

**Amoebiasis levels in relation to sanitation and personal hygiene among patients attending Kauwi and Muthale hospitals in Kitui county, Kenya**

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APPLIED SCIENCES OF KENYATTA UNIVERSITY**

**DECEMBER, 2014**

**DECLARATION**

This thesis is my original work and has not been presented for a degree in any other university or for any other award.

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**DEDICATION**

To my brother Mutisya, you are an inspiration upon which my academic work is established.

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**ABBREVIATIONS AND ACRONYMS**

ALA	Amoebic liver abscess
AMREF	African Medical and Research Foundation
CDC	Centers for Disease prevention and Control
DDP	District Development Plan
DNA	Deoxyribonucleic Acid
ECM	Extracellular matrix
MDG 4	Millennium Development Goal four
mg	Milligram
PCR	Polymerase Chain Reaction
RBC	Red Blood Cell
UNESCO	United Nations Educational, Scientific and Cultural Organization
UNICEF	United Nations Children's Fund
WHO	World Health Organization
µm	Micrometer

## ABSTRACT

*Entamoeba histolytica*, an intestinal protozoan parasite, is the causative agent for amoebiasis which is the third leading parasitic disease causing deaths in humans after malaria and schistosomiasis. Globally, it is responsible for 40,000 to 100,000 deaths annually. Amoebiasis is on the rise in Kitui County and is responsible for diarrheal illnesses and early deaths in children under 5 years. There is no surveillance system in Kenya to combat the disease and its real level of occurrence remains unknown in Kitui west district. It occurs particularly in the tropics largely due to conducive temperatures for the parasite to complete its life cycle and is more common in areas with poor sanitation where barriers between human feces, food and water are inadequate. How this infection associated with age remains unclear. As a result, this study was aimed at determining the occurrence levels of *E. histolytica* infection among persons of various age groups attending Muthale mission and Kauwi sub-district hospitals in Kitui west district. The study involved observation of clinical signs and symptoms as well as microscopic examination of *E. histolytica* trophozoites and cysts in feces. The patients were sampled using simple random method. Fecal samples were collected from a total of 277 subjects attending the Kauwi sub-district and Muthale mission hospitals. To increase the chance of detecting the parasite in the stool, three direct smears were prepared from each sample and examined. Any observation of cyst or trophozoite was recorded. Formal-ether concentration technique was done for each sample that tested negative under direct smear and examined microscopically at low (20x) and high (40x) magnifications. The study collected data on personal hygiene and sanitation level in the study area by use of questionnaires. Laboratory data was obtained based on presence of cysts and / or trophozoites in the stool. Data on level of sanitation and infection by *E. histolytica* in different sexes and age groups were analyzed using Mean square contingency coefficient, Cramer's V and Chi-square. The study revealed that 81 (29.2%) of the sampled individuals tested positive for amoebiasis of which 10.8% were males while females were nearly double at 18.4%. The most affected age group was the under five years at 58.8% and the least infected was 26 to 35 years at 20.6%. Source of water for domestic use played a key role in acquiring the parasite. Washing of hands, fruits and vegetables had no significance since the water used was from unsafe sources highly likely to have been contaminated with *Entamoeba histolytica*. This rendered fruitless the effort shown by the residents in maintaining good sanitation and personal hygiene. The current study findings are critical in preventing amoebiasis in the study area. This would have a long lasting impact in reducing the high child mortality rate in Kitui west district and other regions of the world with similar epidemiological factors. Thus, would put Kenya in an advantage position to achieve Millennium Development Goal number four and her Vision 2030.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background of the study

Amoebiasis is caused by the intestinal protozoan parasite *Entamoeba histolytica* and is the third leading parasitic disease causing death in humans after malaria and schistosomiasis. Globally, it is responsible for 40,000 to 100,000 deaths a year (Sebastiaan *et al.*, 2007). It is distributed worldwide and poses a serious health threat in tropical and subtropical countries (Ohnishi *et al.*, 2004). It is also a problem in developed world among travelers, immigrants, institutionalized persons and homosexuals (Haque *et al.*, 2006). The prevalence of *E. histolytica* infection differs from one age group to the other, with highest prevalence in age group of 1 day to 15 years (Zahida *et al.*, 2010). It also varies with the population of individuals affected, differing among areas with different socioeconomic and conditions. In regions with poor sanitary conditions such as some areas of Central and South America, Africa and Asia, up to 50% of the population is affected (Ryan and Ray, 2004; Al-Harhi and Jamjoom, 2007). In these countries, both sexes are equally exposed to *E. histolytica* infections with increased risk among females working in farms and male homosexuals (Haque *et al.*, 2006). Amoebic colitis affects both sexes equally but invasive amoebiasis is more common in alcoholic males aged 18-50 years (Stanley, 2003).

The causative agent for amoebiasis was initially thought to be a single species, but isoenzyme and molecular studies led to the reclassification of *E. histolytica* into two morphologically identical species: the pathogenic *E. histolytica* and non-pathogenic *E.*

*dispar*. *Entamoeba moshkovskii* is morphologically identical to *E. histolytica* and *E. dispar* but biochemically and genetically different (Stark *et al.*, 2007). Differential diagnosis of these three species in stool samples is not easy on the basis of microscopy alone, except hematophagous trophozoites in acute dysentery (WHO, 1994). Some expensive methods such as amoebic antigen and DNA detection, isoenzyme electrophoretic pattern, and PCR-based methods are available to differentiate them (Tanyukse and Petri, 2003). Inaccessibility of these elaborate diagnostic tools in developing countries such as Kenya, has led to increased deaths as the control and treatment rely on late clinical manifestation of the disease. The aim of this study was to investigate the occurrence levels of amoebiasis in different age groups and sexes among the residents of Kitui west district and the factors possibly linked to its transmission, in order to identify the most affected group in the population which will facilitate treatment and control strategies.

## **1.2 Statement of the problem**

Amoebiasis occur worldwide, causing major health threats in tropical countries (Haque *et al.*, 2006). Diarrhea, a major clinical presentation of intestinal amoebiasis (Haque *et al.*, 2002), is among the leading causes of infant mortality in Sub-Saharan Africa at 9 per cent (UNICEF, 2013). In Kitui County, both maternal and infant mortality rates are higher than the national average of 73 deaths per 1000 live births (AMREF, 2013). An upsurge in water, sanitation and hygiene related diseases such as amoebic dysentery have been reported in Kitui west district (AMREF, 2011). This partially account for the high under-five mortality rate in the district which stands at 8.6% (AMREF, 2013).

However, the number of deaths resulting from amoebiasis remains unknown (DDP, 2002). This is because there are no surveillance systems to monitor levels of amoebiasis in the district. Exposed are the young and the old as well as those living in areas with poor sanitation, that risk developing severe form of the disease with related complications including death (Chandrashekhar *et al.*, 2005). There is therefore need to carry out a study to identify levels of amoebiasis and the associated transmission factors in order to curb the disease in the region.

### **1.3 Justification for the study**

Despite the high prevalence of amoebiasis documented in various parts of world particularly Asia and Africa, there is little or no information regarding amoebiasis in Kenya. Lack of any published work on amoebiasis in Kitui County necessitated retrospective study to be conducted in the study area to observe the disease trend two years preceding the current study. It is in most parts of this view that this study was performed in Muthale and Kauwi hospitals in Kitui County, to determine the occurrence levels of amoebiasis. Kitui west district, as any other region in rural Kenya, has poor sanitation and there is no access to safe drinking water for 94% of the population (DDP, 2002; AMREF, 2011). This act as a fertile ground for transmission of *Entamoeba histolytica*. The outcome of the current study would help to provide basis for the development of control programmes that can improve health status, home environment and personal hygiene.

#### **1.4 Research questions**

- i. What are the levels of *Entamoeba histolytica* infections among different age groups of residents of Kitui West district from <1 years to above 50 years attending Muthale mission and Kauwi sub-district hospitals?
- ii. What is the relationship between sex and *E. histolytica* infections?
- iii. What is the level of sanitation and personal hygiene among the study population?

#### **1.5 Hypothesis**

There are no *Entamoeba histolytica* infections in residents of Kitui west district attending Muthale mission and Kauwi sub-district hospitals.

#### **1.6 Objectives of the study**

##### **1.6.1 General objective**

To establish levels of *Entamoeba histolytica* infection and relationship with sanitation and hygiene practices among residents of Kitui west district attending Muthale mission and Kauwi sub-district hospitals.

##### **1.6.2 Specific objectives**

- i. To determine the occurrence levels of *Entamoeba histolytica* infection in different age groups of patients from Kitui west district attending Kauwi and Muthale hospitals.

- ii. To determine the relationship between sex and *E. histolytica* infection in residents of Kitui west district attending Muthale mission and Kauwi sub-district hospitals.
- iii. To determine the relationship between level of sanitation and personal hygiene and the *E. histolytica* infection levels in Kitui west district.

### **1.7 Significance and output from the study**

This study has documented occurrence levels of *Entamoeba histolytica* in Kitui west district using two referral hospitals, Kauwi and Muthale, which serve patients from the entire Kitui west district. The data will be made available to the Ministry of Public Health and Sanitation and other stake-holders for use in establishing a firm foundation and strategies to prevent and control amoebiasis in the district targeting the more predisposed groups. Improvement of personal hygiene among the school going children in Kitui west district will reduce diarrheal illnesses and facilitate campaigns against high child mortality rate evidenced in the area caused largely due to poor sanitation. This will contribute to the realization of MDG 4 by reducing national child mortality from the current 7.3 per cent to the targeted 3.3 per cent. The data reported in this study could also be applied in designing and implementation of control strategies of amoebiasis in other regions of the world with similar epidemiological factors. The findings of the current study are useful to the learning institutions especially primary schools where hygienic practices such as hand washing could be emphasized in future.

## CHAPTER TWO

### LITERATURE REVIEW

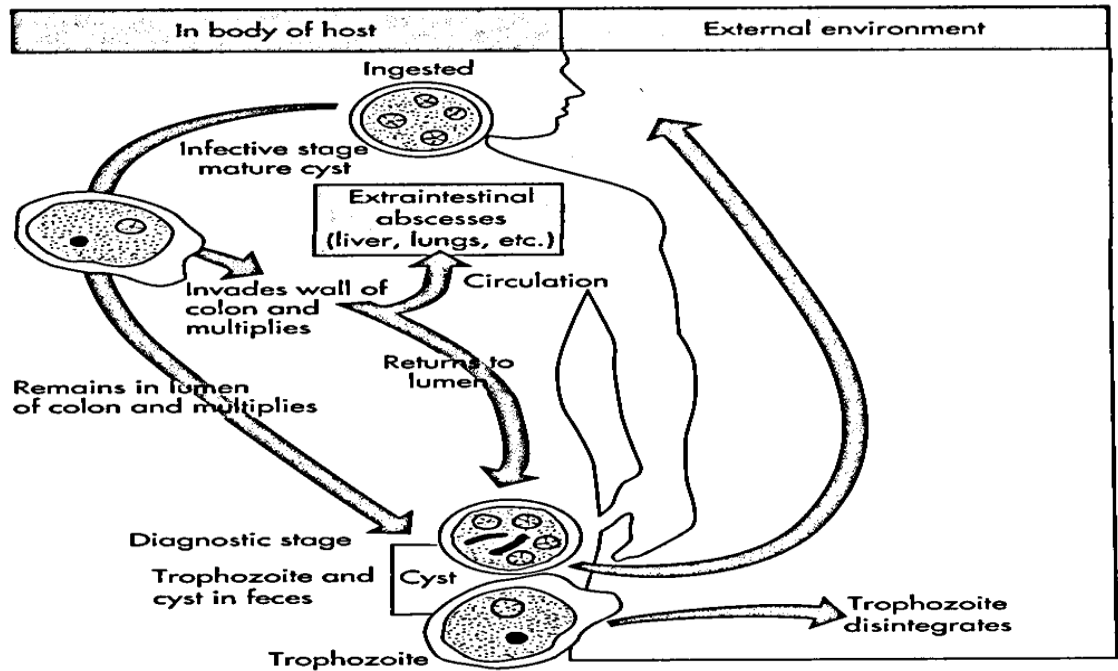
#### 2.1 Morphological features of *Entamoeba histolytica*

*Entamoeba histolytica* exists in two forms namely: trophozoite and cyst. Viable trophozoites vary in size from about 10-60  $\mu\text{m}$  in diameter. Motility is rapid, progressive, and unidirectional, through pseudopodia (Dawit *et al.*, 2006). The nucleus is characterized by evenly arranged chromatin on the nuclear membrane with a small, compact, centrally located karyosome (WHO, 1994). The cytoplasm is usually described as finely granular with few ingested bacteria or debris in vacuoles. In the case of dysentery, RBCs may be visible in the cytoplasm, and this feature is diagnostic for *E. histolytica* (WHO, 1994). Cysts range in size from 10-20  $\mu\text{m}$ . The immature cyst has inclusions namely: glycogen mass and chromatoidal bars. As the cyst matures, the glycogen completely disappears; the chromatoidals may also be absent in the mature cyst (Dawit *et al.*, 2006).

Although *Entamoeba histolytica* has a worldwide distribution, high prevalence rates of more than 10% have been reported in various developing countries such as Cameroon, Zimbabwe, Equatorial Guinea and South Africa (Stanley, 2003). *Entamoeba histolytica*-related diarrheal illnesses have been reported to have a negative impact on the growth of children (Mondal *et al.*, 2006). Despite the availability of effective therapy, morbidity and mortality associated with amoebic infection have persisted, suggesting that interventions designed to control or eliminate the disease may be ineffective (Bercu *et al.*, 2007).

## **2.2 Life cycle of *Entamoeba histolytica***

Intestinal infections occur through the ingestion of a mature quadrinucleate infective cyst in food and / or water contaminated with human fecal material and also by hand to mouth contact. Cysts survive the acidic pH of the stomach and pass into the intestine as the cyst wall is resistant to gastric juice (Stanley, 2003). In the terminal ileum which is alkaline, excystation takes place (Devinder *et al.*, 1996). Trophozoites being actively motile invade the tissues and eventually implant in the sub mucous layer of the colon. Here they grow and multiply by binary fission. Trophozoites are responsible for producing lesions in amoebiasis. Invasion of blood vessels leads to secondary extra intestinal lesions. Gradually the effect of the parasite on the host is toned down together with concomitant increase in host tolerance, making it difficult for the parasite to continue its life cycle in the trophozoite phase (Dawit *et al.*, 2006). A certain number of trophozoites come from tissues into the lumen of bowel and are first transformed into pre-cyst forms (Figure 2.1). Pre-cysts secrete a cyst wall and become a uninucleate cyst. Eventually, mature quadrinucleate cysts form. These are the infective forms. Both mature and immature cysts may be passed in feces. Immature cysts can mature in external environments and become infective. Cysts remain viable for several days in feces and for at least 8 days in soil at 28-34°C (WHO, 1987; CDC, 2007).



**Figure 2.1:** Life cycle of *Entamoeba histolytica* (Adapted from Dawit *et al.*, 2006).

### 2.3 Pathogenesis of *Entamoeba histolytica*

In some cases, the colonic lumen may become colonized by trophozoites, a process that involves interaction of trophozoites with the protective layer of mucin that lines the intestinal epithelium and forms the body's first line of defense (Chadee *et al.*, 1987). This interaction involves adhesion, degradation and subsequent invasion of mucin, which brings the parasite into contact with sub-mucosal epithelium. During such invasion, trophozoites are driven by nourishment derived from intestinal bacteria and food particulates (Devinder *et al.*, 1996).

Amoebapore, a polypeptide that disrupts bacterial and host cell membranes, and cysteine proteases, a group of enzymes that degrade host cells and extracellular matrix

(ECM), are secreted extracellularly by trophozoites at this stage and are important virulence factors that regulate invasion (Laughlin and Temesvari, 2005). Destruction of intestinal epithelium and degradation of ECM that surrounds epithelial cells results in formation of flask-shaped amoebic ulcers (Stanley, 2001). These pathological changes are manifested in the form of watery diarrhea, dysentery, tenesmus and colitic pain in the abdomen thus, causing more suffering especially in children. Highly invasive trophozoites encounter the vascular tissue in the vicinity of the ulcers, and may disseminate through the blood stream to other organs, such as skin, liver, lungs and brain (Laughlin and Temesvari, 2005). Patients of amoebic liver abscess (ALA) present with pain in the abdomen, pyrexia, weight loss and fatigue. In rare instances, other complications may include cutaneous amoebic ulcers, subphrenic abscess, pericarditis and peritonitis (Salles *et al.*, 2003).

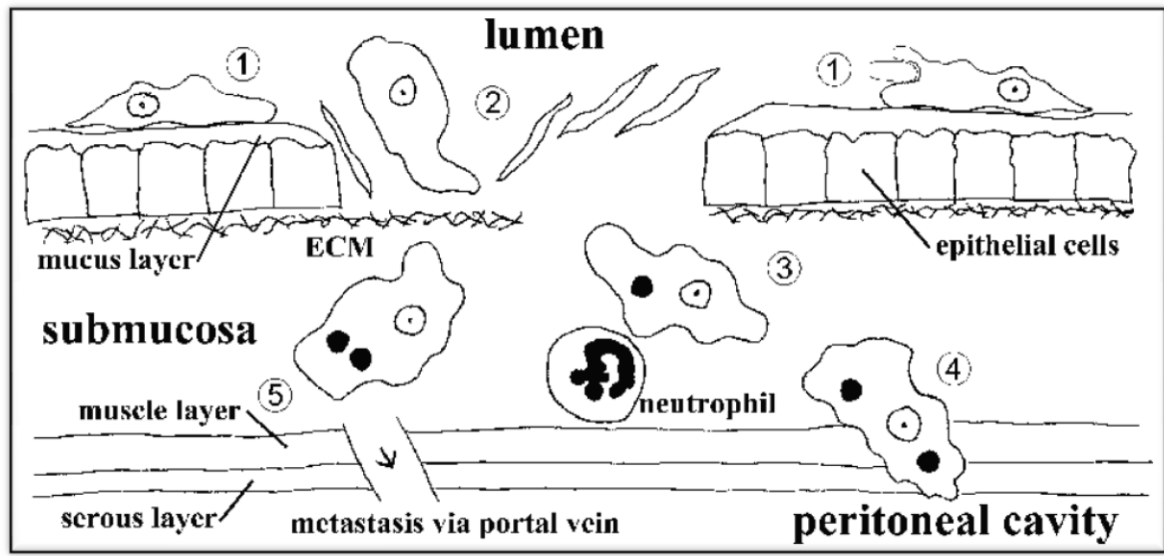
#### **2.4 Invasion mechanism of *Entamoeba histolytica* and the associated pathology**

*Entamoeba histolytica* trophozoites invade the mucosal surface of the colon. The trophozoites attach to the mucus layer where they ingest bacteria and cellular debris from the lumen. The attachment is brought about by the lectin protein which is expressed on the surface of trophozoites (Petri *et al.*, 2006). This non-invasive infection is usually asymptomatic, or may exhibit symptoms ranging from mild abdominal discomfort to diarrhea and cramps (Martinez and Espinosa, 2004). A breakdown in the mucous leads to contact-dependent killing of the epithelial cells. Proteases secreted by the trophozoites play a key role in weakening the mucus, breaking down the host tissues and ECM (Huston, 2004). Apoptotic and necrotic mechanisms are probably involved in

the killing of the host cells. This necrosis of the mucosa will lead to an invasive disease characterized by dysentery (Devinder *et al.*, 1996).

The trophozoites continue to advance laterally and downward into the sub-mucosa producing a 'flask-shaped' ulcer. Necrotic materials occur in the center of the ulcer. Immune effector cells such as the neutrophils are also killed. The trophozoites start to ingest host cells instead of bacteria (Figure 2.2). At this step, the severity of the dysentery increases in terms of amount of mucus and blood in the stool (Leippe *et al.*, 2005). The trophozoites can penetrate the muscle and serous layers leading to intestinal wall perforations, which lead to peritonitis or leakage into the abdominal cavity. Erosion of blood vessels causes massive hemorrhage. An inflammatory thickening of the intestinal wall, called an amoebic granuloma, can appear in response to the *E. histolytica* trophozoites (Bracha *et al.*, 1999).

Trophozoites can gain access to the circulatory system and be disseminated. The liver is the primary site of extra intestinal amoebiasis and hematogenous spread to other organs is rare. Metastasis to the liver involves the portal vein which carries blood from the colon directly to the liver (Figure 2.2). Dissemination to other tissues most often entails the direct extension of hepatic or colonic lesions (Laughlin and Temesvari, 2005).



**Figure 2.2: Pathogenesis of invasive amoebiasis.** 1. Trophozoites adhere to the colon mucosal surface. 2. Breakdown of mucous barrier leading to contact-dependent killing of epithelial cells. 3. Trophozoites advance laterally and downward into submucosa. 4. Intestinal perforation as a result of trophozoites penetration of muscle and serous layers. 5. Metastasis to the liver via portal vein. (Source: Wiser, 2000).

## 2.5 Epidemiology of Amoebiasis

Some of the original epidemiologic descriptions of *E. histolytica* infections originated from studies conducted in South Africa. A community cross sectional survey of asymptomatic individuals found an overall prevalence of the *E. histolytica* of approximately 10%. On further analysis, it was revealed that 90% of individuals were infected with the non-pathogenic *E. dispar* and 10% asymptotically harbored *E. histolytica* (Gathiram and Jackson, 1987). In the same study, it was noted that *E. dispar* occurred more frequently in females than in males due to the difference in their occupation while *E. histolytica* had an equal prevalence and incidence in males and females. Further, it was also interesting to note that although the prevalence of *E. histolytica* infection was equal in all age groups and between sexes, males and

individuals over 16 years of age had a much greater likelihood of invasive disease. It was subsequently noted in this population that approximately 10% of asymptomatic carriers of *E. histolytica* would develop invasive disease while a majority of others spontaneously cleared their infection by 1 year (Blessmann *et al.*, 2002; Haque *et al.*, 2002).

In Africa up to 50% of the population has been reported to suffer from amoebiasis (Al-Harthi and Jamjoom, 2007). A more recent study done in Nigeria however, found 27% of school age children had *Entamoeba histolytica* infection (Reuben *et al.*, 2013). This lower prevalence is further supported by the study done in Kenya among residents of Njoro district where only 21% of patients attending Njoro district hospital tested positive for *E. histolytica* (Kinuthia *et al.*, 2012).

It has been recognized that disease expression varies geographically. For example, invasive disease in Egypt was predominantly amoebic colitis (Abd-Alla *et al.*, 2002), whereas in South Africa there was high rate of amoebic liver abscess (ALA). In fact, in Hue City, Vietnam, an overall estimated frequency of ALA was reported to be as high as 21 cases per 100,000 inhabitants (Blessmann *et al.*, 2002). As part of a study investigating innate and acquired resistance to amoebiasis, 230 Bangladesh children (age 2–5 years) were enrolled in a 2-year observational study (Haque *et al.*, 2002). In this impoverished population 55% of the children acquired *E. histolytica* infection during the 2-year period. Of the 55% who acquired infection, 80% remained asymptomatic while 20% had diarrhea with 4% of these meeting the definition of

amoebic colitis. Further, 17% acquired additional *E. histolytica* infections during the 2-year study which were found to be due to a second genetically distinct strain (determined by polymerase chain reaction (PCR) for the serine-rich *E. histolytica* protein) (Haque *et al.*, 2002).

A study of low socioeconomic status school children in Ecuador shed additional light upon the epidemiology of *E. histolytica* infection. This cross-sectional survey found asymptomatic *E. histolytica* infection present in only seven of 178 children (Gatti *et al.*, 2002). However, it was interesting to note that more than 64% of the children showed high serologic titers implying current or recent infection with *E. histolytica* (Gatti *et al.*, 2002). This high sero-prevalence corresponds to another sero-survey conducted in Mexico (Petri and Singh, 1999). This study summarized some of the difficulties in conducting epidemiological studies as it revealed variation in clinical diagnostic criteria, shortage of trained personnel and reluctance of institutions and governments to provide the necessary support. In addition, antiamoebic antibodies are long lasting and reflect only the cumulative exposure to the infection. The question of what should be considered an adequate test in terms of sensitivity, specificity and cost was revisited and the need for accurate diagnosis for both dysenteric and asymptomatic patients emphasized (Gatti *et al.*, 2002).

Amoebiasis is known to specifically spread within families, institutions such as day care centers and occasionally may cause epidemics (Braga *et al.*, 2001; Barwick *et al.*, 2002). Currently, the standard at most centers is to treat all asymptomatic individuals

with cysts in the stool with an anti-protozoal intestinal agent. It remains to be determined if in addition to the stool ova and parasite examination other diagnostic screening examinations such as stool antigen testing would be more sensitive, specific and cost-effective (Gatti *et al.*, 2002).

## **2.6 Risk factors for amoebiasis**

Poor personal hygiene, particularly common in children such as those in day care centers and institutions such as prison, orphanages as well as among food handlers, are important for transmission. A study conducted in Brazil identified place of residence, age, ingestion of raw vegetables and drinking water quality as important risk factors for amoebiasis (Benetton *et al.*, 2005). In developing countries, poor sanitation coupled with lack of plumbing promotes intestinal protozoa infections. Waterborne epidemics characterized by inefficient water treatment using chlorine or no water treatment at all are common in endemic areas. Male homosexuality brings about oral-anal contact hence depositing cysts directly in the mouth, and promoting fecal-oral transmission for the intestinal protozoa (Haque *et al.*, 2006).

The prevalence of amoebiasis depends on many factors including ignorance, overcrowding, inadequate and contaminated water supplies, poor sanitation (Espinosa-Cantellano and Espinosa-Cantellano, 2000), toilet habit (Oyerinde *et al.*, 1979), low socio-economic status (Chacin-Bonilla *et al.*, 1992), absence of adequate urban services, inadequate hygiene practices, number of rooms per house, and having other protozoan infections (Benetton *et al.*, 2005). The incidence of intestinal parasites is also

closely related to climate, environmental conditions, infrastructure and degree of literacy (Karaman *et al.*, 2006). Prevalence of *E. histolytica* is high among families who eat together from the same plate, those who eat with their fingers, those who eat away from home, workers with high occupational interaction (Oyerinde *et al.*, 1979), in municipal sanitary workers (Karaman *et al.*, 2006), infants, pregnant women and patients who take immunosuppressive medicines. HIV infected patients are at significantly higher risk of amoebiasis than patients from other risk groups largely due to immunosuppression, high-risk sex behavior and multiple exposure (Hung *et al.*, 2008). The greatest risk is associated with carriers of the disease especially when engaged in preparation and handling of food. Carriers discharge up to  $1.5 \times 10^7$  cysts daily (WHO, 1987; Benetton *et al.*, 2005).

## **2.7 Clinical manifestation of amoebiasis**

Gastrointestinal presentations of *E. histolytica* infections range from asymptomatic (carrier) to colitis and the formation of abscesses and intestinal perforations (Martinez and Espinosa, 2004). Although 90% of *E. histolytica* infections remain asymptomatic, approximately 50 million people have invasive disease, resulting in 100,000 deaths per year (WHO, 1997). Individuals harbouring *E. histolytica* (asymptomatic carriers) can develop antibody titers in the absence of invasive disease (Jackson *et al.*, 1985; Gathiram and Jackson, 1987). Asymptomatic colonization with *E. histolytica*, if left untreated, can lead to amoebic dysentery and a wide range of other invasive diseases, but more often the infection resolves spontaneously without the development of diseases (Gathiram and Jackson, 1987).

Natural history of acute amoebic colitis has a gradual onset, with a 1-2 weeks history of mild-to-moderate abdominal pain and tenderness, tenesmus and watery diarrhea, five to seven episodes per day, accompanied by little amounts of feces and abundant mucus with or without blood. About 80% of patients complain of localized abdominal pain; some patients may have only intermittent diarrhea alternating with constipation. Fever is unusual, occurring in less than 40% of patients (Adams and MacLeod, 1977). Other associated symptoms are weight loss and anorexia. This syndrome resolves within a few days following appropriate anti-amoebic treatment. Severe cases of amoebic colitis are characterized by dysenteric stools, diffuse abdominal pain, high fever and severe dehydration; the patient usually appears ill. Patients with fulminant amoebic colitis usually present with profuse bloody diarrhea, fever, pronounced leukocytosis, and widespread abdominal pain, often with peritoneal signs and extensive involvement of the colon (Takahashi *et al.*, 1997).

Another clinical spectrum of intestinal amoebiasis is chronic intestinal amoebiasis and patient with this condition present with intermittent abdominal pain, diarrhea and weight loss. Patients who are at increased risk of severe disease include those who are very young, very old, malnourished, pregnant and those receiving corticosteroid treatment. Acute complications of intestinal disease include bleeding, perforation, peritonitis, perianal skin ulceration and rectovaginal fistulas. Deep ulcer may heal with stricture and adhesion and these will lead to intestinal obstruction. Ameboma results from the formation of annular colonic granulation tissues in the caecum and ascending

colon which may mimic carcinoma of colon, a rare late complication of intestinal amoebiasis (Adams and MacLeod, 1977; Wiser, 2000).

On rare occasions, *E. histolytica* trophozoites enter the bloodstream and thereby are disseminated to other body sites such as lungs, liver and skin. Amoebic liver abscess (ALA) is the most frequent manifestation of extra intestinal amoebiasis (Bruckner, 1992) and occurs in 3 to 9% of patients (Frey *et al.*, 1989). The clinical presentation is highly variable, ranging from weight loss, weakness, and low-grade fever to an acute febrile illness. Patients, who present acutely with symptoms of less than two weeks duration, have more prominent abdominal pain with fevers and rigors (Katzenstein *et al.*, 1982). Cutaneous amoebiasis is a rare extra intestinal manifestation of *E. histolytica* infection (Mhlanga *et al.*, 1992), which in children always occurs in the anogenital or perineal region as a result of direct inoculation of trophozoites from prolonged contact with infected stools in a child's diaper.

## **2.8 Diagnosis of *Entamoeba histolytica* infection**

Several procedures are available to confirm diagnosis, including stools microscopy, cultures, antigen detection, histopathology, serology and molecular biology techniques (Dickson-Gonzalez *et al.*, 2009). Microscopic examination of stools is able to detect trophozoites in amoebic colitis in 33-50%. Several stool samples ( $\geq 3$ ) over no more than 10 days increase the sensitivity to 85-95%. Besides the trophozoite ingesting red blood cells, mostly happening in *E. histolytica* infection, leukocytes may be found (Burchard *et al.*, 1993). Concentration technique is reserved as the best method for

diagnosing intestinal parasites in resource-poor countries such as Kenya where a variety of non-microscopic methods for diagnosing intestinal parasites are unaffordable (Ogvoma and Ekwunife, 2006). Such non-microscopic methods include antigen detection in feces, direct fluorescent antibody methods and molecular biological techniques such as DNA probes and polymerase chain reaction (Parija and Srinivasa, 1999).

Amoebiasis diagnosis can be achieved with cultures in 50% to 70% cases. Culture is not a routine process and is less sensitive than microscopy in detection (Dickson-Gonzalez *et al.*, 2009). Cultures of *Entamoeba histolytica* are primarily research tools rather than diagnostic ones. Antigen detection through enzyme-linked immunosorbent assay (ELISA) is commercially available with multiple kits. In addition, kits using monoclonal antibodies against the GAL/GalNAc-specific lectin and the serine-rich antigen of *E. histolytica* yield an overall sensitivity up to 71% - 100% and specificity up to 93% - 100% (Dickson-Gonzalez *et al.*, 2009).

Histopathological diagnosis of amebic colitis is based on the demonstration of the amoebic trophozoites in the histological sections. Cell populations such as polymorphonuclear neutrophils and eosinophils would be predictive of the parasitic infection (Tanyuksel and Petri, 2003). Multiple serologic assays are currently available. ELISA is the most used of them. It measures the presence of serum IgG antilectin antibodies. Its sensitivity is high for extra-intestinal disease up to 98%, but low for active amoebic colitis particularly in endemic areas due to repeated exposure and

development of some immunity, limiting antibody-based testing for diagnosing currently active disease since antibodies can persist for years after infection (Tanyuksel and Petri, 2003). Other techniques for extra-intestinal disease available include immunofluorescent assay (IFA), indirect hemagglutination (IHA) immunoelectrophoresis, counter-immunoelectrophoresis (CIE), immunodiffusion tests and complement fixation (CF) (Dickson-Gonzalez, *et al.*, 2009).

Molecular biology techniques directly identify RNA and DNA of the parasite, being specific for the species. Multiple targeting genes are used, including a small-sub-unit rRNA gene (18S rDNA), serine-rich protein gene, chitinase gene, 30-kDa antigen gene, hemolysin gene, and extra-chromosomal circular DNA (Mann *et al.*, 1991; Haque *et al.*, 2002).

## **2.9 Impact of sanitation on intestinal parasitism**

Intestinal parasitism infections will remain prevalent as long as people have no easy and convenient access to safe water supplies and acceptable hygienic facilities for the disposal of human excreta. The provision of safe water and better sanitation and their use would make a major contribution to the prevention and control of diarrheal diseases including amoebiasis (WHO, 1985). In many areas, sanitation is the most urgent health need and those concerned with the control of intestinal parasitism infections are urged to promote intersectoral collaboration between health care authorities and those responsible for the provision of sanitation facilities and water supply at the community level.

The benefits expected from sanitation are maximized when such programmes are complemented with provision of safe water and health education (Stanley, 2003). Beside the numerous health benefits of sanitation, the ideal sanitation is rarely achieved as it suffers the following devastating setbacks (WHO, 1985): (a) lack of strict hygienic practices such as washing hands after visiting latrine and before eating which result in auto-infection and transmission through person to person contact, (b) use of contaminated water for personal hygiene and more important for dish washing and cleaning, (c) indiscriminate defecation rather than using latrines by certain group particularly young children which spread parasite cysts in immediate environment and (d) lack of personal hygiene among those who have close contact with domestic animals which promote amoebiasis co-infection with zoonotic diseases. In addition, sanitation efforts can be jeopardized by the contamination of the environment with human excreta through open sewers, the flow of untreated sewage into rivers, canals and ponds, and use of inadequately treated sewage in agriculture, either as fertilizer or for irrigation (Feachem, 1983).

### **2.10 Control measures of *Entamoeba histolytica* infection**

Prevention and control measures of amoebiasis are similar to other diseases transmitted by the fecal-oral route. The major difference is that there is hardly any zoonotic transmission since humans are the only natural hosts for *Entamoeba histolytica* (Stanley, 2003). Pai *et al.* (2003) found *E. histolytica* cysts on the cuticle of 25.4% of 299 American cockroaches (*Periplaneta americana*) suggesting cockroaches may play a potential role in the mechanical dissemination of amoebiasis. Control is based on

avoiding the contamination of food or water with fecal material. Health education in regard to improving personal hygiene, sanitary disposal of feces, and hand washing are particularly effective (Stanley, 2003). Protecting water supplies lowers endemicity and epidemics (Madigan *et al.*, 2003). *Entamoeba* cysts are resistant to standard chlorine treatment, but are killed by iodine or boiling. Sedimentation process and filtration using 0.22 µm filter are quite effective at removing *Entamoeba* cysts. Travelers to endemic regions are normally advised to avoid consumption of unsafe food and water and sexual practices that may lead to fecal-oral transmission (Feachem, 1983; Madigan *et al.*, 2003).

In general, amoebiasis is prevented and controlled by both non-specific and specific measures (Bercu *et al.*, 2007). The non-specific measures to be implemented at community and individual level include (a) improved water supply, excreta disposal and food safety; (b) Health education about possibility of transmitting amoebiasis via sexual contact and to promote hand washing behavior and latrine and (c) General social and economic development. The specific measures employ (a) local epidemiological surveys of amoebiasis; (b) rapid diagnosis and adequate treatment of patients at all levels of health services including community and health centers, and (c) surveillance and control of situations that may favour spread of amoebiasis (CDC, 2007).

### **2.11 Treatment of amoebiasis and prospects for a vaccine**

Therapeutic management of amoebiasis is showing new options. For amoebic colitis, the main choice drug is metronidazole. Treatment requires the use of two groups of antiparasitic drugs: luminal and tissue agents. Asymptomatic intestinal colonization with *E. histolytica* can be treated with luminal agents alone. Drugs used for the treatment of luminal infections and generally prescribed for asymptomatic cyst passers include iodoquinol, diloxanide furoate, and paromomycin (Blessmann and Tannich, 2002). Secnidazole has been used but metronidazole, although having more side effects such as headaches, anorexia, nausea, metallic taste, a disulfiram-like reaction to alcohol, and vomiting, is more effective than secnidazole. Ivermectin is a new drug that has shown potential use in treating amoebiasis (Gonzalez-Salazar *et al.*, 2009).

In a Cochrane Database Systematic Reviews, tinidazole was found to be more effective in reducing clinical failure compared with metronidazole and has fewer associated adverse events (Isea *et al.*, 2012). Combination drug therapy was found to be more effective in reducing persistence of the parasite in stool samples compared with metronidazole alone (Isea *et al.*, 2012). The addition of broad-spectrum antibiotics to the treatment of acute amoebic colitis may be appropriate in case of secondary infection by bacteria and when perforation is suspected. The possibility of coexisting bacteria causing dysentery such as *Escherichia coli* must always be considered (Mackey-Lawrence and Petri, 2011). Amoebic colitis and some of its complications, such as ameboma, generally respond to treatment without surgical intervention, but for acute

necrotizing colitis with toxic megacolon, partial or complete colectomy may be necessary (Isea *et al.*, 2012).

Although not currently a significant problem, it is hypothesized that in the future, antiparasitic drug resistance may emerge as a threat in the therapeutic management of amoebiasis. Recently, the genome of *Entamoeba histolytica* has been sequenced, which has widened the scope to study additional virulence factors (Lejeune *et al.*, 2009). *Entamoeba histolytica* genome-based approaches have now confirmed the presence of Golgi apparatus-like vesicles and the machinery for glycosylation, thus improving the chances of identifying potential drug targets for chemotherapeutic intervention. Gallectin-based vaccines are under development, but additionally, promising vaccine targets such as serine-rich *E. histolytica* protein have yielded encouraging results (Mann *et al.*, 1991). Considerable efforts have also been made to skew vaccination responses towards appropriate T-helper cell immunity that could augment the efficacy of vaccine candidates under study (Stanley, 2006).

Sequencing of the *E. histolytica* genome will provide additional information that will contribute to identification and characterization of important potential vaccine and drug targets and lead to effective immunologic strategies for the control of amoebiasis. Progress in vaccine development has been facilitated by new animal models that allow better testing of potential vaccine candidates and the application of recombinant technology to vaccine design (Stanley, 2006). Oral vaccines and DNA-based vaccines have been successfully tested in animals' models for immunogenicity and efficacy

(Seydel *et al.*, 1997). There has been significant progress on a number of fronts, but there are unanswered questions regarding the effectiveness of immune responses in preventing disease in man and, as yet, no testing of any of these vaccines in humans has been performed. In addition, there are strong economic barriers to developing an amoebiasis vaccine and questions about how and where an effective vaccine would be utilized (Stanley, 2006).

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Study area**

The study was carried out in Kitui west district in Kitui County, Kenya (Appendix I). This region has experienced high incidences of amoebiasis in the last two years preceding the study. Muthale mission hospital and Kauwi sub district hospital laboratories were used for this study because they are the only hospitals in the entire Kitui west district with modern facilities and trained personnel to diagnose parasitic infections. Muthale serves the population to the East and Kauwi serves the population to the West of the district.

#### **3.2 Research design**

Before this study commenced, amoebiasis cases were extracted from the laboratory records for a period starting from April 2011 to March 2013 in Muthale and Kauwi hospitals to ascertain the disease trend. The current was a cross sectional study involving a combination of survey and laboratory investigation with collection of data from a cross-section of respondents randomly sampled. Laboratory investigation was conducted through stool samples and analysis for trophozoites and cysts done by use of a microscope. Questionnaires were used for the survey (Appendix III). They enabled inexpensive collection of data on sanitation and personal hygiene from 277 study subjects during a three months period from April to June 2013. One questionnaire was issued to each respondent and with the help of an interpreter, in case of illiterate respondent, requested to tick only one of the choices given under each question. After

filling the questionnaire, each subject was orally interviewed on clinical symptoms of amoebiasis before being requested to provide fresh fecal sample for laboratory analysis.

### 3.3 Study population

The study population consisted of people attending Muthale mission hospital and Kauwi sub-district hospital.

### 3.4 Sample size determination

Medical out-patients residing in Kitui west district and attending the two hospitals were sampled using simple random sampling method. Seven out-patients were sampled per day where 14 uniform pieces of paper with 7 marked “YES” and the other 7 marked “NO” were used to sample the first 14 out-patients to report in the hospital in the morning. Patients selecting “YES” were recruited into the study.

From the medical out-patients records in the two hospitals, 23.5% of the study population was infected with *Entamoeba histolytica* in the year 2012 where a total of 11,243 medical out-patients visited the two hospitals from April to June 2012 with 6,746 and 4,497 patients in Kauwi and Muthale hospitals respectively giving a ratio of approximately 3:2. The sample size formula by Smith (2010) was used.

$$n = \frac{p(1-p)z^2}{ME^2}$$

Where:

n= the sample size

$z$  =  $z$  score for 95% confidence interval

$p$  = the prevalence of *E. histolytica* taken as 23.5% from the hospital health records.

$1-p$  hence is 0.765

ME = Margin of error corresponding to 95% confidence interval

Therefore;

$$n = \frac{0.235 \times 0.765 \times 1.96^2}{0.05^2}$$

$$= 277$$

Thus the number of patients attending the two hospitals recruited into the study was 277. Based on above ratio, a total of 161 out-patients were sampled at Kauwi and the remaining 116 at Muthale hospital.

### **3.5 Inclusion and exclusion criteria**

Persons attending the two hospitals who agreed to sign the consent form and had been residents of Kitui west district for at least one year before the study or infants less than one year old but whose either of the parent or guardian had been resident, were included in the study.

Non-residents of Kitui west district and those who had not been residents for a period of at least one year and infants less than one year old whom none of the parent or guardian had been a resident, were excluded from the study. Non-medical out-patients and those who failed to sign the consent form were also excluded.

### **3.6 Data collection techniques**

The study collected data on the existing occurrence of *E. histolytica* infections among people of different age groups from <1 year to 50 years and above attending Muthale mission and Kauwi sub-district hospitals in the Kitui west district to assess whether age is related to disease status. Questionnaires were administered and data on sources of water for drinking and cooking, sanitation, personal hygiene, and occupation were collected. The subjects or guardians were required to fill questionnaires after signing a consent form with full explanation about the purpose of the study. Brief patient history and observation of signs and symptoms was used to obtain clinical data. Laboratory diagnosis using fecal samples gave data on *E. histolytica* infections in the subjects. Presence or absence of symptoms and the sanitation level were noted and correlated with positive microscopic test.

#### **3.6.1 Collection of clinical data**

Research assistants who were trained medical laboratory technicians and acquainted with clinical features of amoebiasis, asked subjects oral questions relating to clinical symptoms which included 1-2 weeks history of mild-to-moderate abdominal pain and tenderness, tenesmus and watery diarrhea with five to seven episodes per day and little amounts of feces, abundant mucus with or without blood as well as localized abdominal pain or intermittent diarrhea alternating with constipation (Takahashi *et al.*, 1997). The responses were recorded for analysis. The subjects were not kept in the queue but treated first of their ailments before they participated in this study.

### **3.6.2 Laboratory techniques applied on stool samples**

#### **3.6.2.1 Collection of stool sample**

World Health Organization (1994) guidelines on stool collection were used. A clean screw-top plastic container bearing the patient number and date was issued to the subjects together with a clean newspaper, plastic bag and a pair of latex gloves. The patients were instructed to spread the newspaper over the rim of the toilet to make sure the stool does not touch the inside of the toilet. The urine free stool was then transferred in a screw-top container and lid shut. The used gloves and newspaper were then put in a plastic bag, tied up and put in waste bin. Patients were instructed to wash their hands thoroughly with soap and submit the stool sample to laboratory not more than ten minutes after collecting. The stool consistency and presence of blood and mucus was noted (WHO, 1994).

#### **3.6.2.2 Preparation of direct fecal smears using saline and iodine**

Patient's identification number and date were written at the left hand side of the microscope slide using pencil. A drop of saline was then placed in the centre of the left half of the slide and a drop of iodine solution placed in the centre of the right half of the same slide as per WHO (1994) bench aid for the diagnosis of *E. histolytica*. Using an applicator stick, approximately 2 mg of the stool sample (about the size of match stick head) were picked up and added to the drop of saline. A similar portion was added to the drop of iodine. The feces were mixed with the drops to form suspensions. Each drop was covered with a coverslip by holding the coverslip at an angle, touching the edge of the drop, and gently lowering the coverslip onto the slide so that air bubbles were not

produced. The entire coverslip area was examined for trophozoites and cysts with the 10X objective lens and then switching to high magnification for detailed morphology. *E. histolytica* trophozoites were identified based on their characteristic movement (definite directional motility) and the presence of hematophagous trophozoites in the stool sample. To increase the chances of detecting cysts and trophozoites, tests were done in triplicate for each stool sample (WHO, 1994). The positive samples were recorded and those tested negative were concentrated using formal-ether concentration techniques before they could be declared negative.

#### **3.6.2.3 Concentration techniques for stool samples that tested negative with direct smears**

Concentration procedures by World Health Organization (1994) bench aid for diagnosis of intestinal parasites were used. One gram of feces was added to 10 ml of 10% formalin in a centrifuge tube using an applicator stick, and stirred to form a suspension. The suspension was strained through two layers of formalin wetted surgical gauze directly into a different centrifuge tube. The gauze was then discarded. The suspension was then topped up with 10% formalin to bring the volume to 10 ml. Three milliliter of ether was added and mixed well shaking vigorously for 10 seconds. The tube was then placed in the centrifuge and centrifuged at 400 g for 2 minutes. The tube was removed from the centrifuge having four layers: top layer of ether, a plug of fatty debris adherent to the wall of the tube, a layer of formalin and sediment. The plug of debris was gently loosened with an applicator stick by a spiral movement and the top 3 layers poured off in a single movement. The tube was inverted for 5 seconds to drain. The residual fluid

from the walls of the centrifuge tube was then mixed with the sediments using disposable glass pipette. A drop of the suspension was transferred to a slide for examination under a coverslip using iodine stain. The preparation was examined with the 10X objective for identification of amoebic cysts in a systematic manner so that the entire coverslip area is observed every time switching to higher magnification to see more detailed morphology of the parasites (WHO, 1994). The samples that tested negative after concentration techniques were declared negative for *E. histolytica*.

### **3.6.3 Use of questionnaires**

Questionnaires were used to collect data on personal hygiene and sanitation level and responses to questions were scored on a scale of 1 to 5. The factors were given scores in an increasing order depending on how likely a factor exposes one to the disease such that the higher the score the more the expected risk of exposure to infection. For example, on the sources of water for drinking and cooking, tap water scored 1, borehole 2, shallow well 3, earth dam 4 and river 5. Demographic characteristics and occupational activity of the patients/ guardian of infants were also captured by aid of questionnaire.

### **3.7 Ethical consideration and confidentiality**

Clearance for research was sought from Kenyatta University. The permission to collect hospital data was obtained from hospital administrators. Informed consent was sought from the sample population and participation in the study was voluntary. All data were treated confidentially and used only for the purpose of this research.

### **3.8. Data analysis**

Quantitative data was coded and processed using SPSS version 17.0. The difference in amoebiasis occurrence level in different age groups was analyzed by Chi square test. The relationship between level of sanitation and personal hygiene and *E. histolytica* infection levels was determined using Mean square contingency coefficient and Cramer's V. Relationship between latrine use and *E. histolytica* infection was analyzed using Fisher's exact test. A Chi square test was used to determine the relationship between sex and *E. histolytica* infection in residents of Kitui west district attending Kauwi sub-district and Muthale mission hospitals. A significance level of 0.05 was used. Frequency tables, graphs and pie charts were used to present quantitative data.

## CHAPTER FOUR

### RESULTS

#### **4.1 Amoebiasis levels in Kitui west district two years preceding the current study**

From the hospital records at Muthale and Kauwi hospital, it was observed that people of all ages and sex were infected with *Entamoeba histolytica* (Table 4.1). Between the years 2011 to 2013, a total of 3,896 patients in Kitui west district visited the hospital laboratories in the district for diarrheal related complications. Of these, 19.68% had *E. histolytica* infection. Among the 1,100 children under the age of five who visited the facilities, 27% of them tested positive for amoebiasis compared to the least infected age groups of 16-25 and 26-35 years where 13% were infected.

#### **4.2 Demographic characteristics of the patients**

Male patients were 119 and female patients were 158 in number. Out of the total patients (277) studied 161 were from Kauwi hospital while the number from Muthale hospital was 116.

**Table 4.1: Amoebiasis incidences in Kitui west district from April 2011 to March 2013**

Time (Months)	Sex	Age (Years)												Sub total	Total
		Under 5		6_15		16-25		26-35		36-49		50 and above			
		-	+	-	+	-	+	-	+	-	+	-	+		
2011 April	M	24	6	17	4	17	2	7	0	5	1	11	3	97	191
	F	21	7	11	3	14	2	9	1	9	2	13	2	94	
May	M	19	9	14	3	17	1	12	1	2	0	9	1	88	181
	F	16	6	16	5	16	0	11	2	5	2	11	3	93	
June	M	25	11	18	5	16	3	10	2	10	1	13	4	118	235
	F	23	13	21	7	14	4	8	0	13	1	11	2	117	
July	M	26	8	21	4	16	1	13	1	14	2	14	3	123	230
	F	23	4	20	3	13	2	16	0	11	1	12	2	107	
August	M	18	7	17	5	15	2	6	0	7	1	10	5	93	172
	F	21	5	14	3	11	0	4	1	9	2	8	1	79	
September	M	18	4	13	2	8	1	8	2	4	1	6	1	68	139
	F	19	3	14	4	6	1	9	3	5	2	4	1	71	
October	M	16	5	7	5	9	2	10	1	8	1	9	4	77	156
	F	18	8	9	2	12	3	11	2	5	1	7	1	79	
November	M	11	2	14	1	22	1	13	1	3	0	6	3	77	148
	F	9	2	9	1	21	0	18	1	5	1	3	1	71	
December	M	15	9	9	4	16	2	11	1	13	2	8	3	93	159
	F	12	6	6	1	11	0	13	0	8	1	6	2	66	
2012 January	M	11	8	13	4	15	3	10	2	6	2	5	3	82	163
	F	13	7	14	5	14	3	11	4	4	1	3	2	81	
February	M	12	6	13	4	13	4	13	3	11	3	8	5	95	168
	F	10	4	11	1	12	3	9	1	8	3	7	4	73	
March	M	18	9	12	3	9	2	16	3	6	3	5	1	87	169
	F	13	6	14	2	11	5	11	1	7	5	5	2	82	
April	M	11	5	11	2	13	1	6	2	3	1	5	2	62	119
	F	13	3	12	2	10	2	3	1	6	2	3	0	57	
May	M	16	5	12	4	11	2	9	0	6	1	5	1	72	155
	F	15	7	18	3	13	1	5	2	9	2	7	1	83	
June	M	21	10	13	2	7	3	4	3	5	1	6	2	77	142
	F	17	8	11	3	4	2	5	1	8	1	4	1	65	

<b>July</b>	M	23	14	17	4	5	3	10	3	13	1	7	2	102	181
	F	19	10	14	2	7	1	7	2	8	2	4	3	79	
<b>August</b>	M	19	4	12	3	9	0	9	1	7	0	8	2	74	159
	F	16	9	14	6	11	2	14	2	4	1	5	1	85	
<b>September</b>	M	18	5	13	3	10	2	9	3	12	1	5	2	83	159
	F	16	7	15	1	14	1	4	2	9	3	3	1	76	
<b>October</b>	M	10	2	21	4	12	1	9	1	12	1	5	1	79	164
	F	18	3	16	1	10	2	7	0	18	2	7	1	85	
<b>November</b>	M	16	4	19	1	9	1	5	2	3	1	3	2	66	132
	F	13	1	14	3	11	0	16	1	4	0	2	1	66	
<b>December</b>	M	9	2	12	3	1	1	1	0	4	1	4	1	39	108
	F	17	5	11	4	14	2	2	2	3	1	6	2	69	
<b>2013 January</b>	M	16	6	15	3	9	2	7	1	10	1	6	3	79	186
	F	23	9	21	5	12	1	14	3	11	2	4	2	107	
<b>February</b>	M	15	8	22	4	8	1	7	1	11	1	3	2	83	166
	F	20	8	19	6	6	2	4	1	9	2	4	2	83	
<b>March</b>	M	19	3	8	2	4	0	11	2	6	1	4	1	61	114
	F	12	4	10	3	6	1	8	1	4	0	3	1	53	
<b>Sub total</b>		80	29	67	15	54	8	43	7	36	6	30	9	<b>3896</b>	
		3	7	7	5	4	1	5	0	3	8	7	6		
<b>Total</b>		1100		832		625		505		431		403			

Data obtained from Kauwi and Muthale hospital laboratory records in Kitui west district  
Key: M- Male; F- Female; + Amoebiasis positive; - Amoebiasis negative

#### 4.2.1 Age and sex of the respondents

Of the 277 patients, 75 (27%) were between 16-25 years old which was the group with the highest number of individuals. Most of the female patients (47 out of 158) were also aged between 16-25 years. The under 5 years of age were 17 (6.14%) of the total sampled individuals of whom 8 were males. The oldest person in the  $\geq 50$  years age group was a 67 years old female while a nine months old infant was the youngest (Table 4.2).

**Table 4.2: Distribution of age and sex of the respondents at Kauwi and Muthale hospitals**

	Age (Years)						Total
	< 5	5-15	16-25	26-35	36-49	$\geq 50$	
<b>Male</b>	8	30	28	15	16	22	119
<b>Female</b>	9	30	47	19	28	25	158
<b>Total</b>	<b>17</b>	<b>60</b>	<b>75</b>	<b>34</b>	<b>44</b>	<b>47</b>	<b>277</b>

#### 4.2.2 Rate of infection with *Entamoeba histolytica*

The occurrence level of amoebiasis recorded in the study area was 29.2% with 81 out of 277 sampled individuals testing positive for cysts and / or trophozoites. It was found that 96.3% of patients who had no symptoms of amoebiasis tested negative for the disease when their fecal specimens were examined in the laboratory. Out of 140 patients who exhibited symptoms of amoebiasis, 76 (54.3%) had cysts and trophozoites in their stool while 64 (45.7%) did not. Five patients (3.7%) who showed none of the clinical symptoms of amoebiasis were actually infected with the parasite as they had cysts or

trophozoites in their stool (Table 4.3). Therefore the 3.7% of the study subjects were the true asymptomatic carriers of the disease and active cysts passers since they harbored the parasite without showing the symptoms.

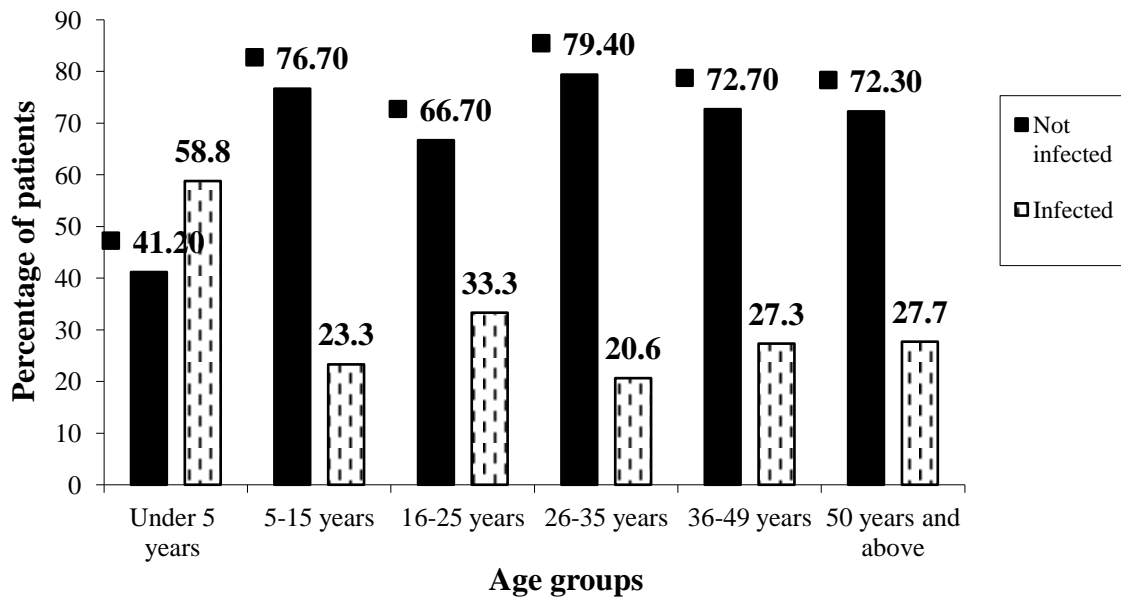
**Table 4.3: Distribution of patients with amoebiasis in Kitui west district**

	<b>Asymptomatic</b>	<b>Symptomatic</b>	<b>Totals</b>
	<b>No. (%)</b>	<b>No. (%)</b>	<b>No. (%)</b>
<b>Cysts and trophozoites present</b>	5 (3.7)	76 (54.3)	81 (29.2)
<b>Cysts and trophozoites absent</b>	132 (96.3)	64 (45.7)	196 (70.8)
<b>Total</b>	137	140	277

#### **4.2.3 Occurrence levels of *Entamoeba histolytica* in different age groups**

The results revealed that the under five years of age were the most affected group (58.8%) followed by the 16-25 years old (33.3% infected) and 27.7% individuals aged  $\geq 50$  years. The least infected age group was 26 to 35 years at 20.6% (Figure 4.1).

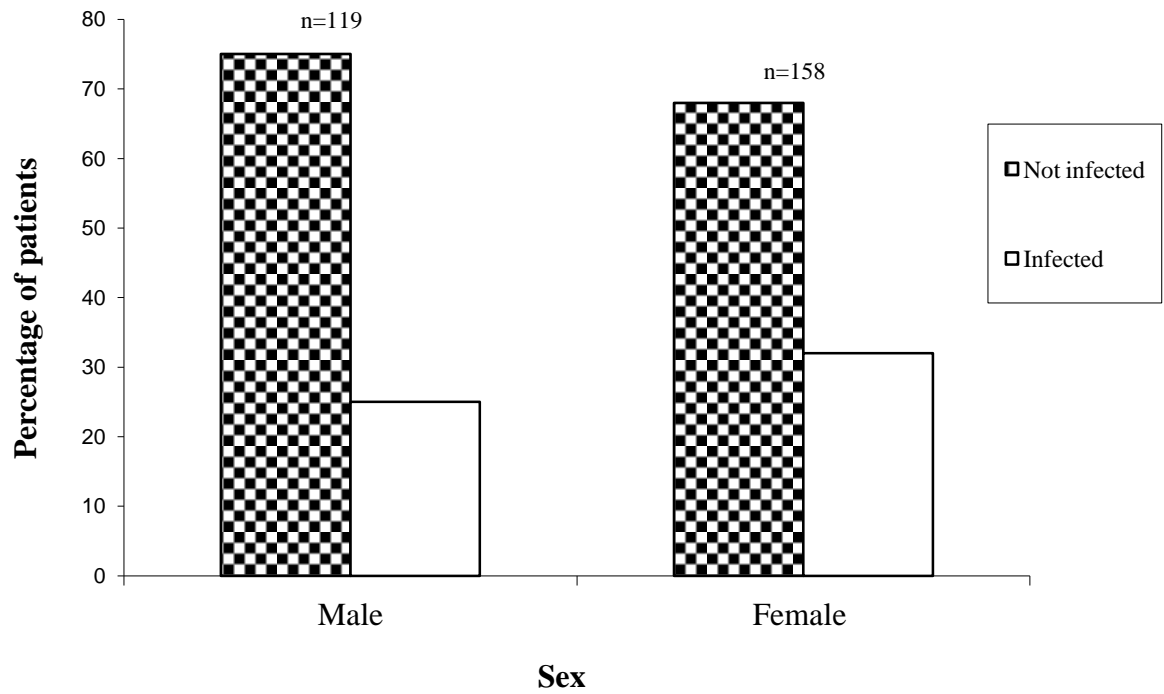
The Chi square test revealed a significant relationship between the under five years age group and the *E. histolytica* infection ( $\chi^2$  (5, n=277) = 16.68; p= 0.005; Cramer's V = 0.25). These results at 0.05 level of significance, showed that the level of infection in the under 5 was significantly higher than 16-25 age group while the infection rates in the other age groups was statistically comparable (p>0.05).



**Figure 4.1: Occurrence levels of *E. histolytica* in different age groups (n=277).**

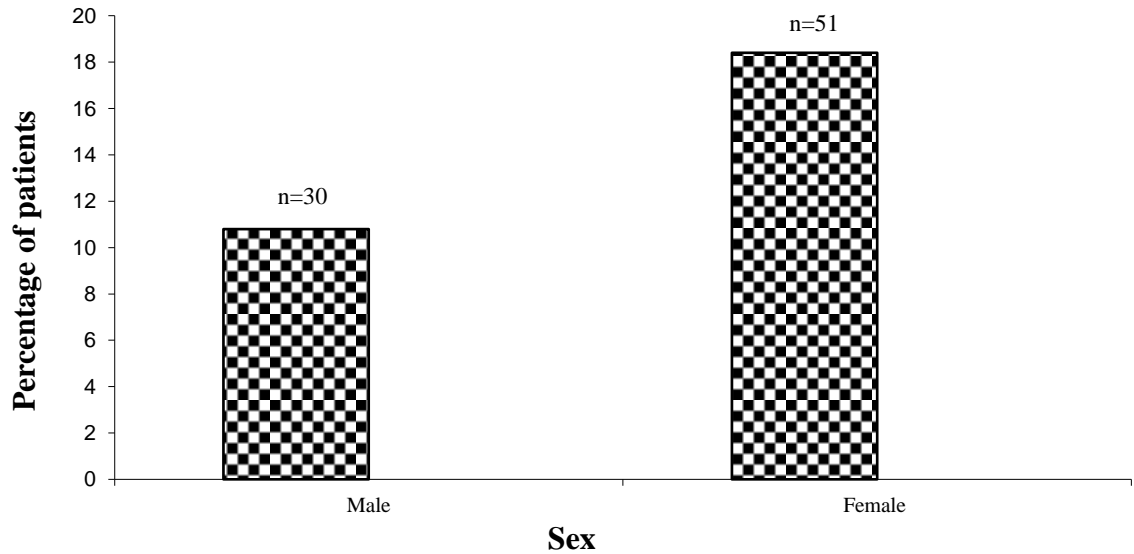
#### **4.2.4 Relationship between sex and *E. histolytica* infection in residents of Kitui west**

Between the sexes, a higher percentage (32%) of 158 female patients were infected with *E. histolytica* compared to 25% of 119 male patients (Figure 4.2) although the difference was not statistically significant ( $\chi^2$  (5, n=277) = 1.35; p=0.251; Phi = 0.078).



**Figure 4.2: Infection rates in male and female patients**

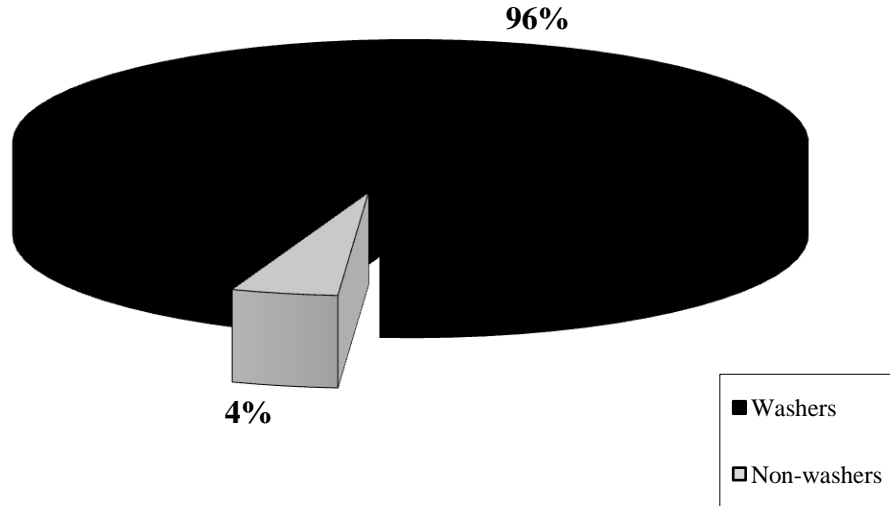
According to overall infection in the 277 individuals sampled, it was found that 18.4% or 51 were females infected by *E. histolytica* parasite compared to 30 (10.8%) males. The difference between male and female infection rate was not significant ( $p > 0.05$ ). This gave an overall infection rate of 29.2% in the study area (Figure 4.3).



**Figure 4.3: The overall percentage of *E. histolytica* infected individuals by sex in Kitui west district**

#### **4.2.5 Relationship between washing of fruits and vegetables and *E. histolytica* infection**

The results showed that majority of the patients, 265 (96%) always washed their fruits and vegetables while only 12 (4%) did not (Figure 4.4). The fruits available in the district included mango, pawpaw, oranges and guavas all of which were grown locally in the farms. Fisher's exact test yielded a non-significant relationship ( $P=0.519$ ) which showed that washing fruits and vegetables did not reduce chances of getting infected with *E. histolytica*.



**Figure 4.4: Fruits and vegetables washing habits among residents of Kitui west district**

#### **4.2.6 Relationship between hand washing behavior and amoebiasis**

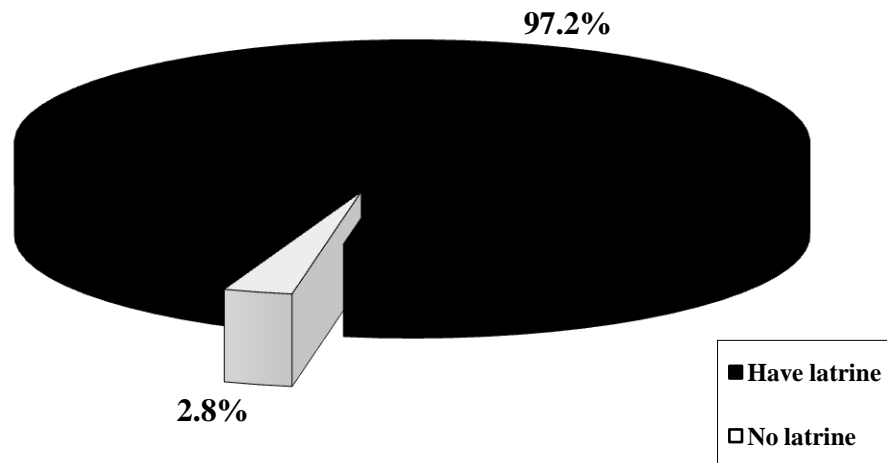
Hand washing behavior of the respondents was observed as one of the critical parameters in personal hygiene. All the 277 individuals interviewed washed their hands after visiting toilets and before eating. However, 39 of the 277 respondents did not use soap while 86% of the patients washed their hands with soap and water. Among those who washed their hands with soap, 28.6% tested positive for *E. histolytica* compared to 33.3% infected among those who did not use soap (Table 4.4). Washing hands with soap and water had no significant effect on the risk of getting infected compared with washing without soap. ( $\chi^2 (1, n=277) = 1.73$ ;  $p= 0.677$ ;  $\Phi = 0.036$ ).

**Table 4.4: Infection rates among patients who washed their hands with soap and patients who did not**

	Washes hands with soap	Washes hands without soap
Negative for <i>E. histolytica</i>	170 (71.4%)	26 (66.7%)
Positive for <i>E. histolytica</i>	68 (28.6%)	13 (33.3%)
<b>Total</b>	<b>238</b>	<b>39</b>

#### 4.2.7 Latrine use and *E. histolytica* infection

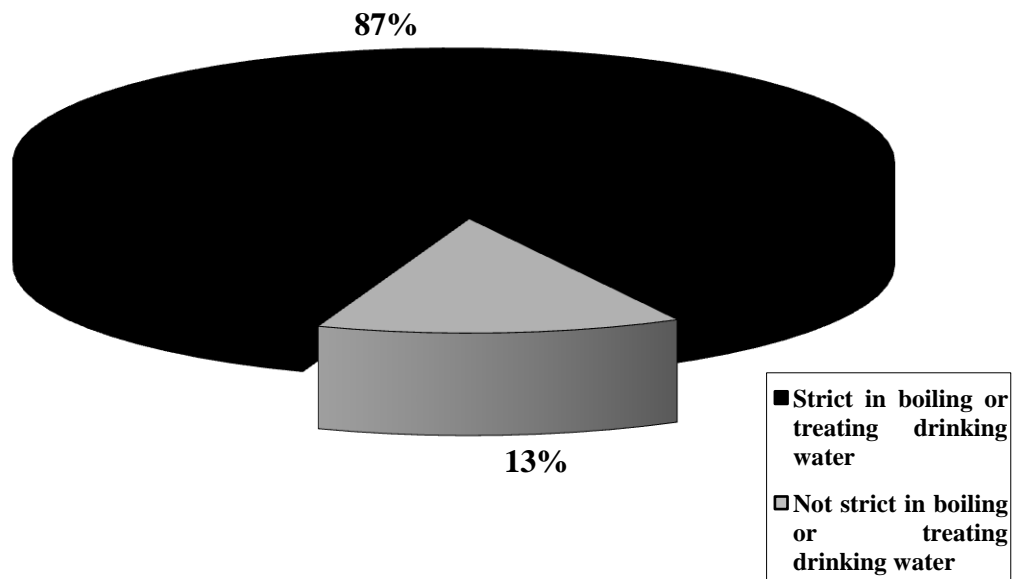
Only 8 of 277 patients (2.8%) did not have a latrine at their home for disposing human waste (Figure 4.5). Fisher's exact test yielded  $p=0.696$  indicating that using latrines did not lower chances of getting *E. histolytica* infection.



**Figure 4.5: Proportion of respondents who had latrine at home**

#### 4.2.8 Treatment of drinking water and its relation to acquiring amoebiasis

Patients were asked whether they drank boiled or treated water. The results revealed that there was no strict raw water drinker. Majority, (87%) of patients used treated or boiled water for drinking but the rest (13%) were not strict in boiling or treating drinking water (Figure 4.6). These patients (13%) did not discriminate drinking water. However, the type of drinking water was found not to have any effect on probability of getting *E. histolytica* infection ( $\chi^2$  (1, n=277) = 0.005; p= 0.944; Phi = 0.004) using a Chi square test of independence.



**Figure 4.6: Proportion of patients who always used treated or boiled water compared to those who did not**

#### **4.2.9 Source of drinking water as a risk factor for amoebiasis**

Patients were also asked to state their sources of drinking water. Most (220) of the patients obtained their drinking water from the river, 14 from earth dams and only 43 patients had access to tap water (Table 4.5). The source of drinking water significantly influenced the risk of infection ( $\chi^2$  (2, n=277) = 8.02; p= 0.018; Phi = 0.17) according to a Chi square test of independence. Among the sources of drinking water, river water was the most commonly used and more likely to be contaminated and unsafe hence predisposing residents to the risk of contracting the infection. Interestingly, out of the 220 patients who used river water, 73 of them (33.2%) tested positive for *E. histolytica* infection while only two (14.3%) tested positive out of 14 patients using water from the earth dam. Forty three patients used tap water and only six (14%) among them tested positive (Table 4.5). Therefore, the patients who used river water were more infected by the disease. Among the river water users, close to two thirds (142) always boiled or treated their drinking water out of which 23 (16.2%) were symptomatic.

**Table 4.5: Infection rates among patients using river, earth dam and tap water at Kitui west district in Kitui County**

	Source of drinking water		
	River	Earth dam	Tap
Negative for <i>E. histolytica</i>	147 (66.8%)	12 (85.7%)	37 (86.0%)
Positive for <i>E. histolytica</i>	73 (33.2%)	2 (14.3%)	6 (14%)
<b>Total</b>	220	14	43

#### 4.2.10 Levels of education among age groups

Level of education highly determines personal hygiene practices. All the respondents in age group 26 to 35 had at least primary education with 23 (67.6%) of them with post-secondary training. Majority of the residents (117 out of 277) interviewed had acquired secondary education. Of the 47 patients in  $\geq 50$  years age group, 4 (8.5%) had tertiary education. The 26 to 35 years age group comprised of a number of young adults in colleges or had just completed tertiary education (Table 4.6). This was the least infected group despite their number being the highest (Figure 4.1). A total of 23 out of 34 patients in this age group had post-secondary education (Table 4.6) where health education is expected to have been acquired.

**Table 4.6: Respondents' educational level**

Age group (Years)	Highest level of education acquired				Total
	No formal education	Primary education	Secondary education	Post-secondary education	
<5	8	9	0	0	17
5 to 15	5	21	34	0	60
16 to 25	4	23	42	6	75
26 to 35	0	4	7	23	34
36 to 49	3	12	18	11	44
≥50	8	19	16	4	47
<b>Total</b>	28	88	117	44	277

### 4.3 Rate of infection in Kauwi and Muthale hospitals

The number of patients from the two hospitals, Kauwi and Muthale, and the proportion of patients whose stool samples tested positive for the presence of *E. histolytica* cysts or trophozoites varied markedly (Table 4.7). The percentage of patients with *E. histolytica* cysts and trophozoites was higher in Muthale hospital at 50% compared to 14.3% at Kauwi hospital. This was despite the fact that Kauwi hospital received more patients than Muthale hospital. The difference in infection levels at the two hospitals was found to be statistically significant ( $\chi^2$  (1, n=277) = 39.85; p= < 0.001; Phi = 0.38). About 90.5% of out-patients who attended Muthale hospital obtained water from either earth dam or river. Out of these, 28.9% did not always boil or treat it. At Kauwi hospital,

19.9% of patients used tap water while those using river water at the same hospital, 3.3% boiled or treated it sometimes (Table 4.7).

**Table 4.7: Rate of *E. histolytica* infection among out-patients attending Kauwi and Muthale hospitals and their water source**

Hospital	Laboratory test results		Source of drinking water		
	No. of out-patients	Positive cases No. (%)	River No. (%)	Tap No. (%)	Earth dam No. (%)
<b>Kauwi</b>	161	23 (14.3)	123 (76.4)	32 (19.9)	6 (3.7)
<b>Muthale</b>	116	58 (50.0)	97 (83.6)	11 (9.5)	8 (6.9)
<b>Total</b>	277	81	220	43	14

## CHAPTER FIVE

### DISCUSSION

#### **5.1. Differences in occurrence levels of *Entamoeba histolytica* in different age groups**

The under five years age group were the most infected with *Entamoeba histolytica* both in retrospective and current study findings similar to report by Zahida *et al.* (2010) that age is an important risk factor for many infectious diseases especially those that are transmitted orally such as *E. histolytica*. The current study findings are also in consistence with previous studies done in Pakistan and Bangladesh which reported that infection with *E. histolytica* is most common among young children who are likely to come into contact with infected material as they crawl on the ground or play games outdoors (Haque *et al.*, 2006; Zahida *et al.*, 2010). In addition, children are likely to put play items in their mouths and eat with unwashed hands.

Children are also less acquainted with hygienic habits such as washing hands before eating or after using the latrines which also makes them more vulnerable to infection (Stanley, 2003). It is worth noting that the current study found people from all age groups were infected with the disease even though the levels of occurrence differed among the age groups (Figure 4.1). This finding is consistent with the assertion by Zahida *et al.* (2010) that although young children are more susceptible to infection, people of all ages in developing countries are exposed to *E. histolytica* infection. The nearly uniform infection rates can be attributed to poor socioeconomic standards and poor sanitation which are risk factors in developing countries (Chacin-Bonilla *et al.*, 1992; Al-Harthi and Jamjoom, 2007).

The 26-35 years age group that had highest number of individuals with post-secondary education was the least infected with *Entamoeba histolytica*. Education is viewed as one of the parameter of determining personal well-being especially in improving hygiene. Ross *et al.* (2003) argued that people with higher levels of educations tend to be healthier than those of similar income who are less educated because they seek medical attention early. Espinosa-Cantellano and Espinosa-Cantellano (2000) and Karaman *et al.* (2006) found ignorance, toilet habit and degree of literacy as serious risk factors for amoebiasis. It is less likely that individuals with post-secondary education had not overcome these risks. The 20.6% infected in 26-35 age group was less than half the infected population in Africa according to Al-Harthi and Jamjoom (2007) who found that up to 50% of Africans suffered from amoebiasis.

## **5.2 Relationship between sex and *E. histolytica* infection**

Sex has been found to be a risk factor for infection with *E. histolytica* particularly in developing countries where both sexes are equally exposed but the risk is reportedly higher for females working in farms and homosexual men (Haque *et al.*, 2006). Kitui west is a rural farming area and women are more likely to be found working in farms than men. Women are also culturally expected to be involved more in domestic chores like changing babies' diapers and cooking. These may bring them into constant contact with contaminated soil and water which potentially promote oral transmission of the disease through contaminated hands. This might account for the slightly higher level of infection among the female population similar to report by Haque *et al.* (2006).

### **5.3 Relationship between level of sanitation and personal hygiene and the *E. histolytica* infection**

Most of the patients (84.5%) obtained water from the river or earth dams both of which are likely to be contaminated with the *E. histolytica* cysts. The river water was highly likely to be contaminated because common practice among locals is to use river waters for washing clothes, relieving themselves and dumping used water into the river. If residents used the same water to wash fruits and vegetables, they ended up contaminating rather than cleaning their fruits and vegetables. Likewise, even if they used the water to wash their hands, they only contaminated rather than cleansing their hands. The source of drinking water had a statistically significant relationship ( $p=0.018$ ) with *E. histolytica* infection and an important risk factor for water and food borne diseases such as amoebiasis (Espinosa-Cantellano and Espinosa-Cantellano 2000; Ryan and Ray, 2004).

Boiling water for drinking is an effective way of controlling diarrheal diseases such as amoebiasis as those who used river water but boiled it before drinking were likely to be safe from the disease. The symptomatic cases among those who boiled drinking water (23 out of 142 or 16.2%) could be due to other sources of contamination such as soiling their hands or washing utensils with contaminated water keeping in mind that Espinosa-Cantellano and Espinosa-Cantellano (2000) emphasized use of safe water for all domestic uses to remain free from diarrhea. Boiling or treating water would go a long way in reducing infection but if it is not done hygienically, there is still a possibility of contaminating the water. Most of the residents of Kitui west in Kitui County, which has

poverty level of 63%, are poor and illiterate (Ministry of Finance and Planning, 2000) hence they may not know the most suitable ways of disinfecting water and storing it safely for use. These account for non-relationship established by the current study between boiling or treating drinking water and the levels of *E. histolytica* infection among the patients in Kauwi and Muthale hospitals.

It might seem that washing fruits and vegetables, drinking boiled or treated water, washing hands with soap and water and using a latrine would drastically reduce the rates of infection with *E. histolytica*. However, that was not the case in this study. The current study suggests that despite the latrines being ideal for disposing off human waste matter, if people continue to use contaminated water for drinking, cooking and cleaning, latrine use alone does not mitigate against the disease.

#### **5.4 *Entamoeba histolytica* infection level among the respondents in Kauwi and Muthale hospitals**

The source of water for drinking for the patients in the two hospitals differed significantly with Muthale, located in the remote part of the district using water from unsafe sources; either earth dam or river and not strictly boiling or treating it. Kauwi is located at the district's headquarters and supplied with piped water where patients got their drinking water from. At Kauwi, more patients were found to boil or treat drinking water than those at Muthale. This difference in the source of water for drinking and its treatment in the two hospitals may account for the different rates of *E. histolytica* infection observed ( $p < 0.001$ ). This is in accordance with Madigan *et al.* (2003) and

World Health Organization (1987) who observed protecting and improving water supplies as well as boiling or treating drinking water as the main methods of controlling amoebiasis. A study in Brazil identified quality of drinking water as an important risk factor for amoebiasis (Benetton *et al.*, 2005).

The findings from this study were contrary to those of the World Health Organization that a large percentage of patients with *E. histolytica* are asymptomatic carriers (WHO, 1997). This is because only 3.7% of asymptomatic patients tested positive. According to Gathiram and Jackson (1987), if asymptomatic colonization with *E. histolytica* is left untreated it can eventually lead to amoebic dysentery and other invasive diseases. It is probable that most of the patients seeking treatment at the two hospitals only did so after the symptoms of the disease had begun manifesting while those who were asymptomatic carriers remained at home and did not seek out-patient services at the hospitals.

## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

- a) The current study found that 29.2% of the patients attending Kauwi and Muthale hospitals in Kitui west district were infected with *Entamoeba histolytica*.
- b) Children aged five years or below were the most infected (58.8%) compared to all other ages. The least infected were 26-35 years old at 20.6%.
- c) There was higher proportion of females infected with *E. histolytica* than males. Among the 29.2% infected patients, 10.8% were males while females were nearly double at 18.4%.
- d) The use of river water was associated with higher levels of *E. histolytica* infection compared with earth dam and tap water. Even though majority of patients practised hygiene habits such as hand washing with soap and latrine use, these practices did not seem to reduce the infection.

## **6.2 Recommendations**

- a) To curb the relatively high levels of amoebiasis in the district there is need for surveillance systems and health education targeting parents and guardians of children under five years aimed at early and proper treatment of the disease.
- b) The Ministry of Health should intensify health campaign especially in children less than five years of age and their parents / guardians, particularly females, on ways to improve hygiene practices at home to avoid infection.
- c) There is need for residents in Kitui west district to emphasize use of safe water for all domestic chores if the benefit of personal hygiene is to be realized. Consequently, supplying all residents in Kitui west district and other rural areas in Kenya with treated piped water will go a long in helping Kenya realize MDG 4 and vision 2030.

## **6.3 Suggestions for further research**

- a) There is need for evaluation of waters in Kitui west district to determine all the contaminating pathogens in water used by residents.
- b) Studies to be done on other causes of diarrhea in the population targeting the 45.7% patients who had symptoms of amoebiasis but tested negative.

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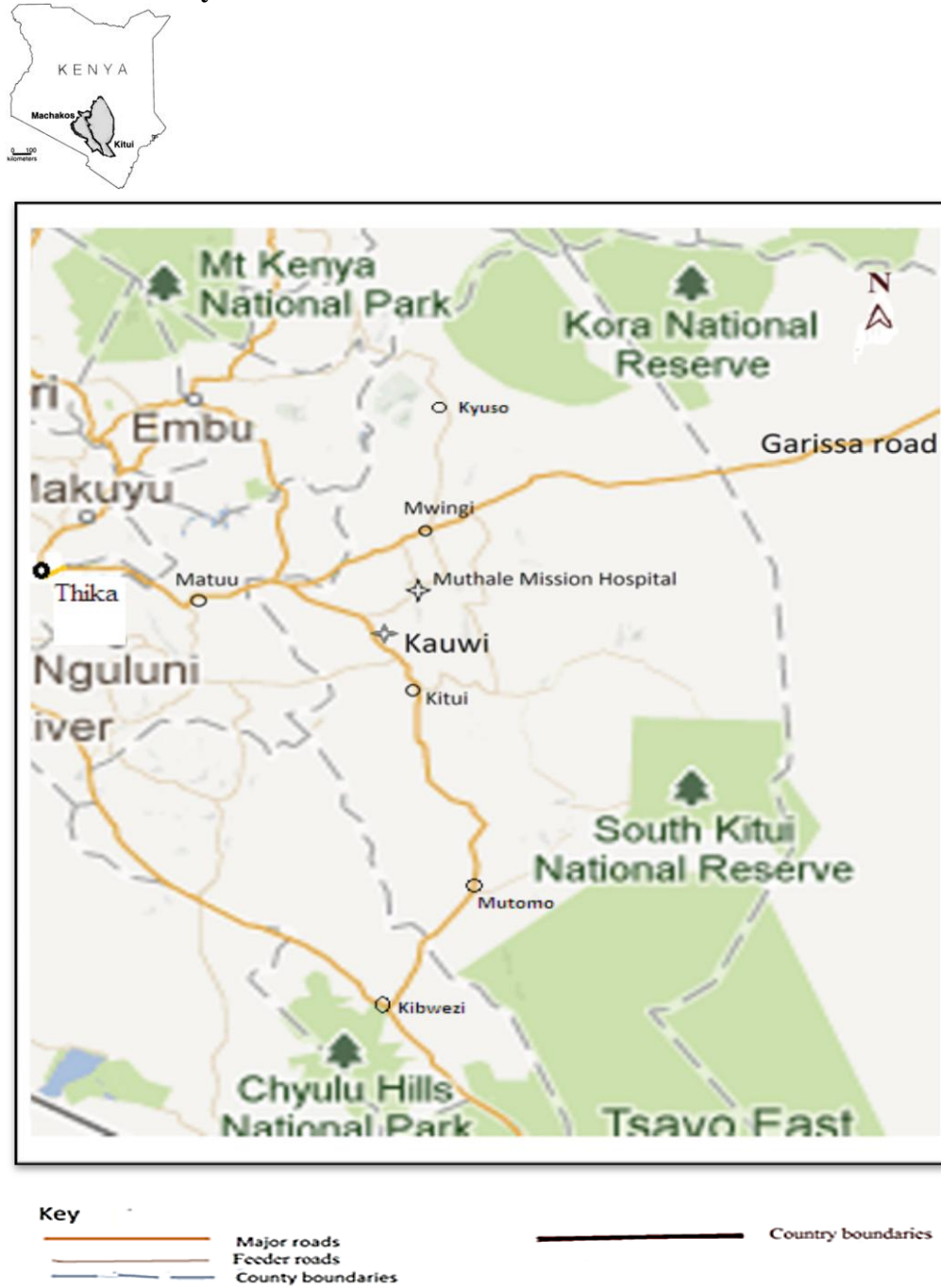
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APPENDICES

Appendix I: Map of the Kitui County. Inset: map of Kenya showing the location of the Kitui County



(Source: Google maps, April 12<sup>th</sup>, 2013)

## Appendix II: Consent form

My name is Elijah Mung'ang'a, a student at Kenyatta University pursuing a degree in Master of Science (Applied Parasitology). As part of fulfillment of the degree requirement, I am carrying out a study to determine the occurrence levels of amoebiasis in Kitui west district. I will ask you to fill a questionnaire to enable me assess the extent to which amoebiasis affect residents of Kitui west district. I will also ask you to allow my research technicians take your stool sample so as to test the presence or absence of *Entamoeba histolytica*, the causative agent of amoebiasis. The results of this diagnosis will be made available to you so as to advise on seeking appropriate control and treatment.

### Declaration

I understand that participation in this study is purely voluntary and I hereby accept to:

- (a) Fill the questionnaire.
- (b) Allow laboratory technician take my stool sample.

Interviewee's/Parent's/Guardian's name.....

Signature.....

Date.....

Interviewer's name.....

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

**Appendix III: Questionnaire****1. Relationship to patient**

i. Self.....

ii. Parent.....

iii. Guardian.....

**2. Sex**

i. Male.....

ii. Female.....

**3. Age**

i. Under 5 years.....

ii. 5-15 years.....

iii. 16-25 years.....

iv. 26-35 years.....

v. 36-49 years.....

vi. 50 years and above.....

**4. Level of education**

i. No formal education.....

ii. Primary education.....

iii. Secondary education.....

iv. Post-Secondary.....

**5. Occupation/economic activity**

- i. Civil servant.....
- ii. Self-employed/business person.....
- iii. Casual worker.....
- iv. Peasant farmer.....
- v. Unemployed.....

**6. Do you have latrine/toilet at home?**

- i. Yes.....
- ii. No.....

**7. Do you drink treated or boiled water?**

- Yes, always.....
- Yes, sometimes.....
- No.....

**8. Where do you get water for drinking and cooking?**

- i. River.....
- ii. Earth dam.....
- iii. Tap.....
- iv. Shallow well.....
- v. Borehole.....

**9. Do you always wash your hands before eating and after visiting toilet?**

i) Yes, with soap.....

Yes, without soap.....

ii) No.....

**10. Do you always wash fruits and vegetables before eating and cooking?**

i. Yes.....

ii. No.....

**11. What do you use at your home for enriching the soil in your garden?**

i. Compost pit fertilizer.....

ii. Animal manure.....

iii. Human wastes.....

iv. Inorganic fertilizer.....

v. Nothing.....

**Thank you for participating in this study.**



## Appendix V: Authorization to collect data at Kauwi sub-district hospital

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REPUBLIC OF KENYA



MEDICAL SUPERINTENDENT  
KAUWI SUB-DISTRICT HOSPITAL  
P O BOX 19-90205,  
KABATI

E-mail: kauwisdh@yahoo.com

All correspondence address to  
"The Medical Officer of Health"  
When replying please quote

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MINISTRY OF MEDICAL SERVICES

14<sup>th</sup> March, 2013

FROM: KAUWI SUB-DISTRICT HOSPITAL

TO: ELIJAH MATIVO MUNG'ANG'A

RE: AUTHORITY TO CARRY OUT RESEARCH AT KAUWI SUB-DISTRICT HOSPITAL.

The fore mentioned person who is a student at Kenyatta University has been permitted to carry out his Masters research work at our Laboratory.

Dr. Rabut

M.O I/C

KAUWI SDH

A handwritten signature in blue ink, appearing to be 'Dr. Rabut'.



**Appendix VI: Authorization to collect data at Muthale mission hospital**

*Muthale Hospital*

P.O BOX 532 Kitui, Kenya, Along Kitui, Muthale Mwingi road, Tel: 020 3581723  
Email: muthalehospitalcdk@yahoo.com

Administrator  
Muthale Mission Hospital  
P.O. Box 532 – 90200  
KITU!

25<sup>th</sup>. November 2014

**FROM:** Muthale Mission Hospital

**TO:** ELIJAH MATIVO MUNG'ANG'A

**RE: AUTHORITY TO CARRY OUT RESEARCH AT MUTHALE MISSION HOSPITAL**

This is to confirm that the above mention student in Kenyatta University had been permitted to carry out his research work in our facilities for a duration of 3 months i.e. From April to June 2013.

Yours sincerely

  
SR. STELLAMARIS MWELU  
HOSPITAL ADMINISTRATOR

*Muthale Hospital*