CO-INFECTION OF *Salmonella typhi* AND SOIL-TRANSMITTED HELMINTHS AND ANTIMICROBIAL PROFILES IN INDIVIDUALS ATTENDING UKWALA SUB-COUNTY HOSPITAL, KENYA

BY

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DECLARATION

I declare that this thesis is my own original work and has not been presented to any other university or institution for the award of a degree or any other award.

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DEDICATION

This work is dedicated to my dad James Waganda and mom Seline Otambo and brothers Onyango and Nelson and sisters Mildred, Mercy and Grace for their moral support and understanding during the entire period of my study.

ABSTRACT

Gastrointestinal infections, such as soil-transmitted helminths (STHs) and *Salmonella typhi* infection, are a major cause of morbidity and mortality globally. In rural areas of Kenya, 9.1 million people are at risk of STHs infection with Western Kenya having the highest burden of both STH and *S. typhi* infection. Siaya County is more prone to *S. typhi* and STHs infection with Ukwala hospital records estimating the burden of infection at 30%. Given that STHs cause intestinal ulcers that may serve as entry points for enteric bacteria, there is need to determine the frequency of STHs and *S. typhi* among co-infected individuals and the demographic patterning of co-infection. *Salmonella typhi* isolates from Western Kenya exhibit widespread antibiotic resistance. It is known that co-infection weaken natural immunity thus compromising efficacy of the drugs in use. Whether co-occurrence of STHs and *S. typhi* affects efficacy of commonly used antibiotics, should therefore be investigated. This study was based in Ukwala Sub-County Hospital, Siaya County and aimed to investigate co-infection of *S. typhi* and STHs and antimicrobial profiles in individuals attending Ukwala Sub-County Hospital. The specific objectives were to determine: differences in frequencies of STHs in *S. typhi* positive and negative individuals; whether age and gender predict co-infection between *S. typhi* and STHs; and antimicrobial profiles of *S. typhi* isolates in individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs. Each stool sample cross-sectionally obtained from 325 individuals recommended for typhoid fever test was examined for the presence of eggs or larvae of *Ascaris lumbricoides*, *Trichuris trichiura* and *Necator americanus* by direct smear, and negative results were confirmed by formalin-ether concentration technique. Antimicrobial susceptibility test was based on disc-diffusion method on Mueller-Hinton agar. *Salmonella typhi* isolates were tested on four antibiotics commonly used to treat typhoid fever: ampicillin, tetracycline, chloramphenicol and ciprofloxacin. The frequency of individuals infected by STHs was higher among *S. typhi* negative than *S. typhi* positive individuals but there was no association between STHs and *S. typhi* infections ($\chi^2 = 0.348$, $P > 0.05$). Similarly, neither age nor gender significantly predicted occurrence of co-infection between *S. typhi* and STHs (logistic regression model, $\chi^2 = 2.804$, $P > 0.05$). Lastly, there was no difference in the antimicrobial resistance profiles of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs. The results suggest that infection by STHs does not appear to facilitate *S. typhi* infection. Alternatively, the level of STH infection in the population was very low, hence may have masked any patterns that may be salient under heavy disease burdens. Therefore, individuals recommended for *S. typhi* testing in Western Kenya, should also be tested for STHs. Furthermore, drugs of choice for the treatment of *S. typhi* as a single infection in Ukwala area should be used by the clinicians in treatment of individuals co-infected with *S. typhi* and STHs.

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ACRONYMS AND ABBREVIATIONS

CDC- Centre for Disease Control

GI- Gastrointestinal

HIV- Human Immunodeficiency Virus

MDR- Multidrug Resistant

STHs- Soil Transmitted Helminths

TB- Tuberculosis

WHO- World Health Organization

XLD- Xylose-Lysine –Desoxycholate

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Gastrointestinal (GI) infections are an important cause of morbidity, mortality and economic loss throughout the world (Dudlová et al., 2016). Globally, about 5 billion people are affected by GI infections with about 1.4 million deaths reported annually (Lozano et al., 2013). When GI infections co-occur, their immunopathogenic response may interact synergistically or antagonistically (Cunin et al., 2003). These interaction may complicate GI infections by increasing disease severity, altering single infection resulting in variation in the pathogen establishment and ultimately increasing morbidity and mortality (Gendrel et al., 1984). For example, infections by soil-transmitted helminths (STHs) downregulate the host's immunity making the host vulnerable to enteric bacteria such as *S. typhi* that causes typhoid fever (Su et al., 2014).

Annually, typhoid fever affects 13.5 million people globally with about 100,000 cases reported in East Africa (Buckle et al., 2012). Population-based studies from rural sites in Africa have reported incidence of typhoid fever where the *S. typhi* is an important pathogen (Breiman et al., 2012). Untreated typhoid fever cases results in mortality rates ranging from 12-30% while in treated cases survival is 99% (Pollack, 2003). People infected with *S. typhi* live in regions with poor health monitoring where opportunity to ingest the bacterium with contaminated food and drinks is high (Parry et al., 2002).

Salmonella typhi may enter the host circulatory system through ulcer spots in the intestine caused by the feeding STHs (Chan, 1997). Helminth infection have been linked to increased susceptibility to subsequent microbial infection through helminth-mediated immunomodulation characterized by inducing T helper (Th) 2 immune response and regulatory T-cell (Treg) response (Maizels & Yazdanbakhsh, 2003). The main species of STHs infecting humans are round worm (*Ascaris lumbricoides*) infecting an estimated 804 million people; whip worm (*Trichuris trichiura*) infecting an estimated 477 million people; and hook worms (*Necator americanus* and *Ancylostoma duodenale*) infecting an estimated 472 million people (Collaborators Global Burden of Disease Study 2013, 2015). The prevalence of STHs in Western Kenya with minimum estimate as at 2010 was at 68% (Riesel et al., 2010). The implementation of the government deworming programs has seen the infection levels of STHs decreasing to an estimated prevalence of 42% in Western Kenya (Andereck et al., 2014; Pullan et al., 2011). Presently, the differences in the frequencies of STHs as a function of *S. typhi* infection is not known, yet such knowledge would advance intervention by targeting efforts that have the most prevention and treatment benefits.

Demographically, STHs infections are common in school-going children in at-risk communities where the worms negatively affect growth, cognitive development and immunity to other infections (Galvani, 2005). Hook worm infection is an important cause of blood loss that leads to iron deficiency and protein malnutrition resulting in anemia in children, women of reproductive age and pregnant women (Brooker et al., 2004; Adegnika et al., 2010). Hook worm infection is common in hosts aged between 10 to 14 years in endemically infected communities (Galvani, 2005). In the case of *S. typhi* infection, children and the old aged individuals are at a higher risk of

the infection (Conner & Schwartz, 2005) and males are normally more affected than the females (Blaser & Feldman, 1981). However the influence of the host's demographic traits such as age and gender on the occurrence of co-infection between *S. typhi* and STHs is not known. Yet, knowledge of epidemiological dynamics of concurrent infections will reveal the demographic patterning in co-infected individuals and have potential to guide policy on management of *S. typhi* and infections caused by STHs.

Many isolates of *S. typhi* are resistant to antibiotics. Emergence of antibiotic resistance has been attributed to development of Multidrug-resistance strains, wide spread misuse of antibiotics and self-medication (Kariuki et al., 2004; Owour et al., 2015). However, recent studies appear to suggest that loss of naturally acquired immunity may enhance emergence of resistance. For example, in malaria infection, weakening of natural immunity has been shown to compromise efficacy of the drug in use (Bijker & Sauerwein, 2012). It follows that exposure to conditions that compromise natural immunity may create conducive conditions that enhance emergence of resistance to drugs. Helminths have the ability to down-regulate the host immune's response (Abruzzi & Fried, 2011) thus increasing disease severity in subsequent infections (Gendrel et al., 1984). Whether the modulation results in loss of efficacy of antibiotics, remains an open question. Thus it is important to examine resistance profiles of *S. typhi* isolates in single and in co-infection scenarios.

Population-based surveillance reports for estimating disease burden in rural area in Kenya have reported high disease burden of typhoid fever and intestinal parasitic infection in Siaya County (Breiman et al., 2012; Feikin et al., 2010, 2011). With Ukwala area being prone to STHs infections and typhoid fever (Decker, 2015). This study, therefore, aimed to investigate co-infection of *S. typhi* and STHs and antimicrobial profiles in individuals attending Ukwala Sub-County Hospital.

1.2 Problem statement

Soil-transmitted helminths cause intestinal ulcers that may serve as entry points for enteric bacteria, but no study has investigated whether STHs exacerbate the occurrence of *S. typhi* infection. Therefore, there is need to assess the differences in frequencies of individuals with STHs as a function of *S. typhi* infection.

Infection by STHs and Salmonella has been shown to differ by age and gender, with STHs being more common in females and school going children. Salmonella infection on the other hand is more prevalent among children and the old aged individuals. However, the influence of the host's demographic traits such as age and gender on the occurrence of co-infection between *S. typhi* and STHs is likely to be different from the pattern seen in single infections hence requires investigations. As such, this study determined whether age and gender predict co-infection between *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital.

Salmonella typhi isolates from Western Kenya exhibit widespread antibiotic resistance. It is known that co-infection weaken natural immunity thus compromising efficacy of the drugs in use. Whether the co-occurrence of STHs and *S. typhi* co-infection affects the efficacy of the commonly used antibiotics needs to be investigated. Therefore, the current study investigated co-infection of *S. typhi* and STHs and antimicrobial profiles in individuals attending Ukwala Sub-County Hospital, Kenya.

1.3 Justification

Co-infections are a major problem in Africa and may complicate development of therapeutics. It was envisaged that the results of the study would highlight any differences in the frequencies of STHs as a function of *S. typhi* infection; explicate the demographic patterning of co-infection between *S. typhi* and STHs; and shed light on the antimicrobial profiles of *S. typhi* isolates under single and co-infection scenarios. In sum, it was anticipated that the findings from the study would inform clinical management of *S. typhi* in Ukwala, Ugenya Sub-County, a rural area that has a high prevalence for *S. typhi* and STHs. In addition, the findings may help shape policy on management of *S. typhi* and infections caused by STHs.

This study was conducted in Ukwala which is in Siaya County that has heavy burden of *S. typhi* and STHs infections (Breiman et al., 2012; Decker, 2015; Feikin et al., 2011; Riesel et al., 2017) . Individuals in the area are more prone to typhoid fever and STHs infection (Decker, 2015) and according to the Ukwala Sub-County Hospital health record (2016) data, the prevalence of *S. typhi*

is estimated at 30%. In Ugenya Sub-County, Ukwala Sub-County Hospital is the main hospital that most individididuals in the area seek medical services.

1.4 General objective

To investigate co-infection of *S. typhi* and STHs and antimicrobial profiles in individuals attending Ukwala Sub-County Hospital, Kenya.

1.5 Specific objectives

i. To determine differences in frequencies of individuals with STHs among *S. typhi* positive and *S. typhi* negative individuals attending Ukwala Sub-County Hospital, Kenya.
ii. To determine whether age and gender predict co-infection between *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital, Kenya.
iii. To determine the antimicrobial profiles of *S. typhi* isolates in individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs attending Ukwala Sub-County Hospital, Kenya.

1.6 Hypotheses

i. There is no difference in the frequency of individuals with STHs between *S. typhi* positive and *S. typhi* negative individuals attending Ukwala Sub-County Hospital, Kenya.
ii. Age and gender do not predict co-infection between *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital, Kenya.

iii. There is no difference in the antimicrobial profiles of *S. typhi* isolates between individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs attending Ukwala Sub-County Hospital, Kenya.

1.7 Significance of the study

The study will provide useful information that can advance intervention by targeting efforts that have the most prevention and treatment benefits, reveal the demographic patterning in co-infected individuals and will have potential to guide policy on management of *S. typhi* and infections caused by STHs. In addition, the results will help inform the development of therapeutics to manage *S. typhi* infections either singly or in combination with other infections.

1.8 Limitation of the study

Selected individuals in the study may be the ones seeking hospital services and may not, therefore, represent the general population in the Sub-County. Under the category of ages, some individuals were grouped under the 20 and above years of age. Consequently, the age groups may not be mutually exclusive particularly from the perspective of mass drug administration for the treatment of helminthic infections among school-going children.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Gastrointestinal infections affect approximately 5 billion people globally with about 1.4 million deaths reported annually (Lozano et al., 2013). Under co-infection scenarios, the immunopathogenic responses in GI infections may interact synergistically or antagonistically (Cunin et al., 2003). In synergistic interactions, the virulence of one pathogen will increase the virulence of the other, while in antagonistic interactions, the virulence of one pathogen will limit the severity of the other. The interactions may be either via the host's resources or immune mediated interactions or both. In co-infected hosts, the interactions may alter the transmission, clinical progression and control of multiple infectious diseases (Chiodini, 2001).

2.2 *Salmonella typhi* infection

Salmonella typhi is the causative agent of typhoid fever. The global burden of typhoid fever is estimated at 13.5 million cases and 100,000 deaths annually (Buckle et al., 2012). In Africa, the overall burden of *S. typhi* is unknown partly because the facilities capable of the blood culture tests essential for diagnosis of *S. typhi* are lacking in many regions or health facilities across the continent (Crump et al, 2004). *Salmonella typhi* infections are more common in densely populated areas where sanitation and hygiene is compromised with high number of infection occurring in children living in densely populated areas (Crump et al, 2004). A population-based study in a rural site in Africa reported high incidence of typhoid fever (Breiman et al., 2012). But knowledge on

the role of host traits such as age and gender on *S. typhi* infection concurrently occurring with STHs is lacking.

Transmission of *S. typhi* occurs when the bacterium enters the host system upon ingestion of food or water that has been contaminated with infected feces. The bacteria use the intestine as a portal entry and pass through the intestinal mucosal layer and ultimately enter macrophages and multiply (Ryan & Ray, 2004). Thereafter, the bacteria are carried through the reticuloendothelial tissues where they continue to multiply and eventually release lipopolysaccharide endotoxins that result in typhoid fever (Ryan & Ray, 2004). Within 1-3 weeks after exposure to *S. typhi*, symptoms of high fever, malaise, headache, constipation or diarrhea, rose-colored spots on the chest and enlarged spleen and liver are observed and about 5% of typhoid patients become chronic carriers and present with persistent gall bladder infection (Bhan et al., 2005). Without prompt treatment, *S. typhi* infection can cause serious complications that can be fatal (Parry et al., 2002);mortality rates range from 12-30% (Pollack, 2003). Much research has been on *S. typhi* as a single infection (Crump et al, 2004; Ryan & Ray, 2004) but not its co-occurrence with STHs.

2.3 Soil-transmitted helminthic infections

Soil-transmitted helminthic infections affect almost one-sixth of the global population (Hotez, 2007). The main species of STHs infecting humans are round worm (*Ascaris lumbricoides*) infecting an estimated 804 million people; whip worm (*Trichuris trichiura*) infecting an estimated 477 million people; and hook worms (*Necator americanus* and *Ancylostoma duodenale*) infecting an estimated 472 million people with an estimated 300 million people suffering from heavy

infection of STHs; out of these, 150,000 die annually (Collaborators Global Burden of Disease Study 2013, 2015).

Infections by STHs are common in tropical and subtropical areas with high infection levels in sub-Saharan Africa, Asia, Latin America and Caribbean regions where the prevailing social environmental risk factors include poor sanitation, unsafe drinking water and poverty (Brooker et al., 2006; Horton, 2003). Transmission of STHs varies from species to species. For instance, in *T. trichiura*, that is responsible for trichuriasis, transmission is fecal-oral with high prevalence occurring in areas with tropical weather and poor sanitation (Bethony et al., 2006). *T. trichiura* eggs are passed in the stool and must incubate in the soil for 2-3 weeks before becoming embryonated and infectious. Upon ingestion, the larvae hatch in the small intestine and invade the intestinal villi where they grow before they move to the large intestine where they penetrate the mucosa and develop into adults. The attachment of the worm to the colonic mucosa and their subsequent feeding activities produce localized ulceration and hemorrhage which may provide enteric bacteria such as *S. typhi* with a portal of entry to the bloodstream (Chan, 1997).

In *Ascaris lumbricoides* that is responsible for ascariasis, transmission is fecal-oral and affects individuals both in the rural and urban areas. Its distribution is largely determined by the in-hygienic practices such as open defecation, which allow its eggs to reach the soil and incubate (Kightlinger et al., 1998). Fertile eggs embryonate and become infective after 18 days to several weeks, depending on environmental conditions. After infective eggs are swallowed, the larvae

hatch, invade the intestinal mucosa, and are carried via the portal to the lungs. The larvae mature further in the lungs (10-14 days), penetrate the alveolar walls, ascend the bronchial tree to the throat, and are swallowed; upon reaching the small intestine, they develop into adult worms. In the small intestine, *A. lumbricoides* interferes with digestion and absorption of food by decreasing micro-nutrients and vitamin A absorption, probably by causing structural abnormality of the intestinal mucosa (Bundy et al., 1995). In children, it is associated with malnutrition that leads to growth retardation, cognitive impairment, poor academic performance, which ultimately is associated with poorer quality of life (Bethony et al., 2006).

Hookworm infection results in ancyclostomiasis (caused by *A. duodenale*) and necatoriasis (caused by *N. americanus*). *Ancylostoma duodenale* is the more common type in Middle East, north Africa, India and Southern Europe, while *N. americanus* is the more common type in the America, Sub-Saharan Africa, Southern Asia, China and Indonesia, affecting poor rural communities (Silva et al., 2003). Hook worm eggs are passed in the stool, and under favorable conditions in the soil, the larvae hatch within 1 to 2 days to become rhabditiform larvae. The rhabditiform larvae feed on the stool and undergo 2 successive molts; after 5-10 days, they become infective filariform larvae. These infective filariform larvae go through developmental arrest and can survive in damp soil for as long as 2 years. On contact with the human host, the larvae penetrate the skin and are carried through the blood to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall with resultant blood loss by the host leading to malnutrition and anemia (Hotez et al., 2004).

2.4 Factors that influence STHs and *Salmonella typhi* infections

Transmission of STHs and *S. typhi* is associated with poverty, poor hygiene practices, lack of clean water and inadequate waste disposal and sanitation (Bethony et al., 2006). At the individual level, host factors that influence susceptibility to infection by *S. typhi* are mainly age and gender (Sayasone et al, 2011). Children and adolescents experience the greatest burden of illness by *S. typhi* infection with individuals in regions with poor sanitation and unsafe food and water mostly affected (Crump et al., 2004). Children aged less than 5 years and individuals aged 70 years and above are more prone to *S. typhi* infection (Misra et al., 1997; Ryan & Ray, 2004). Old aged individuals have higher incidences of infection and their higher susceptibility to infection has been attributed to age-associated deregulation of immune system to pathogens (Misra et al., 1997). Typhoid fever is mainly predominant in rural areas where the likelihood of infection is due to exposure to predisposing factors (Butler et al., 1990).

Demographically, infection rates of gastrointestinal parasites are highest in children living in sub-Saharan Africa, Asia, Latin America and Caribbean regions where approximately a quarter of the total population is infected with one or more STHs (Brooker et al., 2009). An estimated 1.0 billion school-aged children live in areas of stable transmission for at least one STHs species (Brooker et al., 2006) and the greatest public health burden occurs in developing countries, particularly in sub-Saharan Africa (Bethony et al., 2006). In Kenya, 9.1 million people are at risk of STH infections and majority are children (Brooker et al., 1999). The burden of STHs in the former Nyanza province is at 68% (Riesel et al., 2017). Children in the areas experience the greatest morbidity which limit their school academic potential and physical development with the high-worm burden

stunting physical and mental maturation, thereby lacking the energy requirements needed for successful involvement in school activity. Individuals in Ukwala area are more prone to STHs and *S. typhi* infections (Decker, 2015). However, the prediction of the demographic traits such as age and gender on co-infection with STHs and *S. typhi* in Ukwala area is unknown.

Socio-economic factors such as level of education and occupation influence the prevalence of STHs (Mokua et al, 2015) where lower socio-economic status such as unemployment, low education, poor living environment, living in overcrowded spaces, and generally low family social status have been associated with high prevalence of STH infections (Östan et al., 2007). The prevalence and distribution patterns of STHs indicate that children from low-income families living in poor hygiene environments and in the rural areas have a high risk of infection (Gunawardena et al., 2008) with highest prevalence and intensity of infection observed in school-aged children where the boys have slightly higher infection rates than girls (Obala et al., 2013). For instance, the distribution of STHs exhibits an age-dependence with maximum worm burdens for *Ascaris* and *Trichuris* infections occurring in children approximately at 5-10 years of age, but with a subsequent decline among adults (Bundy et al., 1995). In contrast, hookworm infections occur in childhood, but the frequency and intensity commonly remain high in adulthood, even in elderly people (Bethony et al., 2006). The higher prevalence of hookworm infection in adulthood may be explained by the nature of the parasite-host relationship because unlike other STHs, hookworms secrete many bioactive polypeptides, which dampen down host immune responses (Hotez et al., 2003).

According to studies in western Kenya among preschool-age children (below five years), majority of the children were found to be infected by *T. trichiura* and *A. lumbricoides* with *A. lumbricoides* having higher intensity of 25% representing parasitic burden (Mokua et al., 2015; Obala et al., 2013). In children of ages between 5-9 years, the overall prevalence for the STHs are 2.5% lower as compared to those in younger children below five years (Riesel et al., 2017).

In adults, women of reproductive age and pregnant women are vulnerable to hookworm infection which is a major contributor to gastrointestinal blood loss, iron and energy deficiencies, protein and zinc deficiencies, malnutrition and anemia (Brooker et al., 2004). Approximately 44 million pregnancies are complicated by maternal hookworm infections with estimates at 30% of Kenyan, 41% of Nepalese, and 53% of Vietnamese women (Hotez et al., 2004); this places both mothers and children at higher risk of death during pregnancy and delivery (Bundy et al., 1995).

2.5 Co-infection with *Salmonella typhi* and soil-transmitted helminths

Co-infection complicates gastrointestinal infections (Griffiths et al., 2011). The co-infecting pathogens interact synergistically with each other where the presence of one pathogen enhances the abundance and virulence of the other (Cunin et al., 2003). These interactions maybe through the helminth direct modulation of the host immune response facilitating subsequent bacterial infection. For example, helminth infections appear to worsen human health by increasing disease severity in subsequent infections (Gendrel et al., 1984; Brooker & Utzinger, 2007) and downregulate immune response by impairing the host's innate immunity against bacterial infection such as infection by *S. typhi* (Su et al., 2014). More directly, STHs produce localized

ulceration and hemorrhage in the intestine that provides entry points for enteric bacteria such as *S. typhi* (Chan, 1997).

During co-infection, the host may down-regulate its response towards one parasite or pathogen in order to mount a potent immune response towards another co-infecting parasite or pathogen (Cox, 2001). The Th (T helper) cells play a key role in protection against helminthic and bacterial infections (Abruzzi & Fried, 2011). Accordingly, worm infections are controlled by Th2 responses yet the worms are effectively armed to evade it and on the other hand most bacterial infections are controlled by Th1 responses (Maizels & Yazdanbakhsh, 2003). In case of a co-infection, Th cell response is determined by whichever infection is most detrimental to host fitness (Cunin et al., 2003) and mounting a very strong response toward one infection may leave the host vulnerable to infection by another infection type (Fenton et al., 2008). In the case of helminthic infections, regulatory T cells that downregulate Th1 responses where responses to bacterial infection are likely to be suppressed by helminthic infection and may bring about the exacerbation of concurrent infections or failure to respond to vaccination (Abbas et al., 1996). In sum, helminth infection exacerbates concurrent bacterial infection by inducing Th2 immune response characterized by the interleukin (IL 4, IL-5, IL-10 and IL-13) and Treg response that impair the protective Th1 immunity against the bacteria (Maizels & Yazdanbakhsh, 2003).

Further helminth infection associated with increased susceptibility to subsequent infection has been attributed to the parasite disruption and alteration of the small intestinal metabolic profile

which enhance bacterial pathogenicity (Reynolds et al., 2017). For example, Reynolds et al. (2017) found that *Heligmosomoides polygyrus* promotes *Salmonella typhimurium* colonization of the small intestine in mice where chronic *H. polygyrus* infection alters metabolic profile of the small intestine. The metabolites are therefore altered, upgraded and suppressed hence enhancing *Salmonella* pathogenicity. *Salmonella typhi* can multiply in and adhere to some helminths such as schistosome worm which may be the vehicle through which the infectivity of *Salmonella* is enhanced and prolonged (Abruzzi & Fried, 2011). However, differences in frequencies of individuals with STHs as a function of *S. typhi* infection and the prediction of age and gender on the co-infection between *S. typhi* and STHs are undocumented.

2.6 Antimicrobial resistance by *S. typhi*

Resistance of pathogens to commonly used antibiotics is a growing global concern, which may be exacerbated under co-infection scenarios (Abruzzi & Fried, 2011). For instance, in a suppressed immune system, there may be reduced immune-mediated killing of pathogens which lead to high replication of pathogen hence increase in chances of resistance as in the case of co-infection by HIV and malaria (Van et al., 2008). The suppression of the immune response may also result in increase in symptomatic infections and thus concomitant increase in the use of drugs which will increase the selective pressure of the resistant strains of pathogens (Bijker & Sauerwein, 2012).

The emergence of antibiotic resistance has resulted to problems with the clinical treatment of typhoid fever with the development of multidrug-resistant (MDR) strain of *S. typhi* (Kariuki et al., 2004). Chloramphecical, ampicillin, and cotrimoxazole has been the drug of choice for the

treatment of typhoid fever. However, there has been the emergence of MDR of *S. typhi* to ampicillin and chloramphenicol has prompted the widespread use of fluoroquinolones, such as ciprofloxacin and ofloxacin as an alternatve drug of choice for the treatment of *S. typhi*. (Kariuki et al., 2004). There has been reduced susceptibility to fluoroquinolones by *S. typhi* and since, there has been a wide spread (Parry & Beeching, 2009). Therefore, the widespread of MDR and fluoroquinolone resistance by *S. typhi* has presented pharmaceutical and therapeutic clinical challenges.

In Africa, the widespread occurrence of antimicrobial-resistant strain has limited treatment options of *S. typhi* (Beeching & Parry, 2011). In Kenya, the trend in MDR *S. typhi* has been on the rise since 1997 (Kariuki et al., 2004) and problems are emerging with clinical treatment of typhoid fever antibiotics chloramphenicol, ampicillin, tetracycline and cotrimoxazole that have formed the mainstay antibiotics (Kariuki et al., 2010). For instance, resistance of *S. typhi* has been reported for ampicillin, tetracycline, streptomycin and cotrimoxazole (Kariuki et al., 2004). Ciprofloxacin remains the best option for treatment of *S. typhi* in areas where resistance is uncommon and has been used as the drug of choice and azithromycin as an alternative for treatment of resistant *S. typhi* (Parry & Beeching, 2009). However, the antimicrobial profile of *S. typhi* isolates in patients co-infected with *S. typhi* and STHs is unknown. There is a wide spread misuse of antibiotics and self-medication in regions like Nyanza (Owour et al., 2015). Increase in symptomatic infections may result to increased use of drugs which increases selective pressure of the resistant strain of pathogen (Bijker & Sauerwein, 2012). However, antimicrobial profile of *S. typhi* in co-infection scenario with STHs needs to be investigated.

2.7 Prevalence of parasitic infections in Siaya County

Morbidity complications associated with co-infection may be exacerbated in populations that are immunocompromised. In Kenya, western Kenya including counties of Homa Bay, Kisumu and Siaya have the highest prevalence of HIV-AIDS, TB, malaria, diarrhea and typhoid fever (Odhiambo et al., 2012). According to the preliminary report for the Kenya AIDS Indicator Survey (2012), the national prevalence among adults aged 15 years to 64 years in 2012 was 5.6% from 7.2% in 2007 and HIV prevalence among children aged 18 months to 14 years was 0.9% with the incidences commonly occurring in counties of Homa Bay, Kisumu and Siaya (Kenya AIDS Indicator Survey, 2012).

With regards to STHs in Western Kenya, the prevalence is at 68% (Riesel et al., 2010). The delays and staggering implementation of the deworming exercise in Kenya has seen the infection levels of STHs being high despite government guideline policy to manage STHs through mass anti-helminth drug administration (Andereck et al., 2014; Mwandawiro et al., 2013; Pullan et al., 2011).

Siaya County has been the focus of a number of studies under the Kenya Medical Research Institute/ Centre for Disease Control. According to the KNBS, the total number of reported infections in Siaya County is at 455,923 with new cases of GI infections at 5,266 and typhoid fever at 525 patients (Kenya National Bureau of Statistics, 2012). Population-based surveillance reports for estimating disease burden in rural area in Kenya have reported high disease burden of typhoid fever and intestinal parasitic infection in Siaya County (Breiman et al., 2012; Feikin et al., 2010, 2011). Individuals in Ukwala area are more prone to typhoid fever and STHs infection (Decker,

2015) and according to the Ukwala Sub-County Hospital health record (2016) data, the prevalence of *S. typhi* is estimated at 30%. In Ugenya Sub-County, Ukwala Sub-County Hospital is the main hospital that most individuals in the area seek medical services.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted in Ukwala Sub-County Hospital located at latitude $0^0$11'25.4"N, longitude $34^0$11'20.7"E, which lies to the east side Lake Victoria, Figure 1. The hospital is located in an area that is prone to *S. typhi* and STHs infections where (Decker, 2015).

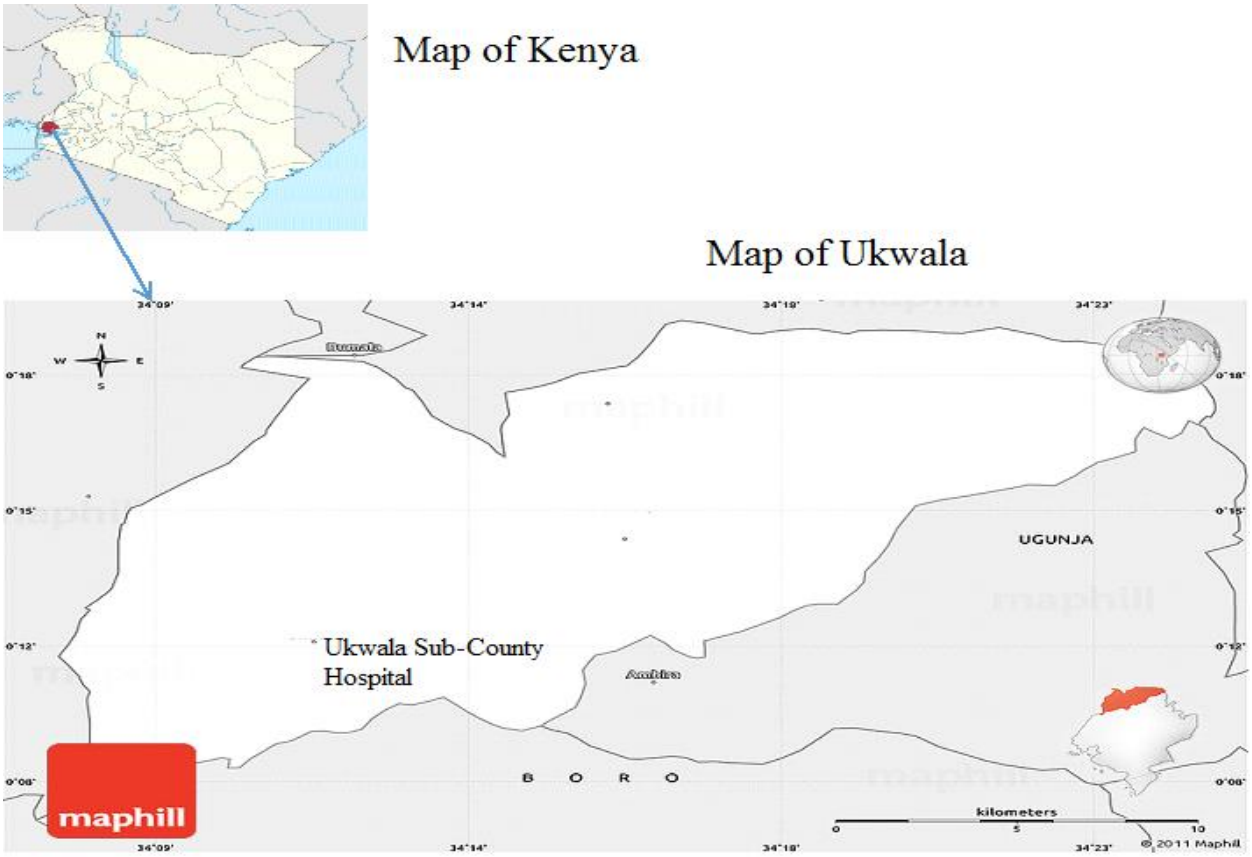

Figure 3.1.1. Map of Kenya showing the location of Ukwala, the catchment area for the study population

3.2 Study population

Ukwala Sub-County Hospital is located in Ugenya Sub-County. The 2009 Kenya census indicated Ukwala population stands at 49,802 with urban population in Ukwala town being 671 (Kenya National Bureau of Statistics, 2010). The study targeted individuals of all ages, both males and females; a consideration in the study objectives. The participants were grouped in two age categories, the school-going and the non-school going as the school going individuals were targeted for the mass drug deworming program by the Kenyan government (Mwandawiro et al., 2013) to capture all the individuals to avoid bias.

3.3 Study design

A cross-sectional study design was used to collect data on patients recommended for typhoid fever test who were attending Ukwala Sub-County Hospital.

3.4 Sample size determination

The formula by Daniel (1999) was used to determine the sample size:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where n = sample size,

Z = Z value, 1.96, at 95% confidence interval

P = expected prevalence of *S. typhi* is 0.3; (Ukwala Sub-County Hospital, 2016)

d = precision (in proportion of one; if 5%, d = 0.05)

Together, the values resulted in an estimated sample size of 325 as indicated below.

$$n = \frac{1.96^2 0.30(1 - 0.30)}{0.05^2}$$

n = 325

The sample size for the study is 325

According to Naing, Winn and Rusli (2006), it is appropriate to have a precision of 5% if the prevalence of the disease is between 10% and 90%.

3.5 Inclusion and exclusion criteria

Only residents of Ugenya Sub-County who were recommended for typhoid fever test in Ukwala Sub-County Hospital were eligible for the study. Individuals of every age and gender were included as participants. Individuals who did not consent to participate in the study were excluded, children whose parents/guardians did not give consent to participate were also excluded from the study. Individuals who had lived a period of less than two weeks in Ukwala area were not included in the study as the period was not adequate for identification of *S. typhi* which required at least two weeks to incubate.

3.6 Collection of stool samples

Stool samples were collected according to World Health Organization (1991) procedure. In brief, each patient recommended for typhoid fever test was provided with a clean, dry stool container

and the following instructions were followed: a plastic wrap was placed over the back ¾ of the toilet bowl, onto which the patient deposited stool. The specimen was picked with a paper plate and a small sample scooped into the container. The stool sample was taken to the clinician (a qualified laboratory clinician in-charge of Ukwala Sub-County Hospital) who labelled it with patient's number, date of collection; age and gender. In the hospital laboratory, the stool specimen underwent subsequent processing and parasitological analysis. Appendix VIII shows a flow chat for processing stool sample for parasitological and microbiological analysis.

3.7 Processing of stool specimen for the identification of STHs

Stool samples were examined for the presence of eggs and larvae of the STHs by direct smear, and negative results were confirmed by formalin-ether concentration technique as per the standards by Beaver et al. (1984). To carry out the direct smear procedure, two drops of saline were placed in the center of right and left half of the slide then 5g of stool was taken by a clean wooden stick and thoroughly mixed. One drop of iodine (Unilab Kenya Limited, Nairobi) was placed on one of the saline drops and mixed to prepare an iodine mount. This was then covered with a cover slide and mounted on the microscope for examination as summarized in Appendix IX. The negative results were confirmed by formalin ether concentration technique where approximately 1g of stool sample was emulsified in 2ml of 10% formalin (Unilab Kenya Limited, Nairobi) then filtered onto a double layer of gauze in a funnel. The stool was then washed through the gauze with 10% formalin into a 15ml tube. About 7 ml of the filtrate was mixed by hand with 3 ml of ethyl acetate (Unilab Kenya Limited, Nairobi) for 30seconds then centrifuged for 5 minutes at 2000revolutions per minute at 37^0 C. the supernatant was discarded, the sediments mixed well. A drop of sediment

was placed in a glass slide under a coverslip and examined under low power (x10 magnification) objective lens then high power (x40 magnification) objective lens for identification of eggs and larvae of STHs. Figure 3.7.1 summarizes the formalin ether concentration techniques for parasitological assay while Figure 3.7.2 shows identification of soil-transmitted helminths.

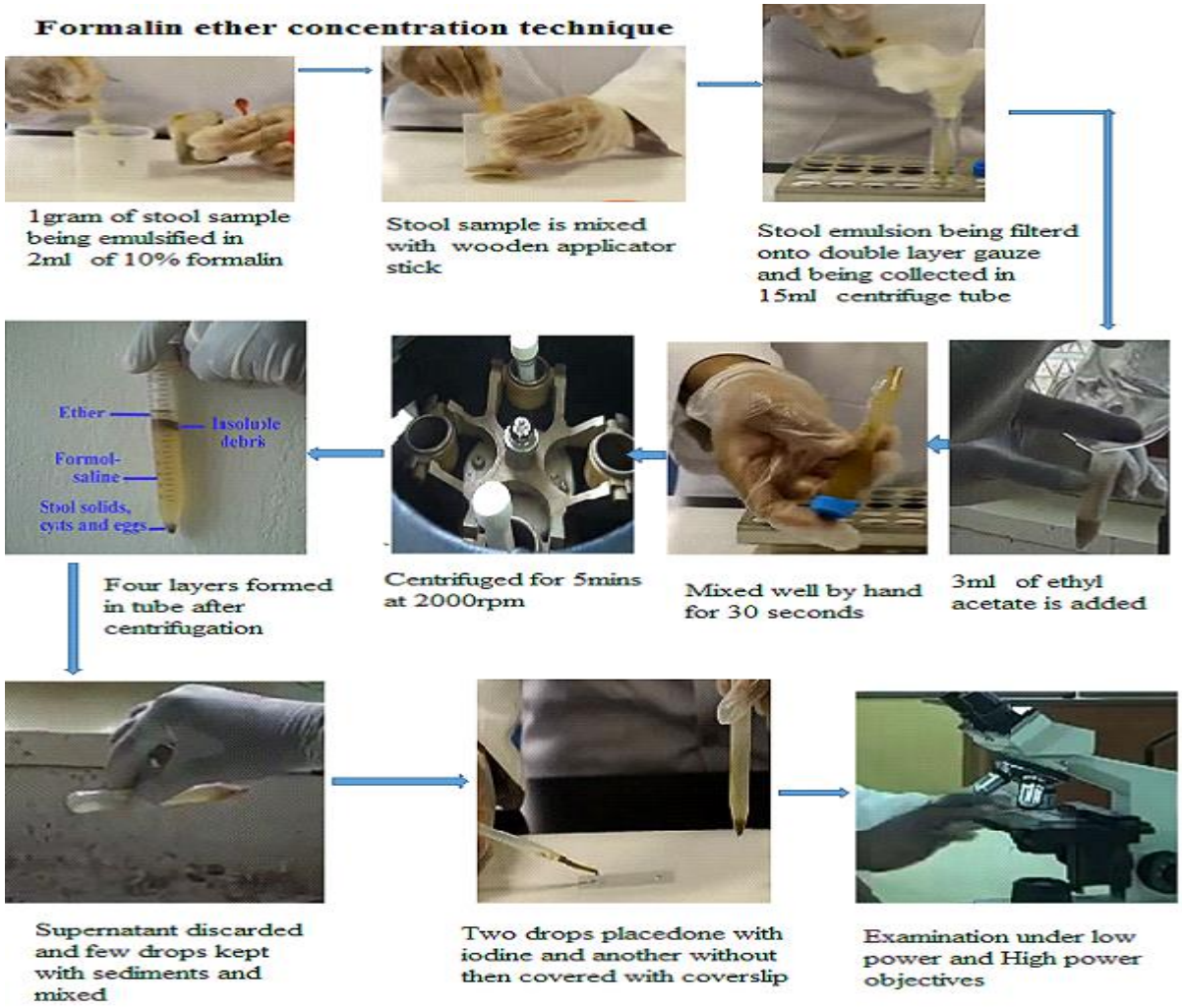

Figure 3.7.1. Formalin ether concentration technique for examining presence of eggs and larvae of STHs

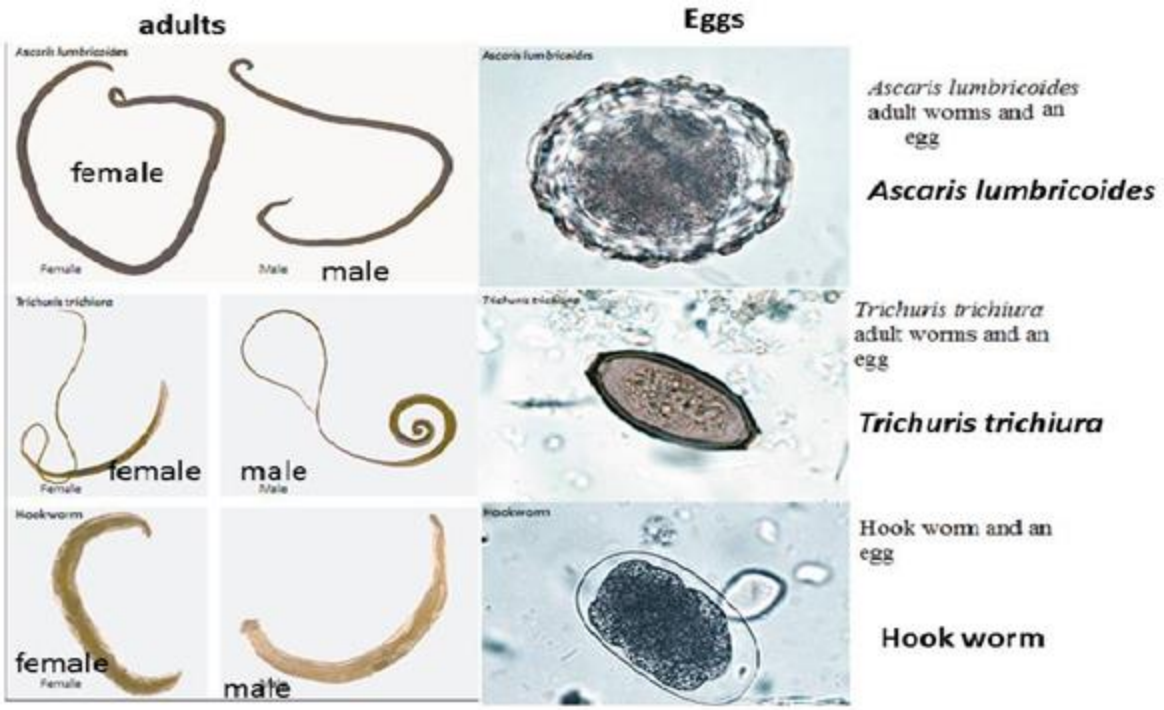

Figure 3.7.2. Identification key for STHs (Bethony et al., 2006)

3.8 Culture of *Salmonella typhi*

Salmonella typhi isolates were obtained from the stool samples confirmed for typhoid fever by the *S. typhi* Rapid Test Device (*Salmonella* Antigen Test; rapid visual immunoassay for the qualitative presumptive detection of *S. typhi* antigen in human stool specimen; the test has a high degree of sensitivity and specificity). Part of the stool sample was transferred using a cotton swab and inserted into a container of Carry-Blair transport medium (HiMedia Laboratories Private Limited Mumbai, India) then transferred to Siaya County Referral Hospital laboratory for culture.

A total of 19 stool specimen were successfully cultured, 10 from *S. typhi* infection alone and 9 from co-infection with STHs and *S. typhi*. Some specimens were rejected because as some cultures turned negative for *S. typhi*.

In Siaya County Referral Hospital Laboratory, the sample was added to 100 ml buffered peptone water (HiMedia Laboratories Private Limited Mumbai, India) and incubated at 42^0C for 24 hours. Thereafter the mixture was transferred into selective enrichment media (Selenite broth that contains additives that selectively permit *Salmonella* to grow while inhibiting the growth of other bacteria obtained from HiMedia Laboratories Private Limited Mumbai, India) and cultured at 37^0 C for 24 hours. The sample was then plated onto a selective agar media, Xylose-Lysine – Desoxycholate (XLD, from HiMedia Laboratories Private Limited Mumbai, India) agar inhibits growth of bacteria other than *Salmonella*), and incubated at 37^0 C for 24 hours. Five presumptive *Salmonella* colonies were then streaked from the agar plate and inoculated on nutrient agar and cultured at 37^0 C for 24 hours. Biochemical analysis was done by transferring 5 ml of 24 hours Tryptophane broth (HiMedia Laboratories Private Limited Mumbai, India) culture to a clean test tube and 0.3 ml Kovac's reagent (HiMedia Laboratories Private Limited Mumbai, India) was added. Negative test which is absence of deep red color at the surface of the broth confirmed *Salmonella*.

3.9 Antimicrobial sensitivity testing

The antimicrobial agents that were tested were ampicillin, tetracycline, ciprofloxacin and chloramphenicol which are commonly available drugs for treatment of typhoid fever (Parry & Beeching, 2009). The antimicrobial discs tested and their minimal inhibition concentration were 25µg ampicillin, 25µg tetracycline, 30µg ciprofloxacin and 30µg chloramphenicol (HiMedia Laboratories Private Limited Mumbai, India). Antimicrobial susceptibility tests were performed

on Mueller-Hinton agar (HiMedia Laboratories Private Limited Mumbai, India) by disc-diffusion method as per the standards by Bauer et al. (1966). Five isolated colonies of the same type were swabbed to pick up each one. The colonies were rubbed on side of the test tube containing 5 ml sterile saline. The suspension was then diluted to obtain turbidity equivalent to the 0.5 McFarland test standard where the diluted tube and the 0.5 McFarland test standard hold against a black-lined McFarland reference card to accurately rate the turbidity. A sterile swab was then dipped into the suspension and excess liquid squeezed on side of test tube. The entire surface of the Muller-Hinton agar plate was swabbed turning the plate 60^0 as the swabbing process was repeated to obtain an even inoculation. The lid was closed and let to set for 15 minutes before placing antibiotic disc on the agar by a pair of sterile forceps. The disc was lightly pressed down with a sterile swab to make contact with the agar surface then incubated at 37^0C. The plate was examined after 18 hours of incubation. The zones showing inhibition was measured (in mm) by holding a ruler over the back of the inverted plate. The inhibition values obtained were compared with those on the Disk Diffusion Chat to determine the susceptibility level to the antibiotics used based on criteria according to Clinical Laboratory Standard Institute (2015). For the antibiotic that stopped the bacteria from growing or killed the bacteria, there was circular areas around the disc where bacteria had not grown (zone of inhibition). In antimicrobial sensitivity tests, the size of the inhibition zone depends on how effective the test antibiotic is at stopping the growth of the bacterium. If the antibiotic was effective against *S. typhi*, no colonies grew where the concentration of the antibiotic was greater than or equals to the effective concentration (zone of inhibition). The zone of inhibition was used to estimate *S. typhi* sensitivity to the particular antibiotic. Plate 3 summarizes the disc diffusion susceptibility procedure for *S. typhi* antimicrobial sensitivity test.

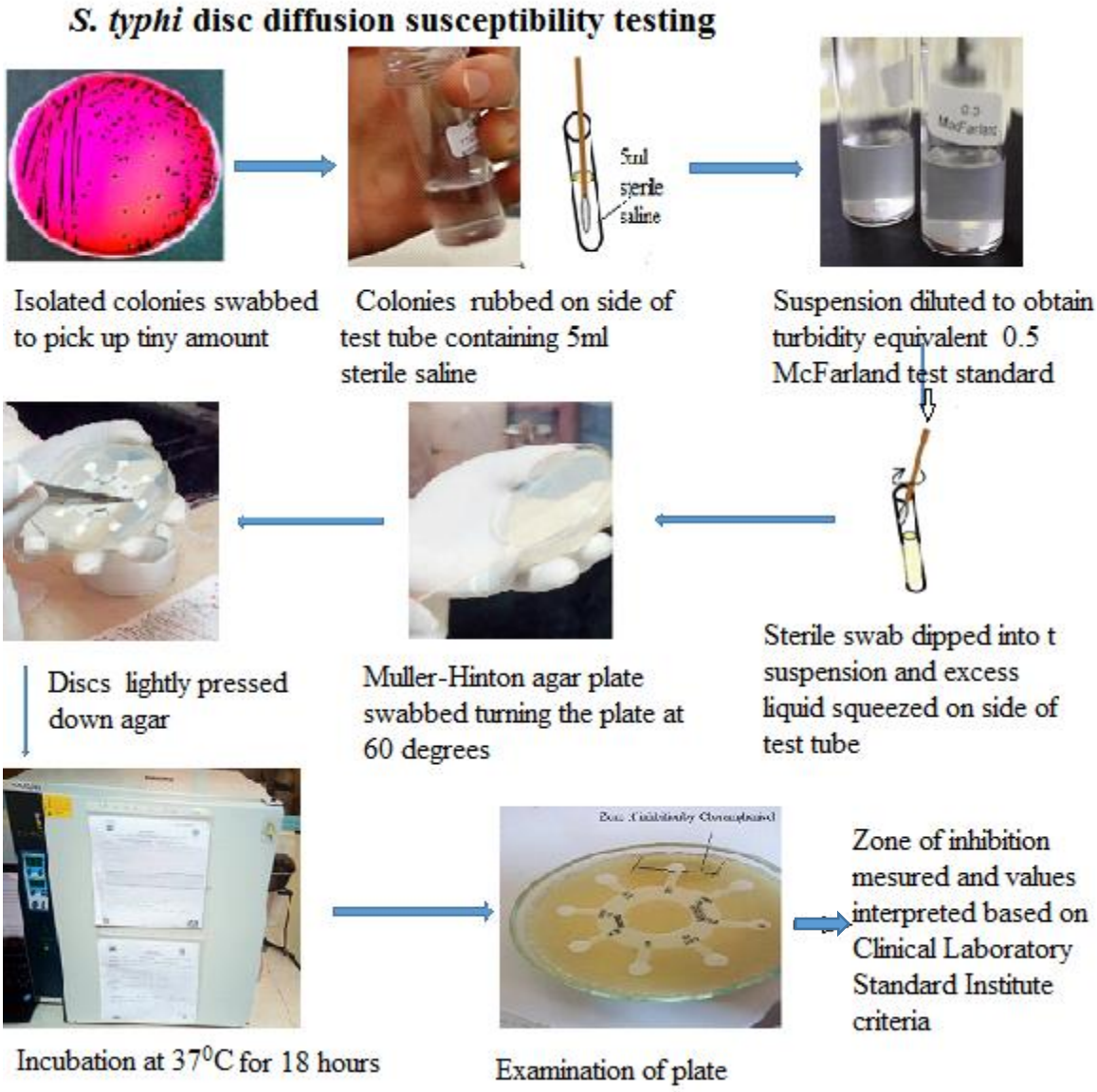

Figure 3.9.1. Disc diffusion susceptibility procedure for antimicrobial sensitivity test

3.10 Data analysis

Chi-square test of goodness-of-fit was used to determine the differences in frequencies of individuals with STHs among *S. typhi* positive and *S. typhi* negative individuals. The extent to which age and gender predicted co-infection between STH and *S. typhi* was determined using

logistic regression. Similarly, Chi-square test of goodness-of-fit was used to determine the differences in the antimicrobial profile of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs.

3.11 Ethical consideration

Ethical approval for the study was obtained from Maseno University Ethics Review Committee. Permission to conduct the study in the hospital was obtained from Siaya County Director of Health (Appendix III; Appendix IV), Ugenya Sub-County Deputy County Commissioner (Appendix V), Medical Superintendent Ukwala Sub-County Hospital (Appendix VI) and Medical Superintendent Siaya County Referral Hospital (Appendix VII). Written informed consent for all the respondents was sought before the study was conducted and for the minors, consent was provided by the parents/guardians. Participants were informed that there was no or minimal risks to participating in the study. They were also informed that refusing to participate will not affect the usual services they normally access at hospital. The data collected was coded with the demographic aspects needed being just sex and age and minimal collection of personal information was needed as there was no questionnaires needed and all information was stored in password secured files. In addition, before the study was conducted, the purpose and objectives of the study was carefully explained to the respondents by the lab technician and it was emphasized that samples collected from them would be treated with uttermost confidentiality. The potential bias in the study was the different socio-economic class of the study participants but the bias was controlled by random sampling of the individuals who were recommended for typhoid fever test.

CHAPTER FOUR

RESULTS

A total of 325 individuals aged 1 year and above were consented for the study. Table 4.1 summarizes demographic characteristics of study participants.

Table 4.1.0. Demographic information of study participants

Demographic variable	Number (Percentage of total)
Total individuals in the study	325 (100%)
Females	204 (62.8%)
Males	121 (37.2%)
Individuals aged 1 – 19 years	129 (39.7%)
Individuals aged 20 years and above	196 (60.3%)

4.1 Differences in frequencies of STHs in *Salmonella typhi* positive and negative individuals

The results showed no significant association between the geo-helminth and *S. typhi* infection (χ^2 = 0.348, P = 0.555, Table 4.1.1). However, a follow-up analysis for potential differences in frequency of individuals infected by each of the STHs among *S. typhi* positive and *S. typhi* negative individuals revealed that the frequency of individuals infected by each of the STHs, was higher among *S. typhi* negative than *S. typhi* positive individuals (Table 4.1.2).

Table 4.1.1. Frequency of individuals with STHs and those without STHs among *S. typhi* positive and *S. typhi* negative individuals attending Ukwala Sub-County Hospital, Kenya

STHs	*S. typhi* positive	*S. typhi* negative	Total
Positive	36(11.1%)	121(37.2%)	157(48.3%)
Negative	34(10.5%)	134(41.2%)	168(51.7%)
Total	70(21.5%)	255(78.5%)	325(100.0%)

Chi square test was used to determine an association between STHs and *S. typhi* infections; χ^2 = 0.348, P = 0.555

Table 4.1.2. Frequency of individuals with STHs among *S. typhi* positive and *S. typhi* negative individuals attending Ukwala Sub-County Hospital, Kenya

STHs	*S. typhi* Positive	*S. typhi* Negative	χ^2 Value	P- Value
A. lumbricoides	17(5.2%)	53(16.3%)	18.514	0.001
T. trichiura	8(2.5%)	25(7.7%)	8.758	0.003
N. americanus	11(3.4%)	43(13.2%)	18.963	0.001
All STHs	36(11.1%)	121(37.2%)	46.019	0.001

4.2 Demographic correlates of co-infection between STHs and *Salmonella typhi*

Neither age nor gender significantly predicted occurrence of co-infection between *S. typhi* and STHs (logistic regression model, $\chi 2$ = 2.804, P > 0.05, Table 4.2.1). However, males were 0.971 times likely to be co-infected than females and individuals aged 20 years and above were 0.526 times more likely to be co-infected than individuals aged 1-19 years. Details of logistic regression are contained in Table 4.2.2.

Table 4.2.1 Demographic information on co-infection between STHs and *S. typhi* among individuals attending Ukwala Sub-County Hospital, Kenya

Demographic variable	*S. typhi*	STHs	Co-infection
Males	9(2.8%)	41(12.6%)	12(3.7%)
Females	25(7.7%)	80(24.6%)	24(7.4%)
Individuals aged 1 – 19 years	9(2.8%)	37(11.4%)	16(4.9%)
Individuals aged 20 years and above	25(7.7%)	84(25.8%)	20(6.2%)

Logistic regression used to whether age and gender predict co-infection between *S. typhi* and STHs (logistic regression model, $\chi 2 = 2.804$, $P > 0.05$).

Table 4.2.2 Logistic regression variables; age and gender prediction on co-infection between STHs and *S. typhi* attending Ukwala Sub-County Hospital, Kenya

	B	Wald	Degree of freedom	Significance	Exp(B)
Gender	-0.030	0.006	1	0.940	0.971
Age	-0.643	2.840	1	0.526	0.526
Constant	-1.044	9.936	1	0.352	0.352

Key: '**B**' values are the logistic coefficients that can be used to create a predictive equation
Wald statistic provide an index of the significance of each predictor in the equation.
The Exp(B) presents the extent to which raising the corresponding measure by one unit influences the odds ratio. (Interpreted in terms of the change in odds).

4.3 Antimicrobial profile of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs

To assess differences in antimicrobial profiles of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs, Chi-square test of goodness-of-

fit was used. The study found no difference in antimicrobial profiles of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs (Table 4.3.1; Table 4.3.2).

Table 4.3.1 Antimicrobial resistance profile of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital, Kenya

Antibiotic Resistance	*S. typhi*	Co-infection	χ^2 Value	P- Value
Ampicillin	9	7	0.250	0.617
Tetracycline	7	5	0.333	0.567
Ciprofloxacin	1	2	0.333	0.567
Chloramphenicol	1	1	0.001	1.000

Table 4.3.2 Antimicrobial sensitivity profile of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital, Kenya

Antibiotic Not Resistant	*S. typhi*	Co-infection	χ^2 Value	P- Value
Ampicillin	1	2	0.333	0.567
Tetracycline	3	4	0.143	0.705
Ciprofloxacin	9	7	0.250	0.617
Chloramphenicol	9	8	0.059	0.808

CHAPTER FIVE

DISCUSSION

5.1 Association between soil-transmitted helminths and *S. typhi* infection

The present study found no association between STHs and *S. typhi* co-infection. This was rather surprising, since previous reports suggests that helminthic infections downregulate host immunity hence making host more susceptible to other infections (Gendrel et al., 1984; Brooker & Utzinger, 2007; Su et al., 2014). However, a number of human studies have reported similar findings. First, Dowling et al. (2002) found no association in co-occurrence of *Salmonella* infection with intestinal helminths; *A. lumbricoides* and *Strongyloides stercoralis* in HIV positive individuals. Second, in a study in Ethiopia involving out-patients presenting with diarrhea and other gastrointestinal complications at local health centers, Eguale et al. (2015) found no association between *Salmonella* infection with co-occurring parasitic infections. The absence of immunomodulatory effects of helminths is not only seen when they co-occur with *S. typhi* but also with other bacteria. For example, Chatterjee et al. (2014) also found that despite the immunomodulatory effects of helminths, the co-morbid STH infection, did not increase the incidence of active pulmonary TB.

In contrast to this study, Abruzzi and Fried (2011) reported that schistosome infection suppresses immunity thus facilitates infection by *S. typhi*. Su et al. (2014) also found that co-infection with *Heligmosomoides polygyrus* impairs the host's innate immunity against *S. typhimurium* and enhanced susceptibility to *Salmonella* through a mechanism involving intestinal tissue injury. Chan (1997) found that STHs feeding produces localized ulceration and hemorrhage in the intestine that provides entry points for enteric bacteria such as *S. typhi*. The absence of association

between *S. typhi* and STHs co-infection reported in the current study may possibly suggest that perhaps STHs severity was too low to cause severe ulceration therefore availing few entry points for *S. typhi* bacteria. Taken together, these studies suggests that the interaction between helminths and bacteria differ depending on host, bacteria and helminth species.

Other studies have pointed to the chronicity of infection having an effect on the subsequent infection (Zaiss et al., 2015; Reynolds et al., 2017). Erb et al. (2002) found that one week of co-infection of mice with *Nippostrongylus brasiliensis* and *Mycobacterium bovis*, had no effect on the bacteria bacillary load from the lungs of the mice but after four weeks the *M. bovis* bacillary load increased significantly. A study by Reynolds et al. (2017) reported that chronic intestinal infection by *H. polygyrus*, altered the metabolic profiles of the small intestine enhancing *Salmonella* colonization hence subsequent pathogenicity in the small intestine with greatest impact of the co-infection at sites proximal to *H. polygyrus* colonization. In addition , Zaiss et al. (2015) study reported that chronic infection with murine *H. polygyrus* altered the intestinal habitat allowing for an increased short chain fatty acid production altering the *Salmonella* microbiota resulting to changes to the mucosal environment. The current study design did not allow for collection of data on how long the participants had had helminthic infections, however it is possible that the participants may not have had continuous STHs infection since the area had been targeted by the Kenyan government campaigns on the mass administration of anti-helminthic drugs over the study period and previous to the data collection (Mwandawiro et al., 2013). Therefore, it is likely that the immunomodulation effect of the helminths that the study expected did not occur. In addition, majority of the study population was aged 20 years and above, hence less likely to be infected with STHs (Gunawardena et al., 2008) implying that infection severity of the STHs among

the current study participants was low. Alternatively, the varied ages of study participants and thus of different susceptibility to STHs and *S. typhi* infections may have obscured any associations between the two pathogen categories.

5.2 Demographic correlates of co-infection between STHs and *Salmonella typhi*

The present study showed that age and gender did not predict co-infection between STHs and *S. typhi*. The results are consistent with those of Motazedian et al. (2015) who found no association between parasitic infections (*Hymenolepis nana, Giardia lamblia* and *Entamoeba histolytica*) and *S. typhi* in different age groups among food-handlers in Iran. Similarly, Eguale et al. (2015) found that there was no difference in co-infection with *S. typhimurium* and gastrointestinal parasites (*E. histolytica*, *G. lamblia*, *H. nana* and *S. stercoralis*) between the gender as well as the age groups among patients attending health centers in Ethiopia. In contrast, Abera et al. (2016) found that gender predicted co-infection between *S. typhi* and intestinal parasites (hookworm and *G. lamblia*) among food handlers in Ethiopia. Results from the present study suggest that no likelihood of either gender being co-infected with STHs and *S. typhi*. This contrasts the findings by Abera et al. (2016) who found that male food handlers in Ethiopia were more concurrently infected with *S. typhi* and intestinal parasites (hookworm, *A. lumbricoides*, *T. trichiura* and *G. lamblia*). Likewise, Gunawardena et al. (2008) found that male school-aged children had slightly higher infection rates with STHs as compared to the females. Also, Damen et al. (2015) found that females were more concurrently infected with *Salmonella* and intestinal parasites (*E. histolytica*, *G. lamblia*, *A. lumbricoides*, *T. trichiura*, hookworm, *S. mansoni* and *H. nana*). Inconsistency in results of studies on co-infection and demographic variables such as age and gender could be attributed to

the heterogeneity in ages of study participants as well as due to low burden of both STHs and *S. typhi* infections in the study population and also to potential differential exposure to risk factors between the sexes and the age groups. For instance, the participants are from rural area who are perhaps equally likely to be exposed to predisposing factors such as; going to the river barefooted to fetch water under unhygienic conditions, farming and handling of farm produce without protective gear (Butler et al., 1990; Agwu et al., 2008; Mukhopadhyay et al., 2016).

5.3 Antimicrobial profile of *Salmonella typhi* isolates

The present study showed that there was no difference in antimicrobial resistance profiles of *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs. The results from the current study are inconsistent with findings from Eguale et al. (2015) who found an association in the antimicrobial resistance patterns of *S. typhimurium* from patients with diarrhea and other gastrointestinal parasites (*E. histolytica*, *G. lamblia*, *H. nana* and *S. stercoralis*) where the resistance of *S. typhimurium* to antibiotic was increased. Similarly, Zhu, et al. (2016) found difference in the antimicrobial resistance pattern of *Mycoplasma hominis* in single infection and co-infection with *Ureaplasma urealyticum* where in the case of co-infection, the resistance of *M. hominis* to antibiotic was increased. The inconsistency in the results in the present study with other studies may be attributed to the individuals living in the endemic area for the bacterial infection. For example, Sztein et al. (2014) found that individuals in endemic areas for enteric fevers rarely experience life threatening complications caused by diseases such as typhoid fever due to the development of the naturally acquired protection. Co-infection may weaken natural immunity in ways that might compromise efficacy of drugs in use as reported by

Bijker and Sauerwein (2012) on malaria infection, the same possibility can be assumed for the case of co-infection between STHs and *S. typhi*. Durão et al. (2016) reported that helminths' downregulation of immune response may influence the spectrum of antibiotics in use in a way that bacteria may either become more resistant or more sensitive to a given rage of antibiotics. The absence of difference in the antimicrobial resistance profiles of *S. typhi* isolates from individuals infected with *S. typhi* alone and those co-infected with *S. typhi* and STHs may be attributed to suspected low severity of the helminthic infection in the study population. Therefore, the predominant factors which may have affected the results of the current study is perhaps the low burden of typhoid fever in Ukwala Sub-County Hospital.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Findings from the study showed that there was no association between STHs and *S. typhi* infections suggesting that STHs did not facilitate nor exacerbate *S. typhi* infection. Neither age nor gender predicted occurrence of co-infection between *S. typhi* and STHs. With regards to antimicrobial resistance patterns as a function of co-infection, there was no difference in resistance patterns by *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs.

6.2 Conclusions

i. There were no differences in frequency of STHs infections between individuals who were positive and those who were negative for *S. typhi* infection.

ii. Age and gender did not predict the occurrence of co-infection between *S. typhi* and STHs in Ukwala Sub-County Hospital.

iii. There were no differences in resistance patterns by *S. typhi* isolates from individuals infected with *S. typhi* alone and individuals co-infected with *S. typhi* and STHs in Ukwala Sub-County Hospital.

6.3 Recommendations

6.3.1 Recommendations from current study

i. The frequency of individuals infected by each of the STHs was higher among *S. typhi* negative than *S. typhi* positive individuals, suggests that individuals recommended for *S. typhi* testing should also be tested for STHs.

ii. The marginal likelihood of males to have concurrent infections between STHs and *S. typhi,* suggests that all males presenting with clinical symptoms of *S. typhi* should be tested for STHs.

iii. The drugs of choice for the treatment of *S. typhi* as a single infection should be used by the clinicians in the study area in the treatment of individuals co-infected with *S. typhi* and STHs.

6.3.2 Recommendations for future studies

i. The observation that a disproportionate number of individuals who were negative for *S. typhi* were infected by STHs, raises the question of whether *S. typhi* immunologically antagonizes geo-helminthic infections. It would be important to investigate the immunogenicity of *S. typhi* with regards to those STHs.

ii. Any gender differences in the occurrence in co-infection may become apparent among subjects of comparable age. Future studies could focus on individuals of the comparable age groups in investigating the duration of co-infection and measuring the burden of the co-infection between *S. typhi* and STHs.

iii. The impact of co-infection should be taken into account when designing and prescribing antimicrobial drug treatments. Research needs to be done on the underlying mechanism involved in the antimicrobial resistance patterns in co-infection between *S. typhi* and STHs.

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APPENDICES

Appendix I Consent to participate in the study

Introduction

I am Wilfred Ouma Otambo a postgraduate student at Maseno University, School of Physical and Biological Sciences in Zoology Department. My supervisors are Dr. Patrick Onyango and Dr. Cyrus Ayieko both of Maseno University, Zoology Department. My study is on co-infection and antimicrobial profiles of *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital, Kenya. I am requesting you to be part of the study as a sample donor.

Aim of the study

The study aims to investigate co-infection and antimicrobial profiles of *S. typhi* and STHs in individuals attending Ukwala Sub-County Hospital, Kenya. It is envisioned that results of the study will inform clinical management of co-infections between *S. typhi* and STHs and help shape policy on management of *S. typhi* and infections caused by STHs.

Requirements

In case you accept to be a study participant, you will be provided with stool container where the stool samples will be collected based on instructions from the clinician. The clinician will then write your number, date of collection, time you passed the stool, your gender and age on the side of the container. In the hospital laboratory, the stool specimen will undergo subsequent processing, bacterial and parasitological analysis. Unauthorized test for the study will not be carried out on the stool samples. Three hundred and twenty five study participants will be required for the study.

Benefits

There are no direct benefit to be given to the study participants but the findings of the study will help shape policy on management of *S. typhi* and infections caused by STHs and inform the development of therapeutics to manage such infections.

Risks

There may be contamination during stool collection but this will be taken care of by the clinician on strict supervision during stool collection.

Confidentiality

All the samples will be treated with utmost confidentiality i.e. the study participants' stool containers will be labeled with unique identifiers instead of the participant's names.

Study participation

You have the right to decline in the participation of the study.

Questions and complains

Any questions and complains to the study will be addressed to Wilfred Ouma Otambo through the contact +254728583180 or an email at oumaotambo@gmail.com. In case of any questions with regards to your right you may contact Maseno University Ethics review secretariat on 057 351221 or mail them at P.O.BOX Private Bag, Maseno or email at muercsecretariant@maseno.ac.ke.

Consent statement:

I have read the above statements and agree to participate in this experiment under the terms outlined above. I understand that if any questions or concerns regarding this project I can contact the investigator Wilfred Otambo or the Maseno University Ethics Review secretariat. (The above statements will be recited and then taped as proof of consent).

You have been given copy of this consent form to keep.

Participant signature: _____ Date: _____

OR

Parent/legal guardian's signature: _____ Date: _____

Principal investigator: _____ Date: _____

Maseno University Ethics Review Committee: _____Date: _____

Informed consent in Luo

Yie donjo e nonro

Yangruok

An Wilfred Ouma Otambo, japuonjre e Mbalariany mar Maseno. Watiyo kanyakla gi Daktari Patrick Onyango kod Daktari Cyrus Ayieko mag Mbalariany ma Maseno. A da timo nonro e twuche ariyo machando jotuenche madhi hospital ma Ukwala Sub-County. Akwayi mondo ibedie achiel kuom joma bobetie enonrowani

Tiend nonro

Tiend nonroni en mondo wanon touché mankuom dhano mikelo gi kudini makelo ich kach gi makelo tuo mar typhoid to gi kaka yath tiyo gi kudinino makelo tuo mar typhoid e del kuom jotuo madhi hosipital mar Ukwala. Osene ni duoko mag nonroni bokonyo jo hosptande kuom gengo' touché mag ich gi mi jothieth rieko matut mar geng'o tuo mag ich gi typhoid.

Chenro

Kapo ni iyie mondo idonji e nonro, ibo miyi gir keto ooko, ae to ookoni ibogolo kalure gi kaka ibokoni gi jathieth. Jathieth bondiko nambi, saa migologo ookoni iki gi kata kain dichuo kata nyako e bath gir keto ooko. Ei kar pim mar hospital ma Ukwala, ooko ibonon. Onge pim maok ondik e nonroni mibiro timni. Jii mia dek gi piero ariyo ga bich ibiro timgo nonro.

Chiwo mar nonro

Onge chiwo mibiro mi joma odonjo e nonro, mak mana ni duoko mar nonro ibiro tigo e medo lony mar thieth gi konyo kuom loso yath mag tuochegi mek ich gi typhoid.

Hinyruok manyalo betie

Nyalo bedie gi kikruok mar chilo mokdwa gi ooko to mano jathieth bo nyiso jii maber kaka ooko ibiro gol maber mondo kik chid.

Pando weche

Ooko mogol ibiro pand nying jononro manogolo ooko to kata gik golo ooko ibomana migi nying magalala mondo jonro kik ng'egi.

Joma bodonjo e nonro

Donjo en hero mar ng'ato.

Penjo gi yuagrok

Penjo mora mora gi yuagruok ei nonroni inyalo neno Wilfred Ouma Otambo e namba simu 0728583180 kata e mbui oumaotambo@gmail.com. Penjo kata yuak malure gi ratiro mari inyalo gocho ni jochiu ratiro ma Mbalariany ma Maseno e namba simu 057351221 kata indik e sanduk mar posta Private Bag, Maseno kata e mbui muercsecretariant@maseno.ac.ke.

Ogiri miti mar donjo e nonro

Asesomo gik moko duto mondik to ayie donjo e nondro madhi timre kalure gi chike mantie. Ang'eyo ni kapenjo kata ywak mora mora manyalo betie anyalo chopo ne Wilfred Otambo e number simu kata joma chiwo oboke mar timo nonro e Mbalariany ma Maseno (weche man malo ibosom matek b mak kaka ranyisi ni idonjo e nonroni adier).

Omiyi oboke mar winjruok mondo ikan.

Sei mari: _____ **Tarik: _____**

KATA

Sei mar janyuol/Jarit Moyangi_____ **Tarik: _____**

Wuon Nonro: _____ **Tarik: _____**

Maseno University Ethics Review Committee: _____Tarik: _____

Appendix II Ethical Approval Letter

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 18th January, 2017

TO: Wilfred Ouma Otambo
PG/MSc/00128/2014
Department of Zoology
School of Physical and Biological Sciences
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/0338/16

RE: Co-Infection and Antimicrobial Profile of *Salmonella typhi* and Soil Transmitted Helminthes in individuals attending Ukwala Sub-County Hospital, Kenya. Proposal Reference Number: MSU/DRPI/MUERC/00338/16

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 18th day of January, 2017 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 17th January, 2018. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 18th December, 2017.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 18th December, 2017.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

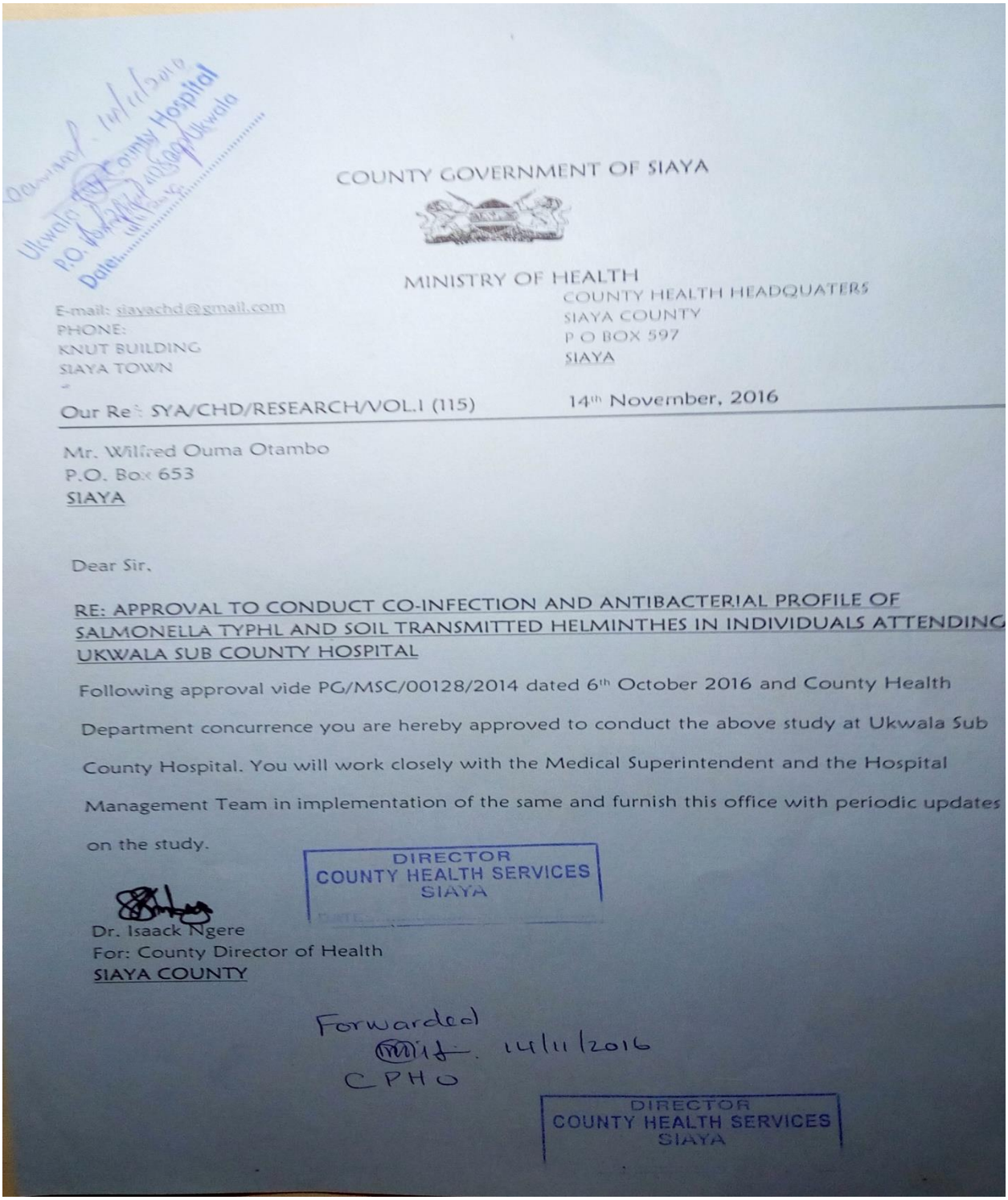
DIRECTORATE OF RESEARCH PUBLICATION & CONSULTANCIES 18 JAN 2017 MASENO UNIVERSITY

Yours faithfully,

Dr. Bonuke Anyona,
Secretary,
Maseno University Ethics Review Committee.

Cc: Chairman,
Maseno University Ethics Review Committee.

Appendix III Authorization from County Director of Health, Siaya



COUNTY GOVERNMENT OF SIAYA

MINISTRY OF HEALTH

COUNTY HEALTH HEADQUATERS
SIAYA COUNTY
P O BOX 597
SIAYA

E-mail: siayachd@gmail.com
PHONE:
KNUT BUILDING
SIAYA TOWN

Our Ref: SYA/CHD/RESEARCH/VOL.I (115) 14th November, 2016

Mr. Wilfred Ouma Otambo
P.O. Box 653
SIAYA

Dear Sir,

RE: APPROVAL TO CONDUCT CO-INFECTION AND ANTIBACTERIAL PROFILE OF SALMONELLA TYPHI AND SOIL TRANSMITTED HELMINTHES IN INDIVIDUALS ATTENDING UKWALA SUB COUNTY HOSPITAL

Following approval vide PG/MSC/00128/2014 dated 6th October 2016 and County Health Department concurrence you are hereby approved to conduct the above study at Ukwala Sub County Hospital. You will work closely with the Medical Superintendent and the Hospital Management Team in implementation of the same and furnish this office with periodic updates on the study.

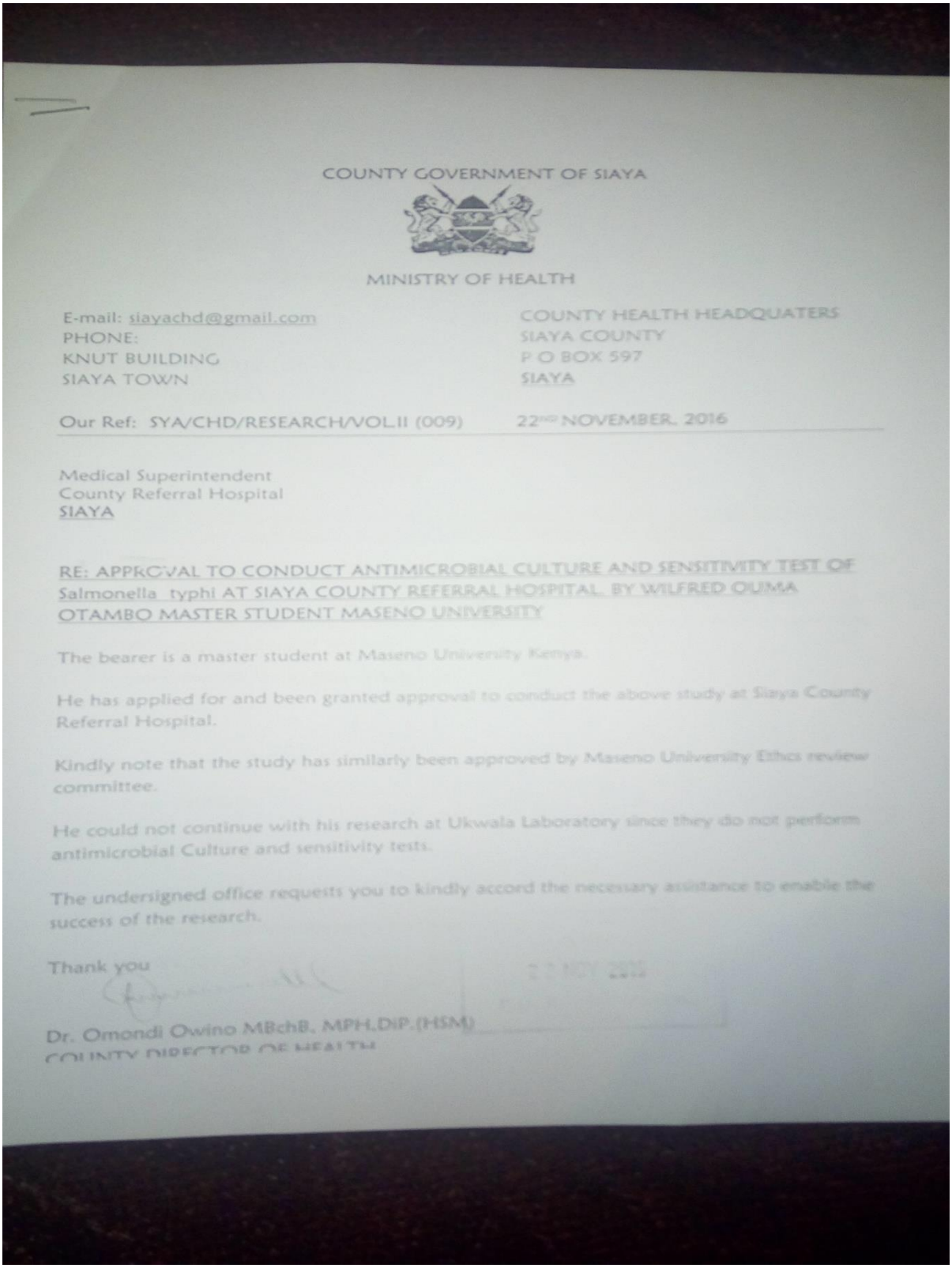
DIRECTOR
COUNTY HEALTH SERVICES
SIAYA

Dr. Isaack Ngere
For: County Director of Health
SIAYA COUNTY

Forwarded
14/11/2016
CPHO

DIRECTOR
COUNTY HEALTH SERVICES
SIAYA

Appendix IV Authourization from County Director of Health, Siaya



COUNTY GOVERNMENT OF SIAYA

MINISTRY OF HEALTH

E-mail: siayachd@gmail.com
PHONE:
KNUT BUILDING
SIAYA TOWN

COUNTY HEALTH HEADQUATERS
SIAYA COUNTY
P O BOX 597
SIAYA

Our Ref: SYA/CHD/RESEARCH/VOL.II (009) 22ND NOVEMBER, 2016

Medical Superintendent
County Referral Hospital
SIAYA

RE: APPROVAL TO CONDUCT ANTIMICROBIAL CULTURE AND SENSITIVITY TEST OF Salmonella typhi AT SIAYA COUNTY REFERRAL HOSPITAL BY WILFRED OUMA OTAMBO MASTER STUDENT MASENO UNIVERSITY

The bearer is a master student at Maseno University Kenya.

He has applied for and been granted approval to conduct the above study at Siaya County Referral Hospital.

Kindly note that the study has similarly been approved by Maseno University Ethics review committee.

He could not continue with his research at Ukwala Laboratory since they do not perform antimicrobial Culture and sensitivity tests.

The undersigned office requests you to kindly accord the necessary assistance to enable the success of the research.

Thank you

Dr. Omondi Owino MBchB, MPH,DiP.(HSM)
COUNTY DIRECTOR OF HEALTH

Appendix V Authorization from Sub-County Commissioner, Ugenya

Received. 14/11/2016
Medical Supt.
Ukwala Sub-County Hospital
P.O. Box 247 - 40600, Ukwala
Date: Ref: Lab.

OFFICE OF THE PRESIDENT
MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT

The Deputy County Commissioner
Ugenya Sub-County
P.O Box 260,
UKWALA

Email:dcugenya@yahoo.com
When replying please quote:

REF NO: RES. 21/1/VOL.1/78
and date

14th November,2016

MOH
UKWALA SUB COUNTY HOSPITAL

RE:RESEARCH AUTHORIZATION - WILFRED OUMA OTAMBO

The above named researcher who is a student of maseno University school of physical and Biological Sciences has been authorized to undertake research on "**Co-infection and Antimicrobial profile of salmonella typhi and soil-Transmission Helminthes in Individuals Attending Ukwala Sub County Hospital, Kenya**" for a period of three months with effect from 15th November, 2017.

Kindly accord him all the necessary assistance.

DEPUTY COUNTY COMMISSIONER
UGENYA SUB-COUNTY

BENSON M. KARANI,
FOR: DEPUTY COUNTY COMMISSIONER
UGENYA SUB-COUNTY.

Cc.
County commissioner
Siaya County.

Appendix VI Authorization from Ukwala Sub-County Hospital

REPUBLIC OF KENYA

MINISTRY OF HEALTH
UKWALA SUB-COUNTY HOSPITAL

UKWALA SCH
P.O BOX 247
UKWALA
14/11/2016

E-mail: ukwalahmt@gmail.com
Our Ref: UKW/MED-SUP./APPROVAL/VOL.1 (1)

- **Wilfred Ouma Otambo**

Dear Sir,

APPROVAL TO CONDUCT CO-INFECTION AND ANTIBACTERIAL PROFILE OF SALMONELLA TYPHI AND SOIL TRANSMITTED HELMINTHES IN INDIVIDUALS ATTENDING UKWALA SUB-COUNTY HOSPITAL.

Following approval vide SYA/CHD/RESEARCH/VOL.1 (115) of 14th November 2016 and county health department concurrence, you are hereby approved to conduct the above study at Ukwala Sub-County Hospital for a period of 3 months

Ukwala Sub-County Hospital
P.O. Box 247 - 40600, Ukwala

DR. OLOO FELIX
Medical Superintendent
Ukwala Sub-County Hospital

Cc - Laboratory i/c – Ukwala Sub-County Hospital.

Appendix VII Authorization from Siaya County Referral Hospital

MINISTRY OF HEALTH

Email: siayacountyRh@gmail.com
Telephone: Siaya 0717197349
When replying please quote
Ref Per:25528313

SIAYA COUNTY REFERRAL HOSPITAL,
P .O. BOX 144,
SIAYA.
23RD NOVEMBER, 2016

WILFRED OUMA OTAMBO
MASENO UNIVERSITY

RE: APPROVAL TO CONDUCT ANTIMICROBIAL CULTURE AND SENSITIVITY TEST OF *SALMONELLA TYHI* AT SIAYA COUNTY REFERRAL HOSPITAL

The bearer of this letter Mr.Wilfred Ouma Otambo has been granted the permission to do a study at the SCRH Laboratory. Vide approval from the County Director of Health's office **Ref:SYA/CDH/Research/VOL II (009)** of 22nd November,2016.

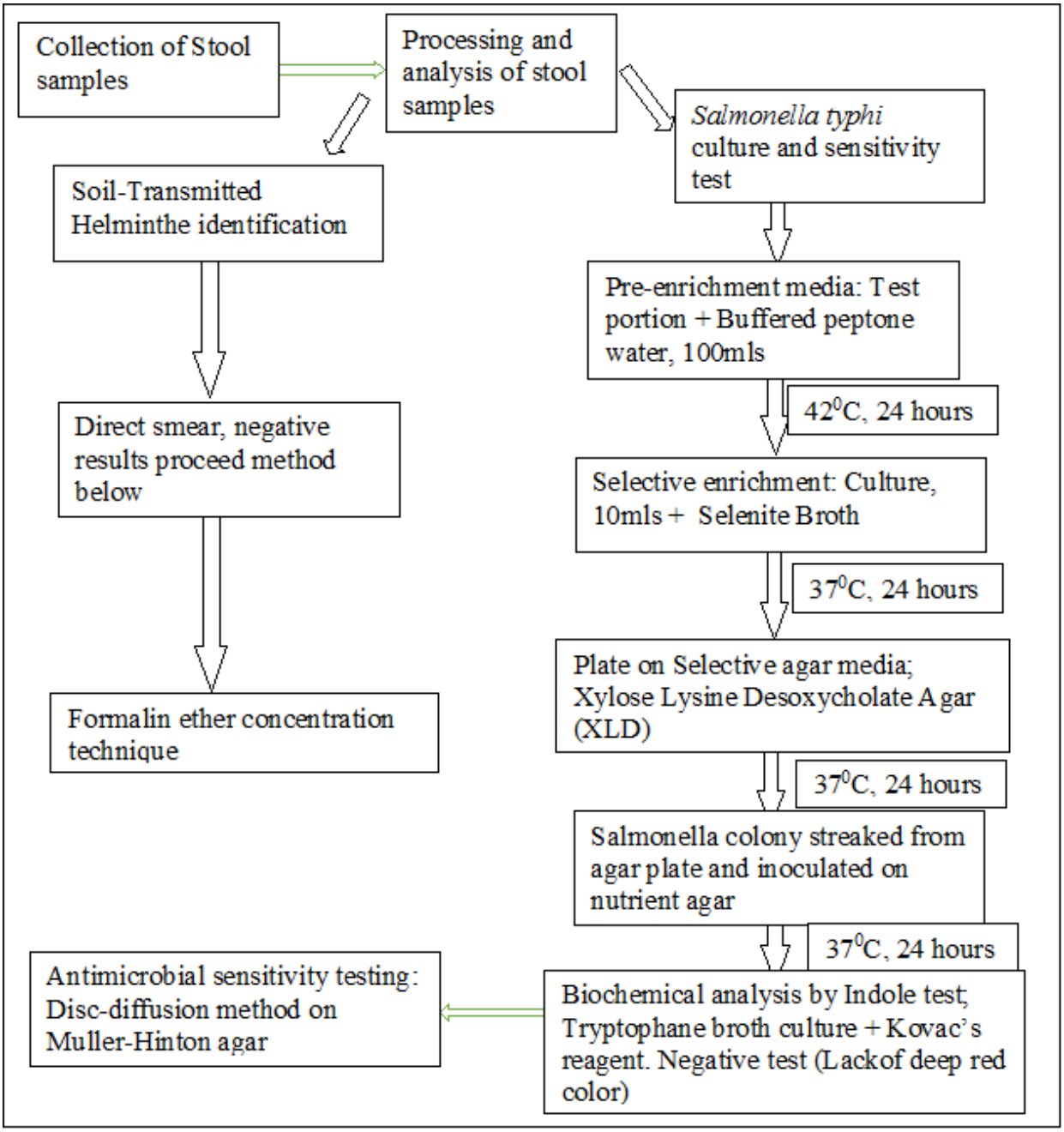
Concerned departments to give him the necessary support.

MEDICAL SUPERITENDANT
SIAYA COUNTY REFERRAL HOSPITAL
P. O. BOX 144 - 40600
SIAYA

ANDREW HONGO
SENIOR HEALTH ADMINISTRATIVE OFFICER
FOR: MEDICAL SUPERINTENDENT
SIAYA COUNTY REFERRAL HOSPITAL

CC.
Laboratory Incharge
Dr. Calleb Otieno, IRC Committee.

Appendix VIII Flow chart for processing of stool sample



Appendix IX Direct smear technique for examining of eggs and larvae of STHs

Stool samples examined for presence of eggs and larvae of the STHs by Direct smear

Drop of saline placed at the centre then stool placed by a wooden stick and then iodine added and mixed.

Both mounts are coverd then mounted on the microscope for examination for the presence of eggs or larvae of STHs

Appendix X Age and gender prediction on co-infection with *S. typhi* and STHs

Table 6.3.1 Logistic regression model chi square for prediction of age and gender on co-infection between STHs and *S. typhi* attending Ukwala Sub-County Hospital, Kenya

	Chi- square	Degree of freedom	Significance
Step	2.804	2	.246
Block	2.804	2	.246
Model	2.804	2	.246

Table 6.3.1 Logistic regression model chi-square test the overall significance. (χ^2 = 2.804, df= 2 P = 0.246)