PREVALENCE OF INTESTINAL HELMINTH INFECTIONS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINICS AT THE KITALE DISTRICT HOSPITAL, KENYA

WEKESA ANTONY   WANYONYI

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DEDICATION

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

ACKNOWLEDGEMENT

ABSTRACT

Intestinal helminth infections with parasites such as *Ascaris lumbricoides, Necator americanus*, *Ancylostoma duodenale* and *Trichuris trichiura,* during pregnancy are associated with adverse outcomes including, poor nutritional status, growth, low birth weight and perinatal mortality. They present major public health problem in developing countries, but the prevalence, intensity and predisposing factors are not adequately documented in many areas of Kenya. The purpose of this study was to determine the prevalence of intestinal helminth infection, their association with maternal haemoglobin levels, and Socio economic factors that predispose pregnant women, to the infection. A hospital based survey was carried out for four months of 2012. A total of 153 pregnant women participated in the study. Stool samples were examined using Kato Katz technique. For each stool sample two Kato slides were prepared and average of the total number of eggs calculated. Haemoglobin levels and eosinophil count were measured using the Coulter counter machine. Socio economic factors that predispose pregnant women to intestinal helminth infection were assessed using a semi structured questionnaire. Data was analyzed using SPSS for windows version 16.0. Prevalence of intestinal helminths was analysed using descriptive statistics. Chi-square was used to analyze the association of intestinal helminths infection and maternal haemoglobin level. Bivariate analysis was used to identify socio-economic factors that predispose pregnant women to intestinal helminth infection. Level of statistical significance was chosen at 5% in this study. The overall prevalence of intestinal helminth infection was 21(13.8%). *Ascaris lumbricoides* was most prevalent parasite 10 (6.5%), *Necator americanus* 6 (3.9%), *Trichuris trichiura* 2 (1.3%), *Enterobius vermicularis* 1(o.7%), *A.lumricoides* and *T.trichiura* 2(1.3%). A significant negative association was observed between heavy infection of *Necator americanus* and low maternal haemoglobin level (P-value 0.013). Pregnant women aged below 29 years had a higher risk of helminth infection (P=0.08) as compared to their older counter parts. Living in permanent house had lesser likelihood of getting helminth infection as compared to those living in semi permanent house (P=0.001). Use of flash toilets had lesser chances of getting helminth infection as compared to pit latrines (P=0.047). The study findings suggested that intestinal helminth infection is prevalent among pregnant women at the Kitale District Hospital. These findings reinforce the need to create public health awareness and screening of all pregnant women for intestinal helminths as part of their routine antenatal care.

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LIST OF ABBREVIATIONS AND ACRONYMS

EPG   :   Eggs per Gram

Hb   :   Haemoglobin

PBF   :   Peripheral Blood Film

WBC   :   White Blood Cell

MOH   :   Medical Officer of Health

WHO   :   World Health Organization

x10   :   Ten Times Magnification

x40   :   Forty Times Magnification

CHAPTER ONE

INTRODUCTION

1.1   Background Information

Intestinal helminths are among the most common and widespread of the human infections. They contribute to poor nutritional status, anaemia and impaired growth Dickson *et al.,* (2000). They are among neglected tropical diseases (Hotez *et al.,* 2007). About 2 billion harbour these infections worldwide, of which 300 million suffer associated severe morbidity. An estimate by WHO states that soil transmitted helminthiasis represented more than 40% of the disease burden due to all tropical diseases WHO (2003). Data from the early 1990s suggest that 44 million of the developing world's 124 million pregnant women harboured hookworm infection Huddle and Gibson, (1999). Numbers like this have led to an increased interest in the topic of hookworm-related anaemia during pregnancy (Gyorkos *et al.*, 2006)

The global burden by disability-adjusted-life year (DALY) of soil transmitted Helminthic diseases is comparable to tuberculosis and malaria (Jex *et al.*, 2011). Creeping impact of these parasitic infestations insidiously affects women and children's health, reduce work productivity of adults and thus, impair economic growth of poor countries WHO, (2012). Worm infestations cause anaemia and poor pregnancy outcome in women and, www.ccsenet.org/gjhs. Global Journal of Health Science Vol. 6, No. 3; 2014 10malnutrition, poor physical growth, and psychological cognitive underdevelopment in children (Yap *et al.*, 2012). Those impacts are often less visible and usually have a low priority.

Intestinal helminthiasis are among neglected tropical diseases which primarily affect low-income populations, causing chronic conditions, learning disabilities, and reduced productivity and income earning capacity in later life. Morbidity control and, where resources allow, local elimination are now recognized as a priority for achieving the millennium development goals (Hotez *et al.,* 2008).

The high rates of helminths infection among pregnant women are mostly indicative of faecal pollution of soil and domestic water supply around homes, due to poor sanitation and improper sewage disposal (Van Eijk *et al.,* 2009; Bundy *et al..,* 1995;). Pregnant women are at high risk of infection because of their close relationship with children (Van Eijk *et al.,* (2009; Bundy *et al..,*

1995). Intestinal helminths are transmitted through the soil. The practice of soil- eating (geophagy) is common amongst pregnant women in many communities in developing countries (Brooker *et al.,* 2008).

Globally, the most common cause of low haemoglobin is believed to be iron deficiency, probably due to helminths infection and inadequate dietary iron intake, physiologic demands of pregnancy and rapid growth Ayoya *et al.,* (2006); Dreyfuss *et al.,* (2000). The prevalence of helminth infection is especially high in developing countries, particularly among populations with poor environmental sanitation Van Eijk *et al.,* (2009). Other practices such as improper disposal of refuse, personal hygiene, and walking bare foot may contribute to infection (Stoltzfus *et al.,* 1997).

Low haemoglobin level is shown by complex signs and symptoms such as dizziness, tiredness, headache, and paleness. Haemoglobin levels less than11g/dl is referred to as anaemia WHO, (1996). The relative contribution of helminth infections to iron deficiency varies by sex, age, and population and is not well described in many populations (Dreyfuss *et al.,* 2000). Helminth infection and dietary intake is responsible for 95% of the cases of low haemoglobin levels or anaemia during pregnancy (Porter and Kaplan., 1999). The most important cause of pathological chronic loss of blood and iron during pregnancy in the tropics is hookworm and other soil-transmitted helminths (Brooker *et al.,* 2008). At a hospital in Kathmandu, Nepal, hookworm infection was associated with severe but not moderate anaemia among women receiving antenatal care Bondevik *et al.,* (2000).

Data on the epidemiology of iron deficiency anaemia in East Africa and elsewhere, point to the important contribution of hookworm infection to this condition (Ayoya *et al.,* 2006). Hookworm infection has been established as a strong predictor of iron deficiency (Stoltzfus *et al.,* (1997), but a limited number of studies have examined their potential patho-physiology in pregnant women (WHO, 1993).

Previous surveys showed variations in the prevalence and intensity of intestinal helminths among pregnant women in Kenya, An additional challenge is exclusion of this group from deworming programmes without considering the risks-benefits ratio (Van Eijk *et al.,* 2009).

The burden, prevalence, and species distribution of intestinal helminths in pregnant women in the study area have not been previously documented. The research questions therefore are: what

is the prevalence of intestinal helminthic infection among this population and what is the worm species commonly encountered. This study investigated prevalence and intensity of intestinal helminth infection, their relationship with maternal haemoglobin concentration and socio-economic factors that predisposes pregnant women attending antenatal services at the Kitale District Hospital to infection. This aimed at getting aanswers to assist policy makers to formulate desirable intervention measures.

1.2 Statement of the Problem

Intestinal helminths in pregnant women are among the neglected tropical diseases. They cause 300 million deaths annually. Kitale District Hospital records of women who attend antenatal clinic show that 200 pregnant women attend the clinic monthly. Majority are anaemic, and do eat soil. Information on the cause of anaemia is lacking. Intestinal helminths have been incriminated. This study was undertaken to determine the prevalence and intensity of intestinal helminth infection in pregnant women attending antenatal clinic at Kitale District Hospital.

1.3   Objectives.

1.3.1   General Objective.

Asses the prevalence and intensity of intestinal helminth infection in pregnant women, attending antenatal clinic at Kitale, district hospital in Western Kenya.

1.3.2   Specific objectives

1. To determine the prevalence of intestinal helminth species in pregnant women attending antenatal clinic at Kitale District Hospital.

2. To evaluate the association between intestinal helminth infection and maternal haemoglobin level.

3. To determine socio-economic factors that predisposes pregnant women to intestinal helminth infection.

1.4   Null Hypotheses

1. Various species of intestinal helminths are not prevalent in pregnant women attending antenatal clinic.

2. Intestinal helminth infections do not affect maternal haemoglobin levels.

3. Socio-economic factors do not predispose pregnant women to intestinal helminth infection.

1.5 Justification

Pregnant women are excluded from deworming programs without considering the risks-benefits ratio. Stool examination is not done routinely on pregnant women for early diagnosis of intestinal helminths. Information on prevalence of intestinal helminths that cause anaemia in the study area is lacking. This study was undertaken to identify intestinal helminths, their prevalence including socio-economic factors among pregnant women attending antenatal clinic at Kitale District Hospital.

2.1   Aetiology of intestinal helminth infections in humans

The most common intestinal helminth infections are caused by hookworm (*Necator americanus* and *Ancylostoma duodenale), Trichuris trichiura* and *Ascaris lumbricoides*. The intestinal helminthiasis is an ancient disease that continues to cause misery and disability in poor populations. Thousands of rural and impoverished villagers throughout the tropics and subtropics are often chronically infected with several different species of parasitic worms (Hotez., 2007). About 2 billion harbour these infections worldwide, of which 300 million suffer associated severe morbidity, (Bundy *et al,* 2000). In 1999, WHO estimated that soil transmitted helminthiasis represented more than 40% of the disease burden due to all tropical diseases (WHO, 2003).

According to WHO estimates, globally there are 800-1000 million cases of round worms (*Ascaris lumbricoides),*700-900 million cases of the hookworm(*Necator americanus* and *Ancylostoma duodenale)* and 500 million cases of the whipworm(*Trichuris trichiura)* (WHO, 1986). Although acute symptoms of infection are uncommon, numerous studies have shown a consistent association between intestinal nematode infection, such as *Ascaris lumbricoides* and diminished food intake and weight loss (Gyorkos *et al.*, 2004). The amount of work done by a woman per day definitely decreases when she is infected by helminths, which produce a double burden for a woman in some rural farming community. Women may even acquire helminth infections in the process of growing family's food (Stephenson *et al.*, 2002).

Geohelminth infections in pregnancy have been associated with maternal iron deficiency, and impaired nutritional status, decreased infant birth weight, intra-uterine growth retardation, and adverse birth outcomes (WHO, 2002). Data from the early 1990s suggest that 44 million of the developing world's 124 million pregnant women harboured hookworm infection (Huddle and Gibson, 1999). Numbers like this have led to an increased interest in the topic of hookworm-related anaemia during pregnancy (Gyorkos *et al.*, 2006). The gastrointestinal blood loss, mal-absorption and appetite inhibition may further aggravate the iron, zinc and protein energy deficiencies and the anaemia of pregnancy (Huddle and Gibson., 1999).

Studies have revealed an association between the presence of hookworm eggs in stool, in the last trimester of pregnancy and low haemoglobin concentration. Studies in Kenya and Zanzibar also revealed an association between the presence of hookworm and low haemoglobin in both children and adults, and this was particularly significant in pregnant women (Mary and Akamori., 2005). In a previous study, moderate and heavy hookworm infection and co-infection of moderate and heavy hookworm and Trichuris infections were found to have a statistical significant association with low haemoglobin concentration (Renee *et al.*, 2005).

A study conducted among pregnant women at Jimma university hospital in Ethiopia showed a statistical significant difference between anaemic and non-anaemic cases with hookworm infection Belachew and Legese. (2006). With the understanding that chronic hookworm infection can lead to anaemia, many people are now questioning the treatment of hookworm and its effects in severe anaemia rates and also on maternal and child health. Evidence suggests that the contribution of hookworm to maternal anaemia merits that all women of child-bearing age living in endemic areas be subjected to periodic antihelmintic treatment. So far studies have validated recommendations to treat infected pregnant women for hookworm infection during pregnancy. Helminthic infected pregnant mothers present significant lower levels of haemoglobin, and obviously higher levels of eosinophilia (Alfonso *et al.*, 2006).

Gastrointestinal blood loss; mal-absorption and appetite inhibition may further aggravate the iron, zinc and protein energy deficiencies and low haemoglobin concentration in pregnancy (Huddle and Gibson., 1999). Heavy hookworm and Trichuris infections were found to have a statistical significant association with low haemoglobin concentration in pregnancy (Renee *et al.*, (2005). The World Health Organization even recommends that infected pregnant women be treated after their first trimester (Bethony *et al.*, 2006). Regardless of these suggestions, only Madagascar, Nepal and Sri Lanka have added deworming to their antenatal care programs (Brooker *et al.*, 2008). The lack of deworming of pregnant women is explained by the fact that most individuals still fear that anthelmintic treatment will result in adverse birth outcome.

A study done comparing a group of pregnant women treated with mebendazole with a control placebo group, AQillustrated rather similar rates in adverse birth outcomes. The treated group demonstrated 5.6% adverse birth outcomes, while the control group had 6.25% adverse birth outcomes (Gyorkos *et al.*, 2006). Furthermore, another study illustrated that treatment for

hookworm infection actually led to positive health results in the infant. The study concluded that treatment with mebendazole plus iron supplements during antenatal care significantly reduced the proportion of very low birth weight infants when compared to a placebo control group. So far, studies have validated recommendations to treat infected pregnant women for hookworm infection during pregnancy (Larocque *et al.*, 2006).

2.2 Life cycle of intestinal helminths
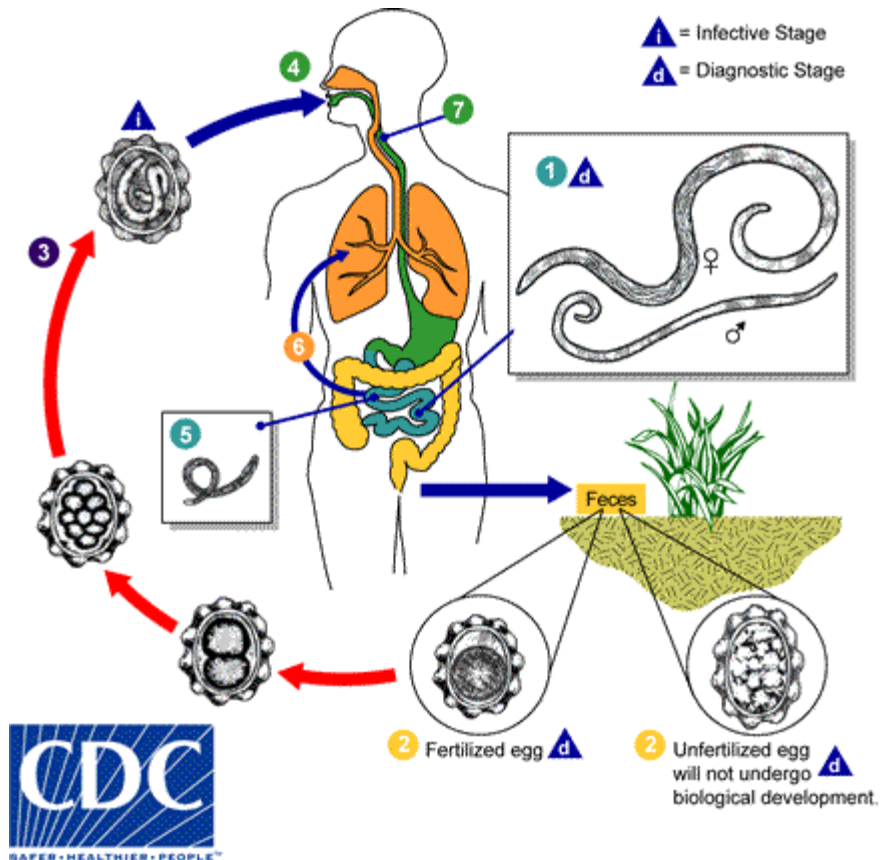
Plate 1: Life Cycle of *Ascaris lumbricoides* (Giant intestinal roundworm) (courtesy: CDC)
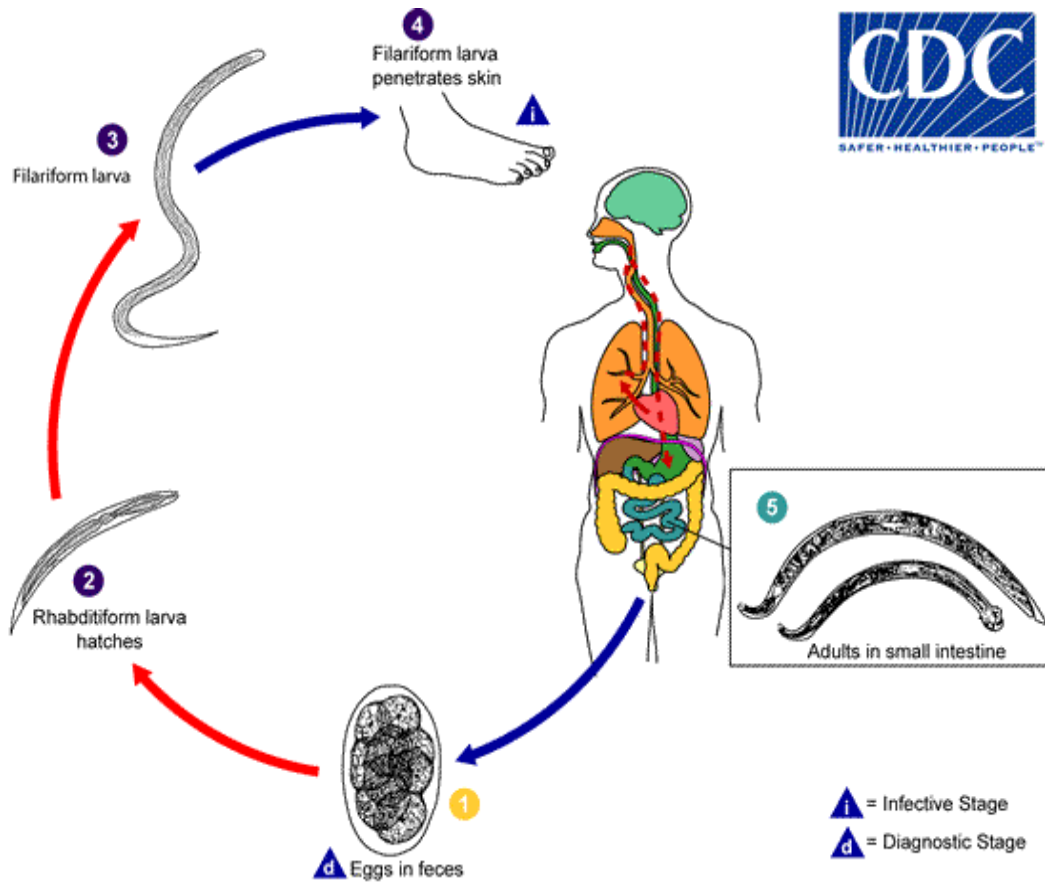
Plate 2 : Life cycle of (Hookworms) *Necator americanus* and *Anclostoma duodenale*(Courtesy: CDC)
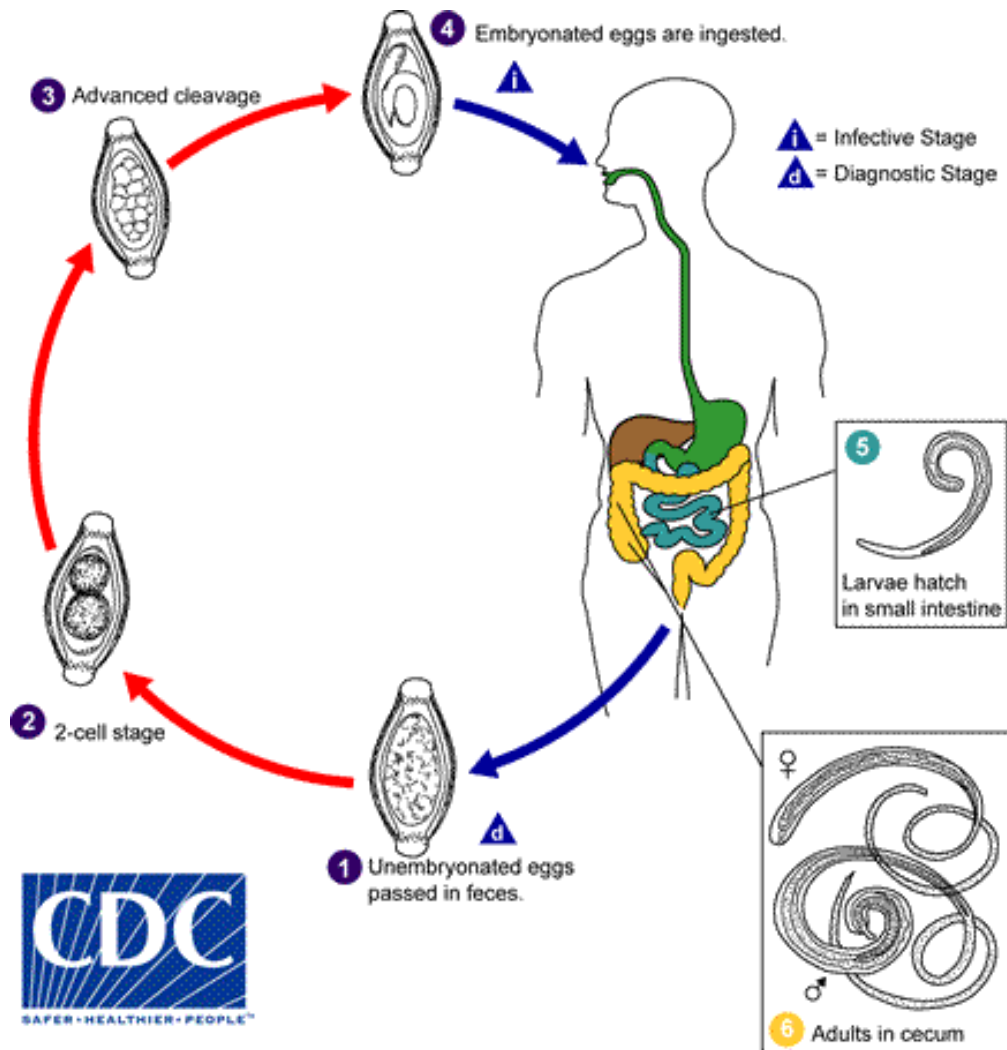
Plate 3: Life Cycle of *Trichuris trichiura*, also called the human whipworm (Courtesy: CDC)

Adult hookworms of the genera *Necator* and *Ancylostoma* parasitise the upper part of the human small intestine, whereas Ascaris roundworms parasitise the entire small intestine and adult whipworms (*Trichuris trichiura*) live in the large intestine, especially the caecum (Despommier *et al.,* 2001). The parasites can live for several years in the human gastrointestinal tract. Human beings are regarded as the only major definitive host for these parasites, although in some cases Ascaris infections can also be acquired from pigs (Crompton, 2001).

Intestinal helminths vary greatly in size, and female worms are larger than males, (Despommier *et al.,* (2005). After mating, each adult female produces thousands of eggs per day, which leave the body in the faeces. People become infected with *T trichiura* and *a lumbricoides*

by ingesting the fully developed eggs. After ingestion of Trichuris eggs, the released larvae moult and travel to the colon where they burrow into the epithelia and develop into adult whipworms within about 12 weeks. Despommier *et Al.,*(2001). Ascaris larvae penetrate the intestinal mucosa and after an obligatory extra intestinal migration, they enter the liver then the lungs, before passing over the epiglottis to re-enter the gastrointestinal tract and develop into egg-laying adult worms about 9–11 weeks after egg ingestion (Jeffrey *et al.,* 2006).

N americanus and *A duodenale* hookworm eggs hatch in soil. The larvae moult twice to become infective third-stage larvae, which are non-feeding but motile organisms that seek out higher ground to improve the chance of contact with human skin. After skin penetration, they enter subcutaneous venules and lymphatic vessels to access the host's afferent circulation. Ultimately, the larvae become trapped in pulmonary capillaries, enter the lungs, pass over the epiglottis, and migrate into the gastrointestinal tract (Hotez *et al.,* 2004). About 5–9 weeks are needed from skin penetration until development of egg-laying adults. *A duodenale,* larvae are also orally infective, and lactogenic transmission during breastfeeding has been postulated. Soil-transmitted helminths do not reproduce within the host. This feature is crucial for understanding of the epidemiology and clinical features of intestinal helminth infections, as well as the approaches to their control.

2.3   Epidemiology of intestinal helminths.

Intestinal helminth infections are widely distributed throughout the tropics and subtropics. Climate is an important determinant of transmission of these infections, with adequate moisture and warm temperature essential for larval development in the soil (Brooker and Michael., 2000). Equally important determinants are poverty and inadequate water supplies and sanitation. (Desilva *et al.,* 2003). In such conditions, intestinal helminth species are commonly co endemic. With the exception of *Strongyloides stercoralis*, helminths do not replicate within the human host. This fundamental aspect of helminth biology establishes a set of transmission dynamics quite different from those for viruses, bacteria, fungi, and protozoa. For example, prevalence which is the proportion of persons in a defined population at a given time point infected with the helminth Fletcher and Fletcher.,(2005), is seldom used as the only measure to assess the epidemiological situation for that intestinal helminth infection. This is because morbidity is

associated with the number of worms infecting the host (worm burden) rather than the absence or presence of infection.

Prevalence is commonly combined with worm burden also referred to as the intensity of infection, which is commonly measured by the number of eggs per gram (EPGs) of faeces for intestinal helminths (Anderson, 1982). Based on EPGs and their association with morbidity, individuals are classified into categories of light, moderate, and heavy infection (Montresor *et al.,* 1998). Climate and topography are crucial determinants of the distribution of intestinal helminth infections (Brooker., 2007). Soil-transmitted helminths are highly affected by surface temperature Brooker., (2003), altitude, soil type, and rainfall (Appleton and Gouws, 1996). An increasing number of studies of helminth epidemiology have shown that it is common for individuals to be infected with more than one species of helminths (Brooker., *et* al., 2000). A number of epidemiological studies have indicated that individuals infected with multiple species of helminth often harbour heavier infections than individuals infected with a single helminths species (Booth., 1998; Needham., 1998).

An important consequence of simultaneous infection with the parasites (Ancylostomiasis) is severe anaemia (Brooker., 2006); Mwangi., 2006). Much of epidemiological research has focused on heterogeneity in the intensity of helminth infection by age. Changes with age in the average intensity of infection tend to be convex, rising in childhood and declining in adulthood. For *A. lumbricoides* and *T. trichiura,* the heaviest and most frequent infections are in children aged 5–15 years, with a decline in intensity and frequency in adulthood (Chan *et* al, 1996). In contrast, hookworm frequently exhibits a steady rise in intensity of infection with age, peaking in adulthood (Bethony, 2002).

2.4 Pathology of intestinal helminths

2.4.1 Hookworm infection

Infection with hookworm is generally considered to be asymptomatic, but it is an extremely dangerous infection because its damage is silent and insidious. There are general symptoms that an individual may experience soon after infection. Ground-itch, which is an allergic reaction at the site of parasitic penetration and entry, is common in patients infected with *N. americanus*. Additionally, cough and pneumonitis may result as the larvae begin to break into the alveoli and

travel up the trachea. Once the larvae reach the small intestine of the host and begin to mature, the infected individual will suffer from diarrhoea and other gastrointestinal discomfort. However, the "silent and insidious" symptoms are mainly related to chronic, heavy intensity hookworm infections (Hotez and Prichard, 1995).

Major morbidity associated with hookworm is caused by intestinal blood loss, iron deficiency anaemia, and protein malnutrition (Hotez and Pritchard, 1995). They result mainly from adult hookworms in the small intestine ingesting red blood cells, rupturing erythrocytes, and degrading haemoglobin in the host (Hotez *et al.,* 2005). Hookworm infection causes mechanical laceration and enzymatic damage to the mucosa of the small intestine leading to approximately 0.05 ml/dl of blood loss per adult *N. americanus* and approximately 0.25 ml/dl per adult *A. duodenale* (Huddle *et al.,* 1999). This long term blood loss can manifest itself physically through facial and peripheral. Oedema and eosinophilia caused by iron deficiency anaemia.

Recent research has focused on the potential of adverse maternal-foetal outcomes when the mother is infected with hookworm during pregnancy (Bethony *et al,* 2006). In contrast to most intestinal helminthiasis where the heaviest parasitic loads tend to occur in children, hookworm prevalence and intensity can be higher among adults. The explanation for this is that hookworm infection tends to be occupational, plantation workers, coalminers and other groups maintain a high prevalence of infection among themselves by contaminating their work environment. However, in most endemic areas, adult women are the most severely affected by anaemia, because they also have much higher physiological needs for iron such as menstruation and repeated pregnancy (Bethony *et al.,* 2006).

2.4.2 Infection with *Trichuris trichiura*

Adult *T. trichiura* (whipworms) live preferentially in the caecum, although in heavy infections, whipworms can be seen throughout the colon and rectum. The adult parasite leads both an intracellular and an extra cellular existence, with the anterior end embedded in epithelial tunnels within the intestinal mucosa and the posterior end located in the lumen. Inflammation at the site of attachment from large numbers of whipworms, results in colitis. Longstanding colitis produces a clinical disorder that resembles inflammatory bowel diseases including chronic abdominal pain, diarrhoea, and the sequelae of impaired growth, low haemoglobin and finger

clubbing. Trichuris dysentery syndrome is an even more serious manifestation of heavy whipworm infection which results in chronic dysentery and rectal prolapse (Bundy and Cooper, (1989). Blood loss can be a feature of *Trichuris trichiura* infection, but it is less prominent than in hookworm.

2.4.3   Infection with *Ascaris lumbricoides*

The presence of large numbers of adult ascaris worms in the small intestine can cause abdominal distension and pain. They can also cause lactose intolerance and malabsorption of vitamin A, and possibly other nutrients Taren *et al.,* (1987) which might partly cause the nutritional and growth failure. Adult worms can enter the lumen of the appendix, leading to acute appendicular colic and gangrene of the appendix tip, resulting into a clinical picture indistinguishable from appendicitis. By contrast with intestinal obstruction, hepatobiliary and pancreatic ascariasis occurs more commonly in adults especially women than in children, presumably because the adult biliary tree is large enough to accommodate an adult worm. *Ascaris lumbricoides* infections are commonly asymptomatic, although clinical complications of extra-intestinal or high numbers of *ascaris* have been well described. *A. lumbricoides* infection has been associated with impaired fat digestion, reduced vitamin absorption, and temporary lactose intolerance. However, treatment has shown to improve nutritional status (Stephenson *et al.,* 2000; WHO, 2002)**.**

2.5 Prevention and control of intestinal helminths.

To achieve sustainable control of worm infections while maintaining high coverage of anthelminthic treatment, it is essential to ensure access to safe water, adequate sanitation facilities and good hygiene at the community level (WHO, 2004). Combination therapy with drugs with differing modes of action is an alternative strategy to improve efficacy and lower the risk of resistance.

A comprehensive control strategy according to (WHO, 2004) for helminth infection should include: ensuring wide availability of anthehelminthics for helminth infections in all health services in endemic areas; ensuring good management of symptomatic cases; regular treatment of all children at risk - including adolescent girls through school and community base initiatives; treating pregnant women at risk, through antenatal care and other women's health programme;

ensuring a safe water supply and adequate sanitation facilities in all schools, ensuring provision of adequate water and sanitation facilities at household/ community level; promoting good hygiene and sanitation practices among schoolchildren; caregivers and to the communities (hand-washing, use of latrines; use of footwear) through community capacity development activities.

Vaccination remains the method of choice to control intestinal helminth infection, because it offers the possibility of a simple, single step for the interruption of infection, disease, and transmission. Several substantial obstacles impede vaccine development against intestinal helminths Maizels *et al.,* (1999), including the lack of good animal models, and a poor understanding of the events that permit intestinal helminths to endure for years, in their human host in the face of a potent immune response.

CHAPTER THREE

MATERIALS AND METHODS

3.1   Study area

This study was conducted in Kitale District Hospital located in Kitale town, whose Population is 20,000 people as per 2009 census. Kitale town is located between Latitude $1^0\ 01^{'}\ 58^{''}$ North and Longitude $35^0\ 00^{'}\ 02^{''}$ East. This is an agricultural area with high rainfall. Majority of women spent most of their time tilling land bear feet, this exposes them to helminth infection especially *Necator americanus.* It consists of slum settlements such as Kipsongo, Folkland and Shimo la Tewa. There are also town residents and rural set ups such Bikeke, Cherengany, Kiminini and Mois Bridge. This area was chosen for study because previous research in western Kenya has reported a high prevalence of Hookworm in comparison with *A. lumbricoides and T. trichiura* infections (Luoba *et al.,* 2005; Brooker *et al.*, 2000).
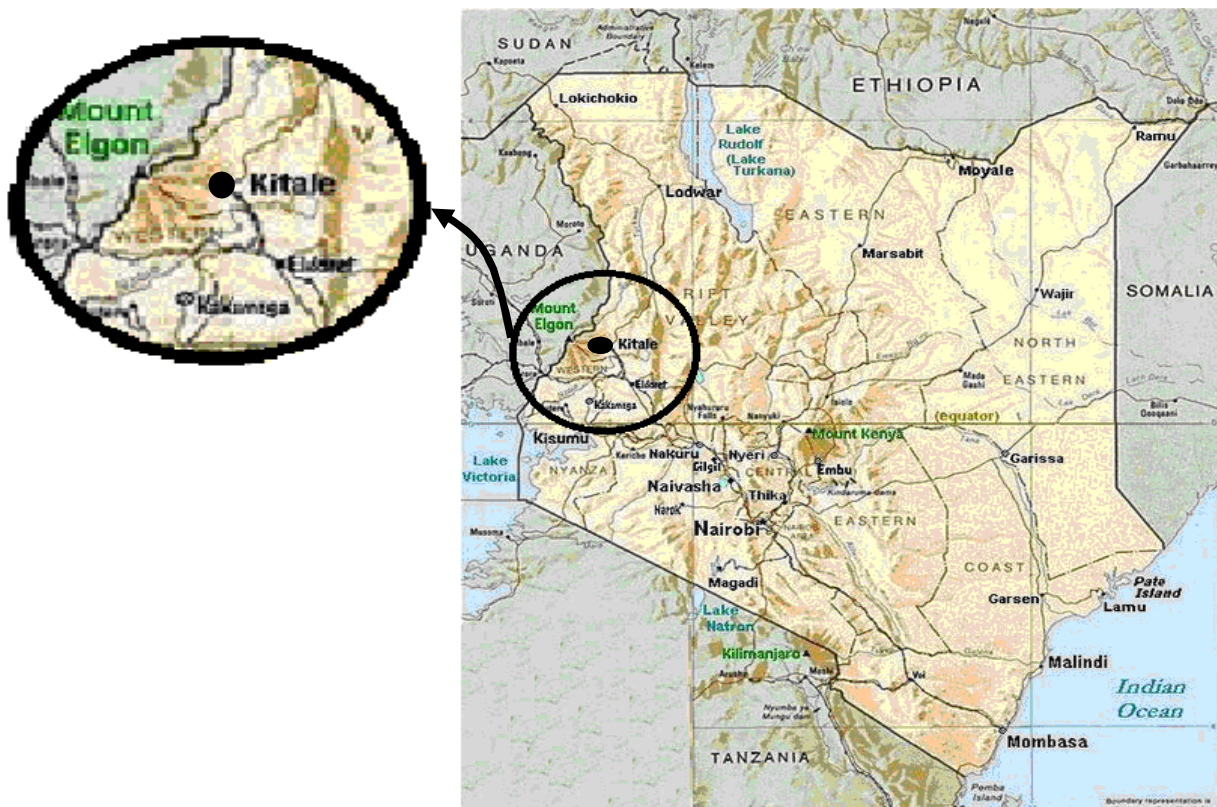
Plate 4 : Location of the Kitale District Hospital, Kenya

Source: http//upload.wikimedia:org/wikipediacommons/4/49/Kenya-relief-map-towns

3.2   Study Design

A hospital-based descriptive cross-sectional survey was carried out. Consecutive sampling was used to recruit participants that met the required criteria.

3.3   Target Population

The target population recruited in the study were pregnant women of ages between 18 and 5years, seeking antenatal services at the Kitale District Hospital, residents of the study area, who had not received anthelmintics treatment for the last 3 months. They were requested to give consent. Those with ova of *Schistosoma mansoni* in their stool and malaria parasites in their peripheral blood films were excluded from the study because these are blood parasites but are also associated with low haemoglobin concentration.

3.4   Sample size determination

The required sample size for this study was calculated based on the prevalence rate of 11.2% of Hookworm Luoba *et al.*, (2005). The 95% confidence level and 5% marginal error, sample size (n) was determined using the formula Mugenda and Mugenda., (1999).

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

Whereby:

D is margin of error (0.05)

n is the minimum sample size

P is the estimated prevalence (11.2%)

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

$$n = \frac{(1.96)^2 \times 0.112 (1 - 0.112)}{0.05^2} = 152.828 \text{ rounded up to } 153$$

Therefore 153 pregnant women were recruited in the study. This was the minimum number of sample required for the study based on 11.2% prevalence of Hookworm which was the highest

compared to the prevalence of *A. lumbricoides* and *Trichuris trichiuria*, in a study done in Nyanza province of western Kenya, to determine the effect of earth-eating and reinfection with intestinal helminths among pregnant and lactating women Luoba *et al.*, (2005). Consecutive sampling was done at the health facility whereby every pregnant woman who fitted the inclusion criteria and consented was recruited into the study.

3.5   Data collection

3.5.1 Methodology: Objective 1 Prevalence and intensity of intestinal helminth infection

Stool Collection and Processing

A sample of fresh stool specimen was collected from all the 153 participants who consented. Subjects were provided with a labeled leak proof stool container (polypots), toilet paper, and applicator stick. Approximately 5gm of stool specimens was collected in to each polypot, using applicator stick. The stool specimens were examined microscopically within 24 hours of collection using the Kato-Katz technique (WHO, 1991). The procedure measures the prevalence and intensity of infection since it provides an accurate measure of the number of eggs present per gram of stool. For each stool sample two Kato slides were made and the average of the total number of eggs was taken. The magnifications of x10 and x40 were used respectively to visualize and identify the ova/eggs of intestinal helminths. Haradamori test tube filter paper method Garcia., (2001), was employed to culture hookworm eggs to filariform larvae. *Necator americanus* and *Ancylostoma duodenale,* filariform larvae were differentiated microscopically based on differences in their body morphology.

Kato Katz Technique

Each stool specimen was prepared using a sieve and a calibrated template to contain 47.1mg of stool. The preparation on the glass slide was covered with glycerin/malachite green impregnated cellophane. The preparation was then turned upside down on a flat surface and pressed gently to spread the stool sample ready for reading. The slides were examined within one hour to avoid over clearing of hookworm eggs by glycerine. All eggs in each preparation were counted to determine the number of eggs per gram. The egg counts were classified as Light infection, Moderate infection, and Heavy infection WHO, (1987), as follows Ascaris light infection (1-4999 eggs/gram), Moderate infection (5000-49,999 eggs/gram), and Heavy infection (> 50,000 eggs/gram). Hook worm-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) eggs/gram.

Haradamoi technique

Narrow filter paper strips of about 5 inhes that slightly tappered at one end was cut for each stool specimen that had hookworm eggs. 1gram of faeces was smeared at the centre of the strip. 4 millilitre of distil water was added to 15 millilitre conical centrifuge tube. The paper strip was inserted into the tube so that the tappered end was near the bottom of the tube. The water level was at slightly 0.05 inches below the faecal point. The tube was plugged using cotton wool and allowed to stand upright in a rack at 25 degrees centigrade 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 10 x objectives. Filariform larvae were examined for typical morphological features to differentiate between the two species of hookworm.

3.5.2 Methodology: Objective 2 Association between intestinal helminth infection and maternal haemoglobin level.

Blood Sample Collection and Analysis

To evaluate the association between intestinal helminth infection and maternal haemoglobin level, blood samples were collected from pregnant women using sterile syringe and needle, after sterilizing the cubital vein on the arm using 70% methylated spirit. The blood was transferred to heperanised vials to avoid clotting. Hemoglobin levels were estimated using coulter counter machine. This was done by placing heparanised blood sample in an automated coulter counter machine, that processed and red out the results in two minutes. Low haemoglobin may result from other causes apart from intestinal helminths. In order to differentiate this from other causes, white blood cell count (Eosinophils) was done using the coulter counter machine. Raised eosinophil of more than 6% is a positive indicator of parasitic infection (Cheesbrough, 2005).

Intestinal helminths (Hookworms and *Trichuris),* suck blood leading to low haemoglobin concentration that results to iron deficiency anaemia. To diagnose iron deficiency anaemia, 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 x100 objective. The presence of anisocytosis, poikilocytosis and microcytic,

hypochromic red cells suggested iron deficiency anaemia (Cheesbrough, 2000). Presence of Malaria parasites was determined in this film to exclude malaria infected clients from the study.

3.5.3 Methodology: Objective 3 Socio-economic factors that predisposes pregnant women to intestinal helminth infection.

A semi structured questionnaire was developed and administered to pregnant women prior to stool and blood sample collection to obtain age, marital status, geophagy, educational level, occupation, home environment and sanitation. This was to address socio economic factors that pre dispose pregnant women to intestinal helminth infection. The use of questionnaire is justified for it enables coverage of a wide area and extensive contents within a short period of time. Quality control was performed by daily review of each questionnaire by the researcher for immediate remedy of any error or problems.

3.6   Data analysis

Data was analyzed with the Statistical Package for Social Science (SPSS version 16.0). In order to determine prevalence and intensity of intestinal helminth infection, data generated through egg count was analysed using descriptive statistics (percentage scores). Helminth infection was determined by counting and data generated was categorised into 3 groups (low, moderate, and heavy).

In order to evaluate association between intestinal helminth infection and Haemoglobin levels plus eosinophil count, Chi-square test was used. In order to determine Socio-economic factors that predispose pregnant women to helminth infection bivariate motel was used. Since helminth infection was the outcome of interest, it was categorized in to a binary variable where those with helminth infection were recorded as 1 and those without condition of interest as 0. Independent factors considered for bivariate model were, age category, hand washing, education level of the mother, type of housing, wearing of shoes, mode of waste disposal, source of domestic water, type of residence and occupation status. Level of statistical significance was chosen as 1% in this study.

3.7   Ethical Consideration

The study sought ethical clearance from Egerton University ethics review committee, and consent from Kitale District Hospital. A written informed consent was sought from the respondents to participate in the study. Questionnaire was administered to every participant before stool and blood collection. Special codes were used to maintain confidentiality.

Completed questionnaires were kept under a key and lock cabinet accessible only to the principal researcher. Pregnant women infested with intestinal helminths got an appropriate treatment at the hospital.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Objective 1: : Prevalence of Intestinal Helminth Infection.

Table 1: Prevalence of Intestinal Helminth Infection.

No. of samples positive for helminths	No. positive for *Ascaris lumbricoides*	No. positive for *N. Americanus*	No. positive for *Trichuris trichura*	No. positive for *Enterobius vermiclaris*	No. positive for Mixed infection *A.lumbricoides/ T.trichiura*
21/153	10 (47.6)	6 (28.6)	2 (9.5)	1 (4.8)	2 (9.5)

A total of 153 pregnant women were recruited in the study. Overall prevalence was 21 (13.7%). Number not infected were 132(86%). The intensity of intestinal helminth infection among (21) infected pregnant women, was categorized as heavy 3 (14.3%), moderate 9 (42.9%) and low 9 (42.9%), respectively. Plate 5 below is intestinal helminths eggs as they were seen in the microscope.
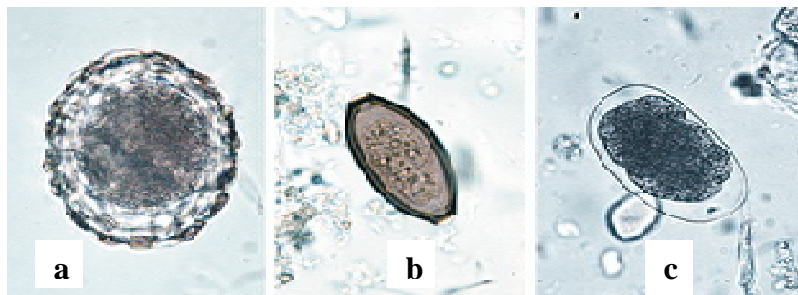
a) Egg of *Ascaris lumbricoides*   .
b) Egg of *Trichuris trichura*
c) Egg of *Necator americanus*

Plate 5: Photographs of the 3 helminth eggs

This study was able to identify *Necator americanus* as hookworm species infecting pregnant women attending antenatal clinic at Kitale District Hospital. Haradamori test tube filter paper method Garcia, L., (2001), was employed to culture hookworm eggs to filariform infective stage. Differentiation between *Necator americanus* and *Ancylostoma duodenale* was done microscopically basing on differences in their body morphology i.e. *Necator americanus* has the following body features. Head is rounded, mouth spears are clear and divergent, has sheath, anterior end of intestine, is as wide as the oesophageal bulb, there is gap between the oesophagus and intestine, length of the oesophagus is 1/3 in proportional to the whole body and total larval length is about 590*um*. Plate 6 below is the filariform larvae of *N.amercanus* it was seen under the microscope.

Plate 6: Filariform larvae of *Necator americanus*.

4.1.2: Objective 2: Association between intestinal helminth infection and maternal Hb Levels

Table 2 : Association between intestinal helminth infection and maternal Hb Levels

VARIABLE		HB LEVELS		
		< 11g%	>11g%	P value
		No. (%)	No. (%)	
Helminth infection	Infected	7 (28%)	14 (10.9%)	0.032
	Not infected	18 (72%)	114 (89.1%)	
Type of helminth infection	*Ascaris lumbricoides*	0 (0%)	10 (100%)	0.013
	N. americanus (Hookworm)	5 (83.3%)	1 (16.7%)	
	Trichuris trichura	1 (50%)	1 (50%)	
	Enterobius vermicularis	0 (0%)	1 (100%)	
	Multiple(mixed) infection *(Ascaris/T trichiura)*	1 (50%)	1 (50%)	

Significance level, (P=0.01)

Moderate and heavy intensities of *Necator americanus* were found to be associated with low maternal haemoglobin levels. There was an association between increasing *Necator americanus* (hookworm) egg counts and decreasing haemoglobin levels, however (P=0.013). No association was found between Ascaris*, Trichuris or Enterobius* egg counts and haemoglobin level 0 (0%). Egg threshold count of *Necator americanus*, with haemoglobin was found at > 2,000 eggs per gram

Table 3: Peripheral blood film (Pbf) and eosinophil count of helminth infection

VARIABLES		*Ascaris lumbricoides*	*Necator americanus*	*Trichuris trichiura*	*Enterobius vermicularis*	Mixed infection (*Ascaris/ Trichuris*)	p-value
Peripheral Blood Film	Microcytic hypochromic	0 (0%)	4 (66.7%)	0 (0%)	0 (0%)	0 (0%)	0.015
	Normocytic normochromic	10 (100%)	2 (33.3%)	2 (100%)	1 (100%)	2 (100%)	
Eosinophil count	>6 Raised	9 (90%)	4 (66.7%)	0 (0%)	0 (0%)	5 (50%)	0.06
	1-6 Normal	1 (10%)	2 (33.3%)	2 (100%)	1 (100%)	5 (50%)	

Significance level, (P=0.01).

The peripheral blood film observations in this study (Table 3), revealed that pregnant women infected with heavy intensities of *Necator americanus* had microcytic hypochromic red blood cells at (P=0.015). The coulter eosinophil count results revealed that 9 (90%) of the women with heavy ascarid infection had eosinophilia (>6) and 4 (66.7%) *Necator americanus* infected women, especially those with moderate and heavy infections had elevated eosinophils. Eosinophil reading in pregnant women infected with *Trichuris trichiura* and *Enterobius vermicularis* was not raised because the intensity of the infection was low.

4.1.3 Objective 3: Socio-economic factors predisposing pregnant women to helminth infection.

Table 4: Socio-economic factors predisposing pregnant women to helminth infection.

Variable		Helminth infection		P-value
		Infected	Not infected	
		No. (%)	No. (%)	
Geophagy	Eats soil	12 (57.1)	0(0)	
	Does not eat	9 (42.9)	132 (100.0)	xx
Age category	< 29years	18(85.7)	73(55.3)	0.008**
	>30years	3(14.3)	59(44.7)	
Waste disposal	Pit latrine	20 (95.2)	98 (74.2)	0.047**
	Toilet	1 (4.8)	34 (25.8)	
Hand washing	Washes hands	11 (53.4)	116 (87.9)	
	Does not wash	10 (47.6)	16 (12.1)	0.001**
Use of Protective Shoes	Wears shoes	2 (9.5)	19 (14.4)	
	Does not wear shoes	19 (90.5)	113 (85.6)	0.547
Domestic water source	Tap	14 (66.7)	101 (76.5)	
	Well/river	2 (9.5)	13 (9.9)	
	Spring	5 (23.8)	18 (13.6)	0.475
Education level	Primary and below	8 (38.1)	13 (9.8)	0.002**
	Secondary and above	13 (61.9)	119 (90.2)	
Housing	Permanent	3 (14.3)	71 (53.8)	0.001**
	Mud/semi permanent	18 (85.7)	61 (46.2)	
Residence	Rural	10 (47.6)	50 (37.9)	
	Urban	11 (52.4)	82 (62.1)	0.396
Occupation	Employed	7 (33.3)	73 (55.3)	
	Not employed	14 (66.7)	59 (44.7)	0.061

P=0.01 denotes level of significance. .xx denotes that one of the variables was 0 hence the values were not able to be computed. ** means the value is significant

At bivariate analysis, factors such as age, waste disposal, hand washing, education level of the pregnant women, and type of housing were significantly associated with helminth infection. (Table 4). Younger pregnant women aged below 29 years had a higher proportion of being infected with helminth infection when compared to their counterparts not infected (85.7% vs. 55.3%) respectively (P=0.05). Women having waste disposal as pit latrines were more likely to have helminth infection when compared to those who were using toilet as source of waste disposal (95.2% vs. 4.8%), (P=0.047). Similar trend was also seen among those with primary education level where women with primary education level were at a higher risk of helminth infection as compared to their counterparts with secondary level (38.1% vs. 9.8%) respectively. Living in a semi permanent house had a higher chance of getting helminth infection (14.3% vs. 53.8%) (P=0.001) Being employed or unemployed was not significantly associated with helminth infection in this population (P=0.061).

CHAPTER FIVE:

DISCUSSION

This study was undertaken to identify intestinal helminths, and their prevalence including socio-economic factors among pregnant women attending antenatal clinic at Kitale District Hospital. Out of 153 stool samples, examined from pregnant women, 21 (13.7%) were diagnosed with intestinal helminths. They were identified as *Ascaris lumbricoides* 10(6.5%), *Necator americanus* 6(3.9%), *Trichuris trichiura 2*(1.3%), and *Enterobius vermicularis* 1(0.7%). These parasites have been reported in some parts of Kenya Van eljik *et al.,* (2009); Luoba *et al.* (2005,), and other parts of the world (Artemis *et al.,* 2008).

In this study, an overall prevalence rate of 13.7% reported, was higher than some findings from previous studies. For example a study in Congo revealed (9%), and in Nigeria (12.5%) Mordi *et al*., (2007); Alfonso *et al.,* (2006) reported a value of 0.7% in Edo state Nigeria. The overall prevalence reported in our study was lower compared to findings from other studies Luoba *et al.,* (2005) reported prevalence of 19.6% among 827 pregnant women in Nyanza Province Kenya).

Alfonso *et al.,* (2006), reported prevalence of 45.1% in Brazil (Sao Paulo State), and 69.7% in Indonesia. Godwin *et al*., (2010) reported prevalence of 23% among 300 pregnant women in Kassena-Nankana District Ghana. The difference in this study and other studies could possibly be attributed to a relatively small sample size of 153 pregnant women for example compared to 827 in the report by Luoba *et al* (2005). However, this could be verified if a larger sample size is used subsequently in a similar study at the Kitale District Hospital.

The predominance of *Ascaris lumbricoides* compared to other intestinal helminth agreed with some previous reports by Adeyeba and Akinlabi., (2002), as well as Agbolate *et al*., (2004). It is well established that the infective stages of *A. lumbricoides*, have enormous capacity in withstanding environmental extremes.Furthermore, *Ascaris lumbricoides* eggs are coated with mucopolysaccharide substance which makes these eggs adhesive to different body surfaces. This accounts for their adhesiveness to door handles, dust, fruits and vegetables, paper money and coins (Omorodion *et al.,* 2012). Consequently, due to poor observance of personal hygiene, pregnant women in the study area inadvertently get infected through these means.

The 6.5% prevalence value reported for *Ascaris lumbricoides* in our study was however low compared to what was reported in other areas by different workers. An examination of 2,394 stool samples from Indonesia showed the prevalence of *A. lumbricoides* as 73.7% Widjana *et al*., (2000). This study value was significantly higher than what was reported by Omudu *et al*., (2004), who reported a prevalence of 1.8% in Markurdi, Benue State, Nigeria and Ramos *et al.,* (2005), who reported a value of 0.7% in a rural community in Mexico.

Human ascariasis is spread through faecal pollution of soil, and so the intensity of infection depends on the degree of soil pollution (Mordi *et al.*, 2007). Man acquires infection by accidentally ingesting embryonated eggs in contaminated food, drink or soil. Ascaris ova are also spread through the agents of flood and coprophagous animals, and can thus be transported to locations far from the defecation sites (Obiamiwe *et al.,* 1990). The eggs are passed unaltered through the intestine of coprophagous animals. The well-protected eggs can withstand drying and can survive for very lengthy periods in soil. This infection is distributed throughout the world.

It is well established that the infective stages of *A. lumbricoides*, have enormous capacity of withstanding environmental extremes. Furthermore, *Ascaris* eggs are coated with mucopolysaccharide substance which makes these eggs adhesive to different body surfaces. This accounts for their adhesiveness to door handles, dust, fruits and vegetables, paper money and coins (Awolaju *et al*., 2009). Consequently, due to poor observance of personal hygiene especially lack of hand washing before eating, inadvertently leads to ingesting infective eggs leading to infection. However prevalence of *Ascaris lumbricoides* recorded in this study indicates some unhygienic practices among some pregnant women which enhanced transmission.

Necator americanus was the second most common parasite species identified in this study, with prevalence value of 3.9%. This value is low compared to the values from other studies from other parts of the country and outside Luoba *et al*., (2005), reported 11.2%, Hookworm prevalence in pregnant women who eat soil in Kisumu county Kenya, Brooker *et al*., (2008) reported 74.9% among pregnant women attending antenatal clinic at Kilifi Kenya. Elsewhere Brooker *et al*., (2008) reported 56.6% in 128 pregnant women at Ukerewe Island Tanzania, 44.5% of the 2507 pregnant women in Entebbe Uganda, and 8.1% of the 1038 pregnant women attending antenatal clinic at Peru, Nine states Venezuela. A study by, Egwuyenga *et al*., (2004) reported infection rate of 22.5% at Eku in Delta State of Nigeria. Hookworm infections occur by skin

penetration of the L3 stage infective larvae. Poor sanitary disposal of human faeces and indiscriminate defecation are the principal factors in the aetiology of hookworm infections, (Mordi *et al.,* 2007).

Prevalence of *Trichuris trichiura* 1.3% was lower compared to, Luoba *et al* (2005) who reported 4.6% among 827 pregnant women in Nyanza province Kenya, and also 1.7%, reported by, Alakija *et al.,* (1986), in Nigeria. It is however higher than, 0.9% reported by, Baidoo *et al.,* (2010) among pregnant women in Ghana.

Transmission occurs through poor sanitary habits of indiscriminate defecation. Infections usually occur through ingestion of infective ova from contaminated hands, food or drinks. Flood and copraphagous animals play some part in the transportation of the ova to locations other than the defecation site. The low prevalence value generally recorded for this organism supports the claim that it is less common in the tropics than in the temperate regions, (Mordi *et al.*, 2007).

The prevalence of *Enterobius vermicularis* (0.7%) reported in this study was close to the values 0.9% recorded in Ghana among pregnant women, Baidoo *et al,* (2010). Also, Mordi *et al.,* (2007) reported a value of 1.0% in Edo State Nigeria. However, this value was lower compared to 3.5% reported by, Alli *et al.,* (2011) in Nigeria. The study findings support the fact that this infection is distributed throughout the world but less common in the tropics, (Mordi *et al.,*2007). Intestinal helminth infections in this study carry a significant impact for both maternal health and foetal development. Differences in the percentage of the results observed in this study and other studies could be due to the study population, methodology used, geographical location, sample size used, environmental factors and variations of economic status, of the subjects.

It is well-established that human hookworm infection results in intestinal blood loss which, in turn, can contribute to low haemoglobin levels. What has remained unclear and hindered public health policy and planning is the extent to which hookworm is associated with low haemoglobin level during pregnancy, Brooker *et al.*, (2008). The results of our systematic review quantify this relationship and confirm that heavy intensities of *Necator americanus* (hookworm) infection are associated with lower levels of haemoglobin than light infection intensities (P=value 0.013) Other studies identified four conditions necessary to show an association between hookworm infection and haemoglobin, a large sample size; quantitative measures of haemoglobin and hookworm infection; sufficient variation in infection levels; and few other competing causes of

anaemia (Brooker *et al.,* 2008). These conditions are also relevant to interpreting the current results.

The study results showed that ppregnant Women infected with *Necator americanus,* 5 (83.3%) had haemoglobin below 11g%, compared to pregnant women who were not infected with *N. americanus*. Various studies in Kenya and other parts of the world have shown similar findings Mary *et al.*, (2005), found an association between heavy intensity of hookworm infection and low haemoglobin in their study carried out in Kenya and Zanzibar. This was significant, both in children and adults, particularly, among pregnant women. A similar association was confirmed by Balachew *et al.,* (2006), among pregnant women attending antenatal clinic at Jimma University, south western Ethiopia. The study results also corroborate those of Larocque *et al.*, (2005) in their study to establish the relationship between intensity of soil-transmitted helminth infections (Hookworm) and low haemoglobin levels during pregnancy in Peru. They reported a significant association between increasing hookworm egg counts and decreasing haemoglobin levels. A study by Godwin *et al.,* (2010), among pregnant women in Kassena-Nankana District, Ghana also indicated that worm infections have greater impact on the haemoglobin levels of pregnant mothers in the district especially hookworm infections.

The study findings corroborate Stoltfus *et al.,* (1997), who identified Hookworm as important contributor to low haemoglobin levels in many resources limited settings. Previous studies, (Van Eijk *et al.*, (2009); Brooker *et al.,* (2008); Bondevik *et al.,* (2000), showed that heavy intensities of hookworm infection are inversely related to Hb concentrations. Our results also concur with a study done by Ayoya *et al.*, (2006), in Ghana where he found out that heavy infection by hookworm was associated with low haemoglobin levels. At a hospital in Kathmandu, Nepal, hookworm infection was associated with severe but not moderate low haemoglobin among women receiving antenatal care (Bondevik *et al.,* (2000), and in East Africa Stoltzfus *et al.,* (2000); Brooker *et al.*, (1999); found out that egg counts of (200-2000 epg) were associated with low haemoglobin after infection thresholds of 1000 epg and 2000 epg had been reached. In this study, the helminths thresholds were found to be 1,000 epg. These are consistent with the lower category recommendations of moderate hookworm infection WHO (1987), based on data obtained in child populations

The study finding confirms that individuals from poorer households had a significantly higher prevalence and intensity of hookworm infection than individuals from better-off households. This finding is consistent with results from a study carried out among schoolchildren in rural Cˆote d'Ivoire, which employed the same statistical method for measuring socio-economic status Raso *et al*., (2005). Studies conducted in Kenya and Panama also showed that hookworm is associated with the absence of a latrine Olsen *et al*., (2001) and low socioeconomic status Holland *et al*., (1988). In the current study, it is unlikely that differences in hookworm infection according to socio-economic status reflect differences in access to anthelminthic drugs since these are not readily available in local shops. More probable is that observed differences reflect variation in sanitation conditions and housing conditions.

Similar result was obtained by Belachew and Legese., (2006). Most rural pregnant women attending antenatal care walk barefoot. Even those women who have shoe do not wear regularly. They wear shoe when they come to town for antenatal care and for marketing. Walking barefoot may predispose them to hookworm infection and the consequence will result in low haemoglobin especially in pregnant women.

Hookworm sustains its life by blood sucking, a process that ruptures the host capillaries and arterioles followed by the release of a battery of pharmacologically active polypeptides which induces intestinal blood loss. Adult *N. americanus* worm sucks approximately 0.05 ml/dl of blood and *A. duodenale* approximately 0.25 ml/dl of blood per day Huddle *et al.*, (1999). This long term blood loss can manifest itself physically through facial and peripheral oedema and eosinophilia, resulting in iron deficiency anaemia (Zhan *et al.*, 2002; Stoltzfus *et al*., 1997); Hotez *et al.,* 1995). Pathological studies indicate that *A. duodenale* causes greater blood loss; *N. americanus* also sustains life by sucking blood (Pawlowski *et al*., 1991).

Epidemiological studies among Zanzibari school children suggested that *A. duodenale* is associated with an increased risk of causing low haemoglobin (Albonico *et al.,* 1998). Thus, where hookworm is exclusively *A.duodenale*, such as in Nepal Dreyfuss *et al*., (2000), he observed effect on maternal low haemoglobin might be greater. Bundy and colleagues estimated that in low income countries, 44 million (35.5%) out of 124 million pregnant women were infected with hookworm (Budy *et al*., 1995). A study by Brooker *et al.,* (2008) estimated that 6.9 million (26.7%) out of 25.9 million pregnant women in sub Saharan Africa were infected with

hookworm. These figures could be higher by now especially in rural poor communities where sanitation is not well addressed.

The interaction between *Ascaris lumbricoides* and *Trichuris. trichiura* infection and intensity was not surprising, given the common mode of transmission of these two worm species. The study findings confirm those of previous studies Needham *et al.,* (1998); Booth and Bundy., (1992). Infection with *A. lumbricoides* and *T. trichiura* was not significantly associated with low haemoglobin levels in the expectant mothers. These finding confirm those of previous studies (Needham *et al.,* 1998; Booth and Bundy.,1992; Larocque *et al.,* 2005).

However an increasing number of studies of helminth epidemiology have shown that it is common for individuals to be infected with more than one species of helminths (Brooker., *et* al., 2000). A number of epidemiological studies have also indicated that individuals infected with multiple species of helminth often harbour heavier infections than individuals infected with a single helminths species Booth., (1998); Needham., (1998). In contrast this study found few individuals infected with more than one species of intestinal helminths and the infection was light. This may be attributed to small sample size in this study, geographical location of the study area, individual's personal hygiene and socio economic status of study subjects.

Low haemoglobin in developing countries has multiple causes, including micro-nutrient deficiencies, infectious diseases and inherited disorders (Tolentino *et al.,* 2007). The causal association between haemoglobin and hookworm infection in this study could have been confounded by other causes such as dietary iron insufficiency, sickle cell, a genetic disorder that causes low haemoglobin. The confounders were eliminated through reading of the peripheral blood films. Peripheral blood films of People with low haemoglobin levels, associated with parasitic infection show microcytic hypochromic red cells and their eosinophis are raised, (Cheesbrough., 2005). Peripheral blood films of pregnant women infested with heavy *N.americanus* in this study showed microcytic hypochromic red blood cells (P value 0.015).

The eosinophils were elevated >6% in those infected by *A.lumbricoides* and *N.americanus*. Malaria parasites can be detected in peripheral blood film. This was used to eliminate those infected by malaria parasites from the study, though all the participants were free from malaria. This could be because all antenatal mothers are given treated mosquito nets free of charge by the

Kenya government. Few studies have examined these relationships in pregnant women. In this study the results indicates that heavy intensities of *N. americanus* (hookworm) infection have an impact on the haemoglobin levels in pregnant women attending antenatal services at Kitale District Hospital.

Findings from this study revealed that pregnant women predisposed to intestinal helminth infection included those bellow 29years (P value 0.008), compared to their older counter parts. The study results agrees with A study by Traub *et al* (2004) who noted a decrease in hookworm prevalence after approximately 25 years of age. The prevalence of *A. lumbricoides* and *T. trichiura* was reported to peak in childhood and decline there after around 30 years.

Those with basic primary education (P value 0.002) and living in mud/semi permanent houses (P-value 0.001), had higher chance of intestinal helminth infection compared to their counter parts with secondary level education staying in permanent houses. The results of this study revealed that eating of soil, and lack of regular hand washing ((P value 0.001), contributed to infection by intestinal helminths. Our result was in agreement with (uoba *et al*., (2005) and also Larry and Janovy; (1996)., cheesbrough; (2005). Poverty, ignorance, geophagy, personal hygiene, and environmental sanitation were found to predispose the subjects to parasitic infection.

Pregnant women living in rural areas in the present study have higher chances of being infected by intestinal helminths compared with their counterparts in urban areas. This may be due to poor environmental sanitation and low socio-economic status in rural areas. This results was in agreement with other study findings Osumba *et al*., (2002), low socio-economic status and poor environmental sanitation which includes poor methods of refuse disposal predisposes pregnant women to helminth infection.

The cases of intestinal helminths reported in this study may be due to faecal pollution of the soil/environment where the respondents whose stool samples were used. This is in agreement with (Mordi *et al.,* 2007). Their study revealed that soil pollution is thus a major factor in the epidemiology of human helminthiasis. It is well documented for example that *Ascaris* eggs, in particular, have been demonstrated to survive in composted faeces up to 10 months Unimproved pit latrines or random defecation may posse risk of infection. (Feachem *et al*., 1983).

Discrepancies observed in the findings of this study and previous studies by other researchers, could be attributed to differences in place of study i.e. geographical location, study population and sample size.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study established that intestinal helminth infection is prevalent among pregnant women attending antenatal clinic at the Kitale District Hospital. *Ascaris lmbricoides was* the most prevalent, followed by *Necator Americanus*, *Trichuris trichiura* and *Enterobius vermicularis*. Heavy intensities of *N americanus* infection were found to be associated with low maternal haemoglobin levels. Results from this study indicated that eosinophilia suggested presence of intestinal helminth infection in pregnant women

Socio-economic factors that predispose pregnant women to intestinal helminth infection, included, age, pregnant women below 29 were at more risk of getting infected compared to their older counter part. Others factors included primary education, mud housing, eating of soil (geophagy), improper waste (faeces) disposal and lack of hand washing after toilet use. This information is very useful in the control strategy for intestinal helminthiasis during antenatal care.

5.2. Recommendations

The findings from this study thus support the need for the establishment of a health programme for the control of the intestinal helminths in pregnancy. For this reason, measures should be adopted to monitor, control or prevent this tendency of helminths eggs from invading the body system. Many of the parasitic infections are chronic, and many women enter pregnancy with these pre-existing conditions. Therefore based on the result obtained from this study, I recommend that;

    a)   All antenatal clinics and health facilities should offer a routine examination of stool of pregnant women for intestinal helminths and treat the infected women using albendazole.

    b) Health education should be enhanced both in rural and urban slum setups to highlight the principles of basic personal hygiene. These include, the importance of toilet use,

hand washing after visiting the toilet, shoe wearing and effects of eating soil. These will enhance the measures taken to control or prevent infection of intestinal hehminths in pregnancy.

c) This study recommends further study on Risk benefits ratio of intestinal helminths in pregnancy which was not studied here but very relevant to future studies for National Health policy development on control strategy.

d) More funds are needed to study a large sample size on pregnant women in other geographical areas of Kitale and possibly other areas of the country. This would produce evidence of different intestinal helminths in different ratios, intensities and haemoglobin profiles that can assist in arriving at a comprehensive approach in solving the problem of intestinal helminth infection in pregnancy.

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APPENDICES

APPENDIX A: INFORMED CONSENT

You are being requested to participate in a research study because you are eligible for this study and your responses might help in controlling intestinal helminth infection in pregnancy. On the questionnaire your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose. You have every right to accept or refuse participation in this study. You will be requested to give small amount of blood and stool. Blood will be collected from the vein of your arm using sterile syringe and needle There will be some pain during blood removal but not harmful to your health. If you agree to give samples, then you will be required to answer the questionnaire. Based on the laboratory investigation, pregnant women infested with helminths will be treated free of any cost.

CONSENT:

I have read the above information / information have been explained to me and I have fully understood. I have had opportunity to ask questions, and all my questions have been answered to my satisfaction.

Please tick in appropriate box below.

I DO CONSENT (   ). Am willing to participate in this study by giving blood and stool sample and also respond to the questionnaire

I DO NOT CONCENT (   ). I do not wish to participate in the study.

Patient name....................................Signature……………………Date …………………

I certify that I have followed all the study specific procedures described in the standard operation procedure for obtaining informed consent.

Researcher signature…………………………Date……………………..Time……………

Questionnaire Number_____

Date_____

PREAMBLE

My Name is Antony W. Wekesa, an M.Sc student at the Department of Biological Science, Egerton University. I am carrying out a study on intestinal helminth infection and anaemia among pregnant women seeking antenatal services at Kitale District Hospital. I would be very grateful if you responded to my questions, honestly and exhaustively this is very important for the success of this study. The information you provide will be treated with utmost confidentiality and anonymity. This research has been cleared by Egerton University and Kitale District Hospital authority, your cooperation and sincerity will be highly appreciated.

Thank you for your cooperation.

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.

1. Age _____

2. Marital status: Married ( ), Single ( ), Divorced ( ), Widow ( )

3. Highest levels of education achieved

   None ( ), Primary ( ), Secondary, ( ), College ( ), University ( )

4. Where do you live? Urban ( ), Rural ( ), Slum ( ), Other…………..

5. When did you take antihelminth drugs last…………?

6. How do you earn a living? Specify……….….

7. How many children do you have? ( )

8. What type of house do you live in? Specify…………

SECTION B: FACTORS ASSOCIATED WITH HELMITH INFECTION

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

   Other…………………………

10. If yes what do you do after visiting the latrine?

11. Where do you get water for domestic use?

   Tap ( )   Protected spring ( )

   River ( )   Bore hole ( )

   Other source…………………………………………………………….…..

12. Do you wear shoes especially when working on the farm? 1 Yes ( )   2 No ( )

    If yes how frequent………………………………….………………….…

13. Have you ever felt like eating soil? 1 Yes   ( )   2. No ( )

APPENDIX C: CONSENT LETTER

MINISTRY OF MEDICAL SERVICES

KITALE DISTRICT HOSPITAL

Tel: (054) 31593, Fax: 31593
Email:dmsokitale@yahoo.com
When replying please quote

District Medical services Officer,
Trans Nzoia/ Uasin Gishu Districts,
P.O. Box 98,
KITALE.

REF NO KTD/16.12.2011

16th DECEMBER 2011

THE VICE CHANCELLOR,
EGERTON UNIVERTY
SCHOOL OF SCIENCE
DEPARTMENT OF BIOLOGICAL SCIENCES
P O BOX 536
NJORO

ATT. DIRECTOR OF POST GRADUATE SCHOOL

RE: CONSENT LETTER

This is to satisfy that ANTONY W. WEKESA a Master of Science (Medical Parasitology) Student Reg. No. SM 17/2792/10, Egerton university has been given a consent by the Hospital authority to carry out his research work on Helminth related anaemia among pregnant women seeking antenatal services at the Hospital.

His research runs run from 1st December to 30th April 2012.

He will be required to collect, Process and examine stool for helminthes ova, then blood for haemoglobin level, white blood cell count and peripheral blood films(PBF).

Only pregnant women that give concent will be included in the study.

Dr Kasembeli G.
District Medical Services Officer
KITALE DISTRICT HOSPITAL

APPENDIX D: ETHICS REVIEW APPROVAL

EGERTON UNIVERSITY
TEL: 051-2217808
FAX: 051-2217942

P. O. BOX 536
EGERTON, KENYA

OFFICE OF THE DEPUTY VICE-CHANCELLOR
DIVISION OF RESEARCH & EXTENSION

RESEARCH ETHICS COMMITTEE

Ref: EU/DVRE/028

December 6, 2012

Wekesa Antony Wanyonyi
P.O. Box 750
KAKAMEGA

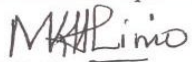**RE: APPLICATION FOR ETHICAL APPROVAL OF RESEARCH PROJECT**

Reference is made to your application for ethical clearance of your research project entitled "*Prevalence of Intestinal Helminth Infection in Pregnant Women Attending Antenatal Clinic at Kitale District Hospital in Kenya.*

This is to inform you that the Egerton University Research Ethics Review Committee met on 4th Dec 2012 and discussed your application. The Committee observed that due consideration was given to the following ethical issues that would arise from the conduct of the study:

i) That participation is based on informed consent.
ii) That the specimen collection and testing is a routine activity done in many hospitals and therefore the participants would not be exposed to risks.
iii) That the identity of the participants would not be exposed.
iv) That the study is beneficial as participants found to be positive with parasites and anemia would be treated at the hospital.

The committee therefore gave ethical clearance to your research project. You are required to note that the authorization to conduct the study has already expired and you will be required to apply for clearance if you plan to continue with the study or analysis beyond this phase.

Please further note that the Standard Operating Procedures (SOPs) requires that you submit a copy of the final report of your study to the Committee.

Prof. M. K. Limo
CHAIRMAN – RESEARCH ETHICS COMMITTEE

c.c. DVC (R&E)
     Director Research
MKL/pao

Egerton University is ISO 9001 : 2008 Certified

Research Article

1. **Intestinal Helminth Infections in Pregnant Women Attending Antenatal Clinic at Kitale District Hospital, Kenya**

A. **W. Wekesa,1 C. S. Mulambalah,2 C. I. Muleke,3 and R. Odhiambo4**

*1 Department of Biological Sciences, Faculty of Science, Egerton University, P.O. Box 536,
Njoro 20107, Kenya
2Department ofMedical Microbiology and Parasitology, School of Medicine, College of Health
Sciences,Moi University,
P.O. Box 4606, Eldoret 30100, Kenya
3Department of Veterinary Clinical Services, Faculty of Veterinary Medicine & Surgery,
Egerton University, P.O. Box 536,
Njoro 20107, Kenya
4Department of Gender Studies, Egerton University, P.O. Box 536, Njoro 20107, Kenya*

Correspondence should be addressed to C. S. Mulambalah; csmulambalah@gmail.com

Abstract

Intestinal helminth infections during pregnancy are associated with adverse outcomes including low birth weight and prenatal mortality. The infections are amajor public health problem in developing countries. A hospital based survey was undertaken for six months to determine the infection prevalence, intensity, and risk factors. The study involved expectant women attending antenatal clinic. Stool samples were screened microscopically for helminth ova using Kato Katz technique. Information on risk factors was collected using semistructured questionnaire and analyzed using SPSS. Epidemiological data was analyzed using descriptive statistics and

multivariate analysis. The overall prevalence of infection was 21 (13.8%). Ascariasis was the most prevalent 10 (6.5%), hookworm infection was 6 (3.9%), and trichuriasis was 2 (1.3%). Pregnant women aged below 29 years (OR = 3.63, CI = 0.87– 11.75) and those with primary level of education (OR = 3.21, CI = 0.88–11.75) were at a higher risk of infection compared to those aged ≥ 29 years with secondary level of education. Hand washing was significantly associated with reduced likelihood of infection (OR = 0.18, 95% CI = 0.06–0.57). It was concluded that intestinal helminth infections were prevalent among pregnant women. We recommended that all expectant women visiting antenatal clinics be screened for intestinal helminth infections and positive casesbe advised to seek treatment.

Original Research Article

   2.   Analysis of hookworm infection intensity and maternal haemoglobin levels

in women attending antenatal clinic at Kitale, Kenya

Wekesa Antony Wanyonyi1, Mulambalah Chrispinus Siteti2

Inyagwa Charles Muleke3 and Orenge Caleb Oburu4

1Department of Biological Sciences, Faculty of Science, Egerton University, P.O. Box 536, Njoro 20107, Kenya.
2Department of Medical Microbiology and Parasitology, School of Medicine, College of Health Sciences, Moi University, P.O. Box 4606, Eldoret 30100, Kenya.
3Department of Veterinary Clinical Services, Faculty of Veterinary Medicine & Surgery, Egerton University, P.O. Box 536, Njoro 20107, Kenya
4Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Medicine & Surgery, Egerton University, P.O. Box 536, Njoro 20107, Kenya.

A b s t r a c t

It is well-established that human hookworm infection results in intestinal blood loss which, in turn, can contribute to low haemoglobin levels especially in pregnant women. One third of all pregnant women in developing countries are infected with low haemoglobin level is a common public health problem in many developing countries and is mainly attributed to parasitic intestinal helminth infections. The parasite species involved and host-parasite outcomes have not

been adequately studied in different specific population segments in Kenya. A six month hospital based study to assess the association between hookworm infection, spatial variation in intensity of infection and maternal haemoglobin levels was undertaken at a district hospital. A total of 153 pregnant women who consented participate were enrolled in the study. Data was analyzed using SPSS windows version 16.0. Chi-square was used to determine the association of Necator americanus infection and maternal haemoglobin level.

21(13.8%) out of 153, had intestinal helminth infections. Ascaris lumbricoides was10 (6.5%) Necator americanus 6 (3.9%). Trichuris trichiura 2 (1.3%). A significant negative association was observed between heavy infection of Necator americanus and maternal low haemoglobin level (P-value 0.13). We concluded that heavy intensities of Necator americanus are associated with low haemoglobin levels in pregnant women. It is recommended that all women of child bearing age living in hookworm endemic areas be subject to periodic antihelmintic treatment and incorporation of de-worming in antenatal care programs.