressing gently, g) slide ready for reading

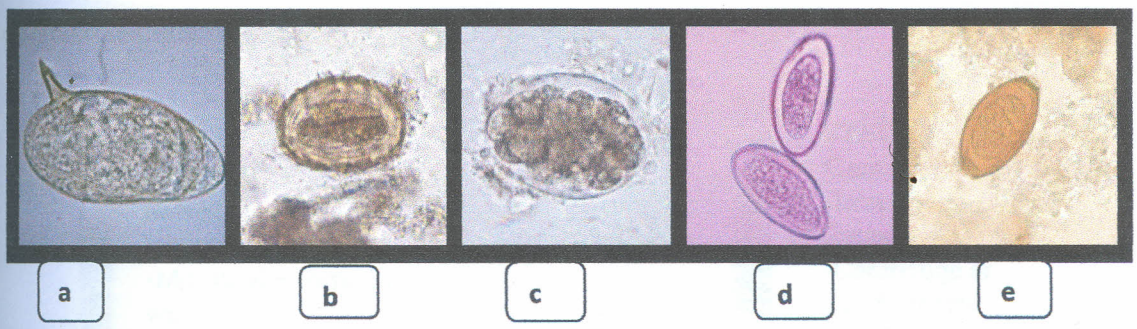


Plate 3: Helminth eggs (Photos courtesy of DPDX). a) *Schistosoma mansoni,* b) *Ascaris lumbricoides,* c) hookworm, d) *Enterobius vermicularis,* e) *Trichuris trichiura*

3.4.2 Mini-Parasep

Mini-Parasep® fecal parasite concentrator (DiaSys, England), is a single-use *in vitro* diagnostic device for fecal concentration of helminth ova and larvae/protozoan cysts and oocysts by sedimentation. It has a flat bottom tube that allows mixing of stool sample, formalin and the solvent (i.e., ethyl acetate), a sedimentation cone, where deposition of helminth ova and protozoan oocysts occur after centrifugation, and a built-in filter. To avoid cross contamination, the Mini-Parasep kits remained closed at all times except when introducing the fecal material and formalin, or when retrieving the final concentrated sediment for examination.

One mL of Ethyl acetate was added to the formalin+ stool sample mixture, and shaken well. The Mini-Parasep kit was then inverted, and centrifuged at 230g for 4 minutes without breaking. All the supernatant in the conical tube was poured off, and the flat bottom tube with filter discarded. The walls of the conical tube were carefully wiped with cotton swabs to remove excess formalin and fatty materials, without disturbing the sediment. The sediment was resuspended to 0.5ml of the conical tube with 10% formalin. 50 µl of the resuspended sediment was loaded onto two

slides, and each slide read at x10 magnification (plate 4). Final EPG (eggs per gram of faeces) was obtained using a factor 10.

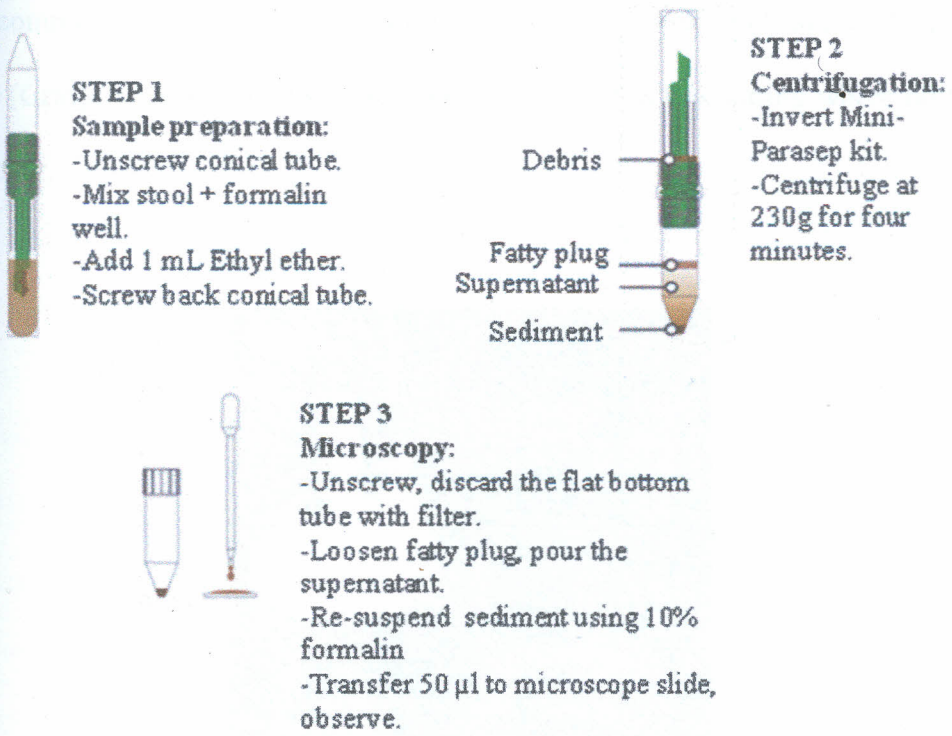


Plate 4: Mini-Parasep procedure (DiaSys instruction sheet DYS001)

3.4.3 Mini-FLOTAC

The Mini-FLOTAC, based on floatation of helminth eggs using two different floatation solutions (saturated saline FS2 and Zinc sulphate FS7), is a new simplified device that has been recently developed to improve the quality of copromicroscopic diagnosis of intestinal parasitic infections (Barda *et al.*, 2013b) (plate 5). The Mini-FLOTAC disc has three components, a key, the base and a reading disc. The base includes two 1-mL floatation chambers labeled 1 and 2 which are designed for optimal examination of fecal sample suspensions and also permits maximum magnification. The Fill-FLOTAC is a reusable sampling device that is part of the Mini-

FLOTAC. It consists of a container, a collector and a filter that facilitates performance of homogenization, filtration and filling processes of the Mini-FLOTAC. Since saturated saline (FS2) is recommended for diagnosis of soil transmitted helminths and saturated zinc sulphate for *S. mansoni* (Cringoli, 2006), the two solutions were tested in this setting where both STH and *S. mansoni* occur.

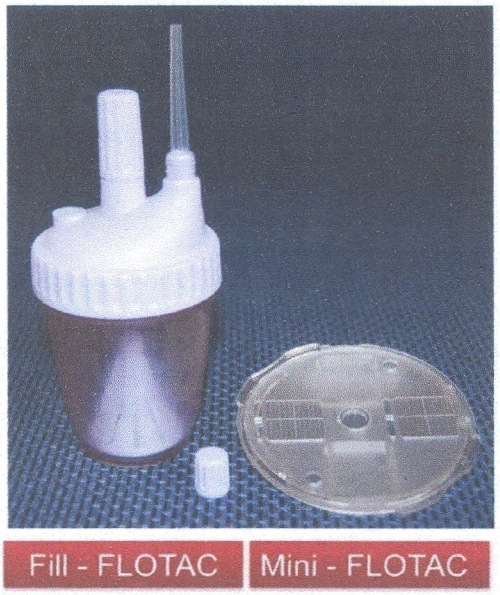


Plate 5: The Fill-FLOTAC and Mini-FLOTAC kit (Barda *et al.*, 2013b)

The preserved stool sample was centrifuged at 230g for 4 minutes without breaking, and the supernatant discarded. 19 mL of the floatation solution, either saturated Sodium chloride, specific gravity 1.2 (FS2) or saturated Zinc sulphate, specific gravity 1.35 (FS7), together with the sediment were transferred to the fill-FLOTAC container, homogenized, and then loaded to the Mini-FLOTAC chambers. Two Mini-FLOTACs were performed for each sample, one filled with the fecal suspension in FS2, and the other with the fecal suspension in FS7. An average of 10 minutes was needed for the helminth eggs to float before translating and reading under the

microscope at x10 magnification. Eggs of intestinal helminthes were identified and counted within the grids and final EPG (eggs per gram of faeces) obtained using a factor 10.

3.5 Data analysis

Performance of the diagnostic techniques was assessed based on the assumption that the combined results of all the tests reflect the true infection status. Sensitivity and specificity of Mini-Parasep and Mini-FLOTAC were estimated using Kato-Katz as the reference standard. The total positives (sensitivity) or negatives (specificity) by either Mini-Parasep or Mini-FLOTAC, and that were also positive or negative by Kato-Katz, were divided by the total positive or negative by the Kato-Katz. Kappa Cohen (k) statistic was employed to assess 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. Infection intensity was calculated using egg counts from the three diagnostic methods by Fisher's exact test. Stratification into light, moderate and heavy infections was according to cutoff values put forward by the World Health Organization (WHO, 2002). Mean egg per gram of stool were calculated using arithmetic mean. Association between the techniques in estimating *S. mansoni* egg counts was calculated using Spearman Correlation. All analyses were performed using SAS statistical software (v. 9.2; SAS Institute Inc., Cary, NC, USA) and P value < 0.05 was considered statistically significant.

3.6 Ethical considerations

The current study was reviewed and approved by the Scientific and Ethical Review Committees (ERC) of the Kenya Medical Research Institute (KEMRI, SSC No. 2185) (appendix 1), and

Maseno University. The Institutional Review Board of the Centers for Disease Control and Prevention also reviewed the study.

CHAPTER FOUR

RESULTS

4.1 Distribution of intestinal helminthes

The distribution of intestinal helminthes in relation to the method used in analysis of fecal samples is shown in table 2. The prevalence of soil transmitted helminthes by all the techniques was very low. Mini-Parasep and Mini-FLOTAC FS7 were able to detect an additional *Enterobius vermicularis* and *Taenia*, respectively.

Table 2. Distribution of intestinal helminthes in relation to the method used in stool analysis

	Number positive (%)			
Helminth species	Kato-Katz (n=282)[2]	Mini-Parasep (n=209)	Mini-FLOTAC FS2 (n=127)	Mini-FLOTAC FS7 (n=127)
S. mansoni	133 (47.2)[1]	124 (59.3)	4 (3.2)	16 (12.6)
A. lumbricoides	7 (2.5)	4 (1.9)	2 (1.6)	3 (2.4)
T. trichiura	6 (2.1)	5 (2.4)	10 (7.9)	9 (7.1)
Hookworm	3 (1.1)	10 (4.8)	8 (6.3)	5 (3.9)
E. vermicularis	-[3]	1 (0.5)	-	-
Taenia	-	-	-	1 (0.8)

[1]For individual helminthes, values in parentheses represent prevalence %

[2] values in parentheses indicate total number of stool salmples analyzed by the technique

[3] Not detected

4.2 Sensitivity and specificity of Mini-Parasep and Mini-FLOTAC

Table 3 is a summary of the sensitivities and specificities of Mini-Parasep and Mini-FLOTAC with reference to the standard Kato-Katz. Mini-Parasep showed a higher sensitivity in detecting *S. mansoni* and hookworm than Mini-FLOTAC. However, its specificity for detection of *S. mansoni* ova was low.

Table 3 Sensitivity and specificity of Mini-Parasep and Mini-FLOTAC using Kato-Katz as the reference standard[1]

Test	Sensitivity			
	S. mansoni	**Hookworm**	***A. lumbricoides***	***T. trichiura***
Mini-FLOTAC FS2	8.5% (2.4-20.4)[2]	-[3]	-	100.0% (15.8-100.0)
Mini-FLOTAC FS7	27.7% (15.6-42.6)	-	-	100.0% (15.8-100.0)
Mini-Parasep	85.0% (76.5-91.4)	33.3% (0.8-90.6)	33.3% (4.3-77.7)	75.0% (31.3-62.5)
Test	**Specificity**			
	S. mansoni	**Hookworm**	***A. lumbricoides***	***T. trichiura***
Mini-FLOTAC FS2	100.0% (95.5-100.0)	93.7% (87.9-97.2)	98.4% (94.3-99.8)	93.6% (87.8-97.2)
Mini-FLOTAC FS7	96.3% (89.4-99.2)	96.0% (90.9-98.7)	97.6% (93.2-99.5)	94.4% (88.8-97.7)
Mini-Parasep	64.2% (54.5-73.2)	95.6% (91.9-97.9)	99.0% (96.5-99.9)	99.0% (96.5-99.9)

[1] The total positives (sensitivity) or negatives (specificity) by either Mini-Parasep or Mini-FLOTAC, and that were also positive or negative by Kato-Katz, were divided by the total positives or negatives by the Kato-Katz

[2] Values in parentheses indicate 95% confidence intervals

[3] The were no positive hookworm or *Ascaris* by Mini-FLOTAC FS2 and FS7 that were also detected as positive by Kato-Katz

4.3 Degree of agreement among the techniques

In table 4, a moderate agreement was found between Kato-Katz and Mini-Parasep for detection of *S. mansoni.* Mini-FLOTAC FS2 and Mini-FLOTAC FS7 had a fair agreement for detection of hookworm. For detection of *T. trichiura*, Kato-Katz and Mini-Parasep had substantial agreement, while FS2 and FS7, MP and FS2 had an almost perfect agreement.

Table 4 Kappa test of agreement among the techniques[1]

	S. mansoni	P value	Hookworm	P value	*A. lumbricoides*	P value	*T. trichiura*	P value
[2]KK vs FS2	0.108	0.0163	-0.014	1.0000	-0.011	1.0000	0.315	0.0057
KK vs FS7	**0.284**[3]	<0.0001	-0.013	1.0000	-0.012	1.0000	0.347	0.0046
KK vs MP	**0.487**	<0.0001	0.135	0.1374	0.385	0.004	**0.659**	<0.0001
FS2 vs FS7	0.157	0.0771	**0.434**	0.0016	-0.019	1.0000	**0.943**	<0.0001
FS2 vs MP	-0.000	1.0000	-0.025	1.0000	-	-	**1.000**	0.0185
FS7 vs MP	0.009	1.0000	0.000	1.0000	0.000	1.0000	0.000	1.0000

[1]Agreement was determined using the following 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.

[2]KK- Kato-Katz, MP- Mini-Parasep, FS2- Floatation solution 2, while FS7- Floatation solution 7

[3]Kappa values in bold indicate values of agreement that are fair, moderate, substantial and almost perfect

4.4 Distribution of light, moderate and heavy infections among the tests

In table 5, Mini-Parasep technique performed marginally better at detecting light *S. mansoni* infections, 70.2% by Mini-Parasep vs 59.4% by Kato-Katz, $P = 0.0896$, using Fisher's exact test, whereas Kato-Katz was better at detecting heavy *S. mansoni* infections, 16.5% for Kato-Katz vs 4.8% for Mini-Parasep, $P = 0.0026$, using Fisher's exact test.

Analyses of the distribution of infection intensities among the tests revealed that of the 87 *S. mansoni* infections that were classified as light by Mini-Parasep, 33 (37.9%) of these were also classified as light by Kato-Katz, 36 (41.4%) were classified as uninfected, whereas 15 (17.2%) and 3 (3.4%) were classified as moderate and heavy by Kato-Katz. The distribution of the moderate infection intensities were fairly even between Mini-Parasep and Kato-Katz. Analyses of the distribution of infection intensities among the tests revealed that although all the 6 *S. mansoni* infections that were classified as heavy by Mini-Parasep were also positive by Kato-Katz, there was a difference in their distribution by Kato-Katz. Of the 6 *S. mansoni* infections classified as heavy infections by Mini-Parasep, 3 (50%) of these were also classified as heavy by Kato-Katz, whereas 2 (33.3%) and 1 (16.7%) were classified as moderate and light by Kato-Katz (Table 5).

Further, all 16 *S. mansoni* infections detected by Mini-FLOTAC FS7 were all classified as light infections. Of these 16, 9 (56.3%) were also classified as light by Kato-Katz, whereas 3 (18.8%),

1 (6.3%) and 3 (18.8%) were classified as uninfected, moderate and heavy, respectively, by Kato-Katz.

Table 5 Summary of the distribution of light, moderate and heavy infections among the tests

Species infection	Overall Prevalence, (%)	Intensity Threshold Prevalence, (%)			Intensity (epg)
		Light	Moderate	Heavy	
Schistosoma mansoni					
Kato-Katz	47.2 (41.2-53.2)[1]	59.4	24.1	16.5	203.6± 313.9[2]
Mini-Parasep	59.3 (52.3-66.1)	70.2	25.0	4.8	104.7± 192.9
Mini-FLOTAC (FS2)	3.2 (0.9-7.9)	100.0	0.0	0.0	17.5 ± 6.5
Mini-FLOTAC (FS7)	12.6 (7.4-19.7)	100.0	0.0	0.0	21.6 ± 13.1

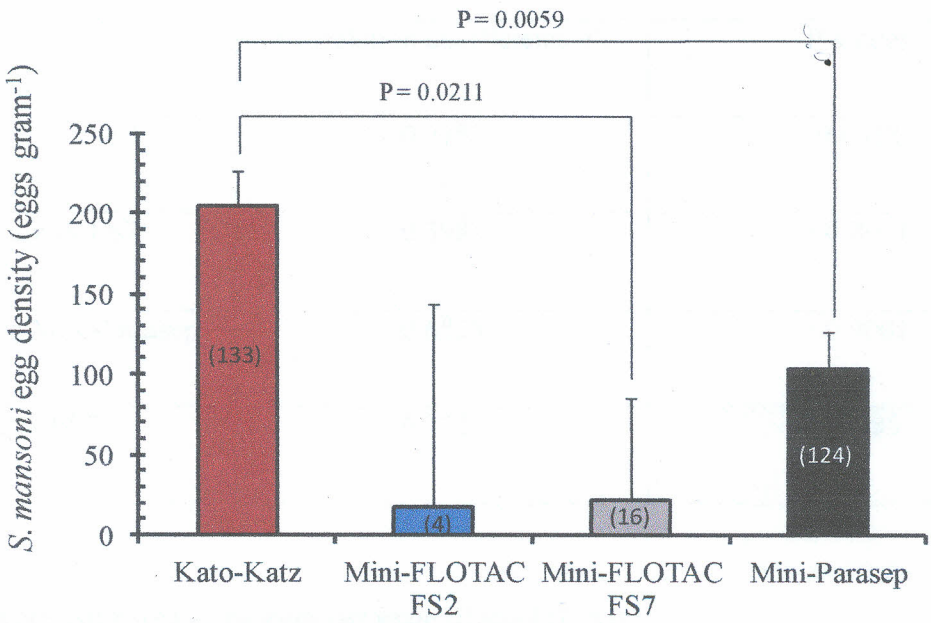
[1] Values in parentheses indicate 95% confidence intervals

[2] Intensity if infection calculated using epg (egg per gram of stool), was expressed as arithmetic mean ± standard deviation

4.5 *S. mansoni* mean egg density

Comparison of the mean egg densities revealed that estimates for *S. mansoni* by Mini-Parasep compared to the standard Kato-Katz was (104.7 vs 203.6, p=0.0059), while that of FS7 compared to Kato-Katz was (21.6 vs 203.6, p=0.0211), and were considered significant (Figure 2).

Figure 2 Comparison of *S. mansoni* mean egg density between Mini-Parasep and Mini-FLOTAC with the standard Kato-Katz



*Values in parentheses represent the number positive by each technique

4.6 Correlation analysis for *S. mansoni* egg counts

There was a significant and positive association between Kato-Katz, Mini-Parasep and Mini-FLOTAC in determining *S. mansoni* egg counts. (Table 6)

Table 6 Summary of correlation coefficients for *S. mansoni* egg counts by the three techniques

	Correlation coefficients Υ_s	P values
Kato-Katz vs FS2	0.2753	0.0018
Kato-Katz vs FS7	0.3982	<0.0001
Kato-Katz vs Mini-Parasep	0.6835	<0.0001
FS2 vs FS7	0.2325	0.0085

*egg counts were estimated using eggs per gram of stool (EPGs)

CHAPTER FIVE

DISCUSSION

To facilitate progress towards control and elimination of helminths, it is necessary to develop new quantitative diagnostic tools that are of low-cost, sensitive, accurate and easy to perform (Barda *et al.*, 2013a). To date, such a method does not exist and suggestions have been put forward to combine more than one technique to achieve a more comprehensive diagnosis (Barda *et al.*, 2013a; Levecke *et al.*, 2011). The Kato-Katz method is a quantitative technique that allows for good visualization of helminth ova/eggs in faeces and has been recommended by the WHO for diagnosis of intestinal helminthiasis (WHO, 2002). However, studies on comparison among techniques have shown that it is not the most sensitive and accurate method for egg counts (Albonico *et al.*, 2012; Barda *et al.*, 2013a; Levecke *et al.*, 2011). Results from this study have shown a difference in the ability of different techniques to detect intestinal helminth eggs in stool. With reference to the standard Kato-Katz, Mini-Parasep showed a higher sensitivity in detecting *S. mansoni,* and hookworm ova than the Mini-FLOTAC technique. There was a fair to almost perfect agreement between Mini-Parasep, Mini-FLOTAC and Kato-Katz in detecting all the intestinal helminths that were investigated. Mini-Parasep detected more light intensity *S. mansoni* infections while Kato-Katz detected more heavy intensity *S. mansoni* infections. Further analysis of the mean egg density and egg counts for *S. mansoni* showed a significant difference and a positive association, respectively between Kato-Katz, Mini-FLOTAC and Mini-Parasep.

The recovery of intestinal helminth eggs by Kato-Katz, Mini-Parasep and Mini-FLOTAC is comparable provided that microscopy is done expertly. Mini-Parasep and Mini-FLOTAC FS7 were able to detect an additional *E. vermicularis* and *Taenia,* respectively. This is an important

observation since concentration techniques have the potential to increase the ability to detect rare infections (Parameshwarappa *et al.*, 2012) and, accurate diagnosis of an infection is the basis for defining the prevalence and understanding the epidemiology and morbidity of a disease.

Using Kato-Katz as the reference standard, the sensitivity of Mini-Parasep in detecting *S. mansoni* and hookworm were all higher than the corresponding values of Mini-FLOTAC FS2 and FS7. Use of ether could be the reason. Studies have shown that it results in a more clearly examinable slide by dissolving organic compounds, hence increasing the chances of detecting helminth ova (Knoop *et al* 2011). Floating organic debris might have prevented accurate detection of transparent hookworm eggs in some stool samples, hence affecting Mini-FLOTAC sensitivity for detecting hookworm eggs (Knoop *et al* 2011). A similar observation was made in stool samples from school children in Cote d'Ivoire and Pemba that were analyzed by the FLOTAC technique (Glinz *et al.*, 2010; Rinaldi *et al.*, 2009). It should be noted that no solvents were included in the Mini-FLOTAC procedure in this study. A comparative study between FLOTAC and two other techniques in Cote d'Ivoire showed that hookworm prevalence as assessed by the FLOTAC, Kato-Katz and ether concentration technique was 65.7%, 51.0% and 28.4% respectively. Moreover, the FLOTAC technique showed a sensitivity of 88.2% compared with 68.4% for the Kato-Katz (Esposito *et al.*, 2013). Results from this study however demonstrate a relatively lower sensitivity by Mini-FLOTAC (a modification of the FLOTAC technique) especially in detecting STHs. Findings to-date are that a single FLOTAC is more sensitive than multiple Kato-Katz thick smears for diagnosing low intensity soil-transmitted helminth infections (Barda *et al.*, 2013a; Knoop *et al.*, 2009; Speich *et al.*, 2010). However, differences in epidemiological settings and period of time taken to preserve stool samples might explain some of the discrepancies in diagnostic sensitivity by the Mini-FLOTAC technique. The

design of the present study did not allow for investigation on the effects of duration of stool preservation on helminth species-specific detection. It is worth noting that stool samples were preserved/fixed in 5% formalin for one month prior to laboratory analyses. It is therefore appreciated that duration of stool fixation may have had a considerable effect on the diagnostic performance of Mini-FLOTAC. Consistent with this observation, Glinz *et al* reported a reduction in FLOTAC sensitivity for detection of hookworm and *A. lumbricoides* from a study in Côte d'Ivoire, day 83 post stool preservation in Sodium acetate-acetic acid-formalin (SAF) (Glinz *et al.*, 2010). Similarly Knoop *et al* observed a higher sensitivity of FLOTAC for hookworm diagnosis at follow-up compared to baseline survey when samples collected at follow-up had at least a 3-week shorter preservation period in 5% formaldehyde than samples preserved at the baseline survey (Knoop *et al.*, 2011). Since the FLOTAC technique examines a large amount of faeces, its analytical sensitivity is expected to be higher and thus it is less likely to give false negative results. This is important because many types of research require highly reliable fecal egg count techniques; for example, studies of the efficacy of anthelmintics, studies of helminthic and host resistance (Esposito *et al.*, 2013). A research in Tanzania has suggested that for qualitative diagnosis of STHs, stool samples can be fixed in 5% formalin for at least 30 days. However, for an accurate quantitative diagnosis of hookworm, a limit of 15 days of preservation would be appropriate (Barda *et al.*, 2015).

The performance of the two floatation solutions in detecting various helminth ova was noted: Mini-FLOTAC FS7 was more sensitive than Mini-FLOTAC FS2 in detection of *S. mansoni*. Additionally, Mini-FLOTAC FS7 detected a higher number of *S. mansoni* eggs than Mini-FLOTAC FS2. Differences in sensitivity are associated with differences in the specific gravities of the solutions, that plays a crucial role in floatation of the eggs (Barda *et al.*, 2013a). The

saturated saline FS2 detected more hookworm than the Zinc sulphate FS7 solution. Similar results were also observed in a study done in Mwanza, Tanzania, (Barda *et al.*, 2013a). Results from this study support other research work that have shown that the saturated saline (FS2) performs better and is recommended for the detection of STHs, while Zinc sulphate(FS7) is recommended for *S. mansoni* detection (Cringoli, 2006). Thus, in endemic areas as around Lake Victoria where the prevalence of *S. mansoni* is high (Mwinzi *et al.*, 2012; Odiere *et al.*, 2012) use of Mini-FLOTAC (FS7) is ideal. However, the low sensitivity of *S. mansoni* as detected by Mini-FLOTAC FS7 requires further investigation before it can be used for surveys and monitoring and evaluation.

Results on degree of agreement among techniques has shown a moderate agreement between Kato-Katz and Mini-Parasep, and a fair agreement between Kato-Katz and Mini-FLOTAC FS7 in detection of *S. mansoni* ova. While concentration techniques increase the detection of helminthic eggs, larvae and protozoan cysts, some like the Formol-ether concentration have the advantage of less alteration of organisms and an increased recovery of schistosoma species and operculated eggs (Parameshwarappa *et al.*, 2012). Hence, in view of the increasing polyparasitism in developing countries, there is the need to supplement the routine methods (Kato-Katz technique) with sedimentation and floatation techniques to concurrently detect different intestinal parasite species in the same stool sample. This will improve diagnostic accuracy as compared to use of the routine methods alone.

The distribution of *S. mansoni* infection intensities by Kato-Katz and Mini-Parasep has several implications for mass treatment programs and in monitoring and evaluation programs. WHO

recommends mass drug administration (MDA) with Praziquantel (PZQ) (for schistosomiasis) wherever the prevalence of infection exceeds 10% (WHO, 2006). Data from the current study suggests that Mini-Parasep technique performed better at detecting light *S. mansoni* infections; whereas Kato-Katz was better at detecting heavy *S. mansoni* infections (1-99 eggs gram^{-1}) (70.2% by Mini-Parasep vs 59.4% by Kato-Katz; whereas Kato-Katz was better at detecting heavy *S. mansoni* infections (≥ 400 eggs gram^{-1}) (16.5% for Kato-Katz vs 4.8% for Mini-Parasep). Further analyses revealed that over 41% of the light infections detected by Mini-Parasep were not detected by Kato-Katz, indicating that Kato-Katz would likely result in a misdiagnosis of infected individuals. Such a misdiagnosis would lead to misestimation of the prevalence of infection in a population, and would consequently affect important Mass Drug Administration (MDA) decisions, specifically the cut-offs prevalence thresholds and frequency of implementing MDA. The WHO also emphasizes on morbidity control. Existing evidence shows that the degree of morbidity is related to the intensity of infection, where individuals with heavy infection intensities tend to experience more severe health outcomes (Jardim-Botelho *et al.*, 2008; Stoltzfus *et al.*, 1996) Use of Mini-Parasep therefore may misclassify heavy infections. Additional studies are therefore needed to further validate the performance of Mini-Parasep in areas of high *S. mansoni* endemicity where heavy infections are likely to be common.

From the study in Mwanza, Tanzania, Mini-FLOTAC and Kato-Katz detected a similar mean eggs per gram, slightly higher with Kato-Katz both for hookworm (455 vs 427) and *S. mansoni* (71 vs 58), but were not statistically different (Barda *et al.*, 2013a). In the present study, similar results were observed where the highest number of hookworm and *S. mansoni* was detected by Mini-FLOTAC FS2 *and* Mini-Parasep, respectively: Kato-Katz detected a higher number of eggs

(calculated as arithmetic mean) compared to Mini-Parasep and Mini-FLOTAC FS7 .This is an important aspect in helminth control as intensity of infections measured as eggs per gram is directly related to morbidity, and is an important indicator to monitor the impact of control programs as well as drug efficacy (Barda *et al.*, 2013b; Levecke *et al.*, 2011). The higher egg counts by Kato-Katz in comparison to all other techniques could be explained by the following reason: when scrapping the plastic spatula of the Kato-Katz kit across the upper surface of the fine-meshed screen placed on top of the stool sample, the faeces are sieved, and helminth eggs are concentrated (Katz *et al.*, 1970), an issue that is under investigation.

CHAPTER SIX

SUMMARY OF STUDY FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of Study Findings

Prevalence of STH infections across all the techniques was very low. With reference to the standard Kato-Katz, Mini-Parasep showed a higher sensitivity for detection of *S. mansoni* and hookworm than the Mini-FLOTAC technique. There was a moderate agreement between Kato-Katz and Mini-Parasep, and a fair agreement between Kato-Katz and FS7 in detection of *S. mansoni* ova. Further, Mini-Parasep detected more light intensity *S. mansoni* infections, while on the other hand, Kato-Katz detected more heavy intensity *S. mansoni* infections. There was a significant positive association between Kato-Katz and Mini-Parasep in counts of *S. mansoni.*

6.2 Conclusions

I. Findings from this study have shown that with reference to the standard Kato-Katz, Mini-Parasep is more sensitive than Mini-FLOTAC in detecting *S. mansoni* and hookworm ova.

II. In estimating degree of agreement among techniques in this study, a moderate agreement between Kato-Katz and Mini-Parasep was observed, and a fair agreement between Kato-Katz and Mini-FLOTAC FS7 was also observed in detecting *S. mansoni* ova.

II. This study has also demonstrated the superior performance by Mini-Parasep technique in detecting light intensity *S. mansoni* infections than Kato-Katz, which detected more heavy intensity *S. mansoni* infections.

6.3 Recommendations

- The high prevalence of *S. mansoni* in this region is certainly associated with specific occupational and other water-related activities such as swimming, and regular use of lake waters for domestic purposes, and is an indication of high fecal contamination of water sources. To mitigate this situation, behavior change and regular treatment of infected individuals (chemotherapy) to prevent reinfection, provision of sanitation facilities in schools and poor rural settings, together with access to clean piped water is recommended.

- The high sensitivity of Mini-Parasep in detecting *S. mansoni* and hookworm ova calls for it inclusion into schistosomiasis and STH control and drug efficacy evaluation programs as an alternative to the Kato-Katz technique.

- Since there was a moderate agreement between Kato-Katz and Mini-Parasep in detecting *S. mansoni* eggs, this study recommends the combined use of Mini-Parasep and Kato-Katz in disease surveillance and epidemiological studies to increase the diagnostic sensitivity of detecting intestinal schistosomiasis.

6.3.1 Recommendations for future research

- Microscopic analysis of stool samples in this study by Mini-Parasep and Mini-FLOTAC identified two individuals with *Taenia* and *Enterobius vermicularis*. The source of these infections is still not clear or whether it is an indication of focal point of transmission of these infections within Mbita. Future research may therefore benefit from further validation and standardization of the Mini-Parasep and Mini-FLOTAC techniques to detect these and other intestinal parasites of public health importance.

- The effect of duration of stool preservation on sensitivity and specificity by the Mini-FLOTAC technique warrants further investigation especially for the hookworm and *A. lumbricoides* eggs.
- Future research may benefit from carrying out a cost analysis of using Mini-Parasep and Mini-FLOTAC techniques with unpreserved stool samples.

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