EFFECTS OF GEOHELMINTHIASIS AND MALARIA CO-INFECTION ON MATERNAL HEALTH AND BIRTH OUTCOMES IN BUNGOMA COUNTY, KENYA

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DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award

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CERTIFICATION

We the undersigned certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a Thesis entitled: **Effects of Geohelminthiasis and Malaria Co-infection on Maternal Health and Birth Outcomes in Bungoma County. Kenya.**

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DEDICATION

I dedicate this work to my beloved wife Lonah A Wekesa, my daughters Nambuye W. Patricia, Nanjala Yvonne W, Nafula W. Sybil and son Matere W. Darius

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ABSTRACT

Geohelminthiasis and malaria are global pandemics closely linked to high morbidity and mortality in Sub Saharan Africa. In Kenya geohelminthiasis and malaria are public health problems especially in reproductive health. Despite theirr enormous effects on maternal health and birth outcomes, information is scarce on the current status of these infections to guide an intervention in the study area. Therefore, this study was conducted to determine the effects of geohelminths and malaria parasites co-infection on maternal health and birth outcomes in Bungoma County. A longitudinal hospital based study was carried out from March 2016 to January 2017. Seven hundred and fifty expectant mothers aged between 18 to 45 years attending antenatal clinic at the Bungoma County Referral hospital were enrolled in the study and followed up to the time of delivery after obtaining ethical clearance. Prevalence and intensity of specific geohelminths infection was determined by stool microscopy and Kato-Katz Technique. Malaria positivity, prevalence and intensity were determined by microscopic examination of Giemsa stained blood smears Haemoglobin levels and eosinophil cell count was done using the Coulter Counter Machine. Peripheral Blood Films for red cell morphology were prepared and stained using Leshman stain then examined microscopically. The fitness of the neonates was evaluated based on the Apgar scale. Socio economic risk factors were determined using a pre-structured and tested questionnaire. Data was analysed using STATA version 12. Point estimation of prevalence and intensity of geohelminths and malaria parasites were calculated based on the stool and blood sample results. The relationship of geohelminths and malaria co-infection and different explanatory variables was done using chi-squire (X^2) test and correlation co efficient. To identify the risk factors for geohelminthiasis and malaria, mixed effects of logistic regression models were fitted in bivariate analysis. Multivariate logistic regressions were employed for those variables that had significant association with disease outcome to determine the main socio economic risk factors of infection. P-value ≤ 0.05 was considered significant. Odds ratios (OR) with a 95% confidence interval was computed to compare the strength of association between explanatory and outcome variables. The results showed that the prevalence of geohelminths, malaria parasites, and co infection was 24.7%, 21.6% and 6.8% respectively. Pregnant women with geohelminthiasis and malaria co infection were four times more likely to have anaemia P-value 0.001 OR 4.137, CI(2.088-8.195) compared to those infected with geohelminths or malaria parasites alone P-value 0.001, OR 0.505, CI (0.360-0.709) and P-value 0.001, OR 0.274, C(I 0.187-0.402) respectively. The odds of having low birth weight P-value 0.001 OR 0.186 CI (0.094-0.369), Preterm deliveries, P-value 0.006 OR 6.896 CI (1.755-27.101), still birth P-value 0.048 OR 3.701 CI (1.008-13.579) were greater in co infected women than in those not infected. Risk factors for co-infection included unemployment P-value 0.002, OR 9.588 CI (2.281-40.304), rural residence P-value 0.752, OR 1.118 CI (0.559-2.235). Expectant mothers in their second trimester of pregnancy were three times at risk of co-infection OR 2.961 CI (0.892-10.060) compared to their counterparts in their first and third trimester of pregnancy. This study demonstrated that pregnant women co-infected with geohelminths and *Plasmodium* parasites were at particular risk of anaemia and adverse birth outcomes. The study recommends routine diagnosis and prompt treatment, provision of safe drinking water, treated mosquito nets, improved sanitation, and health education to be given to expectant mothers during antenatal clinics to improve the quality of life.

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List of Abbreviations and Acronyms

ACT	Artemisinin based combination therapy
ANC	Antenal clinic
APGAR	Appearance, Pulse, Grimace, Activity, Respiration
EPG	Eggs per Gram
Hb	Haemoglobin
ITN	Insectcide Treated Nets
IUGR	Interuterine growth retardation
LBW	Low birth weight
MIP	Malaria in Pregnancy
MLS	Millilitres
MOH	Medical Officer of Health
NPCSIH	National programme for control of Schistosomiasis and intestinal helminths
NTDS	Neglected tropical diseases
NMCP	National malaria control programme
OR	Odds ratio
PBF	Peripheral blood film
IUGR	Inter uterine growth reterdation
SP	Sulphadoxine Pyrimethamine
STH	Soil transmitted helminths
TX	Treatment
WBC	White blood cell
WCP	Webuye Climate Pattern
WHO	World Health Organization

Definition of terms

.**Anaemia** was defined as haemoglobin level < 11.5g/dl of blood.

Birth outcomes were defined as health effefs related to geohelminths and malaria parasites co-infection in expectant women and their their fetus during pregnancy.

-Low birth weight was defined as a birth weight of less than 2,500 g,

-Preterm birth was defined as gestational age of less than 37 weeks.

-Stillbirth has been adapted for this study to mean the death of a fetus prior to during or soon after delivery.

Geohelminths positivity was defined as presence of eggs of geohelminths species in a stool smear.

Malaria positivity was defined as the presence of asexual forms of *Plasmodium* species in a blood smear.

Parasitemia was defined as the presence of parasites particularly malarial forms in blood

Prevalennce was defined as the propotion of expectant women that had geohelminths, malaria parasites or both.

Intensity of parasitic infection or parasite load was defined as ameasure of the number of geohelminths, malaria parasites or both that an individual expectant woman was harbouring.

Unemployment was defined in this study as low income.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Geohelminths (also refered to as soil-transmitted helminths) are intestinal parasites that belong to the Phylum Nematoda. These parasites can be found in the soil, food stuff and water supplies contaminated with infected faeces and can be transmitted through faecal-oral or transdermally (WHO, 2017). The main species of medical importance are the *Ascaris lumbricoides* (roundworm), (*Trichuris trichiura* (whipworm) that are transmitted through ingestion of eggs in contaminated water or food and *Necator americanus* and *Ancylostoma duodenale* (hookworms) transmitted through active skin penetration by infective filariform larvae (WHO, 2017).

Geohelminthiasis are ranked among the most widely distributed neglected tropical diseases affecting people living in areas characterized by poor accessibility to safe water, inadequate sanitation and health facilities (WHO, 2011). It is estimated that about 3.8 billion persons get infected, with geohelminths globally with annual clinical cases and complications of about 720 million and 135,000 respectively (WHO, 2011). Approximately 800–1000 million cases of *A.lumbricoides*, 700–900 million cases of hookworm (*N.americanus* and *A.duodenale*), and 500 million cases of *T.trichiura* are reported worldwide annually (Brooker *et al*., 2010). More than 85% of these cases occur in Africa affecting both children and adults with an annual estimated mortality of 280,000 (WHO, 2010).

In sub-Saharan Africa, women of child bearing age are more vulnerable to the infection with an estimated 40 million expectant mothers reportedly infected with

geohelminths and schistosomes globally and more are at an increased risk of infection (Million *et al*., 2013). The epidemiology of parasitic infections depends on micro geographical variation of location. For instance the risk of *A.lumbricoides, T. trichiura,* and hookworms (*N.americanus* and *A. duodenale*) infection are influenced by environmental conditions and exposure to multiple species of parasites which may vary over slight distances and location, an aspect that has not been adequately addressed by previous studies (Stephen *et al*., 2015).

Most studies that have examined the occurrence of geohelminths have been based on a single stool sample taken at one point in time. Such studies are likely to underestimate geohelminth burden due to low sensitivity (Yatich *et al*., 2010). Therefore stool sample analysis advisably require at least two stool samples taken consecutively since geohelminthes ova are released intermittently (Teixeira *et al.,* 2010).

A study carried out in Western Kenya based on a single stool sample reported geohelminthiases to be common among adults including pregnant women at a prevalence rate of 15.7% (Jonathan *et al*., 2014). Data is inadequate on prevalence of geohelminths among expectant mothers, more so in Bungoma County.

Malaria is still a major global health burden in tropical and subtropical countries, despite the intensive control measures that are carried out worldwide (WHO, 2015). Human malaria is caused by five known parasite species of *Plasmodia; Plasmodium falciparum, P.vivax, P.ovale, P.malariae and P.knowlesi.* Most infections are caused by *P. falciparum*. (WHO, 2015). Each year, 350 to 500 million cases of malaria are reported globally (WHO, 2013). The disease kills more than one million people

wordwide annually most deaths occur in Sub-saharan Africa where it is the leading cause of death for children under five years, pregnant women and those with low socioeconomic status (WHO., 2013).

Malaria transmission in Kenya can be described as stable, unstable and recurrent depending on location and altitude with children and expectant mothers being the most vulnerable (Rachel *et al*., 2015). In western Kenya malaria prevalence of 28% was reported by Rachel *et al*., (2015). Medical records of 2015 from Bungoma County Referral Hospital Laboratory reported the existence of other *Plasmodium* malaria parasite species a part from *P.falciparum* (records officer), this indicate that prevention approaches must be strengthened and this can only be done based on valid data which is currently lacking in the study area.

Geohelminths and malaria parasites are known to cause adverse effects separately or as co-infection in humans. Geohelminths contribute to morbidity characterized by malnutrition, growth retardation, anaemia, vitamin A deficiency and impaired intellectual performance (WHO., 2010). Hookworm infection is often associated with intestinal bleeding, blood loss and anaemia (Dotter *et al.*, 2011). Although geohelminths are an important cause of anaemia in developing countries, there is lack of consensus regarding the risks and benefits of treating geohelminths in pregnancy, and this has, until recently, led to the exclusion of pregnant women from deworming programs (Ntui *et al*., 2014). In Kenya national dewoerming programs forcuses on children and leaves out this vulnerable group that may chronically get infectedbwith geohelminths.

Malaria in pregnancy is a major cause of maternal and fetal morbidity and mortality (Yatich *et al*., 2010) Compared to other malaria parasites, *P. falciparum* is known to be a major contributor to pregnancy anaemia especially in nulliparous women (Yatich *et al*., 2010). The species has been consistently and widely associated with pathologies during gestation. However, the impact of the other malaria parasites *(P.vivax, P.malarie and P. ovale)* particularly in pregnancy is not clear (Bhattacharyya *et al*., 2011).

The overlapping geographic distributions of geohelminthiasis and malaria present a situation where there is a high possibility of cases of co-infection. A study examining malaria and geoelminth co-infection in pregnancy in Nigeria demonstrated that over 45% of *Plasmodium*-infected pregnant women also harbored various geohelminths. This co-infection was associated with low haemoglobin level among pregnant women in Ghana (Yatich *et al*., 2010). Other related studies have posted inconsistent findings. Adegnika *et al.*, (2010) reported positive associations between geohelminths and malaria, suggesting that geohelminths may increase susceptibility to malaria. Mazigo *et al*., (2010) found out that pregnant women infected with hookworm had increased susceptibility to malaria infection. However another study reported no association between geohelminths and malaria parasites in pregnancy (Boel *et al*., 2010). Further studies are necessary to address these inconsistencies.

Individuals infected with geohelminths especially hookworm and malaria parasites have decreased haemoglobin levels leading to anaemia (Degarege *et al*., 2010). Haematological parameters indicating causes and severity of anaemia are paramount for prompt management of anaemia in pregnancy (Mulambalah *et al*., 2014). Data is

scarce on the association of geohelminths and malaria parasites co-infection on maternal red cell morphology and eosinophil levels, more so in expectant women of Bungoma County.

There is no consensus on the impact of geohelminths and malaria parasite co-infection and neonatal outcomes. A study in Nigeria found that geohelminth infections were associated with lower birth weight (Aderoba *et al*., 2015). But in another study Ndibazza *et al*., (2010) revealed that geohelminths alone are not associated with low birth weight. A study done in Ghana found that anaemia in pregnant women, co-infected with geohelminths and malaria parasites was associated with low birth weight, preterm delivery, and small birth weight for gestational age. Much of this effect appeared to be driven by malaria. The only significant effect of geohelminths alone was an increase in the risk of being small for gestational age Yatich *et al*., (2010), another study, women co-infected with geohelminths and malaria had higher birth weights (Fairley *et al*., 2013). Therefore A clear understanding of the association between geohelminths and malaria parasites co-infection in human populations especially in expectant mothers is important.

A hospital-based study of Nigerian women found that geohelminths and malaria parasites co-infection was associated with lower birth weight than malaria infection alone, (Blackwell *et al*., 2016). However no study has looked at the effects of geohelminths and malaria parasites co-infection on neonatal APGAR score particularly in expectant mothers of Bungoma County Kenya.

Geohelminth infections are thought to occur in rural poor communities where the biophysical, cultural and environmental factors favor transmission (Samuel *et al*., 2015). However a study in the Republic of South Africa reported the rate of geohelminths infections in urban slums was alarmingly high (Appleton *et al*., 2009). Generally, low standard of living, poor personal hygiene, waste management, unsafe and inadequate water supply are some of the factors that allow geohelminths, malaria and other communicable diseases to flourish in developing countries (WHO., 2010).

Pregnant women are particularly vulnerable to infections, due to suppression of their immune system during pregnancy (Stephen *et al*., 2015). Participation of pregnant women in agricultural activities with ill equipped farm wear puts them ordinately at higher risk of contracting hookworm infection that is linked to iron depletion (Dotter *et al.,* 2011). A clear understanding of the effects of geohelminths and malaria parasites co-infection in expectant women is inadequate specifically in respect to expectant mothers of Bungoma County, Kenya**.** Thus, this study sought to determine effects of geohelminths and malaria parasite co-infection on maternal health and birth outcomes in this important and vulnerable group. The findings from the study provide useful information for designing strategies for effective control and management of co-infections in expectant mothers in Kenya.

1.2 Statement of the problem

Geohelminths and Malaria parasites are common in Kenya therefore increases the risk of anaemia in pregnancy leading to adverse consequences to the mother and her unborn baby (Jonathan *et al*., 2014, Rachael *et al*., 2015). *P. falciparum* is widely associated with pathologies during gestation including anaemia (Yatich *et al*., 2010).

However, the impact of the other malaria parasites *(P.vivax, P.malarie and P. ovale)* particularly in pregnancy is not clear (Bhattacharyya *et al*., 2011). Medical records of 2015 from Bungoma County Referral Hospital Laboratory revealed that over 350 expectant women attenting antenatal services are anaemic, geohelminths and malaria parasites are incriminated but valid data is questionable.

1.3 Justification of the study

Expectant women face a myriad of challenges ranging from physiological to social and thus form an important part of the society that requires special attention. This is more so in regard to not only her health but also that of the unborn baby. In Kenya, expectant mothers are usually excluded from de-worming programs without considering the risk-benefit ratio. Stool and blood examination for the detection of geohelminths and malaria infection respectively are not routinely done during antenatal clinic (ANC) visists unless expectant mothers are symptomatic, although WHO recommend routine treatment during pregnancy (Savioli *et al*., 2003. This therefore, exposes mothers and the foetus to dangerous effects of geohelminths and malaria co-infection.

Participation in agricultural farm activities without protective footware and geophagy common in expectant mothers make them vulnerable to the infections. There is inadequate information on the effect of other malaria species on maternal health and birth outcomes. A better understanding of the effects of concomitant infections of these parasites among expectant mothers in Bungoma County is important to quite in control intervention. Therefore there was need to determine the

effects of geohelminthiasis and malaria co-infections on maternal health and birth outcomes in Bungoma County.

1.4 Objectives of the Study

1.4.1 General objective of the Study

To determine the effects of geohelminths and malaria parasites on maternal health and birth outcomes among pregnant mothers attending antenatal clinic at Bungoma County referral hospital Kenya

1.4.2 Specific Objectives of the Study

i. To determine the prevalence and intensity of geohelminths and malaria parasites among pregnant women in Bungoma County
ii. To establish the effect of geohelminths and malaria parasites co-infection on maternal health
iii. To evaluate the impact of geohelminths and malaria parasites co-infection with neonatal outcomes
iv. To identify the social economic risk factors associated with geohelminths and malaria parasites in pregnant women

1.5 Research Questions

i What is the prevalence and intensity of geohelminths and malaria parasites among pregnant women in Bungoma County?

ii What is the effect of geohelminths and malaria parasites on maternal health?

iii What is the impact of geohelminths and malaria parastes co-infection and neonatal outcomes?

iv What are the social economic risk factors associated with geohelminths and malaria parasites in pregnant women?

1.6 Significance of the study

The study findings were instrumental in elucidating the effects of geohelminths and malaria parasite on maternal health and birth outcomes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Prevalence and Intensity of Geohelminths and Malaria parasites among Pregnant Women

2.1.1 Global Prevalence of Geohelminth Infections

Approximately 1.5 billion people, or 24% of the world's population, are infected with geohelminths. Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the America, China and East Asia (WHO, 2017). Geohelminthic infection prevalence of up to 50% has been documented in some regions in Sub-Saharan Africa (Sousa *et al.,* 2012). Until the past decade, geohelminthiasis had been neglected due to insufficient knowledge of their impact on human life (Standley *et al.,* 2011). Parasitic infections have no global boundaries (Pullan *et al*., 2014). However, their prevalence is higher in the tropical and sub-tropical regions of the globe where ambient conditions favors their survival and transmission, particularly impoverished areas which are usually coupled with poor personal and environmental hygiene (Samuel *et al*., 2017).

Geohelminths are transmitted to humans via life stages occurring in soil or water. They reside in the intestine, where they produce eggs that are excreted in the faeces. Hookworm is a common name referring to two distinct species, *N. americanus* and *A. duodenale*. These two species of hookworms cannot be distinguished by standard faecal egg counts, and so are frequently referred to simply as hookworms. Hookworm larvae infect their hosts by penetrating through the skin, generally through the feet (Blackwell *et al*., 2016). *A. lumbricoides* or roundworm has the largest proportion of the infection; it is transmitted by ingestion of eggs through

contaminated water or food. Both hookworm and roundworm lifecycle goes through tissue stage. The ingested eggs hatch in the duodenum, larvae penetrate intestinal wall and circulate in blood. From the heart they migrate to lungs, ascend to the trachea, descend to the oesophagus, shallowed and finally reach the small intestines to become adults (Blackwell *et al*., 2016).

Trichuris trichiura (whipworm), infect the gastrointestinal tract directly once the eggs are ingested they hatch into larvae which develop into adults in the small intestines without tissue migration, (Blackwell *et al*., 2016). Other soil-transmitted nematodes such as *Strongyloides stercoralis* (threadworm) infect their hosts by larvae penetrating through the skin, particularly the feet. They have complex life cycles that involve free-living stages and less directed tissue migration (Greaves *et al*., 2013).

2.1.2: Global Prevalence of Malaria

Malaria kills more than one million people annually, most of them in Sub Saharan Africa, where malaria is a leading cause of death for children less than five years, pregnant women and those with low socioeconomic status (Joseph *et al*., 2016). The prevalence of malaria is influenced by a number of factors such as maternal age, parity, use of prophylaxis, nutritional status, host genetics, parasite genetics and transmission rate (Ntui *et al.*, 2014).

Malaria during gestation period is therefore problematic in sub-Saharan Africa; an estimated 24 million expectant mothers are infected every year (Akinboro *et al*., 2010). The risk of death due to malaria is higher in children and pregnant women, malaria indicator surveys tend to focus on children as adult populations acquire

partial immunity (WHO, 2013). Of all human diseases caused by protozoan parasites, malaria has the greatest burden and is responsible for most deaths amongst young children and pregnant mothers in sub-Saharan Africa, accounting for 90% of all global cases (WHO, 2011).

2.1.3: Global Prevalence of Geohelminths and Malaria Co-infection.

In many tropical countries, parasitic co-existence is a common phenomenon with increased potential for co-infection, which may adversely impact the outcome of the diseases they cause (Degarege *et al*., 2010). In a study of parasitic infection in pregnancy conducted at the coastal region of Kenya, from 2000 to 2005, the results revealed that 32% of pregnant women were infected with geohelminths 31% had hookworm, 43% had malaria (*P. falciparum*), while more than 46% of women were co-infected with geohelminths and malaria parasites (Fairley *et al*., 2013). Even though there are efforts aimed at preventing parasitic infections in pregnancy, the challenge still persists, particularly in deprived communities and among impoverished individuals (Samuel *et al*., 2017).

2.2 Effects of Geohelminths and Malaria Parasite Burden on Maternal Health

2.2.1: Effects of Geohelminths on Maternal Health

Infections with geohelminths have been linked to adverse maternal health. Geohelminth infections are the major medical and public health problems in many parts of the world (WHO, 2017). Infection with *A.lumbricoides* increase malabsorption of nutrients in the infected individuals. In addition, they may possibly compete for vitamin A in the intestine and cause loss of appetite and, therefore, a

reduction of nutritional intake and physical fitness. Infection with *T. trichiura* is known to cause diarrhoea and dysentery (WHO, 2017).

Geohelminths cause morbidity which is related to the number of worms harboured by the host. People with infections of light intensity (few worms) usually do not suffer from the infection. Heavy infections can cause a range of symptoms including intestinal manifestations (diarrhoea and abdominal pain), malnutrition, general malaise and weakness, anemia, impaired growth and physical development (WHO, 2017). The most common side effects of infection with geohelminths is anaemia, due to blood loss in the intestine. *Necator americanus* and *A. duodenale* (Hookworm) have been associated with moderate reductions in haemoglobin during pregnancy (Gyorkos *et al*., 2011).

Estimates suggest that more than 25% of pregnant women are infected with *N. americanus* and *A.duodenale* (Hookworm) which causes intestinal bleeding and blood loss, and has been most commonly associated with anaemia (Mclean *et al.*, 2012, Sousa *et al*., 2012). Hookworm (*N.americanus* and *A. duodenale*) infection leads directly and indirectly to a spectrum of adverse maternal and foetal/placental effects (Huchon *et al*., 2013). It is linked to poor pregnancy consequences plus greater danger of foetal death and preterm birth, maternal anaemia, increased risk of maternal mortality because of obstetric haemorrhage, and severe illnesses (Elizabeth *et al*., 2014). Anaemia in pregnancy contributes toward maternal morbidities and

greater chances of death connected with conditions such as postpartum haemorrhage (Huchon *et al*., 2013).

Maternal anaemia may also lead to anaemia in the unborn baby and subsequently to anaemia in the infant. Long-term childhood adverse effects include impaired brain development (Koura *et al.*, 2012). Hookworm (*N.americanus* and *A.duodenale*) infection is also associated with a lower probability of a women becoming pregnant (Blackwell *et al*., 2015). Hookworm (*N.americans* and *A.duodenale*) infection causes maternal anaemia which affect egg maturation, leading to overall lower fecundity (Miller *et al*., 2016). Geohelminth infections is thought to affect fecundity or lead to early loss of pregnancies through multiple pathways, including redirection of resources and alteration of the hormonal milieu (Hernandez *et al*., 2010), or immunological biasing (Blackwell *et al*., 2015).

Even though most morbidity has been seen with high intensity infections, in populations with low iron states, even low-intensity hookworm infection has been associated with great morbidities (Agu *et al*., 2013). Poor nutrition, which contributes to inadequate intake of iron, folate, and other micronutrients, is common in the geographic areas where geohelminth infections are prevalent, and may have an important role in the relationship of infections and anaemia (Bechir *et al*., 2010)

2.2.2 Effects of Malaria parasites on Maternal Health

Malaria during gestation period is problematic in sub-Saharan Africa with an estimated 24 million expectant mothers being infected annually (Akinboro *et al*., 2010). It is estimated that 10,000 women and 200,000 infants die as a result of malaria infection during pregnancy (Joseph *et al*., 2016). Severe maternal anaemia, premature and low birth weight contributes to more than half of the deaths (Joseph *et al*., 2016). Pregnant women are especially prone to severe attacks of malaria, which is known to cause abortion, premature labour and stillbirth, (Bhattacharyya *et al.,* 2011). Annually, 125 million women are at risk of infection by *Plasmodium* parasites which may lead to anaemia (Dellicour *et al*., 2010). *P. falciparum* malaria increases risk for moderate and severe maternal anaemia (Degarege *et al*., 2012).

Many studies have focused on the effects of a single infectious agent on pregnancy outcome and maternal anaemia, while few studies that have attempted to understand the relative effects of multiple agents have posted conflicting results (Agu *et al*., 2013). *P. falciparum* infection is usually asymptomatic during pregnancy, yet the parasites may be present in the placenta and contribute to maternal anaemia even in the absence of documented peripheral parasitaemia (WHO, 2017). Both maternal anaemia and placental parasitaemia can lead to low birth weight, which is an important contributor to infant mortality. In high-transmission settings, the adverse effects of *P. falciparum* infection in pregnancy are most pronounced for women in their first pregnancy (WHO, 2017).

2.2.3 Effects of Geohelminths and Malaria parasites Co-infection on Maternal Health

Parasitic infections are of general public health concern globally and their effects among pregnant women are even more enormous, with adverse outcomes including low pregnancy weight gain (Roberts *et al*., 2011). There is a geographical overlap of malaria endemicity and geohelminth infections resulting into co-infection especially in resource-limited communities and clinical consequences of co-infection are heavily increased, as compared to single infection (Ugbomolko *et al*., 2012).

Plasmodium spp. and helminths affect haemoglobin levels in different ways and exert an additive effect when they co-exist, leading to an increased risk of iron-deficiency anaemia among co-infected individuals (Salazar *et al*., 2014 Geohelminths may also interact with *Plasmodium* species. through other mechanisms including resource competition and direct interference (Knowlesi *et al*., 2011). The occurrence of geohelminths infection could increase susceptibility to *Plasmodium* infection and related clinical outcomes .Geohelminths and *Plasmodium* parasites activate different modes of the immune system in the human body. It is postulated that geohelminth infections can downregulate immune responses to *Plasmodium* pathogens (Salazar *et al*., 2014).

Findings on whether and how geohelminths and *Plasmodium* species interact within humans when there are co-infections are heterogeneous (Degarege *et al*., 2010). Hookworm infection has been associated with increase susceptibility to malaria

parasites (Adegnika *et al*., 2010), while *A. lumbricoides*, may be associated with reduced risk of malaria, (Boel *et al*., 2010). In a randomized trial, treatment of geohelminths led to a short-term increase in malaria parasitemia, but with no long-term effects on malaria symptoms or prevalence. (Wiria *et al*., 2013). There is still controversies concerning the biological association between geohelminthes and malaria parasites (WHO, 2010)

2.3 Impact of Geohelminths and Malaria parasite Co-infection on Birth Outcomes

An estimated 2·6 million stillbirths occur worldwide each year, resulting in substantial psychosocial and economic costs (Heazell *et al*., 2016). A study done by Blencowe *et al*., (2016) revealed that 98% of preventable stillbirths occur in resource-limited settings. Several investigations found that Low birth weight (LBW) risk was associated specifically with malaria infections occurring in early pregnancy (Hynh *et al*., 2011). Data from Thailand did not show a significantly lower birth weight in newborns of mothers with a single treated malaria episode in the first trimester compared to newborns of mothers without malaria infection (Mc Gready *et al*., 2012) Likewise, conflicting results have also been reported on the association between the number of malaria infections and the risk of LBW (Kalilani *et al*., 2010).

Malaria during pregnancy is thought to affect birth outcomes through two mechanisms, intrauterine growth restriction (IUGR) and preterm delivery, which might at least partially explain these discordant findings. It has been estimated that malaria during pregnancy in settings with stable malaria transmission in Africa is potentially responsible for up to 70% of IUGR and 36% of preterm delivery (Risken

et al., 2011). However, accurate determination of gestational age is required to distinguish IUGR from preterm delivery, a determination that is difficult to make in resource-constrained settings where tools such as ultrasound are rarely available. As a result, evidence of the relative importance of IUGR versus preterm delivery due to malaria during pregnancy remains limited (Rijken *et a*l., 2011)

A study in Nigeria found that geohelminth infections were associated with lower birth weight (Aderoba *et al*., 2015). Another study found that in women with anaemia, co-infection with helminths and malaria was associated with low birth weight, preterm delivery, and small birth weight for gestational age (Yatich *et al*., 2010). Geohelminthiasis and malaria co-infection is also associated with an increased risk of anaemia and other negative outcomes during pregnancy, while the effects of geohelminths alone are often less clear (Naing *et al*., 2013).

There is scarcity of data on whether the geohelminth-induced anaemia affects birth outcomes. Severe anaemia during pregnancy is associated with a number of adverse outcomes which include increased maternal mortality, preterm delivery, low birth weight, still birth (Banhidy *et al*., 2011), and increased risk of neonatal anemia (Miller *et al*., 2016).

2.4 Social Economic Factors Associated with Geohelminths and Malaria parasites in Pregnant Women.

Infection and transmission of geohelminthiasis are disseminated by many factors such as improper personal cleanliness, unselective discarding of human and animal

faeces, which permits contact of faeces and microbial agents with soil or water. Generally, geohelminth infection is linked to poverty, lack of sanitation, impaired hygiene and overpopulation (Ojurongbe *et al*., 2013). The acquisition of requisite information on co-morbidity and interactions between malaria and helminthiasis would be invaluable to controlling malaria infection and clinical disease.

The control of parasitic diseases rely on the WHO strategies, which lay emphasis on a combined control approach for malaria infection (NMCP, 2010) and deworming activities for geohelminths parasites (NPCSIH, 2011). Commonly, there is a geographical overlap of regions with high malaria endemicity, and the occurrence of Neglected Tropical Diseases (NTDs), such as geohelminth infections. As a consequence, co-infections are expected to be a common phenomenon, rather than an exception, mainly in resource poor communities (Ugbomoiko *et al*., 2012). In Kenya the occurrence of geohelminth infection differ based on locality and environmental conditions and is indicative of faecal pollution of soil and domestic water supply around homes due to poor sanitation, geophagy and improper sewage disposal (Mulambalah *et al*., 2014).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Bungoma Country Referral Hospital. The Hospital serves patients from all the sub counties of Bungoma County who seek medical attention or are referred from other facilities. Bungoma County Referral Hospital is located in Kanduyi Sub County.

The County lies between latitude 0° 34′0”N and Longitude 34° 34′ 0”E and has an area of 3,032.2sq km.It has an estimated human population of 1,375,063 according to Kenya 2009, national census. It has 9 Sub counties, namely Kanduyi, Bumula, Sirisia, Kabuchai, Kimilili, Webuye East, Webuye West, Tongaren and Mount Elgon (Figure 3.1). It has a tropical climate characterized by hot and humid conditions. The County has two rainy seasons, long rain season (between April and August) and the short rains (October to December). There is a dry season from January to March. The predominant ethnic group are Luhyas (Bukusu) Teso and Sabaot, who practise subsistence and sugarcane farming. Majority of these communities toilet facilities are made up of pit latrines, which are generally poorly constructed and insufficient for the members of each household. It is common to find pools of standing and dirty water close to some households that are prolific for mosquito breeding.

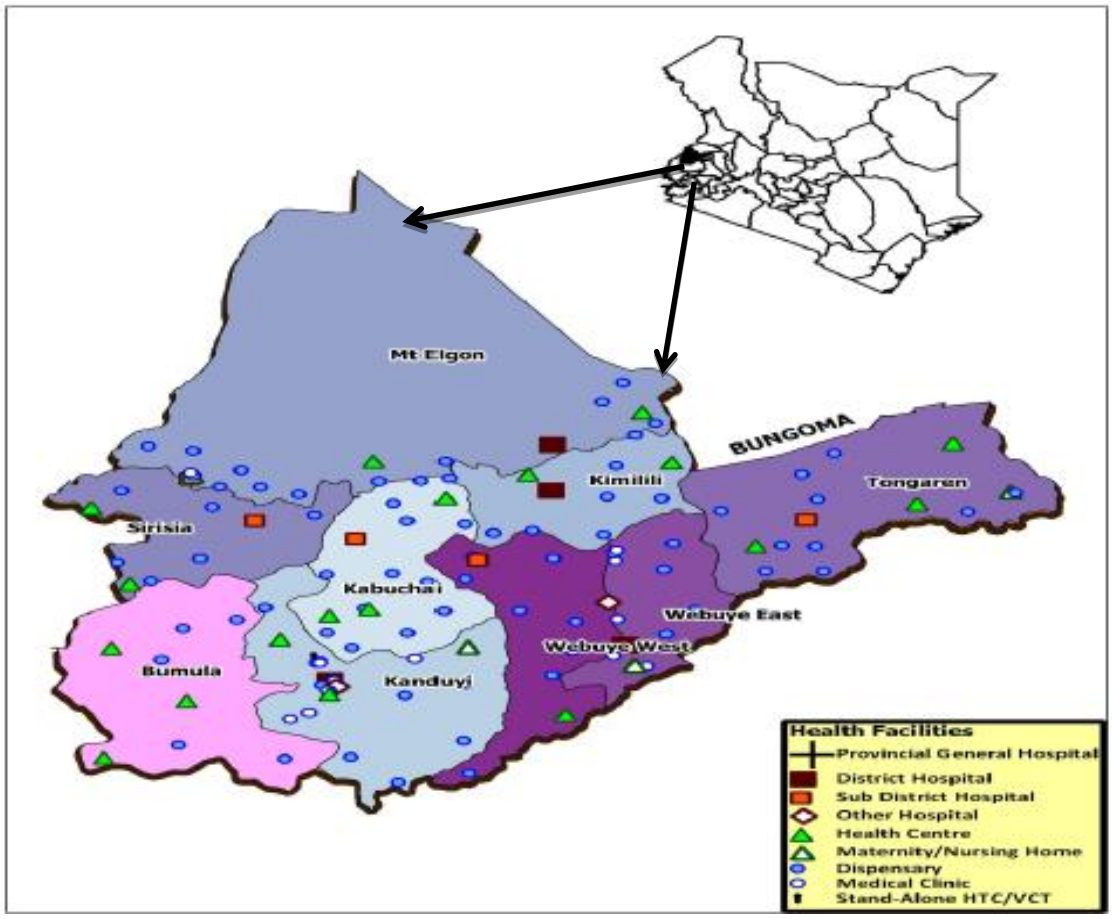

Figure 3.1: Map of Bungoma County showing Bungoma County referral hospital (Adopted from Google Maps, 2016)

3.2 Study Design and Target Population

A Longitudinal cohort hospital based study was adopted. Consecutive sampling was used to recruit participants who met inclusion criteria from March 2016 and followed to delivery. The study population comprised of expectant mothers aged 18 to 45 years. In Kenya a person is considered an adult if he/she attains the age of 18 years and at 45 years she is within the reproductive age bracket. Expectant mothers in their 1st trimester of pregnancy seeking antenatal services at Bungoma county referral Hospital were recruited in the study

3.2.1 Sample Size Determination

The sample size was calculated using Mugenda and Mugenda formula (1999), with 95% confidence interval. The sample size was based on a study done in rural Gem Nyanza, Western Kenya that revealed 76.2% prevalence of geohelminth infections among pregnant women (Van Eijk *et al*., 2009).

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

Where;

D is margin of error (0.05)

n is the minimum sample size

P is the estimated prevalence (76.2%)

Z is the standard normal deviation that corresponds to 95% confidence interval (1.96)

$n = \frac{(1.96)^2 \times 0.762\ (1 - 0.762)}{0.05^2}$ = 278.4. Plus 10%. 27.84, in case of defaulters = 306.24 approximate minimum sample size of 400 pregnant women.

To account for refusals or inability to produce stool specimen when required, the sample size was adjusted to 750. Large sample size is more representative.

3.2.2 Inclusion Criteria

i. Pregnant women aged between 18 to 45 years.
ii. Residents of Bungoma County for the last 6 months.
iii. HIV seronegative
iv. Those willing to sign informed consent to participate in the study preceeding the study period
v. 1st trimester of pregnancy

3.2.3 Exclusion Criteria

i. Pregnant women aged less than 18 years or more than 45 years
ii. Pregnant women who were not willing to participate in the study
iii. Non-residents of Bungoma County
iv. HIV positive expectant mothers
v. Those not willing to sign informed consent to participate in the current study

3.3 Safety Procedures and Data Collection

Strict procedures were followed before sample collection. Personal protective equipment for instance gown, gloves were put on. This was to avoid any contamination due to likelihood of contact with blood, body fluids and stool samples during procedures of specimen collection and processing.

3.3.1 Determination of Geohelminthic Infections Prevalence and Intensity

After getting written consent, pregnant women were provided with labeled screw caped stool container plus a scoop and informed on how to transfer about 5 grams of stool sample into a container. Each container was identified by the code number of the participant.

Kato-Katz thick smear technique (WHO, 1991) was used to collect qualitative and semi-quantitative data on diagnosis of ascariasis, trichuriasis, hookworm, enterobiasis and schistosomiasis. The method was preferred because it is a quantitative technique. Samples were prepared within six hours after collection. Each stool specimen was prepared by sieving it through a sieve to remove debris. Two slides were labeled A and B. Filtered stool sample was put in a hole of a template

mounted on each glass slide. The template has a hole that is calibrated to contain 47.1mg of stool. Each preparation on the glass slide was covered with cellophane soaked in glycerine and malachite green. The preparations were then turned upside down on a flat surface and pressed gently to spread the stool sample evenly. Each slide was read by a separate qualified laboratory technologist to increase the sensitivity of geohelminths detection and verification of negative slides.

The readings were done microscopically using ×10 and ×40 and average number of eggs in the two slides calculated. The slides were examined within one hour after preparation to avoid over clearing of hookworm eggs by glycerine. Specific parasite eggs were identified morphologically, counted and recorded in the prepared laboratory format. As a quality control measure, 10% of already examined stool slides were re-examined by the principal investigator.

Eggs per gram of individual geohelminths were determined by multiplying the number of counted eggs by factor 24 (WHO, 1987) to get infection intensity. The egg counts were categorized as Light infection, Moderate infection, and Heavy infection (WHO, 1987) as follows *A.lumbricoides*, light infection (1-4999 eggs/gram), Moderate infection (5000-49,999 eggs/gram), and Heavy infection (> 50,000 eggs/gram). Hookworm (*N.americanus*) light infection (1-999 eggs/gram), moderate infection (2000-3999 eggs/gram), and heavy infection (>4000 eggs/gram). *Trichuris trichiura*, light infection (1–999), moderate infection (1000–9999) and heavy infection (10,000).

3.3.2 Culturing and identification of Hookworm species

Haradamori technique (Harada and Mori., 1955) was used to culture and hatch hookworm eggs to differentiate *N. americanus* filariform larvae from those of *A. duodenale*. This method was chosen because it is less expensive and equally gives better result.

The procedure involved the use of filter paper strips of about 5 inches slightly tappered at one end for each stool specimen confirmed to contain hookworm eggs. One gram of each faecal sample was smeared at the centre of the strip. Four millilitre of distilled water was added to 15 millilitre conical centrifuge tube. The paper strips were inserted into the tube, such that the tapered end was near the bottom of the tube with water level slightly below the faecal point. The tube was plugged using cotton wool and allowed to stand upright in a rack at 25°C for 10 days. Small amount of the fluid was withdrawn from the bottom of the tube and a smear was prepared on a glass slide. The preparation was cover slipped and examined microscopically using 10x objectives. *Necator americanus* filariform larvae were identified based on following morphological features: average larval length 59μm, pointed head and tail, oesophagus with thistle funnel shape (oesophageal bulb), presence of gap between oesophagus and intestine, oesophagus length approximately 1/3 in relation to entire body length (Harada and Mori., 1955).

3.3.3 Malaria Prevalence, Species Identification and Intensity

Maternal peripheral, blood was drawn at the first antenatal clinic visits. Safety procedures were adopted in the collection of venous blood samples by swabbing the ante cubital fossae with 70% alcohol and 5mls of blood was drawn into EDTA bottle with sterile hypodermic needle. The blood was enough for malaria parasite examination, haemoglobin estimation, peripheral blood film preparation and eosinophil count.

For malaria parasites examination, Thick and thin films were made on clean slides and labeled and then air-dried in a horizontal position. Thick blood film concentrates malaria parasites for easier viewing while thin blood film facilitates malaria species identification by their morphological features. Thin blood films were fixed in 70% methanol for 30 seconds and air-dried. Both thin and thick films were stained using 10% Giemsa for 10 minutes. The blood slides were examined microscopically using oil immersion objective ×100 as recommended by (WHO1991).

All asexual forms of malaria parasites (trophozoites and schizonts) in each preparation were identified microscopically and recorded. Malaria parasites density per microliter of blood was calculated by counting the number of malaria parasites against 200 white blood cells (WBCs) and multiplying by 8,000 to get malaria parasites/µl of blood (Cheesbrough, 1998) At least 100 high-power fields were examined before a film was declared negative. For quality control 10% burfered (PH 7.2) Giemsa stain was prepared for use after evey 6 hours. Known malaria positive

controls with low parasitemia were stained and examined daily to check the quality of the stain. 10% of read blood smears were re-examined for quality control.

3.3.4 Determination of geohelminths and malaria parasites burden on maternal health

Haemoglobin levels of anaemic mothers infected with geohelminths, malaria or both were measured every month until normal haemoglobin level was regained. This was done to determine how long one takes to gain her normal haemoglobin level after treatment.

Maternal haemoglobin levels were measured using a portable haemoglobinometer (Hemocue Lee Diagnostic Inc. Switzerland) system by putting 2 micro litres (ul) of blood in a microcuvette preloaded with stablelising reagents. The micro-cuvette was then immediately placed into a portable spectro-photometric instrument and the digital reading of the haemoglobin concentration of each blood sample was read within 10-20 seconds. Control blood samples with known values of low haemoglobin levels (Hb<11.0g/dl) and high haemoglobin values (Hb 18g/dl) were run daily to validate the accuracy and reliability of the haemoglobinometer results.

Coulter counter machine CBC5 (Coulter corporation Miami, FL, USA) was used to evaluate eosinophil levels. Acryo vial containing 5mls of blood was placed in the coulter machine which sacks 10µl of blood following specific command and each subject results were read within 10 – 20 seconds. To diagnose iron deficiency (presence of hypochromic and microcytic red blood cells) peripheral blood films

(pbf) were made from already collected blood, air-dried and fixed using 70% methanol. Staining was done using 10% Leishman stain for 10 minutes and examined using oil immersion with ×100 objective.

Daily running of known control blood samples to validate accuracy of the Coulter counter and haemoglobinometer machines before running test samples was done. Weekly running of Quality Assurance (QA) to check the effectiveness of quality systems that are in place, example proper recording of the results was also done

3.3.5 Impact of Geohelminths and Malaria parasites Co-infection on Neonatal Outcomes.

Neonatal outcomes were obtained from both infected and non-infected mothers, recorded, evaluated and comparisons made. Gestation period was assessed by palpation before delivery. At delivery, neonatal condition (alive or stillbirth) were assessed, weight was measured to the nearest 0.1kg using an electronic balance, and Apgar score of new borne babies (Normal, high chances of survival, low chances of survival or dead) were assessed on apgar scale.

3.3.6 Social Economic Factors Associated With Geohelminthiasis and Malaria in Pregnant Women

Semi-structured questionnaire (appendix B) was developed and administered to pregnant women meeting the inclusion criteria prior to stool and blood sample collection. This was to identify possible risk factors of geohelminths and malaria parasites co-infection in the study area. The questionnaires addressed the individual's

socio-demographic information, use of malaria and geohelminths preventive measures, housing conditions, residence, trimester of pregnancy, marital status, faecal disposal, level of education hand washing and other issues related to possible risk factors for co-infection.

Quality control was performed by daily review of each questionnaire. After sample collection due procedures for waste segregation and disposal were followed. Sharps, example needles and lancets were disposed in sharp boxes and incinerated. Stool specimen after processing were put in biohazard bags and disposed by incineration. Blood samples after use were put in biohazard bags, autoclaved before incineration.

3.4 Data analysis

Data was checked and cleaned for completeness and consistency then analysed using STATA version 12 (STATA corporation college station TX USA). Both descriptive and inferential statistical tools were used to analyse and present the data. Point estimation of prevalence and intensity of geohelminths and malaria parasites were calculated based on the stool and blood sample results. The relationship of geohelminths and malaria co-infection and different explanatory variables was done using chi-squire (X^2) test and correlation co-efficient.

To identify the risk factors for geohelminthiasis and malaria, mixed effects of logistic regression models were fitted in bivariate analysis. Multivariate logistic regressions were employed for those variables that had significant association with disease outcome to determine the main socio economic risk factors of infection. P-value $\leq$ 0.05 was considered significant. Odds ratios (OR) with a 95% confidence interval

were computed to compare the strength of association between explanatory and outcome variable.

3.5 Ethical Considerations

The study was approved by Masinde Muliro University of Science and Technology Institutional Review Board (approval number MMU/COR403OO9 (57). Further approval was obtained from Bungoma County Referral Hospital. Oral and written informed consent was obtained from all study participants in any of the languages (English, Kiswahili). The purpose of the study was explained to expectant women before they were requested to sign written individual consent form. Expectant mothers consented verbally to access their medical records (ANC profile books) to acertain their HIV status.

Study participants were given the option to withdraw from the study at any time they wished. Data was coded and kept strictly confidential and stored under key and lock. Information of the results was shared with study participants. No compensation was given to expectant women who participated in the study. But those infected were treated with antimalarial, anti-helminthic and haematinic drugs free of charge at the hospital in accordance with clinical guidelines of WHO and Ministry of Health Kenya. Follow up was done after treatment to establish recovery and any reinfection.

3.6 Limitations of the study

The study confined itself to the detection of anaemia resulting from exposure of expectant women to geohelminths and malaria parasites. Other causes of anaemia for instance haemoglobinopathies (sickle cell, thalassemia, G6PD deficiency) were

not investigated. Genetic, nutrional and environmental effects were not part of this study. It was therefore not possible to comment on these factors

CHAPTER FOUR

4.0 RESULTS

4.1: Prevalence and Intensity of Geohelminths and Malaria Parasite Co-infection

4.1.1 Prevalence and Intensity of Geohelminths.

A total of 750 pregnant women were recruited in the study. Geometric mean for gestation age of 24.119 and standard error mean of 0.190. Majority of expectant mothers 541(71.1%) were within the age bracket 18–27 years, followed by those aged 28–37 years 192(25.6%) and those in age group 38-40 years 17(2.3%). The overall prevalence of the geohelminths was 185 (24.7%). *A.lumbricoides* was the most prevalent species 76 (41.1%), followed by hookworm (*N.americanus*) 73 (39.5%), *T.trichiura* 11 (5.9%), *E. vermicularis* 4 (2.2%), *S.mansoni* 4 (2.2%) and mixed infection of *A. lumbricoides* and *N.americanus* 17 (9.2%) (Table 4. 1).

Parasite intensity was expressed as mean egg per gram 95% CI for each species (Table 4. 1) *A.lumbricoides*, 87 (55-124) eggs per gram of faeces, (*N. americanus*)15 (13-17) eggs per gram of faeces and *T.trichiura* 11 (9-13) eggs per gram of faeces.

Table 4.1: Prevalence and intensity of geohelminth infection among pregnant women.

	Prevalence	Intensity of infection			
Geohelminths		Low	Moderate	Heavy	Mean egg/
185 (24.7%)	No. % .	No. (%)	N0. (%)	No. (%)	gram 95% CI
A. lumbricoides	76 (41.1%)	53(69.7%)	20(26.3%)	3(3.9%)	89 (53.3-123.9)
N.americanus	73 (39.5%)	42(57.5%)	24(32.9%)	7(9-6%)	15.2 (13.4-16.7)
T. trichiura	11 (5.9%)	10(90.9%)	1(9.1%)	-	10.9 (8.8-12.9)
E. vermiclaris	4 (2.2%)	-	-	-	-
S. mansoni	4 (2.2%)	-	-	-	-
A.lumbricoides/	17 (9.2%)	-	-	-	-
(*N.americanus*					

- Value for calculation not available in WHO criteria.

4.1.2 Prevalence and Intensity of Malaria Parasites during Pregnancy

The overall prevalence of malaria was 162 (21.6%) with *P. falciparum* being the most prevalent species 135 (83.3%), *P. malariae* 17 (10.5 %), *Plasmodium ovale* 2(1.2 %), and mixed infection of *P. falciparum* plus *P.malariae* 8 (4.9 %). The mean malaria parasite density 95% CI in pregnant women was as follows *P. falciparum* 527.99 (405.03-750.94), *P.malariae* 312.00 (254.15-369.85) and *P.ovale* 132.00 (47.79-741.9) parasites per micro litre of blood (Parasites/µl), based on parasite species (Table 4.2).

Malaria parasite intensity was categorised regardless of the species as follows. Light infection (40-5000) parasites/µl of blood, moderate infection (5001=10,000) parasites/µl of blood and heavy infection > 10,000 parasites/µl of blood (Chesbrough, 1998). Under these categories expectant mothers with light infection were 109 (14.53%), moderate infection 42 (5.60%) and heavy infection 11 (1.47 %) (Table 4.2).

Table 4.2 Prevalence and density of malaria parasites

No. +ve for malaria parasites	*P. falciparum*	*p. malariae*	*P. ovale*	*P. falciparum* and *P. malariae*
162(21.6%)	135 (83.3%)	17 (10.5%)	2 (1.2%)	8 (4.9%)
		Mean malaria parasites/µl of blood		
P.f 528 CI (405-751)		*P.m*312 CI (254-570)		*P.o*132 CI (48-742)

P. f- Plasmodium falciparum, P. m- Plasmodium malariae, P. o- Plasmodium ovale, CI-95% confidence interval

4.1.3 Prevalence of Geohelminths and Malaria Parasite Co-infection

A total of 51 (6.8%) expectant mothers were co-infected with geohelminths and malaria parasites. *P. falciparum* and (*N.americanus*) 27 (52.9%), *P. falciparum* and *A .lumbricoides* 13 (24.9%), *P. falciparum* and *T.trichiura* 2 (3.9%), *P. falciparum* and *S.mansoni* 1 (2%), *P.malaria* and *A. lumbricoides* 4 (7.8%), *P. malariae* and (*N.americanus*) 4 (7.8%) Figure 4. 1. There was no co-infection between any geohelminth and *P.ovale*

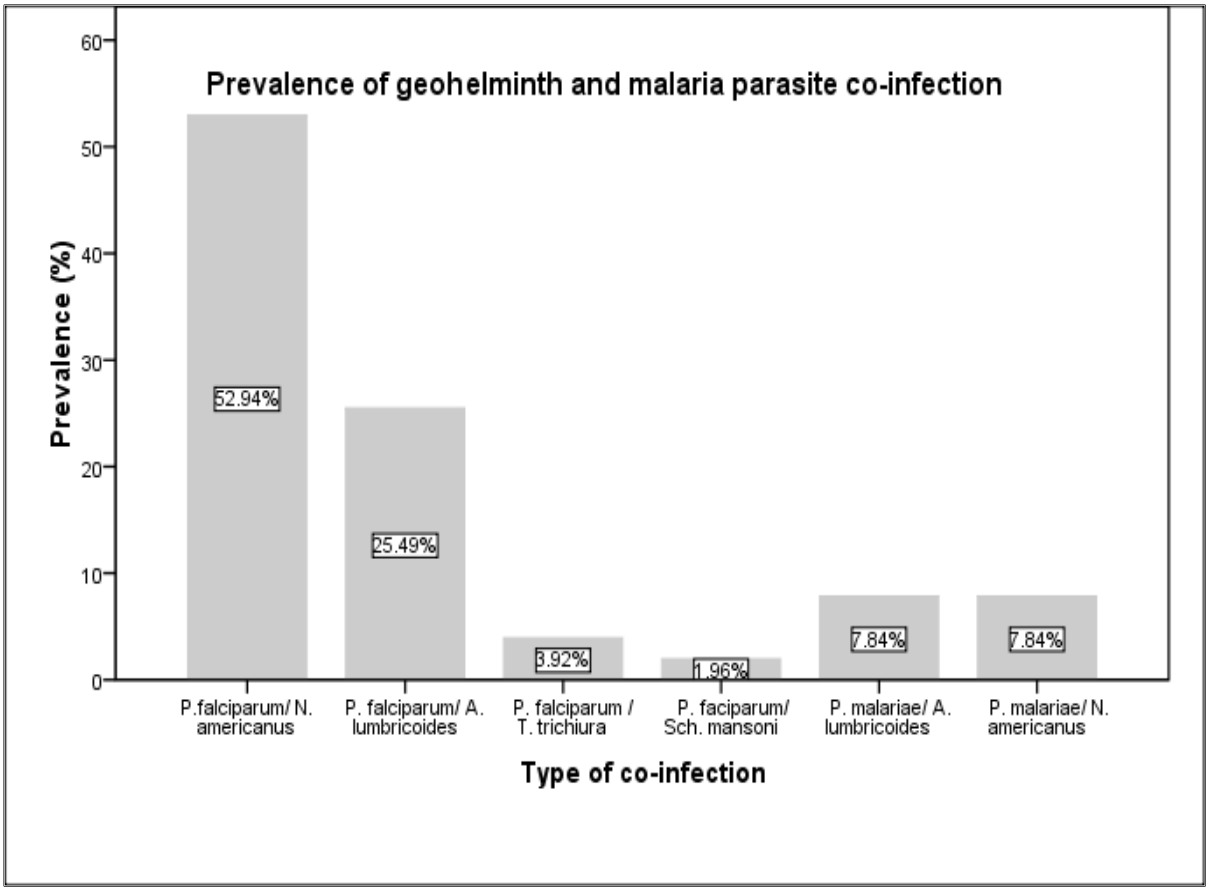

Figure 4.1 Prevalence of geohelminths and malaria parasite coinfection

4.2 Effects of Geohelminths and Malaria Parasite on Maternal Health

4.2.1 Effect of geohelminths on Maternal Health

Overall geometric mean and stardand error of mean haemoglobin level of expectant mothers were 11.47g/dl and 0.066 respectively. Expectant mothers infected with geohelminthiasis alone and had haemoglobin levels less than 11.5 g/dl were 114 (61.6%) mothers. Expectant mothers with microcytic hypochromic red blood cells (anaemia) 62 (33.5%) and those with raised eosinophil 116 (84.3%).

Infection with geohelminths was associated with low maternal haemoglobin levels chi-square 15.822 p=value 0.001 and eosinophilia chi-square 5.806, p=value 0.001 see (Table 4.3)

Table 4.3: Association between Geohelminths on maternal haemoglobin levels, peripheral blood and eosinophil levels.

Variables		Geohelminth infection		Chi-square(χ^2)	p-value
		Infected	Not infected		
Hb level	<11.5gm/dl	114(61.6%)	253(44.8%)	15.822	0.001
	>11.5gm/dl	71(38.4%)	312(55.2%)		
PBF	Microcytic hypochromic	62(33.5%)	31(5.5%)	1.008	0.009
	Normocytic normochromic	123(66.5%)	534(94.5%)		
Eosinophil count	>6 Raised	156(84.3%)	4(0.7%)	5.806	0.006
	1-6 Normal	29(15.7%)	561(99.3%)		

***** χ2 (Chi-square), p<0.05 was considered significant

4.2.2 Effect of Specific Species of Geohelminths on Maternal Health

Pearsons correlation on bivariate model revealed that *N.americanus* infection was negatively correlated to low maternal hemoglobin levels, (r= -0.534, p=0.034), microcytic hypochromic red cells, (r= -0.571, p=0.026), but no correlation with eosinophilia, (r=0.234, p=0.056). Egg threshold count of *N.americanus* with haemoglobin was found at > 2,000 epg. There was no correction between *T.trichiura infection* with maternal haemoglobin level, and microcytic hypochromic red cells, (r= -0.346, p=0.297) and (r= -0.147, p= 0.662) respectively, but no correlation with

eosinophilia (r= 0.194, p=0.568) respectively. *Ascaris lumbricoides* infection was not correlated to maternal haemoglobin level, microcytosis hypochromasia but there was a correlation with eosinophilia, (r=0.094, p=0.417), (r=0.067, p= 0.562) and (r=0.503, p=0.038) respectively.

Multivariate analysis showed that mothers infected with geohelminths were 3 fold likely to have hyochromasia (Table 4.4)

Table 4.4: Effect of geohelminthiasis on maternal health

Variable	Geohelminthiasis		
	P-value	OR	95%CI
Haemoglobin level <11.5gm/dl	0.031	2.505	2.260-5.109
Peripheral blood.film Microcytic hypochromic	0.041	3.683	3.407-7.943
Eosinophil count >6% Raised	0.051	1.540	1.613- 3.700

***** 95% CI=Confidence interval, OR= Odds ratio, p<0.05 was considered significant.

4.2.3 Effect of Malaria on Maternal Health

Malaria in the curent study was associated with low maternal haemoglobin level, microcytic hypochromic red blood cells and eosinophilia, χ^2 47.258 p= 0.002, χ^2 69.262, p= 0.003 and χ^2 15.953, p= 0.006 respectively (Table 4. 5)

Table 4.5: Effect of malaria on maternal haemoglobin levels, peripheral blood and eosinophil levels.

Variable	Malaria infection		chi-square ($\chi 2$)	p-value
	Infected	Not infected		
Haemoglobin level	<11.5gm/dl	118 (72.8%)	249 (42.3%)	47.258	0.002
	>11.5gm/dl	44 (27.2%)	339 (57.7%)		
Peripheral Blood Film	Microcytic hypochromic	51 (31.5%)	42 (7.1%)	69.262	0.003
	Normocytic normochromic	111 (68.5%)	546 (92.9%)		
Eosinophil count	>6 Raised	53 (32.7%)	107 (18.2%)	15.953	0.006
	1-6 Normal	109 (67.3%)	481 (81.8%)		

***** χ^2= Chi-square, $p<0.05$ was considered significant,

4.2.4: Effect of Individual Species of Malaria Parasites on Maternal Health

Pearsons correlation showed that infection with *P.falciparum* was correlated to low maternal haemoglobin level and microcytosis hypochromasia, (r=0.933, p=0.040) and (r=0.689, p=0.051) respectively. The correlation was statistically significant. There was no acorrelation between *P.falciparum* and eosinophilia (r= 0.146, p=0.933). *P.malariae* infection was correlated to low maternal haemoglobin level, (r= -0.622, p= 0.008). Although there was a weak correlation between *P.malariae* infection and microcytic hypocromic red blood cells (r= .364, p=0.779) this correlation was not statistically significant. There was no correlation between *P.malariae* infection and eosinophilia (r= -0.07, p=0.151)

Multivariate analysis revealed that malaria alone was associated with low maternal haemoglobin (anaemia) OR 2.274, p=0.041. Mothers with malaria were 5 fold likely to have microcytic hypochromic red blood as compared to non infected (Table 4.6).

Table 4.6: Effect of malaria alone with maternal health

Variable	Malaria		
	P-value	OR	95%CI
Haemoglobin level <11.5gm/dl	0.041	2.274	1.187-5.402
Peripheral blood.film Microcytic hypochromic	0.051	4.973	3.784-9.429
Eosinophil count >6% Raised	0.054	2.186	1.481-3.22

***** 95% CI=Confidence interval, OR= Odds ratio, p<0.05 was considered significant.

4.2.5: Effect of Geohelminths and Malaria parasite Co-infection on Maternal Health.

Co-infection of *N.americanus* and *P.falciparum* in the current study was associated with low haemoglobin, χ^2 10.288, p=0.006 A. *lumbricoides* and *P.falciparum* co-infection did not show any influence on the low haemoglobin, χ^2 1.680, p=0.891. Expectant mothers 24(47.1%) co-infected with *N.americanus* and *P.falciparum* had microcytic hypochromic red cells depicting iron deficiency anaemia, this association was statistically significant, χ^2 24.866, p=0.001. Although 25(51%) mothers co-infected *A.lumbricoides* and *P.falciparum* had eosinphia, this association was not statistically significant χ^2 0.943, p=0.967

Multivariate regression analysis showed that expectant mothers co-infected with geohelminths and malaria are 6 times likely to have low haemoglobin level as

compared to those infected with either geohelminths or malaria alone p=0.009 OR 4.137 (2.088-8.195) (Table 4.7)

Table 4.7: Effect of geohelminthiasis and malaria co-infection with maternal Health

Variable	Geolminthiasis and Malaria Co-infection		
	P=value	OR	95%CI
Haemoglobin <11.5gm/dl	0.009	6.137	2.088-8.195
Peripheral b.film Microcytic hypochromic	0.031	5.057	4.96-9.016
Eosinophil count >6% Raised	0.051	3.290	3.110-5.414

***** 95% CI=Confidence interval, OR= Odds Ratio, p<0.05 was considered significant.

4.3 The Impact of Geohelminthiasis and Malaria Co-infection on Neonatal Outcomes

Out of 185 pregnant women infected with geohelminths alone, 18(9.7%) had preterm deliveries 7(3.8%) abortions, 11(5.9%) still birth, 27(14.0%) low birth weight and on APGAR score neonates who had Low chances of survival were 3(1.6%), those born dead were 11(5.9%). Out of 162 Mothers with malaria alone 26(16%) had preterm deliveries 5(3%) abortions, 8(4.9%) still birth, 36(22.0%%) low birth weight and on APGAR score neonates who had low chances of survival were 7(4.3%), those born dead were 8(4.9%) (Table: 4.8). Geometric mean of neonates in relation with gestation perid was 1.0555 Kg with starndard error mean of 0.0119 Kg at birth.

Table 4.8: Association of geohelminthiasis and malarian with neonatal outcomes

	Geohelminthiasis		Malaria	
Variable	Infected	Not infected	Infected	Not infected
Gestation period				
>37 weeks Term delivery	160(86.5%)	538(95.2%)	131(88.7%)	567(96.4%)
<37 weeks preterm delivery	18(9.7%)	23(4.1%)	26(16.0%)	15(2.6%)
< 28 weeks abortion	7(3.8%)	4(0.7)	5(3%)	6(1.0)
Chi-square	18.296		49.334	
P-value	0.001		0.001	
Neonatal condition at birth				
Live birth	174(94.1%)	553(97.8%)	154(95.1%)	573(97.5%)
Fresh still birth	2(1.1%)	6(1.1%)	2(1.2%)	6(1.0%)
Macerated still birth	9(4.8)	6(1.1%)	6(3.7%)	9(1.5%)
Chi-square	10.288		3.128	
P-value	0.006		0.209	
Birth weight				
>2500g Normal birth	158(85.4%)	532(94.2%)	126(77.8%)	564(95.9%)
<2500g Low birth eight	27(14.6%)	33(5.8%)	36(22.0%)	24(4.1%)
Chi-square	14.511		56.788	
P-value	0.001		0.001	
APGAR score				
10 Normal	153(82.8%)	531(94%)	130(80.3%)	554(94.2%)
7-9 High chances of survival	18(9.7%)	15(2.7%)	17(10.5%)	16(2.7%)
<6 Low chances of survival	3(1.6%)	7(1.2%)	7(4.3%)	3(0.5%)
0 Dead	11(5.9%)	12(2.1%)	8(4.9%)	15(2.6%)
Chi-square	24.590		36.351	
P-value	0.001		0.001	

*****$p<0.05$ was considered significant**.**

4.3.1 Association of Geohelminthiasis with Neonatal Outcome

Necator americanus infection was associated with low birth weight Chi-square 17.303, p=value 0.003, still birth Chi-square 22.547 p= 0.018 and on APGAR score, neonates with low chances of survival at bith due to infection with *N.americanus*, Chi-square 38.908, p= 0.001. Although 6 % (n=11) expectant mothers had *N.americanus*, this was not significantly associated with Preterm delivery Chi-square 15.529, p=0.114.

Multivariate analysis revealed that mothers infected with geohelminths were 6 fold and 3 fold likely to have preterm deliveries and neonates with low chances survival (Table 4.9)

Table 4.9: Association of geohelminthiasis with neonatal outcome

Variable	Geohelminths		
	P-value	OR	95% CI
Gestation period			
< 37 weeks Preterm delivery	0.045	5.884	1.701-9.356
< 28 weeks Abortion	0.251	2.236	0.566-8.841
Neonatal condition at birth			
Fresh still birth	0.053	4.767	1.673-11.582
Macerated still birth	0.122	4.500	0.670- 10.230
Birth weight			
Low birth weight	0.049	2.755	1.607-4.721
APGAR			
<6 Low chances of survival	0.051	3.181	1.377-7.352
7-9 High chances of survival	0.621	0.764	0.263-2.221
Dead	0.346	2.137	0.440-10.391

***** 95% CI=Confidence interval, OR= Odds Ratio, p<0.05 was considered significant.

4.3.2 Association of Malaria with Neonatal Outcome

Infection with *P.falciparium was* associated with low birth weight Chi square 18.405, p=value 0.006, and on APGAR scale *P. falciparium* infection was significantlly associated with low chances of neonatal survival, Chi square 22.468, p=value 0.049.

Multivariate analysis showed that expectant mothers infected with *Plasmodium* parasites were 3 times likely to have new-borne babies of low birth weight p=value 0.051 OR 3.114 (2.868-8.654) (Table 4.10)

Table 4.10: Association of malaria alone with neonatal outcome

Variable	Malaria		
	P-value	OR	95% CI
Gestation			
< 37 weeks Preterm delivery	0.036	2.607	1.084-6.998
< 28 weeks Abortion	0.286	0.481	0.225-1.848
Neonatal condition at birth			
Fresh still birth	0.039	2.481	0.870-7.076
Macerated still birth	0.476	2.000	.298-13.435
Birth weight			
Low birth weight	0.051	3.114	2.868-8.654
APGAR			
<6 Low chances of survival	0.047	2.273	0.944-5.474
7-9 High chances of survival	0.218	0.502	0.168-1.501
Dead	0.074	0.229	0.046-1.134

***** 95% CI=Confidence interval, OR= Odds Ratio, p<0.05 was considered significant.

4.3.3 Association of Geohelminthiasis and Malaria Co-infection and Neonatal outcome

Multivariate analysis revealed that pregnant women co-infected with geohelminthes (*N. americanus*) and *Plasmodium* parasites were more likely to experience an adverse birth outcome. *N.americanus* and *Plasmodium* co-infection was associated with stillbirth, p=value 0.018 OR 5.701, (4.008-12.579), preterm deliveries p-value 0.006 OR 6.896 (1.755-.27.105) and on APGAR scale, neonates with low chances of survival, p=value 0.025 OR 5.310, (3.860-14.162). (Table 4.11)

Table 4.11: Association of geohelminthiasis and malaria co-infection and neonatal outcome

Variable	Co-infection		
	P-value	OR	95% CI
Gestation period			
< 37 weeks Preterm delivery	0.006	6.896	1.755-12.101
< 28 weeks Abortion	0.897	0.906	0.205-4.012
Neonatal condition at birth			
Fresh still birth	0.018	5.701	4.008-12.579
Macerated still birth	0.750	0.750	0.098-5.768
Birth weight			
Low birth weight	0.004	4.186	3.094-8.369
APGAR			
<6 Low chances of survival	0.025	5.310	3.860-14.162
7-9 High chances of survival	0.478	0.639	0.185-2.204
Dead	0.911	1.111	0.177-6.990

***** 95% CI=Confidence interval, OR= Odds Ratio, p<0.05 was considered significant.

4.4: Social Economic Risk Factors

4.4.1: Socio Economic Characteristics by Participants Infection Status

Expectant mothers of age group 18-27 years were more infected with geohelminths 140 (75.7%), malaria parasites 128 (79%) and co-infection 42 (82.4 %) as compared to their counterparts in age group, 28-37 geohelminths 40 (21.6%) malaria 32

(19.8%) and co-infection 9 (17.6 %). While age group 38-49 geohelminths 5 (2.7%), malaria 2 (1.2%) and co-infection 0 (0%), χ^2 2.151 p=0.341, χ^2 5.086 p=0.079 and χ^2 0.341 p=0.184 respectively.

Mothers staying in rural area were more infected with geohelminths 156 (84.3%), malaria parasites 129 (79.6%) and co-infection 40 (78.4%) as compared to those in urban residence; geohelminths, 29 (15.7%) malaria, 33 (20.4%) co-infection 11 (21.6%), χ^2 2.709 p=0.001, χ^2 0.330 p=0.467, χ^2 0.100 p= 0.435 respectively. Mothers living in semi-permanent houses were more infected with geohelminths 136 (73.5%), malaria 110 (67.9%), co-infection 39 (76.5%) as compared to those staying in permanent houses; geohelminths 49 (26.5%), malaria 52 (32.1%) and co-infection 12 (23.5%), χ^2 8.174 p=0.002, χ^2 0.871 p=0.201, χ^2 3.268 p=0.046 respectively.

Expectant mothers whose water source was well were more infected with geohelminths 81 (43.8%) malaria 61 (37.7%) and co-infection 21 (41.2%) as compared to those using tap water; geohelminths 56 (30.3%), malaria 50 (30.8%) and co-infection 13 (25.5%), χ^2 7.468 p=0.024, χ^2 3.239 p=0.198 and χ^2 2.970 p=0.227 respectively. Mothers with primary level of education were more infected with geohelminths 102 (55.1%), malaria 79 (48.8%) and co-infection 30 (58.8%) as compared to their counterparts who had higher level of education (College); geohelminths 16 (8.7%), malaria 22 (13.6%) and co-infection 2 (3.9%), χ^2 22.786 p=0.001, χ^2_4. 366 p=0.359,and χ^2 11.948 p=0.018 respectively.

Unemployed mothers were more infected with geohelminths 95 (51.4%), malaria 87 (53.7%) and co-infection 37 (72.6%) as compared to employed counterparts geohelminths 23 (12.4%) malaria 23(14.2%) and co-infection 2 (3.9%), $\chi^2$11.999 p=0.007, χ^2 8.738 p= 0.033 and $\chi^2$18.604 p=0.001. Pregnant women in their 2nd trimester were more infected with either geohelminths 137 (74.1%), or malaria 107 (66.1%) and coinfection 40 (78.4%) compared to their counter parts in 1st trimester; geohelminths 30 (16.2%), malaria 31 (19.1%) and co-infection 5 (9.8%). Whose in 3rd trimester; geohelminths 18 (9.7%), malaria 24 (14.8%) and co-infection 6 (11.8%), χ^2 3.478 p=0.176, χ^2 8.327 p= 0.16 and χ^2 4.309 p=0.116 respectively.

4.4.2: Risk Factors for Geohelminthiasis

Risk factors for geohelminths alone included: Not washing of hands after visiting the toilet p=0.001 OR 3.286 (1.669-6.476), Unemployment p=0.001 OR 2.295(1.389-3.990), use of borehole water p=0.008 OR 1.435(0.948-2.173). Not wearing protective shoes while working on the farm, p=0.010 OR 0.0412(2.10-8.080) and staying in semi-permanent house p=0.008 OR 1.650 (1.138-2.394) (Table 4.12)

Table 4.12: Risk Factors for geohelminthiasis

Variable	Geohelminthiasis		
	p-value	OR	95% CI
Waste disposal			
Pit latrine	0.050	2.002	1.001-4.002
Flash toilet	0.078	0.534	0.266-1.072
Shoe wearing			
Doesn't wear	0.010	0.412	2.10-.8.080
Wears shoes	0.630	1.216	0.548-2.699
Hand washing			
Does not	0.001	3.286	1.669-6.476
Use water only	0.003	1.653	1.299-2.777
Water and soap	0.014	0.482	1.109-2.463
Housing			
Semi permanent	0.008	1.650	1.138-2.394
Permanent	0.092	0.286	0.071-1.238
Water source			
Tap water	0.088	1.435	0.948-2.173
Well	0.009	1.694	0.488-1.851
Employment			
Unemployed	0.001	2.295	1.389-3.990
Employed	0.683	1.079	0.748-1.557

***** 95% CI=Confidence interval, OR= Odds Ratio, $p<0.05$ was considered significant.

4.2.3: Risk Factors for Malaria

Risk factors for malaria infection among expectant mothers in this study included unemployment p=0.002 OR 9.588 (2.281-40.304), lack of malaria treatment p=0.015 OR 3.615 (1.285-10.167), not sleeping under treated mosquito net p=0.001 OR 3.220 (2.019-8.138) and 2nd trimester of pregnancy p=0.006 OR 2.126 (1.238-3.651) as ssen in (Table 4.13).

Table 4.13: Risk factors for malaria

Variable	Malaria		
	P-value	OR	95% CI
Malaria Treatment			
No treatment	0.015	3.615	1.285-10.167
More than 6 months	0.078	0.520	.251-1.075
Mosquito net use			
Does not use	0.001	3.220	2.019-5.138
Not always	0.001	0.035	0.004-0.276
Other treatment			
Herbal treatment	0.008	9.931	1.793-48.551
Employment			
Unemployed	0.006	2.034	1.228-3.371
Employed	0.149	1.333	0.92-1.970
Trimester			
1st	0.040	0.708	0.509-0.984\
2nd	0.006	2.126	1.238-3.651
3rd	0.013	2.235	1.186-4.211

***** 95% CI=Confidence interval, OR= Odds Ratio, P<0.05 was considered significant.

4.4.4: Risk Factors for Geo helminthiasis and Malaria Co-infection

Co- infection risk factors included unemployment P=0.002 OR 9.588 (2.281-40.304. Women in their 2nd semester of pregnancy were 2 fold more likely to be co-infected by geohelminths and malaria as compared to their counter parts in their 1st and 3rd trimester P=0.082 OR 2.961(0.872-10.060) as shown in (Tale 4.14).

Table 4.14: Risk factors for geo helminthiasis and malaria co-infection

Variable	Co-infection		
	P-value	OR	95% CI
Residence			
Rural	0.062	1.118	0.559-2.235
Urban	0.743	1.214	0.657-3.436
Housing			
Semi-permanent	0.074	1.832	0.942-3.563
Permanent	0.094	0.546	0.281-1.061
Water source			
Tap water	0.139	1.713	0.840-3.490
Well	0.881	0.950	0.488-1.851
Employment			
Unemployed	0.002	9.588	2.281-40.304
Employment	0.082	2.961	0.872-10.060
Trimester			
1st	0.730	1.712	0.477-2.874
2nd	0.009	1.824	1.254-4.818
3rd	0.082	2.961	0.872-10.060

***** 95% CI=Confidence interval, OR= Odds Ratio, P<0.05 was considered significant.

CHAPTER FIVE

5.0 DISCUSSION

The study provided data on the effects of geohelminths and malaria parasites co-infection among pregnant women in Bungoma County. Geohelminths and malaria parasites were common in the study participants. The parasites were associated with adverse effects both to the mother and the unborn child. Effects of *P. malariae* and *P. ovale* infection on maternal health and her unborn baby were assessed. Effect of geoheohelminths and malaria parasite co-infection on neonatal APGAR score was determined. Potential risk factors for geoheminths and malaria parasites co-infection were determined.

5. 1 Prevalence and Intensity of Geohelminths and Malaria in Pregnant Women

5.1.1 Prevalence and Intensity of Geohelminths

Geohelminths were common among pregnant women in Bungoma County. This study recorded an overall prevalence of 24.7% for geohelminth infections, with *A. lumbricoides* as the dominant parasite species, followed by *N.americanus*. These findings suggest that the study participants reside in areas characterized by high transmission and re-infection. Further more the high prevalence can be explained by the fact that pregnant women are often excluded from national de-worming programme hence remain a persistent source of faecal contamination within their surroundings.

The prevalence was comparable with what was obtained in a similar study in Ghana by Baido *et al* (2014). The study findings are however lower than what was reported by other studies, in Ethiopia by Yeshambel *et al*., (2010), Ghana by Yatich *et al*., (2010) and in Kenya Van Eijik *et al*., (2009) that showed higher prevalence but lower than what was observed in Ghana by Ntui *et al*., (2014). The variations may be attributed to differences in geoghraphical and climatic conditions that influences development, distribution and survival of infective stages of parasites. These finding agrees with a study in Kenya that partialy modeled the transmission and distribution of geohelminthiasis and reported Western and Coastal regions warranted for mass drug administration (Pullan *et al*., 2011).

Geohelminths prevalence by species in this study was higher as compared to those in the study done by Samuel (2017) where *A.lumbricoides* had 8.5%, *N. americanus* 4.0%, %), and in another study in Ephiopia (Millionn *et al*., 2013) species prevalence were *A.lumbricoides* 14.9%, *N.americanus* 29.4%, *T.trichiura* 3.4% and *E.vermicularis* 1.3% . People are exposed to different species of geohelminths differently and thus the prevalence differs in each individual. In addition difference in soil type of a particular area may influence the prevalence of infection. Therefore different control strathegies should target particular areas.

5.1.2 Prevalence and Intensity of Malaria parasites

Malaria is the most serious public health problem in Kenya. In this study malaria prevalence was 21.6% with *P.falciparum* being the most prevalent species. *P.malariae* and *P.ovale* were also common. Malaria prevalence reported in this study

was lower than the results of similar studies done among pregnant women in Cameroon (Francis *et al*., 2014) where the prevalence was 77.2 %.), while at coastal region of Kenya (Fairley 2013), it was 32%. The difference in prevalence may be linked to mosquito treated nets given to all pregnant mothers free of charge during antenatal clinic visits, as one of the Kenya government malaria control strategy. The study results are comparable with a study in Ghana where the prevalence was 25.7% (Yatich *et al*., 2010). The observed prevalence in this study still needs more effort to be intensified in controlling malaria in Bungoma County.

The findings had a higher prevalence than that of of a similar study in Ethiopia 11.6% (Million *et al*., 2013) and 16.5% in Ghana (Samuel *et al*., 2017). Observed differences could be attributed to differences in the geo-ecological and climatic conditions that might influence malaria vector breeding and *Plasmodium* parasite distribution in different areas. For instance rainy season has a lot of stagnant water that favors breeding of *Anopheles* mosquito vectors. A study by Bigoga *et al.*, (2012) showed that mean intensity of malaria species specific infections vary considerably by individuals. The differences in the intensity of malaria infection in this study could therefore be attributed to the difference between the period of infection, the infection rate, and the preventive measures (chemoprophylaxis) at individual level that can influence the infection intensity.

5.1.3 Prevalence of Geohelminths and Malaria parasites Co-infection

Geohelminthiasis and malaria share endemicity in Kenya. Geohelminths and malaria parasites co- infection prevalence of 6.8% in this study was comparable to a study in

Ethiopia 7.7% (Million *et al*., 2013), but lower than 22.1% Cameroon (Francis *et al*., 2014) and 16.6% in Ethiopia (Yatich *et al*., 2009). *P.falciparum* and *N. americanus* in this study constituted the main co-infection contrary to *P.falciparum* and *A.lumbricoides* as the main co-infection (Francis *et al*., 2014). This difference may be attributed to climatic and environmental condition of each community**.** The observed prevalence of parasitic infection in this study is indicative that efforts should be intensified in controlling these infections in pregnant women of Bungoma County. Therefore the study findings support the World Health Organization's resolution for an integrated approach to improve tropical disease surveillance and control (WHO, 2010).

5.2. Effects of Geohelminths and Malaria parasites Burden on Maternal Health

5.2.1 Effects of Geohelminths on Maternal Health

The present study showed that geohelminths infection alone increased the risk of maternal low haemoglobin level (anaemia) and hypochromic microcytic blood cells. Over 61.6% of pregnant women with geohelminthiasis alone were anaemic. The finding of this study is in corroboration with study findings conducted in Nigeria where 61.1% pregnant mothers were anaemic as aresult of geohelminthiais (Tay *et al*., 2013). *N. americanus* infection in the current study was significantly associated with decreased maternal haemoglobin level. The present study findings are in agreement with previous studies which showed that geohelminth infections, specifically infections with Schistosomes, hookworms (*N.americanus* and *A.duodenale*) and *T.trichura,* have been demonstrated to be associated with iron deficiency anemia (Yatich *et al.,* 2010, Ntui. *et al*., 2014). Pregant women are

vulnerable to iron deficiency anaemia because of high demand due to rapid cell and tissue development of the foetus (Burke *et al*., 2014).

This study reported lower *N. americanus* related anaemia in pregnant women than what was reported in a similar study at the Coastal region of Kenya where 71% of pregnant women infectd with hookworm were anaemic (Mac clure *et al*., 2014). The observed prevalence of anaemia in mothers infected with *N.americanus* is attributed to the fact that, majority of women in this County spends most of their time in farms without protective foot and hand ware. This puts them at risk of getting infected with hookworm, whose mode of transmission is through skin penetration by filariform larvae.

The findings of anaemia in mothers due to infection with *N.amerinus* in this study were higher compared to 53.5% observed in Southwest Ethiopia (Gatachew *et al*., 2012). Hookworms are said to be the most important cause of pathological blood and iron loss in the tropics, these worms cause anaemia through blood loss, impaired nutrient absorption, and damage to the mucosal lining (Yatich *et al.*, 2010). The current study findings suggest that iron supliments (haematinics) should be given to all expectant mothers to reduce anaemic cases in this County.

5.2.2 Effects of Malaria on Maternal Health

In this study, a high proportion 72.8% of pregnant women with malaria parasites alone had low haemoglobin (anaemia) and the association was statistically

significant. Most of the anaemia resulted from *P.falciparum* even though *P.malariae* and *P.ovale* infection also contributed to low maternal haemoglobin level. Malaria in pregnancy has been reported as a major cause of maternal anaemia, particularly in malaria endemic region especially in Sub Saharan Africa (WHO, 2017), hence the observation that most malaria parasite positive mother were anaemic.

Malaria contributes to anaemia by various mechanisms, including hemolysis or the direct destruction of parasitized red blood cells, defective red cell production, and shortened red cell survival (Yatich *et al*., 2010). The malaria pathogen acts by directly destroying erythrocytes while malaria pigments and pro-inflammatory mediators may also inhibit erythropoiesis. Malaria pigments and pro-inflammatory mediators may also inhibit erythropoiesis (Quedraogo *et al*., 2012). The study findings suggest that prompt diagnosis and effective malaria treatment among pregnant mothers in this area is important.

5.2.3 Effects of Coinfection on Maternal Health

Geohelminths and malaria parasites co-infection was associated with 6 fold increased risk of anemia. A comparison of co-infected women with women infected with malaria or geohelminthiasis alone showed that co-infection substantially increased the risk of low hemoglobin level (anaemia). These results are in agreement with a study in Ghana which revealed that co-infection between geohelminthiasis and malaria substantially increased the risk of anaemia in pregnancy (Yatich *et al*., 2010).

Francis *et al*., (2014) also observed a high prevalence of anemia in mothers co-infected with Hookworm and *P. falciparum*.

The increase of anaemia in co-infections is due to additive effects in the mechanisms of these infections on total haemoglobin concentration. Hookworm is associated with chronic loss of blood and iron in gastrointestinal tube, while *P.falciparum* is associated with bursting and loss of red blood cells during its blood stage, all contributing to haemoglobin loss and anaemia.(Francis *et al*., 2014). Mechanisms by which geohelminthiasis and malaria affect haemoglobin levels are distinct but their combined manifestation enhances the risk of anaemia in pregnant women.

Maternal anaemia in the current study was as aresult of geohelminthiasis and malaria. Anaemia due to parasitic infection is depicted by microcytic hypochromic red blood cells and also individuals infected with parasites have raised eosinophils (Cheesbrough, 1998), which was the case in the current study. Anaemia due to nutritional factors for example presents with normocytic normochromic red blood cells (Cheesbrough, 1998), a factor that was not studied in the curremt study.

Maternal anaemia due to geohelminths and malaria coinfection adversely affected birth out comes in this study because the variance of treatment which was the parasite load was compared with error variance of non studied factors. If anaemia was due to other factore for instance haemoglobinopathies (sickle cell, thalassemia,

G6PD deficiency) genetics, and environmental factors nutritional status during pregnancy and metabolic diseases, the error variance compared to variance due to parasitic treatment could have been larger which was not the case. Therefore anaemia due to other factors may have been of less significance in this study.

Haematological manifestations such as microcytosis and eosinophilia observed in expectant mothers in this study have been associated with parasitic infections that cause blood loss such as *P. falciparum* infection in a previous study (Kimbi *et al*., 2013). Microcytic hypochromic red blood cells observed in this study suggest iron deficiency anaemia, therefore haematinics should be given to mothers diagnosed with microcytosis. Microcytic hypochromic red blood cells signify iron deficiency anaemia while raised eosinophils denote presence of parasitic infections (Cheesbrough, 1998).

5.3 Effects of Geohelminths and Malaria Co-infection on Neonatal Outcomes

5.3.1 Effects of Geohelminths on Birth Outcomes

In this study expectant mothers infected with geohelminths alone especially *N. americanus* were 3 fold likely to have low birth weight babies, 5 fold still birth, 6 fold preterm deliveries and on APGAR scale 3 fold low chances of neonatal survival. This finding agrees with Ntui *et al*., (2014) whose findings revealed that hookworm infection causes blood loss that result into iron deficiency anaemia which is associated with adverse birth outcomes. These results are in disagreement with the findings of a study done in Ghana that did not find any association between

geolminths infection and stillbirth (Yatich *et al*., 2010). The fact that Yatich's study did not categorize the helminths according to their association with blood loss as was done in this study could explain the absence of such an association.

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5.3.2 Effects of Malaria Parasites on Birth Out come

Results of this study revealed that peripheral malaria infection during pregnancy contribute to preterm delivery and lower birth weight. The study findings are attributed to the fact that preventive efforts, for example insecticide-treated bed nets still leave out a large proportion of pregnant women with parasitaemia that may eventually lead to anaemia. A similar association between malaria infection with fever and an increased risk of miscarriage has been reported in mothers with malaria during the first trimester of pregnancy (McGready *et al*., 2012) though current WHO year policy calls for "the administration of at least two doses of SP during the second and third trimester's of pregnancy" (McGready *et al*., 2012). Likewise, increased infant mortality has been reported after symptomatic malaria infections occurring at the end of the pregnancy (Boardaji *et al*., 2011). These findings therefore suggest prompt malaria screening and treatment to all pregnant women when they present to antenatal care to reduce adverse effects of the infection.

5.3.3 Effects of Co-Infection on Birth Outcomes

The findings of this study revealed that geohelminths and malaria parasite co-infections were associated with adverse birth outcomes. As compared to non co-infected counterparts, expectant mothers co-infected with geohelminths and malaria parasites were 4 times likely to have low birth weight babies, 6 times likely to have

still births, 7 times likely to have preterm deliveries and 5 times likely to have newborn babies with low chances of survival. Geohelminths and malaria parasite co-infection, associated with adverse birth outcomes in this study highlights the serious adverse health impact of these parasites. Therefore expectant mothers should be treated promptly.

The study results are higher compared with findings from Ntui *et al*., (2014), whose study revealed that women who had both malaria parasites and intestinal helminths were 3.0 fold and 2.6 fold at risk of low birth weight and Preterm deliveries respectively. The differences would be attributed to the differences in methodology, area of study sample, size used. Ntuis study compared effects of geohelminths malaria coinfection in treated pregnant women mothers and untreated pregnant women.

Women who began antenatal care in their second trimester had an increased risk of having a low birth weight infant as compared to women who began antenatal care in their first trimester. These findings suggest that women who began antenatal clinic early tend to have more visits and therefore more medical evaluations. This finding differ with Joeseph *et al*., (2016) in Nigeria who did not record any case of still birth and low birth weight among pregnant mothers with co-infection of malaria and geohelminthiasis.

5.4 Risk Factors of Geolminths, Malaria Parasites and Co-infection

Risk factors for geohelminthiasis in this study were unemployment, not wearing protective shoes while working on the farm, staying in semi-permanent house, soil eating and use of untreated well water. These findings agree with (WHO, 2011) which revealed that geohelminthiasis affect the poorest and most deprived communities. This finding also agree with a study in Gabon by Noe *et al*., (2018) that identified risk factors for geohelminthiasis to be drinking untreated water and living in a rural area. Although toilet facility coverage in Kenya was estimated at 78% coverage in urban areas and 48% coverage in rural set ups (WHO, UNICEF 2013), the observed prevalence of geohelminths among expectant mothers may indicate faecal contamination of the soil in the study area.

The presence of eggs in the soil is indicative of faecal pollution corroborating the inadequacy of toilet facilities in the area. It is possible that the use of an unprotected water source example well and the absence of water treatment were markers for soil contamination. Therefore transmission of geohelminths through water contaminated with infected faeces and lack of washing hands after visiting the toilet may be additional sources of infection. Therefore, in addition to the provision of appropriate means of faecal disposal in rural communities, there is a need to incorporate behavioral change and health education in the control programme, if the desired goal of geohelminhth elimination is to be achieved in this County.

Malaria risk factors were low income due to unemployment, lack of mosquito net use, staying in rural area and age group 18-27 years. These findings support the idea that it is essential to scale up malaria prevention efforts in more isolated and deprived

communities as it was highlighted in a meta-analysis of data sets from 25 African countries (Eisele *et al*., 2012). These finding suggests that geo-ecological conditions in the rural area of Bungoma County might influence malaria vector breeding and distribution. High mosquito breeding may influence malaria transmission. Therefore integrated effort of controlling malaria transmission especially in rural areas is essential.

Polyparasitism is a marker of poor sanitation and poverty (Amare *et al*., 2015, Dellaimy *et al.,* 2014). Risk factors for co-infection of geohelminthiasis and malaria in this study were identified as rural residence, semi-permanent house, Primary level of education, 2nd Trimester though not statistically significant. Unemployed expectant mothers with low income in this study were ten times at risk of co-infection as compared to employed mothers. Low income due to unemployment may lead to poverty. Incidences of poverty levels are estimated between 56 and 60% in Bungoma County (WCP., 2012).

In most cases unemployment means low income that could lead to poverty, which can be a predisposing factor to geohelminths and malaria parasites. The study agree with other research findings which found that , inadequate personal hygiene, an unsafe water supply, low levels of education, and being unemployed were associated with multiple parasitic infections (Ngui *et al*., 2011, Njenga *et al.*, 2011, Staundacher *et al*., 2014,). Therefore, targeting the possible risk factors identified in this study may reduce geohelminthiasis and malaria co-infection in pregnant women.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study findings revealed that both geohelminths and malaria parasites are prevalent in expectant mothers of Bungoma County.

Geohelminths and malaria parasites co-infection were associated with low maternal haemoglobin level and consequently, increases the risk for anaemia in expectant women. Anaemic pregnant women co-infected with geohelminthiasis and malaria had microcytosis and hypochromasia indicating iron deficiency anaemia.

Geohelminths and malaria parasites co-infection in this study were associated with still birth and Preterm deliveries. On APGAR scale, co-infection between geohelminths and malaria parasites was associated with low chances of neonatal survival at birth.

Risk factors associated with geohelminths and malaria parasite co-infection among pregnant women in Bungoma County were unemployment (low income), second trimester of pregnancy , age group between 18-27, rural residence, staying in a semi-permanent house, and primary level of education,

6.2 Recommendation

Interventions targeting parasitic co-infections in pregnant women should be prioritized and incorporated into antenatal programs.

Routine screening for geohelminthiases and malaria should be included in antenatal clinic profile. Early diagnosis and treatment of these infections will reduce anaemia related effects both to the mother and her baby.

Geohelminthisis and malaria observed in this study can be contained by provision of safe treated water, improvement of personal and environmental hygiene, community-based health education. To realize the impact of treated mosquito nets supplied to expectant women, they should be educated on the impotance of their use.

6.3 Suggestions for further research

More detailed studies including and not limited to pathogenesis, full blood count would be useful in assessing the impact of geohelminthiasis and malaria co-infection in pregnant women.

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APPENDICES

APPENDIX A: Informed Consent Form for Collecting Stool and Blood Samples Masinde Muliro University of Science and Technology,

Department of Biological Sciences

Title of the study: Effects of geohelminthiasis and malaria co-infection on maternal health and birth outcomes in Bungoma County, Kenya

Greetings: My name is Antony Wanyonyi Wekesa from Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Tel 0712 438 437. You are requested to participate in a study that is being done at Bungoma County referral hospital antenatal clinic. Kindly read this document and ask any question(s) on any issue(s) that may be unclear to you, before consenting to participate in this study.

Purpose and duration of the study:

This study is to investigate Effects of geohelminthiasis and malaria on maternal health and birth outcomes in Bungoma County, Kenya. Part of this study will be the research related tests done on your faecal and blood samples after being provided. The study will take nine months from now and each expectant mother will have a contact period of 20 minutes with the researcher. Otherwise you will receive antihelminthic or antimalaria treatment if found infected.

Study methodology: In the event that you consent to be recruited into the study, you will be examined to determine whether or not you meet the criteria set for recruitment into the study. A little faecal and blood samples will be taken from you here at the clinic. Faeces will be used for geohelminths diagnosis while blood sample will be used for malaria parasites diagnosis and haemoglobin estimation. In case you are diagnosed with geohelminths eggs, malaria parasites or low haemoglobin

(anaemia) then you will be offered treatment and screened again for worms after three months from the day of treatment. Blood samples for those with low haemoglobin levels will be taken on every visit (monthly) and treated until normal haemoglobin level is attained. Each participant will be given a study number. You will not be recruited into the study even if you consent and are below 18 years or above 45 years, do not reside in Bungoma County and have certain diseases example HIV aids that may compromise the data and/or study objective.

Things that may cause harm in the study:

There will be some pain when taking blood sample but this is not harmful to your health. Obtaining faecal samples will not cause physical pain. But in case of any psychological torture the psychologist in the study will address it

Benefits of participating in the study:

You will get free medication for geohelminths, malaria or if anaemic. No other compensation will be provided for your participation in this research.

.**Confidentiality and privacy in the study**:

The identities and or names of those participating in the study will not be in any way revealed during the reporting and discussions of data from this study. On the day of recruitment, you will be assigned a study number to be used on their samples and questionnaires that will be used in all communications and reporting. Filled questionnaires will be kept in a safe cabinet at Masinde Muliro clinic's microbiology laboratory under lock and key and accessible to authorized personnel of the study. All the details of the study will be entered into a computer that is only used by the investigators and his chief investigators. All documents used for this study will be

kept for 4 years after completion of the study. During this period, any findings based on this study and all the stored stool samples will not be used for any other purposes not stated in the consent document, unless authorized by MMUST Ethical Review Committee.

Joining and withdrawing from the study:

Participation in the study is voluntary you will not be forced to join the study. In case you do not want to participate in this study, you will not be denied any medical services in any health facility. You are allowed to withdraw from participating in the study at any time without suffering any consequences or prejudice.

Things that volunteers are not prohibited from doing: You can withdraw from the study at any time without any repercussions whatsoever. Doing that will not deny you the opportunity of

seeking any legal redress/action over whatever issue you want addressed.

I have read the information provided. I have been given an opportunity to ask questions and all of my questions have been answered to my satisfaction. I have been given a copy of this form.

Name of the participant……..……

Signature of research subject:……..……Date…….……

Names and contacts of investigators: In case of any concern/ question(s) about this study contact the investigators using either the telephone or mail address given below. Dr. Elizabeth Omukunda, the Study Supervisor P.O Box 190-50100, Kakamega, : 0724 609 700. Dr David Mulama, the Study Co-Supervisor , P.O Box

190-50100, Kakamega, Mobile 0722968675, Prof Chrispinus Mulambalah the Study Co-Supervisor Mobile no. 0721347269 or Antony wekesa P.O Box 190-50100 Kakamega, Mobile no: 0727239578.Investigator.

Chuo Kikuu cha Masinde Muliro cha Sayani na Technologia,

Idara ya Sayanzi ya Biologia

Ruhusa ya kutoa damu na choo.

(Kiswahili)

Jina la utafiti : Madhara yanayotokana na minyoo pamoja na ugonjwa wa malaria kwa mama mja mzito na mtoto wake atapozaliwa katika Kaunti ya Bungoma inchin, Kenya.

Habari mimi naitwa Antony Wanyonyi Wekesa kutoka chuo kikuu cha Masinde Muliro cha sayanzi na technologia.Anwani ya chuo ni. Sanduku la posta 190-50100, Kakamega. Nambari ya simu Tel 0712 438 437. Tunakuomba ushiriki katika utafiti huu unaofanyika kwenye hospitali kuu ya rufaa ya Kaunti ya Bungoma, kwenye kiliniki ya wamama waja wazito. Tafadhali soma uelewe fomu hii na kuuliza maswali kabla ya kupeana ruhusa ya kushiriki kwenye utafiti huu.

Lengo na muda wa utafiti huu.

Utafiti huu utachunguza madhara yanayotokana na minyoo pamoja na ugonjwa wa malaria kwa mama mja mzito na mtoto wake atapozaliwa. Baadhi ya utafiti huu ni kuchunguza choo yako kama ina minyoo, na damu yako kama ina ugojua wa malaria ama una damu yakutosha. Ikiwa utapatikana na minyoo, malaria ama safura utapewa matibabu ya bure na tena ukipatikana na minyoo utahitajika kupimwa tena baada ya mwezi mitatu baada ya kutibiwa. Na ikiwa huna damu ya kutosha utahitachika kupimwa damu kila ukija kwenye kiliniki yaani baada ya mwezi mmoja baada ya matibabu mpaka damu yako irudi kwa kiwango kinacho takikana. Kila

muhusika atapewa nambari maalum. Huwezi kuhusishwa kwa utafiti huu ikiwa umuri wako uko chini ya miaka 18 au zaidi ya miaka 45, kama wewe si mkaaji wa kaunti hii ya Bungoma na pia ukiwa unaugua maradhi kama ukimwi ambao inaweza kutatanisha matokeo ya utafiti huu.

Vitu vinavyoweza kuleta maumivu

Utasikia uchungu kidogo utakapo tolewa damu lakini hii haina mathara kwa afya yako. Utoaji wa choo hauna maumivu yeyote. Kama utasumbukana kiakili, Daktari wa saikologia atakuhudumia

Manufaa ya kuusika kwa utafiti huu

Ukipatikana na minyoo, malaria ama safura utatibiwa bila malipo, hakuna faida nyingine

Uwekaji siri ya muusika kwenye utafiti huu

Jina lako halitaandikwa au kutumiwa mahalipopote. Utapewa nambari maalum itakayo tumika kwa kuandikwa kwenye sampuli zako utakazo toa na hata kwenye fomu ya maswali utayoulizwa ambayo ikisha jazwa itafungiwa kwenye kabati ilioko chuo kikuu cha Masinde Muliro na ufunguo wake utawekwa tu na wanaohusika na utafiti huu.Maandishi yeyote ihusikanayo na utafiti huu itaingizwa na kuhufadhiwa kenye computa. Rekodi na sampuli zote kuhusu utafiti huu zitahifadhiwa kwa muda wa miaka mine baada ya utafiti na kwa muda huu hazitatumika kwa utafiti mwingine isipokua ruhusa itolewe na Masinde Muliro Ethical Review Committee.

Kuusika au kujitoa kwa utafiti

Kuusika kwa utafiti huu ni kwa hiyari ya mtu na unaweza kujiondoa kwa utafiti huu wakati wowote bila kudhulumiwa. Ukijiondoa hautanyimwa huduma kwa hosipitali yeyote.

Mambo ambayo mhisani hajakataswa kufanya

Unawesa kujiondoa kwa utafiti huu wakati wowote bila tashiwishi ama dhuluma yeyote

Nimesoma/nimeambiwa na kuelewa yote yanayousiana na utafiti huu. Nimepewa nafasi ya kuulisa maswali ambayo yamejibiwa sawa sawa

Jina la muhusika……..……

Saahihi……..……Tarehe…….……

Kama kuna swali ama shaka yeyote pika simu au andika barua pepe kwa watafiti husika. Dkr. Elizabeth Omukunda, Sanduku la posta 190-50100, Kakamega, : Nambari ya simu ya rununu. 0724 609 700 Dkr David Mulama, Sanduku la posta 190-50100, Kakamega, Nambari ya simu ya rununu. 0722968675, Prof Chrispinus Mulambalah., Nambari ya simu ya rununu. 0721347269 ama Antony wekesa Sanduku la Posta 190-50100 Kakamega, Nambari ya simu ya rununu. 0727239578.

APPENDIX B: Informed Consent Form for Questionnaire
(English)

Masinde Muliro University of Science and Technology,

Department of Biological Sciences

Introduction

Greetings. My name is Antony Wanyonyi Wekesa of Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Tel 0712 438 437. Kindly read this document and ask any question(s) on any issue(s) that may be unclear to you, before consenting. Before we proceed, I would like to seek your permission, please read the consent form below.

Purpose of the study is to investigate effects of geohelminthiasis and malaria on maternal health and birth outcomes in Bungoma County, Kenya

Part of this study will be questionnaire related and you will have a contact period of 20 minutes with the researcher

Procedure and confidentiality

Participation in this study is voluntary; the purpose of this form is to obtain your consent to participate. If you choose to take part, I will provide a questionnaire for you to complete. You are not obliged to answer any questions if you do not feel comfortable to do so. The filled questionnaire will be stored in a sealed envelope to ensure confidentiality and will not be accessible to anyone else in this campus in order to ensure confidentiality. To ensure confidentiality, your name will not appear anywhere on the questionnaire and it will not be possible to know which responses came from an individual. After starting the exercise and you wish to stop, you are

free to do so. If you do not intend to participate please inform me now. In case you have any question please do ask.

Benefits of participating

Information from the questionnaire will be used to come up with information that will guide the formulation of intervention measures of controlling geohelmintiasis and malaria infection among pregnant women in Bungoma County. No other compensation will be provided for your participation in this research. Thank you for your cooperation.

I have been informed that completion of this form is voluntary and I therefore make my decision.

Respondent Signature: _____ Date: _____

Research Assistant Signature: _____ Date: _____

Contact Persons:

Dr. Elizabeth Omukunda, the Study Supervisor P.O Box 190-50100, Kakamega, : 0724 609 700; Dr David Mulama, the Study Co-Supervisor , P.O Box 190-50100, Kakamega, Mobile 0722968675, Prof Chrispinus Mulambalah the Study Co-Supervisor Mobile no. 0721347269 or Antony wekesa P.O Box 65-50136, Mobile no: 0727239578.Investigator.

Chuo Kikuu cha Masinde Muliro cha Sayani na Technologia,
Idara ya Sayanzi ya Biologia
Ruhusa ya kuulizwa maswali.

(Kiswahihi)

Utangulizi

Habari, mimi naitwa Antony Wanyonyi Wekesa kutoka chuo kikuu cha Masinde Muliro cha sayanzi na technologia.Anwani ya chuo ni. Sanduku la posta 190-50100, Kakamega. Nambari ya simu ya rununu. Tel 0712 438 437. Tafadhali soma maagizo kwenye fomu hii na kama una swali ama kitu chochote usicho kielewa tafadhali uliza. Naomba ruhusa yako kabla sijaanza kukuuliza maswali.

Lengo la utafiti

Utafiti huu utachunguza madhara yanayotokana na minyoo pamoja na ugonjwa wa malaria kwa mama mja mzito na mtoto wake atapozaliwa katika kaunti ya Bungoma. Sehemu nyingine ya utafiti huu ni kuulizwa maswali fulani. Utakua na mtafiti kwa muda wa dakika 20

Namna ya kujibu maswali na usiri

Kushiriki ni kwa hiari ya mtu kwa hivyo fomu hii inahitaji ruhusa kutoka kwako ndipo ushiriki. Ukikubali nitakupatia fomu hii ujaze. Haulazimishwi kujibu swali lolote ikiwa hujisikii kufanya hivyo. Fomu ya maswali iliyojazwa itafungiwa kwenye kabati na ufunguo kuwekwa na mtafiti mkuu peke ili kuwe na usiri wa kushiriki kwenye utafiti. Una ruhusa ya kuuliza maswali na kama hutaki kushiriki pia unaweza kuniambia ama unaweza kujiondoa wakati wowote ata kama umeanza kushikiri kwenye utafiti

Umuhimu wa kushiriki

Majibu utakayo peana kwa maswali haya yatasaidia kuungamiza, ama kuzuia uambukizaji wa minyoo na malaria kwa wamama waja wazito katika kaunti ya Bungoma. Hakuna umuhimu mwingine. Ahsante kwa kunisikiliza

Nimeelewa kwamba kujaza fomu hii ni kwa hiari ya mtu binasfi kwa hivyo mineamua kushiriki

Sahihi ya mushiriki _____ Tarehe: _____

Sahihi ya mtafiti: _____ Tarehe: _____

Kama kuna swali ama shaka yeyote pika simu au andika barua pepe kwa watafiti husika. Dkr. Elizabeth Omukunda, P.O Box 190-50100, Kakamega, :Nambari ya simu ya rununu: 0724 609 700; Dkr David Mulama, P.O Box 190-50100, Kakamega, Nambari ya simu ya rununu: 0722968675, Prof Chrispinus Mulambalah Nambari ya simu ya rununu: 0721347269 ama Antony wekesa P.O Box 65-50136, Nambari ya simu ya rununu: 0727239578.

QUESTIONNAIRE:

GENERAL INSTRUCTIONS

Please kindly respond by ticking [√] in the itemized questions and write in the spaces provided where applicable . There is no wrong or right answer. Where applicable, more than

One answer to the same question can be ticked.

PLEASE, DO NOT INDICATE YOUR NAME ANYWHERE IN THIS QUESTIONNAIRE.

SECTION A: RESPONDENTS INFORMATION.

Questionnaire number _____

Date_____

1. Age _____

2. Marital status: Married [ ] Single [ ] Divorced [ ] Widow [ ]

3. Highest levels of education achieved

None [ ] Primary [ ] Secondary [ ] College [ ] University [ ]

4. Residence: Where do you live? Urban [ ] Rural [ ] Slum ( ) Other……..

5. Have you ever been treated for geohelminths?

6. When did you take antimalarial drugs last?

7. Do you normally sleep under mosquito net?

8. How many nets are in use?

6. Source of income: How do you earn a living? Specify…….

7. How many children do you have? Total ( ). Boys ( ), Girls ( )

SECTION B: FACTORS ASSOCIATED WITH HELMITH INFECTION

8. What type of house do you live in Specify, Mud ( ), Semi permanent ( ) Permanent ( ), Other ?

9. Where do you dispose your human waste? Pit latrine ( ), Flash toilet, Bush ( ) others ……

10. Do you wash your hands with a detergent (soap) after visiting the latrine?

11. Where do you get water for domestic use?

Tap ( ) Protected spring ( )

River ( ) Well ( )

How far is the nearest water source ……?

12. Which protective gear do you put on when working on the farm? How often to you put on protective gear?

13. Have you ever had the craving for eating soil? 1 Yes. ( ), 2. No ( )

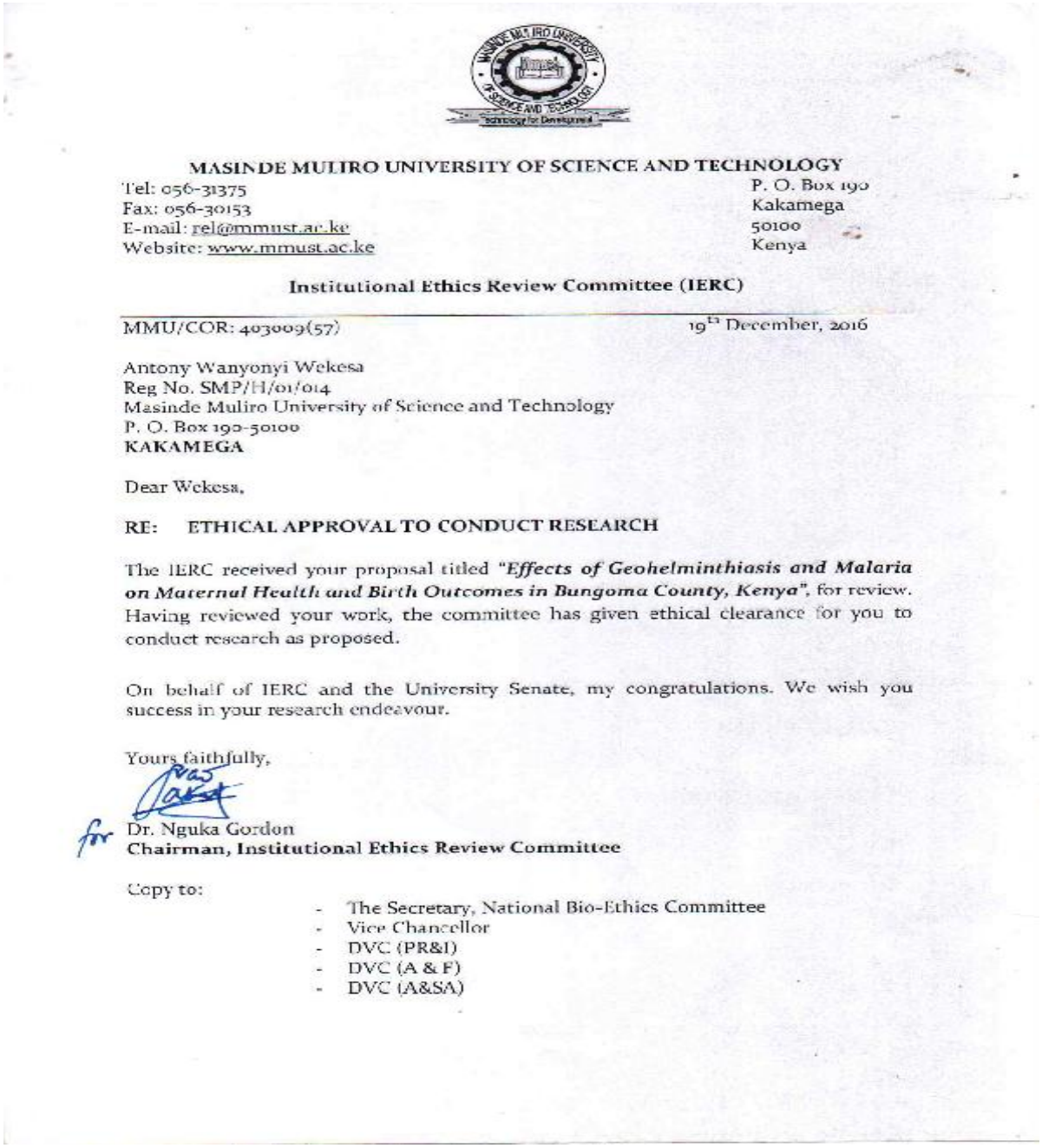
14. How far is your nearest health facility?

15. Do you have the following? 1 Radio ( ) 2 Television ( ) 3 Mobile phone ( ) 4 Motor cycle ( )

16. Do you eat fruits?. If so do you wash them before eating?

17How long have you stayed in Bungoma County 1 more than 6 months( ) 2 Less than 6 months( )

APPENDIX C: Approval of Research by Ethical Review Committee Mainde Muliro University of Science and Technology



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: rel@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190
Kakamega
50100
Kenya

Institutional Ethics Review Committee (IERC)

MMU/COR: 403009(57)

19th December, 2016

Antony Wanyonyi Wekesa
Reg No. SMP/H/01/014
Masinde Muliro University of Science and Technology
P. O. Box 190-50100
KAKAMEGA

Dear Wekesa,

RE: ETHICAL APPROVAL TO CONDUCT RESEARCH

The IERC received your proposal titled ***"Effects of Geohelminthiasis and Malaria on Maternal Health and Birth Outcomes in Bungoma County, Kenya"***, for review. Having reviewed your work, the committee has given ethical clearance for you to conduct research as proposed.

On behalf of IERC and the University Senate, my congratulations. We wish you success in your research endeavour.

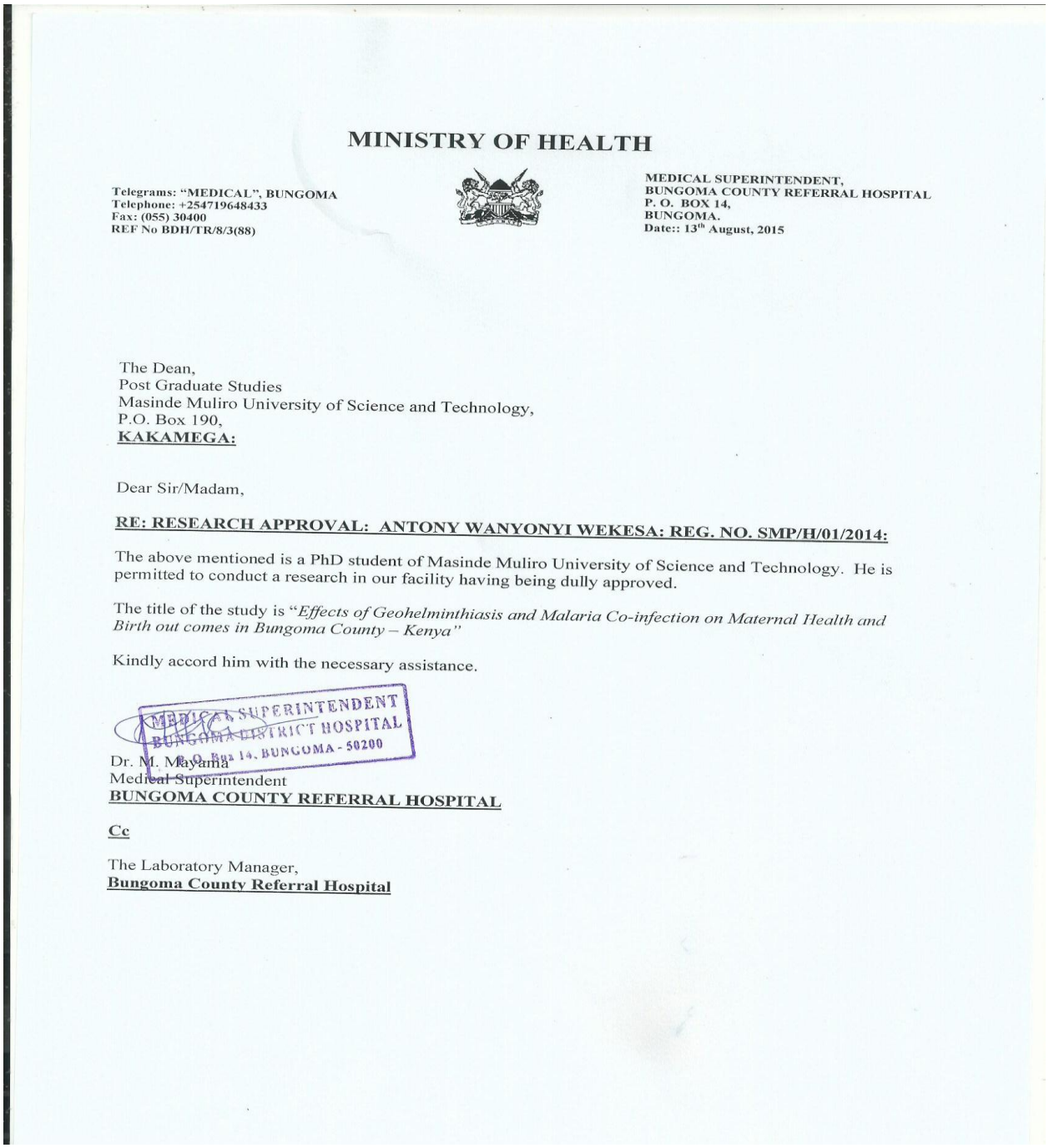
Yours faithfully,

for Dr. Nguka Gordon
Chairman, Institutional Ethics Review Committee

Copy to:

- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)
- DVC (A & F)
- DVC (A&SA)

APPENDIX D: Research Approval by Bungoma County Referral Hospital



MINISTRY OF HEALTH

Telegrams: "MEDICAL", BUNGOMA
Telephone: +254719648433
Fax: (055) 30400
REF No BDH/TR/8/3(88)

MEDICAL SUPERINTENDENT,
BUNGOMA COUNTY REFERRAL HOSPITAL
P. O. BOX 14,
BUNGOMA.
Date:: 13th August, 2015

The Dean,
Post Graduate Studies
Masinde Muliro University of Science and Technology,
P.O. Box 190,
KAKAMEGA:

Dear Sir/Madam,

RE: RESEARCH APPROVAL: ANTONY WANYONYI WEKESA: REG. NO. SMP/H/01/2014:

The above mentioned is a PhD student of Masinde Muliro University of Science and Technology. He is permitted to conduct a research in our facility having being dully approved.

The title of the study is "*Effects of Geohelminthiasis and Malaria Co-infection on Maternal Health and Birth out comes in Bungoma County – Kenya*"

Kindly accord him with the necessary assistance.

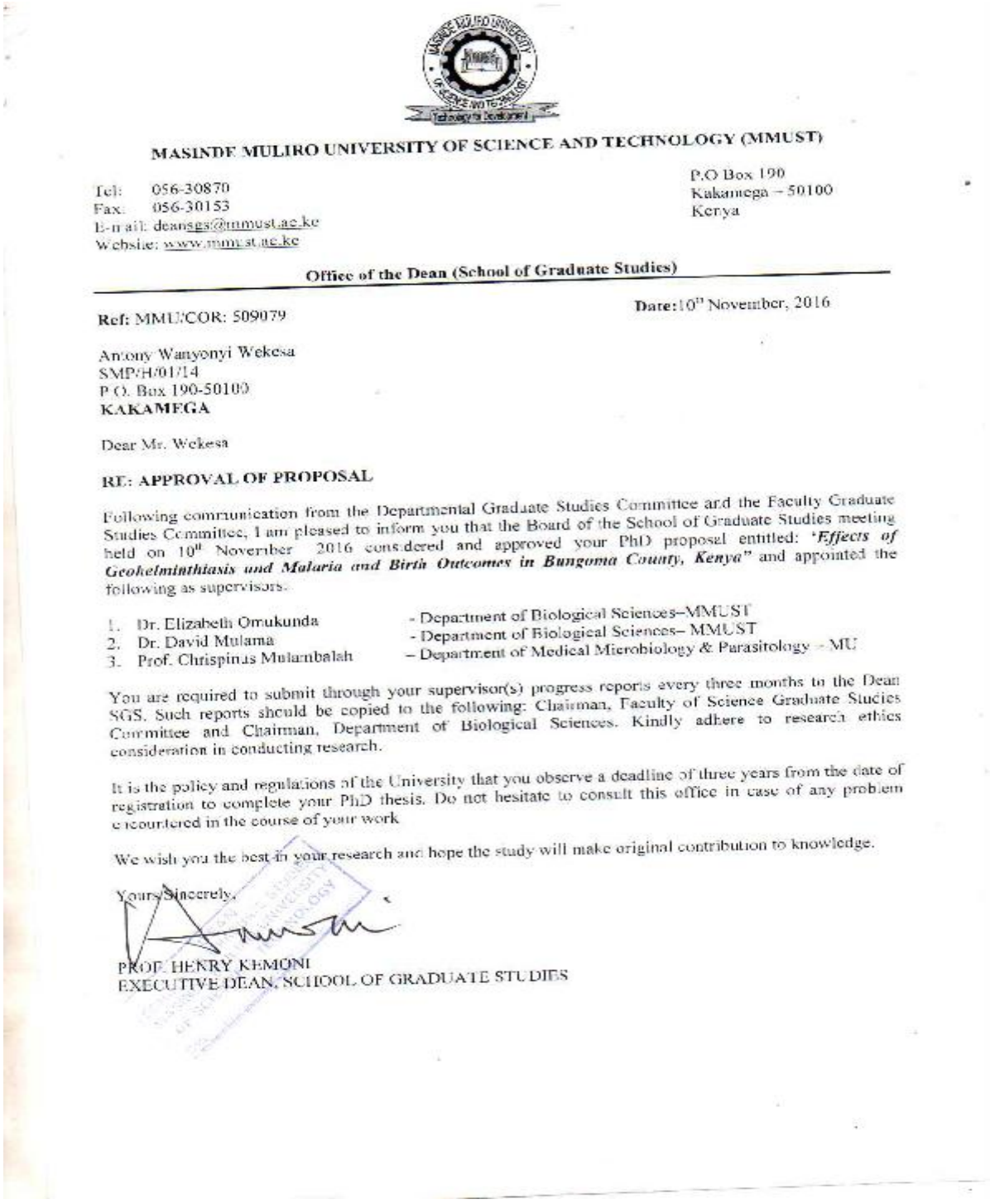
MEDICAL SUPERINTENDENT
BUNGOMA DISTRICT HOSPITAL
P. O. Box 14, BUNGOMA - 50200

Dr. M. Mayama
Medical Superintendent
BUNGOMA COUNTY REFERRAL HOSPITAL

Cc

The Laboratory Manager,
Bungoma County Referral Hospital

APPENDIX E: Proposal Approval by Board of the School of Graduate Studies



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: deansgs@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Office of the Dean (School of Graduate Studies)

Ref: MMU/COR: 509079

Date: 10th November, 2016

Antony Wanyonyi Wekesa
SMP/H/01/14
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Wekesa

RE: APPROVAL OF PROPOSAL

Following communication from the Departmental Graduate Studies Committee and the Faculty Graduate Studies Committee, I am pleased to inform you that the Board of the School of Graduate Studies meeting held on 10th November 2016 considered and approved your PhD proposal entitled: "***Effects of Geohelminthiasis and Malaria and Birth Outcomes in Bungoma County, Kenya***" and appointed the following as supervisors.

1. Dr. Elizabeth Omukunda - Department of Biological Sciences–MMUST
2. Dr. David Mulama - Department of Biological Sciences– MMUST
3. Prof. Chrispinus Mulambalah – Department of Medical Microbiology & Parasitology – MU

You are required to submit through your supervisor(s) progress reports every three months to the Dean SGS. Such reports should be copied to the following: Chairman, Faculty of Science Graduate Studies Committee and Chairman, Department of Biological Sciences. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your PhD thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

PROF. HENRY KEMONI
EXECUTIVE DEAN, SCHOOL OF GRADUATE STUDIES

Appendix F: Publications

International Journal of Healthcare Sciences ISSN 2348-5728 (Online) Vol. 6, Issue 2, pp: (41-48), Month: October 2018 - March 2019, Available at: **www.researchpublish.com**

GEOHELMINTHIASIS: RISK FACTOR ANALYSIS, PREVALENCE AND INFECTION INTENSITY AMONG PREGNANT WOMEN IN BUNGOMA COUNTY, KENYA

Wekesa Antony Wannyonyi.1, Elizabeth Omukunda1, Chrispinus Siteti Mulambalah2, David Mulama1

1Wekesa Antony Wanyonyi. Department of Biological Sciences, Masinde Muliro University of Science and Technology, P.O Box 190 Kakamega Kenya.
E-mail: antony.wekesa@yahoo.com

1David Mulama. Department of Biological Sciences, Masinde Muliro University of Science and Technology, P.O Box 190 Kakamega Kenya.
Email: dmulama@gmail.com

`1Elizabeth Omukunda . Department of Biological Sciences, Masinde Muliro University of Science and Technology. P.O Box 190 Kakamega Kenya.
E-mail: omukundaelizabeth@gmail.com

2Chrispinus Siteti Mulambalah. Department of Medical Microbiology & Parasitology, College of Health Sciences, Moi University P.O Box 4606 Eldoret Kenya
Email: csmulambalah@gmail.com

Abstract: Geohelminthiasis are common in sub Saharan Africa. It is associated with morbidity and mortality in pregnancy. The study aimed at analyzing risk factors, prevalence and infection intensity among pregnant women in Bungoma County, Kenya. A cross sectional hospital based survey that was carried out from March 2016 to January 2017. Consecutive sampling was used to enrol 750 expectant mothers aged 18 to 45 years, seeking antenatal services at the hospital. Kato-katz technique was used to process stool for identification of eggs. Pre-tested structured questionnaire was used to collect data on socio-economic risk factors. Data was analysed using STATA Version 12. Discrete values were analysed using frequencies and percentages. Continuous variables were analysed using central tendency such as mean. Chi-square (*X*2) test was used to determine association between geohelminths and different variables. Multivariable logistic regression was used to analyse the association between geohelminthiases and significant variables at bivariable analysis. Level of significance was set at < 0.05 and 95% CI. Overall prevalence of geohelminthiasis was 185 (24.7%). *Ascaris lumbricoides,* was the most prevalent 76(41.1%), *Necator americanus 73*(39.5.%), *Trichuris trichiura*, 11(5.9%), *Enterobius vermicularis*, 4(2.2%), *Schistosoma mansoni* 4(2.2%) and, mixed infection of *A. lumbricoides* and *Necator americanus* 17(9.2%). Women without geohelminths 565(75.3%). Risk factors included lack of hand washing, (AOR=3.386, 95% CI 1.664-6.079), use of borehole water for drinking (AOR 1.69, 95% CI 1.144-2.508), lack of shoes (AOR=0.412, 95% CI 210-808) and unemployed (AOR=2.295,

95% CI 1.389-3.990). The study showed a moderate prevalence of geohelminthisis in expectant mothers. Routine stool examination, provision of treated water and health education particularly on personal hygiene are important.

Keywords: Geohelminths, Prevalence, Intensity, Risk factors, pregnant women.

Dear Authors,

Our manuscript has been accepted for publication.

I hereby forward the communication from the editor.

Thanks for support and collaboration.

---------- Forwarded message ---------
From: **Medicine Science | International Medical Journal** <ms@ejmanager.com>
Date: Fri, Oct 26, 2018 at 10:13 PM
Subject: Decision Letter to Authors - Acceptance - (MS-2018-08-191)
To: <csmulambalah@gmail.com>

Dear Antony Wanyonyi Wekesa, Chrispinus Siteti Mulambalah, David Mulama, Elizabeth Omukunda,

I am pleased to inform you that your manuscript titled as "Malaria Prevalence and Risk Analysis among Pregnant Women in Bungoma County, Kenya" (Manuscript Number: MS-2018-08-191 was accepted for publication in the Medicine Science | International Medical Journal. You could check your possible publication date at your author page.
You may login to your author account page, and visit accepted articles section in order to get offical/formal acceptance letter as PDF.
I would like to remind that you could send your future manuscripts to Medicine Science | International Medical Journal.

Sincerely yours,

#imagesignature#
Osman CELBIS (MD, Professor)
Editor-in-Chief
Medicine Science
www.medicinescience.org
http://www.scopemed.org/?jid=53

Author Login Page - http://my.ejmanager.com

Reviewer Login Page - http://www.ejmanager.com/reviewers/

http://www.ejmanager.com

Bernard Marchand <jpr@hindawi.com>
To:csmulambalah@gmail.com
Cc:marchand@univ-corse.fr,antony.wekesa@yahoo.com,dmulama@gmail.com,omukundaelizabeth@gmail.com,darwinsiteti@gmail.com
Nov 14 at 10:35 AM

Dear Dr. Mulambalah,

The review process of Research Article 2613484 titled " Malaria and geohelmithiasis co-infections in expectant women: Effect on maternal health and birth outcomes in a malaria endemic region in Kenya." by Antony Wekesa, Chrispinus Mulambalah,

David Mulama, Elizabeth Omukunda and DARWIN INJETE SITETI submitted to Journal of Parasitology Research has been completed. I am pleased to inform you that your manuscript has now been accepted for publication in the journal.

The publication process of your manuscript will be initiated upon the receipt of electronic files. Please log in to the Manuscript Tracking System at the link below using your username and password, and upload the electronic files of your final accepted version within the next 2-3 days.

http://mts.hindawi.com/author/2613484/upload.files/

The electronic files should include the following:

1- Source file of the final accepted manuscript (Word or TeX/LaTeX).
2- PDF file of the final accepted manuscript.
3- Editable figure files (each figure in a separate EPS/PostScript/Word file) if any, taking into consideration that TIFF, JPG, JPEG, BMP formats are not editable.

If you have deposited your manuscript on a preprint server (e.g. arXiv, bioRxiv, chemRxiv), now would be a good time to update it with the accepted version. If you have not deposited your manuscript on a preprint server, you are free to do so.

Thank you again for submitting your manuscript to Journal of Parasitology Research.

Best regards,

Bernard Marchand marchand@univ-corse.fr