PREVALENCE OF AMOEBIASIS AND THE FACTORS ASSOCIATED WITH ITS TRANSMISSION AMONG PATIENTS ATTENDING LONGISA COUNTY HOSPITAL, BOMET COUNTY, KENYA

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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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We Kenyatta University supervisors confirm that the work reported in this thesis was carried out by the candidate under our supervision and that the thesis is submitted for examination with our approval.

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DEDICATION

To Mum and Dad, Mr and Mrs Zakayo Too, for your invaluable love. Without you I would not be whom I am today.
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I thank the Almighty God for keeping me in good health condition throughout this study.

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LIST OF ABBREVIATIONS AND ACRONYMS

CDC Center for Disease Control

GI Gastro-intestinal

HIV Human Immunodeficiency Virus

KNPCKenya National Population Census

MDGsmMillennium Development Goals

MSM Men having sex with Men

NGO’s Non-Governmental Organizations

SPSS Statistical Package for Social Sciences

USA United States of America

WHO World Health Organization

% Percent

°C Degrees Celsius

df Degrees of freedom

χ² Chi square
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ABSTRACT

Amoebiasis is a common life-threatening parasitic disease affecting 12% of the world population. It is the third leading cause of mortality due to parasitic infections worldwide, after malaria and schistosomiasis. It is estimated that about 500 million people are at risk of infection with amoebiasis yearly. In developed countries infection occurs among travelers, recent immigrants from endemic regions, homosexual males, immune-compromised persons and institutionalized individuals. In less developed and developing countries, infection occurs more commonly in areas of low socio-economic status, poor sanitation and nutrition. In Kenya, there is no continuous surveillance system to combat amoebiasis hence its real prevalence remains unknown in most parts of the country including Bomet County. Cases of amoebiasis have been reported in most health facilities in Bomet County. It is with this regard that the study sought to determine the prevalence and transmission risks factors associated with amoebiasis in Longisa County Hospital in Bomet County, Kenya. The study involved a retrospective survey through evaluation of data from hospital records for a period of five years, from January 2009 to December 2013. The study also employed cross sectional survey by collecting 255 stool samples through purposive sampling. Macroscopic and microscopic examination of stool was used to investigate amoebiasis in the laboratory. Macroscopically, stool samples were checked for the presence of mucus, blood, consistency of the stool and colour. Microscopically, direct saline and iodine wet mount was used to identify Entamoeba histolytica trophozoites and cysts. Data on transmission risk factors was collected through questionnaires and from hospital check list. Data were entered in Statistical Package for Social Sciences (SPSS) then analysis carried out to determine relationship between prevalence of amoebiasis and various variables such as age, location, socioeconomic status, and personal hygiene, and water source, waste disposal, diet and level of education. The results are presented using graphs and tables. The study revealed that out of 5480 stool samples examined at the hospital laboratory during the period January 2009 to December 2013, 1574 (28.72%) stool tests were positive for amoebiasis of which 10.47% were adult males while 15.76% were adult females. The least affected age group was the under five years at 2.37% in the retrospective study. In the cross-sectional study, out of the 255 patients interviewed and their stool examined for Entamoeba histolytica trophozoites and cysts, 11.76% adult females, 7.06% adult males and 0.78% children under the age of five years tested positive for amoebiasis. Most of the patients interviewed were farmers (60.8%). Majority of the patients obtained water for domestic use from open earth dams (38.8%) and rivers (29.0%). Source of water for domestic use and unhygienic personal practices played a key role in acquiring the parasite. The findings of this study will be used in informing the County health professionals on the extent of amoebiasis hence help in designing control and preventive measures in Bomet County. It is therefore recommended that, the government through the ministry of health and non-governmental organizations should focus on provisionsafe water and campaigns on improving personal hygiene to combat the disease in Bomet County.
CHAPTER ONE
INTRODUCTION

1.1 Background information

Amoebic infections are a significant health problem worldwide, particularly in tropical countries and places where public hygiene and sanitation are poor. It is the third leading cause of mortality due to parasitic infections worldwide after malaria and schistosomiasis (Park et al., 2007). It has been established that, approximately 500 million individuals are infected with amoebiasis each year and only about 10% experience symptomatic disease (Ravdin, 2005; Stauffer et al., 2006).

Transmission is mainly through ingestion of fecally contaminated food or water containing Entamoeba cysts. The cysts measure 10-18 mm in diameter and contain four nuclei. Cysts are resistant to environmental conditions such as low temperature and the concentrations of chlorine commonly used in water purification but can be killed by heating to 55°C (Ximenez, 2009). After ingestion, the cyst, which is resistant to gastric acidity and digestive enzymes, excysts in the small intestine to form eight trophozoites. These large, actively motile organisms colonize the lumen of the large intestine and may invade the mucosal lining. Once E. histolytica trophozoites invade the intestinal mucosa, the organisms multiply and spread laterally underneath the intestinal epithelium to produce characteristic flask-shaped ulcers (Ximenez, 2009).

Studies have demonstrated that 4-10% of individuals infected with E. histolytica develop amoebic colitis, and less than 1% of individuals develop disseminated disease, such as
amoebic liver abscess (Ximenez, 2009). Amoebic colitis may occur within two weeks of infection or be delayed for months. The onset is usually gradual with colicky abdominal pains, frequent bowel movements and diarrhea associated with blood-stained stools with a fair amount of mucus (Chunge et al., 2008). Amoebic colitis affects all age groups, but its incidence is strikingly high in children, 6-10 years of age (Reuben et al., 2013). Severe amoebic colitis in infants and young children tends to be rapidly progressive with frequent extra-intestinal involvement and high mortality rates, particularly in tropical countries. Occasionally, amoebic dysentery is associated with sudden onset of fever, chills and severe diarrhea, which may result in dehydration and death in infants (Blessmann, 2006).

1.2 Statement of the problem

Amoebiasis remains a public health problem despite the great development in medicine and proper diagnosis. The disease burden is growing with the advent of drug resistant species and environmental degradation (WHO, 2010). It is emerging that though *E. histolytica* is believed to cause small proportion of individuals to develop amebic disease, amoebiasis has been increasing in developing countries, Kenya included. This is mainly due to poverty, characterized by the absence of portable clean drinking water, improper sanitary habits, absence of good fecal disposal system, and poor hygienic practices by the impoverished citizens and overcrowding (WHO, 2010). Cases of the disease have been reported in different health facilities within Kenya which indicates a rise over time,
probably because of clearing of water catchments areas, use of open earth dams prone to
pollution and general poor disposal of fecal waste (WHO, 2008).

Access to hygienic waste disposal methods and portable clean water are fundamental to
the wellbeing of every population. An analysis of the 2009 Kenya population census
shows that a fifth of the rural population (20.9%) and a quarter of the total population
(24.3%) use unhygienic solid waste disposal systems mainly, bucket and bush. This
translates to 9.3 million Kenyans who do not have access to hygienic toilet facilities. The
report shows that 47.5% of the total population use unprotected source of water, mainly
dam, lake and stream, translating to 18.3 million people (KNPC, 2009). In Bomet County
majority of the residents use unprotected water sources mainly open earth dams, rivers
and wells which are prone to infection. More than half (58.7%) of total the population
live below poverty line (KNPC, 2009). It is in this regard that the study established the
prevalence, and the factors associated with disease transmission and trend of amoebiasis
in Bomet County, using Longisa County Hospital as a proxy of the catchment area.

1.3 Justification of the study
Amoebiasis is preventable, yet it continually causes deaths globally, with developing
countries bearing the highest burden. Amoebiasis is a public health problem in Kenya and
although not given much attention, it exacerbates considerable pain and death in the
population. With reference to previous studies on amoebiasis in Kenya, a lot of emphasis
has been on comparison of different antimicrobial drugs on treatment of intestinal (Pamba
etal., 2007) and systemic amoebiasis (Chunge et al., 2008). Some studies focused on
selected practices among rural residents versus the prevalence of amoebiasis and giardiasis (Kinuthia, 2012). These studies have been done on other parts of the country but none has been done in Bomet County. There is need to identify factors associated with amoebiasis transmission, in Bomet County which are key in controlling the disease. Longisa County Hospital being the only district hospital in Bomet County gave a representative picture of the status of amoebiasis in the region. Laboratory documentation provided a basis to investigate temporal changes in the Longisa County Hospital and by extension related to the climatic changes for the five years from January 2009 to December 2013. Understanding the mechanism and dynamism of amoebiasis adds invaluable information needed for planning and policy making towards minimizing the effects of the disease.

1.4 Research questions

i. What is the prevalence of amoebiasis in Longisa County Hospital in Bomet County during the period January 2009 to December 2013?

ii. What is the incidence of amoebiasis in Longisa County Hospital in Bomet County during the period January 2014 to June 2014?

iii. What are the factors associated with amoebiasis transmission among patients attending Longisa County Hospital?
1.5 Hypothesis

i. Amoebiasis is not prevalent among patients attending Longisa District hospital in Bomet County, Kenya.

ii. There are no risk factors associated with amoebiasis transmission among patients attending Longisa County Hospital in Bomet County, Kenya.

1.6 Objectives of the study

1.6.1 General objective

To establish the prevalence of amoebiasis and the factors associated with disease transmission among patients attending Longisa County Hospital in Bomet County.

1.6.2 Specific objectives

i. To determine the prevalence of amoebiasis in Longisa County Hospital during the period January 2009 to December 2013.

ii. To determine the incidence of amoebiasis in Longisa County Hospital for the period January 2014 to June 2014.
iii. To determine the factors associated with amoebiasis transmission among patients attending Longisa County Hospital, Kenya.

1.7 Significance of the study

Amoebiasis has been associated with water contamination and poor personal hygiene and other risk factors, thus the data obtained in this study may be used in informing the county health professionals on the extent of the disease which may help in designing and implementing preventive measures in the County. Information generated from both out and in-patients departments in hospitals can be utilized in providing the initial information needed for planning meaningful public health control programs.
CHAPTER TWO

LITERATURE REVIEW

2.1 General pathology and transmission of amoebiasis

Amoebiasis is an infection caused by the protozoan parasite of species *Entamoeba histolytica*, which is classified in the subphylum Sarcomastigophora, class sarcodina and belonging to a group commonly called amebas. Though there are several protozoans of amebic species, *E. histolytica* is a pathogenic amoeba that can cause invasive intestinal and extra-intestinal disease (Hung *et al.*, 2005). The most frequent manifestations of invasive amoebiasis are colitis and liver abscess (Park *et al.*, 2007). Many infections with *E. histolytica* are asymptomatic, but invasive disease develops in 4%–10% of persons with symptomatic infections which may last over a one year period if not treated (Hung *et al.*, 2005).

Humans are the primary known reservoir for *E. histolytica* (Hung *et al.*, 2005). Amoebiasis is usually transmitted by the fecal-oral route. Stools infected with the cyst form of the parasite may contaminate fresh food or water. It can also be transmitted indirectly through contact with dirty hands or objects (Hung *et al.*, 2005). Infection is spread through ingestion of the cyst form of the parasite found in feces. Trophozoites die quickly after leaving the body but may also be present in stool; these are rarely the source of new infections (Ryan and Ray, 2004). Since amoebiasis is transmitted through contaminated food and water, it is often endemic in regions of the world with limited modern sanitation systems, including México, tropical parts Asia, Central America
and western South America (Ryan and Ray, 2004). In addition, there is a suggestion of zoonotic transmission (Stark et al., 2006). Experimental infections with *E. histolytica* have been produced in some animals such as dogs, cats, rats, monkeys, and other laboratory animals (Stanley, 2003). These animals may also acquire human strains of the parasite as a result of close contact with humans. Natural *E. histolytica* infections with strains morphologically similar to *E. histolytica* have been found in monkeys (Stanley, 2003). In one study, *E. histolytica* was found microscopically in stained fecal smears from six species of non-human primates in Kenya (Muriuki et al., 2002). There may be some animal reservoirs of *E. histolytica* such as dogs, monkeys, and probably pigs but they represent a very small source of human infection compared with humans themselves. Infective cysts may be spread by arthropods such as cockroaches and flies, suggesting that these insects are able to play a rare but important role in transmission (Nozaki et al., 2006).

### 2.2 Life cycle of *Entamoeba histolytica*

The life cycle of *Entamoeba histolytica* consists of an infective cyst stage and a multiplying trophozoite stage (Fig 1.1). Humans are infected by ingesting these infective cysts, which travel through the gut lumen to the small intestine (terminal ileum), where each excysts to form eight daughter trophozoites. The trophozoites are motile forms, which adhere to and invade intestinal epithelial cells lining the gastrointestinal tract. Trophozoites move by extending creeping projections of cytoplasm, called pseudopodia, which pull them along. They also use these projections to surround and engulf food.
particles. The trophozoites of *Entamoeba histolytica* always have a single nucleus. Trophozoites are easily destroyed in the outside environment, degenerating within minutes (Stark *et al.*, 2006). The trophozoite of *Entamoeba histolytica* can convert to pre-cyst form with a nucleus, and this form matures into a tetranucleated cyst as it migrates down and out of the colon. It is the mature cyst that, when consumed in contaminated food or water, is infectious. In the process of becoming tetranucleated, the nucleus of the cyst divides twice (Ogunlesi *et al.*, 2005). Cysts can remain alive outside the host for weeks or months, especially under damp and warm conditions (Chunge *et al.*, 2008), but are rapidly destroyed at temperatures under −5°C and over 40°C (Stark *et al.*, 2006). Cysts are not invasive, but trophozoites can penetrate the gastrointestinal mucosa, from where they migrate to other organs, causing extra intestinal infections (Nozaki *et al.*, 2006).
Ingestion of infective cysts

Excystation in the small intestine

Division of quadranuclate cyst into four and then eighth trophozoites

Trophozoites move to colonize the colon

Encystation

Excretion of cysts

Ingestion of cysts by patient

*Fig 1.1:* Life of cycle *E. histolytica* (CDC, 2010).
2.3 Identification of Entamoeba histolytica

The trophozoite measures 20 to 40 µm and may contain red blood cells. It has one nucleus. If examined quickly under a microscope in a fresh fecal sample or a swab from a rectal ulcer, the colourless motile trophozoites can be seen. Motility disappears when cooled, and the parasites are then difficult to recognize. Once stained the nucleus is moderately visible (Meserati et al., 2002). Lugol staining kills the parasite almost immediately and motility disappears. Stained Entamoeba histolytica trophozoites have a transparent outer border and an opaque inner border (CDC, 2010).

The cysts have 1, 2 or 4 nuclei and measure 8 to 15-20 µm (Plate 2.1). The nuclei are revealed by means of an iodine stain. They have a dark circumference and a dark central point (karyosome). Iodine staining can also detect glycogen (brown) in young cysts. Fresh cysts of Entamoeba histolytica also contain chromatoid bodies. In active dysentery, often no cysts are found in the faeces, but if there is little diarrhea, the parasites have time to encyst (CDC, 2010).
Plate 2.1: Immature *Entamoeba histolytica* cyst (CDC, 2010).

2.4 Pathogenesis of Amoebiasis

Amoebiasis is classified into intestinal and extra intestinal types; the intestinal amoebiasis is further subdivided into asymptomatic carriers, amoebic colitis, fulminant colitis and amoeboma. In asymptomatic carriers, trophozoites can sometimes remain in the intestinal lumen for years without causing any damage. The majority of patients (90%) fall into this group. Asymptomatic carriers have no symptoms of amoebiasis. These persons can be detected by faeces analyses, which for unknown reasons are not invasive (Ryan and Ray, 2004). The incubation period of amoebic colitis varies greatly.

When *Entamoeba histolytica* penetrates the intestinal mucosa, it produces ulcerations of the colonic mucosa. This is expressed clinically as abdominal pain, diarrhea with blood in the faeces, and moderate or no fever, with good general condition. When the rectum is
affected there are painful cramps in the anus. Peri-anal ulcers may occur via direct spread from rectal amoebiasis. The ulcers develop rapidly and are painful. After suffering from amoebic colitis there may be persistent intestinal problems, the aetiology of which is unclear (Moran et al., 2005). There is sometimes a fulminant course with high fever, a severe illness, intestinal bleeding or perforation of the colon. A slow infiltration of intestinal content into the peritoneum is very likely in a severely ill whose condition deteriorates progressively, with the formation of intestinal paralysis and a distended abdomen. In 1% of patients an inflammatory thickening of the intestinal wall occurs. A mass may then be palpated (amoeboma). The diagnosis may be made via biopsy. The inflammatory mass may mimics colon carcinoma. Countless trophozoites are found in the tissues (never cysts). Correct therapy reduces the volume of trophozoites immediately (Moran et al., 2005).

The extra-intestinal amoebiasis is the type that involves the liver, brain and spleen as well as other organs of the human body. Clinical presentation of the intestinal infection may include abdominal discomfort, weakness, malaise, constipation that may alternate with diarrhea, dysentery with the passage of exudates, blood and mucus as well as colicky abdominal pain. Systemic signs of infection include fever, rigors and polymorphonuclear leukocytes while liver abscess results from infection via the intra-hepatic portal vessels. The incubation period of amoebiasis variable, ranging from a few days to months or years, but is usually one to four weeks (Stark et al., 2006).
2.5 Amoebiasis in relation to age and sex

Symptomatic intestinal amoebiasis occurs in all age groups. Liver abscesses due to amoebiasis are 10 times more frequent in adults than in children. Very young children seem to be predisposed to fulminant colitis (Davies, 2006). Amoebic dysentery is very rare in children under the age of two years. Amoebic colitis is common in young to middle-aged adults of between six years to twenty-four years (WHO, 2011).

Amoebic colitis affects both sexes equally (Davies, 2006). However, invasive amoebiasis is much more common in males than females. In particular, amebic liver abscess is 7-12 times more common in men than in women, with predominance among men aged 18-50 years. The reason for this disparity is unknown, though hormonal effects are implicated, as the prevalence of amebic liver abscess is also increased among postmenopausal women (Khairnar et al., 2007). Among prepubertal children, amebic liver abscess is equally common in both sexes (Davies, 2006). Acuna-Soto and co-authors, 2012, noted that asymptomatic E. histolytica infection is distributed equally between sexes and that higher proportion of males with invasive amebiasis may be due to a male higher susceptibility to invasive disease.

2.6 Global status of amoebiasis

In developed countries, the infection occurs primarily among travelers, recent immigrants from endemic regions, homosexual males, immuno-compromised persons and institutionalized individuals (Swords and Canytey, 2002). The prevalence of E.
*Entamoeba histolytica* and *E. dispar* was reported as 4% in the USA (Park *et al.*, 2007). *Entamoeba histolytica* is a major cause of morbidity worldwide, causing approximately 50 million cases of dysentery and 100,000 deaths annually (Ravdin *et al.*, 2005). *Entamoeba histolytica* associated dysentery is common in the less developed and developing countries of the world but is more common in areas of low socio-economic status, poor sanitation and nutrition, especially in the tropics (Ravdin *et al.*, 2005).

Majority of *E. histolytica* infections, morbidity and mortality occur in Africa, Central and South America and the Indian Sub-continent, where the prevalence of *Entamoeba* infection is as high as 50% (Haque *et al.*, 2006). *Entamoeba histolytica* seroprevalence studies in Mexico revealed that more than 8% of the population was positive (Park *et al.*, 2007). In endemic areas, as many as 25% of patients may be carrying antibodies to *E. histolytica* as a result of prior infections, which may be largely asymptomatic. The prevalence of asymptomatic *E. histolytica* infections seem to be region-dependent; in Brazil, for example, it may be as high as 11%. In Egypt, 38% of individuals presenting with acute diarrhea to an outpatient clinic were found to have amebic colitis (Park *et al.*, 2007). In Hue City, Vietnam, the annual incidence of amoebic liver abscess was reported to be 21 cases per 100,000 inhabitants (Park *et al.*, 2007). An epidemiologic study in Mexico City reported that 9% of the population was infected with *E. histolytica* in the 5-year to 10-year period preceding the study. A prevalence of 39% was recorded in Bangladesh and 33% in Columbia (Park *et al.*, 2007).
2.7 Regional status of amoebiasis

In some African countries, 6% to 75% of the population had the parasite (Stark et al., 2006). These studies were conducted using microscopic examination, and therefore only indicate distribution of the disease. Such results require confirmation by techniques that clearly differentiate *E. histolytica* from *E. dispar*, which is not pathogenic. Other studies in parts of Africa reported prevalence rates of 22% and 21% in South Africa and Egypt respectively (Stauffer et al., 2006). In Nigeria, prevalence rates were 22.3% in Calabar (Ozumba, 2001), 21.6% in Enugu and 13.7% in Ilesa (Ogunlesi et al., 2005). In Uganda the prevalence was 1.4% (Brink et al., 2002), Ethiopia was 10.3% (Hailemariam et al., 2004), and Dakar, Senegal was 5.1% (Gassama et al., 2001) and South Africa was 12.4% (Samie et al., 2006). Cases of the disease are reported in different health facilities in Kenya, for example, in Njoro PCEA health center, Nakuru County, prevalence rate of 21% was reported (Kinuthia, 2012).

2.8 Transmission risk factors associated with amoebiasis

Poor personal hygiene, particularly common in day care centers and institutions such as prison, orphanages as well as among food handlers, are important for transmission (WHO, 2012). Age is a risk factor for amoebiasis, young children aged between six to ten years being mostly susceptible. This can be attributed to the fact that they are very active and are ignorant about hygiene. The playing areas are main sources of diseases because they form disposal sites for homes and industries (Davies, 2006). It is also due to immature immunity and a lack of prior antigen exposure, which results in a high
frequency and more severe infections than mature individuals (Davies, 2006). A study conducted in Brazil identified place of residence, ingestion of raw vegetables and clean drinking water as important risk factors for amoebiasis (Benetton et al., 2005).

In developing countries ignorance, poverty, poor sanitation, overcrowding coupled with lack of plumbing, characterized by lack of safe portable drinking water promotes intestinal protozoa infections, inadequate and contaminated water supplies. Inefficient water treatment using chlorine or no water treatment at all has been associated with amoebiasis. Men having sex with men (MSM) brings about oral-anal contact hence depositing cysts directly in the mouth, and promoting fecal-oral transmission for the intestinal protozoa (Stark et al., 2005).

The prevalence of amoebiasis depends on many factors including 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 temperature changes and seasonal variations (Karaman et al., 2006). Prevalence of amoebiasis is high among families who eat together from the same plate, those who eat with their fingers and those who eat away from home (Oyerinde et al., 1979), in municipal sanitary workers (Karaman et al., 2006), pregnant women and patients who take immunosuppressive drugs (Hung et al., 2008). Alcohol is considered to be an important risk factor (Braga et al., 2001).

Various factors such as individual general condition, undernourishment, stress, overwork, diet (especially cholesterol), surgery, inter-current viral/ bacterial/ bacterial infections,
and hormonal / neural disorders have been found to weaken resistance or increase risk of amoebiasis infection (Tsai et al., 2006).

Several studies indicate that HIV infection is a risk for invasive amoebiasis (Hung et al., 2005; Tsai et al., 2006). It is suggested that severe invasive amoebiasis may develop in HIV-positive patients (Moran et al., 2005) and that susceptibility and clinical factors differ between HIV-positive and HIV-negative patients because of differences in immune status. However, the effect of HIV infection on these risk factors for invasive intestinal amoebiasis remains unclear (Moran et al., 2005).

2.9 Treatment of amoebiasis

Antiamoebic drugs are divided into three categories; luminal, tissue and mixed amoebicides. Metronidazole is the main drug of choice. Tinidazole, secnidazole and ornidazole are also effective. Diloxanide furoate, diiodohydroxyquin, paramomycin, emetine and chloroquine have also been used as alternative drugs (Blessmann and Tanni, 2002). Diloxanide furoate is used mainly in treating asymptomatic cyst carriers. Chloroquine can be used along with Metronidazole or emetine in cases of hepatic amoebiasis. Metronidazole, tinidazole, secnidazole and ornidazole kill trophozoites by alterations in the protoplasmic organelles of the amoebic. Chloroquine acts on the vegetative forms of the parasite and kills it by inhibition DNA synthesis and emetine kills the trophozoites mainly by inhibiting protein synthesis (Stanley, 2006).
Metronidazole is given for five days for amoebic dysentery and for four to ten days in liver abscess or extra intestinal spread. Large abscesses in liver may require drainage. In case of severe dehydration oral fluids or intravenous fluids may be given and for intestinal perforation, surgical procedures may be required (Stanley, 2006).

2.10 Prevention and control measures

The basic approach for preventing amoebic infection is by improvement of living conditions. This can be done by; (i) improved environmental sanitation including, the protection of water supplies against fecal contamination, food safety and safe disposal of human feaces (CDC, 2007).(ii) early detection and treatment of the disease (Stanley, 2003). iii) E. histolytica cysts are resistant to chemical disinfectants such as chlorination, and can survive a wide range of pH values and osmotic pressures. They die rapidly if heated at above 40°C or frozen at below -5°C (WHO, 2012) iv). Fecal-oral transmission via hands or food is most common. Therefore, proper sanitation is the key to avoiding ameobiasis which can be achieved through the following measures when preparing food and eating food: Thoroughly washing fruits and vegetables before eating, thoroughly washing hands with soap and water after toilet use and before handling food, sticking to bottled water and soft drinks, drinking boiled water or water that has been treated with iodine and avoiding ice cubes or fountain drinks, milk, cheese, or other unpasteurized dairy products and food sold by street vendors (WHO, 2012).

Prevention can be also achieved through Health education on amoebiasis which should form part of the general education programme for controlling infections transmitted by
the fecal-oral route. This can be done through mothers, schoolchildren, and homes through periodic campaigns and mass media (WHO, 2011).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted at Longisa County Hospital which is located in Longisa market, Bomet District in Bomet County. The hospital is situated 15 km South of Bomet town along Bomet-Narok road, 180 km south-west of Nairobi. The facility is a level 4 hospital with a bed capacity of 144. The hospital deals with diagnosis, treatment and control of various illnesses ranging from common diseases such as malaria, typhoid, cholera, TB, skin infections, helmithiasis, accidents, surgeries, mother and child health care and HIV and AIDS management among others. The hospital is capable of dealing with emerging health issues as it is well equipped with modern facilities and trained personnel. At the hospital is Longisa Medical Training College which is a teaching and training center for nurses. The hospital was selected for the study because being a government health center where services are affordable to most of the local population. It is also the only County hospital in Bomet County.

Bomet County is an agricultural area and mainly inhabited by Kipsigis community. The residents are normally in constant contact with the soil as they cultivate their farms. The County lies in plains and highlands. The temperature is moderate throughout the year ranging from 18-25°C. The region has two rainy seasons, short rains between the months of November to January and long rains between the months of March to August. Most roads are tarmac and housing is heterogeneous, ranging from scarce well-constructed homes to wood and grass thatched houses. Only a small number of homes have piped
water. Longisa County Hospital serves this area. The hospital laboratory was used for this study and receives approximately 800 patients per month according to hospital records. A map for the location of Longisa County Hospital is shown in figure 3.1 below.

Fig 3.1: Map showing Bomet County
3.2 Study population

Bomet County has a total population of 730,129, and about 111,288 households. It covers an area of 1997.9 km$^2$. The population density is 367 people per km$^2$ and 58.7% of the population live below the poverty line (KNPC, 2009). About 800 patients are referred to Longisa County Hospital laboratory per month (Hospital records).

3.3 Study design

The study was two folds; one was a retrospective study that examined the hospital laboratory records on cases of amoebiasis contained in hospital databases between the periods January 2009 to December 2013. This database was not created for research but purely for hospital purposes hence minimized bias. The other part of the study was a cross-sectional study, involving microscopic examination of stool from patients attending the facility from January 2014 to June 2014. Questionnaires and hospital check list was used to collect data on factors associated with amoebiasis transmission.

3.4 Inclusion and Exclusion Criteria

Patients attending Longisa County Hospital in Bomet County with persistent diarrhea, dysentery or presumptive amoebiasis during the period January 2014 to June 2014 were enlisted after giving a written consent to participate. Patients not presenting with diarrhea or dysentery and those on antimicrobial agents/regimen were excluded.
3.5 Sample size determination

The sample size was determined using the formula (Stauffer et al., 2006)

\[ \text{Sample size} = \frac{n}{1 + \left(\frac{n}{N}\right)} \]

Where; \( N \) = the expected population of patients attending the hospital for the period January 2014 to June 2014. The hospital laboratory receives approximately 800 patients per month. The target population was approximately 5000 patients in six months.

\( n \) = desired sample size

\[ n = z^2 \frac{p(1-p)}{d^2} \]

\( p \) = the expected frequency of the factor under study is 22%. This based on similar studies (Stauffer et al., 2006; Kinuthia, 2012).

\( Z = 1.960 \) confidence limit of 95%.

\( d \) = normal deviation of 0.05.

Mathematically; \( n = 1.96^2 \frac{0.22(1-0.22)}{0.05^2} \quad n = 263.68 \)

\[ \text{Sample size} = \frac{263.68}{1 + \left(\frac{263.68}{5000}\right)} = 250.47 \]

A total 255 stool samples were collected from patients at the hospital for the period January 2014 to June 2014.
3.6 Retrospective study

Data was collected from hospital records for the period January 2009 to December 2013, to check on amoebiasis cases. The records showed age, gender of the patient, date and year of investigation and the results under investigation. Amoebiasis prevalence was then calculated for the study period. Percentage proportions of positive cases for adult females (six years and above), adult males (six years and above) and children under the age of five years as well as monthly and yearly proportions were also analyzed.

3.7 Sampling

Purposive sampling was used to capture patients presenting with diarrhea in the hospital during the period, January 2014 to June 2014, through simple random sampling, in order to complement the information obtained from the laboratory records. The hospital received an average of ten patients presenting with diarrhea each day. Willing patients were requested to fill a written consent form. Four patients were systematically sampled on three randomly selected days of the week for a period of six months from January 2014 to June 2014. Incidence rate of amoebiasis was obtained from the data collected and analysis made with the factors associated with amoebiasis transmission.

3.7.1 Collection of data using questionnaires

The pre-tested questionnaires were used to collect data on factors associated amoebiasis transmission with emphasis on access to clean drinking water, fecal waste disposal, diet and personal hygiene. Indicators of economic status of the respondents such as
occupation and monthly earning were also assessed. The questions were asked by the researcher at the time the patients were visiting the hospital.

3.7.2 Stool samples

A total of 255 stool samples were collected from 255 patients presenting with diarrhea over a period of about six months from January 2014 to June 2014. The patients were advised to pass stool directly into a clean container labeled with the patient’s code number at the hospital. Patients were asked to ensure that the stool specimen was not contaminated with urine. Patients who could not produce stool at the hospital were advised to place about 30-50 grams of the morning fecal specimen directly into the container and deliver it to the laboratory immediately.

3.7.3 Examination of stool

Analysis of stool samples was carried by the researcher assisted by qualified laboratory technicians. The consistency of the stool was checked and noted as F (formed), L (loose), W (watery), M (with mucus) or B (with blood) (Chessbrough, 2005). Wet mount microscopy was used to analyze the samples. A drop of physiological saline was placed at the centre of the left half of a microscope slide and another drop of Dobell’s iodine in the centre of the right half of the slide. About 2 grams of stool sample was picked using an applicator stick and mixed with the Dobell’s iodine and an equal amount with normal saline until a smooth thin preparation was obtained. Each preparation was covered with a cover slip. The entire preparation was examined at a magnification of x40 under a
light microscope, systematically. The iodine preparations assist in the identification of the cysts. Normal saline preparation was used to examine for trophozoites on fresh dysenteric samples to avoid their encysting. The grading of cysts found per preparation was reported as follows: Scanty (1–3), few (4–10), moderate (11-20), many (21–40) and very many (>40) (Chessbrough, 2005).

3.8 Ethical consideration

The confidentiality of patients’ records was upheld with care and the study sought clearance Kenyatta University and the hospital administration before commencement of the study. All patients included in the study were required to sign a written consent. Patients below the age of 15 years, consent were sought from their parents or guardians before inclusion in the study.

3.9 Data analysis and presentation of results

The data collected from hospital records and stool samples was tabulated and analyzed. Association between prevalence of amoebiasis and level of education, age, occupation, place of residence, water source, monthly spending and hygienic practices of the respondents were analyzed using Chi-square statistics. Line graphs and frequency tables have been used in presentation of results. A P value < 0.05 was considered statistically significant.
CHAPER FOUR

RESULTS

4.1 Prevalence of amoebiasis during the study period

Out of 5480 stool samples analyzed for amoebiasis during the period January 2009 to December 2013 at Longisa County Hospital, 1574 tests were positive for *E. histolytica*. This translated to 28.72% stool tests which were positive for *E. histolytica* while 1.73% was positive for intestinal worms. The stool samples that tested positive for amoebiasis gave a ratio of approximately 7: 4: 1 for adult females, adult males and children respectively implying that more female adults were prone to infection (Table 4.1).

Table 4.1: Total number and ratio of positive cases of amoebiasis during the period, January 2009 to December 2013

<table>
<thead>
<tr>
<th>Year</th>
<th>Total patients</th>
<th>Total stool sample</th>
<th>Positive cases of amoebiasis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Female adults (%)</td>
</tr>
<tr>
<td>2009</td>
<td>11,807</td>
<td>1,263</td>
<td>145(11.5)</td>
</tr>
<tr>
<td>2010</td>
<td>12,360</td>
<td>1,308</td>
<td>226(17.3)</td>
</tr>
<tr>
<td>2011</td>
<td>10,094</td>
<td>1,247</td>
<td>253(20.3)</td>
</tr>
<tr>
<td>2012</td>
<td>12,390</td>
<td>638</td>
<td>112(17.6)</td>
</tr>
<tr>
<td>2013</td>
<td>14,051</td>
<td>1,024</td>
<td>131(12.9)</td>
</tr>
<tr>
<td>Total</td>
<td>60,702</td>
<td>5,480</td>
<td>867(15.8)</td>
</tr>
<tr>
<td>Average/year</td>
<td>12,140</td>
<td>1,098</td>
<td>173</td>
</tr>
</tbody>
</table>

| Ratio | 7 : 4 : 1 | prevaletence(%) | 15.76 | 10.47 | 2.37 | 1.73 |

The table shows data obtained from Longisa County Hospital. Amoebiasis prevalence for the study period was calculated by; total positive stool samples/total stool analyzed x100

Percentage proportions of positive stool samples were calculated by; average number of positive stool samples per year /average number of stool sample analyzed per year x 100

Females $= \frac{173}{1098} \times 100 = 15.76$, Males $= \frac{115}{1098} \times 100 = 10.47$, Children$= \frac{26}{1098} \times 100 = 2.37$
4.2 Amoebiasis monthly and annual trends for the period January 2009 to December 2013

The percentage proportion per month for all the five years was calculated. Overall, the month of February for the five years recorded the highest proportion of stool samples positive for amoebiasis (47.1%). The lowest proportion of positive samples was recorded in the month of August (20.1%). Out of the 1574 stool samples that tested positive for amoebiasis in the five years, the year 2010 recorded the highest positive cases of amoebiasis of 449 (28.5%) as compared to 203 (12.9%) positive cases of amoebiasis registered in the year 2012. Data was missing in the records for the months of September and October 2009 and the months of January and February 2011 (Table 4.2).
### Table 4.2: Monthly and annual trends of amoebiasis cases for the period, January 2009 to December 2013

<table>
<thead>
<tr>
<th>Month</th>
<th>Stool samples positive for amoebiasis</th>
<th>Total Stool samples positive for amoebiasis</th>
<th>Total samples analyzed</th>
<th>Percentage proportion of positive stool samples(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
<td>2010</td>
<td>2011</td>
<td>2012</td>
</tr>
<tr>
<td>Jan</td>
<td>15</td>
<td>39</td>
<td>*</td>
<td>9</td>
</tr>
<tr>
<td>Feb</td>
<td>51</td>
<td>40</td>
<td>*</td>
<td>20</td>
</tr>
<tr>
<td>March</td>
<td>69</td>
<td>50</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>April</td>
<td>26</td>
<td>63</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>May</td>
<td>30</td>
<td>50</td>
<td>29</td>
<td>13</td>
</tr>
<tr>
<td>June</td>
<td>23</td>
<td>40</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>July</td>
<td>23</td>
<td>30</td>
<td>24</td>
<td>30</td>
</tr>
<tr>
<td>Aug</td>
<td>26</td>
<td>20</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>Sep</td>
<td>*</td>
<td>22</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td>Oct</td>
<td>*</td>
<td>28</td>
<td>74</td>
<td>24</td>
</tr>
<tr>
<td>Nov</td>
<td>35</td>
<td>37</td>
<td>86</td>
<td>30</td>
</tr>
<tr>
<td>Dec</td>
<td>20</td>
<td>30</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>318</td>
<td>449</td>
<td>386</td>
<td>203</td>
</tr>
</tbody>
</table>

*Data was missing in the records.

Percentage proportions of positive stool samples were calculated by; total number of positive stool samples /total number of stool samples analyzed x 100.

For December it is \( \frac{117}{495} \times 100 = 23.6\% \).
4.2.1 Seasonal trends of amoebiasis

During the dry season, January to March, there was an increase in amoebiasis infection rate and slight decline during the long rainy season, April to August. There was also a slight increase in amoebiasis infection rate during the short rainy season, September to November (Fig 4.1).

Fig 4.1: Seasonal distribution of amoebiasis for the period 2009 to 2013
4.2.2 Annual trends of amoebiasis

The highest number (34.3%) of amoebiasis cases was recorded in the year 2010 and the least during the year 2013 (21.3%) percentage cases of amoebiasis analyzed per year. Out of 5480 stool sample analyzed during the five years, 1263 stool sample were analyzed during the year 2009 and only 638 stool samples during the year 2012 (Table 4.3).

Table 4.3 Annual distributions of amoebiasis cases during the study period

<table>
<thead>
<tr>
<th>YEAR</th>
<th>Positive case (%)</th>
<th>Negative cases (%)</th>
<th>Total stool tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>318(25.2)</td>
<td>945(74.8)</td>
<td>1263</td>
</tr>
<tr>
<td>2010</td>
<td>449(34.3)</td>
<td>859(65.6)</td>
<td>1308</td>
</tr>
<tr>
<td>2011</td>
<td>386(31)</td>
<td>861(69)</td>
<td>1247</td>
</tr>
<tr>
<td>2012</td>
<td>203(31.8)</td>
<td>435(68.2)</td>
<td>638</td>
</tr>
<tr>
<td>2013</td>
<td>218(21.3)</td>
<td>806(78.7)</td>
<td>1024</td>
</tr>
<tr>
<td>Total</td>
<td>1574(28.7)</td>
<td>3906(71.3)</td>
<td>5480</td>
</tr>
</tbody>
</table>

4.3 Incidence of amoebiasis during the study period (January to June 2014)

The incidence of amoebiasis infection was determined during the study period. Out of the 255 stool samples tested, 50 (19.6%) were positive of amoebiasis and 205 (80.4%) were negative. Among the positive individuals, it was found out that 30 out of 129 (23.3%) were adult females, 18 out of 114 (15.8%) were adult males and 2 out of 12 (16.7%) were children under the age of five years. This gave a ratio of 15:9:1 in adult females, adult males and children respectively. This is relatively similar to the findings of the retrospective study were the ratio was 7:4:1. Adult females (23.3%) recorded slightly higher infection rate compared to adult males and children under the age of five years. The same case in the retrospective study, were a slightly higher prevalence of amoebiasis
in adult females of 15.76% was recorded compared to adult males (10.47%) and children under the age of five years (2.37%). The incidence was slightly lower than the overall prevalence for the five years in the retrospective study which was 28.72%(Table 4.4).

4.4 Factors associated with amoebiasis transmission

Among the factors that were accessed in the current study, source of water for domestic use and monthly earning were found to be significant and statistically different in relation to amoebiasis infection. Personal hygiene and solid fecal disposal systems were also significant and statistically different in risk of amoebiasis infection.

4.4.1 Sources of water versus amoebiasis infection

Among the sources of drinking water, dam/ pond (38.8% n=99) was the most commonly used by the patients compared to those who obtained from wells (23.5% n=60) and river (29% n=74) among the 255 patients interviewed. Piped water was considered safe for drinking, however only 22 (8.6%) patients out of 255 patients interviewed had access to piped water.

Out of 99 patients using dams/ ponds as their source of water, 22 (22.2%) were infected with E. histolytica. Out of 74 patients who obtained water from rivers 15 (20.3%) were positive for E. histolytica. Out of 22 patients interviewed using piped water, 8 (36.4%) tested positive for E. histolytica. The source of drinking water significantly influenced the
risk of infection as those using piped water were less exposed to amoebiasis infection ($\chi^2=1.636$ d.f= 1, p= 0.201) (Table 4.4).

4.4.2 Monthly earning versus amoebiasis infection

Most of the patients interviewed earned between 1001-5000 Kshs per month (191 out of 255). Only 36 (14.12%) patients earned over 5000 Kshs per month. Out of 36 patients who earned over 5000 Kshs per month, 14 (39.9%) were infected with *E. histolytica*. Out of 191 patients who earned between 1001-5000 Kshs per month, 20 (10.5%) were infected with *E. histolytica* and out of 28 patients who earned less than 1000 Kshs per month, 16 (57.1%) were positive for amoebiasis. Those who earned between 1001-5000 Kshs were at risk of amoebiasis infection ($\chi^2=119.377$, d.f=1, p=0.000) (Table 4.4).

4.4.3 Hygiene factors versus amoebiasis infection

Irregular washing of hands before eating and after visiting the toilet was found to be a risk factors for amoebiasis (p<0.05). Out of 255 patients interviewed, 201 (78.8%) patients did not wash their hands regularly after using the toilet and 159 (65.4%) patients did not wash their hands regularly before eating. Washing hands using water in a basin was also found to be a risk factor for amoebiasis. Washing hands using soap was found to reduce the risk of amoebiasis infection ($\chi^2=3.846$, d.f=1, p=0.050).
4.4.4 Waste disposal system versus amoebiasis infection

Majority of the patients interviewed possess a solid fecal disposal system (n =249; 97.65%) in their homes only a few did not (n = 6; 2.35%). The absence of a solid fecal disposal system did not pose as risk factor for amoebiasis ($\chi^2=0.667$, d.f=1, p=0.414) (Table 4.4).

4.4.5 Age, education level, occupation, locality, water treatment and diet versus amoebiasis infection

The other factors were age, education level, occupation, locality, water treatment and diet. There was an association between these factors and amoebiasis infection(p<0.005) although they were not statistically different. Out of the 255 patients included in the study, 117(45.9%) had attained tertiary level of education and above. It was also found out that, individuals with this level of education had a slightly higher infection rate (29.1%) compared to primary and secondary levels of education. Most of the patients interviewed were farmers (60.8%). A slightly higher infection rate occurred in patients who were in formal employment(34.2%, n=76) to farmers (12.9%, n=155) and traders (16.7%, n=24) (Table 4.4).

Rural residents in the study area presented a higher infection rate (25.3%, n=178) compared to urban residents (6.5%, n=77). Most patients did not treat water (60%) for domestic use. For those who treated water for domestic use, they mainly boil or use chlorine. Patients were unaware of using solar system for purifying water for drinking which it is considered cheap and effective. Out of 255 patients included in the study,
141 (55.3%) considered a balanced diet and 114 (44.7%) did not although higher infection rate occurred in patients who considered a balanced diet (29.1%).

Table 4.4: Factors associated with amoebiasis transmission (January to June 2014, n=255).

<table>
<thead>
<tr>
<th>Factors/Categories</th>
<th>Total respondents</th>
<th>Infected (%)</th>
<th>Uninfected (%)</th>
<th>Chi-square</th>
<th>d.f</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children(0-5yrs)</td>
<td>12</td>
<td>2 (16.7)</td>
<td>10 (83.3)</td>
<td>5.33</td>
<td>1</td>
<td>0.021</td>
</tr>
<tr>
<td>Adult females( 6yrs and above)</td>
<td>129</td>
<td>30 (23.3)</td>
<td>99 (76.7)</td>
<td>36.91</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Adult males( 6yrs and above)</td>
<td>114</td>
<td>18 (15.8)</td>
<td>96 (84.2)</td>
<td>53.37</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Education level:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary level and below</td>
<td>72</td>
<td>4 (5.6)</td>
<td>68 (94.4)</td>
<td>56.889</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Secondary level</td>
<td>66</td>
<td>12 (18.2)</td>
<td>54 (81.8)</td>
<td>26.727</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Tertiary level and above</td>
<td>117</td>
<td>34 (29.1)</td>
<td>83 (70.9)</td>
<td>20.521</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Occupation:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>155</td>
<td>20 (12.9)</td>
<td>135 (87.1)</td>
<td>85.323</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Formal employment</td>
<td>76</td>
<td>26 (34.2)</td>
<td>50 (65.8)</td>
<td>7.579</td>
<td>1</td>
<td>0.006</td>
</tr>
<tr>
<td>Trader</td>
<td>24</td>
<td>4 (16.7)</td>
<td>20 (83.3)</td>
<td>10.667</td>
<td>1</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Locality:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>178</td>
<td>45 (25.3)</td>
<td>133 (74.7)</td>
<td>43.506</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Urban</td>
<td>77</td>
<td>5 (6.5)</td>
<td>72 (93.5)</td>
<td>59.282</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Monthly earning (Kshs):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over 5000</td>
<td>36</td>
<td>14 (39.9)</td>
<td>22 (61.1)</td>
<td>1.778</td>
<td>1</td>
<td>0.182</td>
</tr>
<tr>
<td>1001-5000</td>
<td>191</td>
<td>20 (10.5)</td>
<td>171 (89.5)</td>
<td>119.377</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Less than 1000</td>
<td>28</td>
<td>16 (57.1)</td>
<td>12 (42.9)</td>
<td>0.571</td>
<td>1</td>
<td>0.450</td>
</tr>
<tr>
<td><strong>Source of water:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>74</td>
<td>15 (20.3)</td>
<td>59 (79.7)</td>
<td>26.162</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Well</td>
<td>60</td>
<td>5 (8.3)</td>
<td>55 (91.7)</td>
<td>41.667</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Pond/dam</td>
<td>99</td>
<td>22 (22.2)</td>
<td>77 (77.8)</td>
<td>30.566</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Piped</td>
<td>22</td>
<td>83 (6.4)</td>
<td>14 (63.6)</td>
<td>1.636</td>
<td>1</td>
<td>0.201</td>
</tr>
<tr>
<td><strong>Water treatment:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>102</td>
<td>23 (22.5)</td>
<td>79 (77.5)</td>
<td>30.745</td>
<td>1</td>
<td>0.000</td>
</tr>
<tr>
<td>Untreated</td>
<td>153</td>
<td>27 (17.6)</td>
<td>126 (82.4)</td>
<td>64.059</td>
<td>1</td>
<td>0.000</td>
</tr>
</tbody>
</table>
**Waste disposal system:**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pit latrine</td>
<td>225</td>
<td>42(18.7)</td>
<td>88.360</td>
<td>0.000</td>
</tr>
<tr>
<td>Flash toilet</td>
<td>24</td>
<td>4(16.7)</td>
<td>10.667</td>
<td>0.010</td>
</tr>
<tr>
<td>Absent</td>
<td>6</td>
<td>4(66.7)</td>
<td>0.667</td>
<td>0.414</td>
</tr>
</tbody>
</table>

**Hygiene:**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregularly wash hands after visiting the toilet</td>
<td>201</td>
<td>24(11.9)</td>
<td>116.463</td>
<td>0.000</td>
</tr>
<tr>
<td>Irregularly wash hands before eating</td>
<td>159</td>
<td>36(16.4)</td>
<td>52.082</td>
<td>0.000</td>
</tr>
<tr>
<td>Wash hands using water in a basin</td>
<td>225</td>
<td>22(9.8)</td>
<td>145.604</td>
<td>0.000</td>
</tr>
<tr>
<td>Always wash hands using soap</td>
<td>26</td>
<td>8(30.8)</td>
<td>3.846</td>
<td>0.050</td>
</tr>
</tbody>
</table>

**Considered a balance diet:**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percentage</th>
<th>Chi-square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>141</td>
<td>41(29.1)</td>
<td>24.688</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>114</td>
<td>9(7.9)</td>
<td>80.842</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Chi-square was calculated by comparing the number of infected persons versus the uninfected persons using SPSS version 16.
CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of amoebiasis at Longisa County Hospital

The current study recorded relatively high prevalence of amoebiasis at 28.7% for the period, January 2009 to December 2013. The high prevalence rate at hospital could be attributed to the fact that the Longisa Hospital uses microscopic examination which does not differentiate \textit{E. histolytica} from \textit{E. dispar} which is nonpathogenic. It can also be attributed to poverty, water sources that may be contaminated and inadequate water supply, type of houses, ignorance and poor personal hygiene. In some African countries 6% to 75% of the population had the parasite (Stark \textit{et al.}, 2006). For example, in Kenya, a prevalence rate of 21% has been reported in Nakuru County based on data from Njoro PCEA health center (Kinuthia \textit{et al.}, 2012). In Nigeria a prevalence rate of 27% was reported in school age children (Reuben \textit{et al.}, 2013) and in Egypt a prevalence rate of 38% was reported by Park \textit{et al} (2006). Majority of \textit{E. histolytica} infections occur in Africa, Central and South America and the Indian sub-continent where the prevalence of \textit{Entamoeba} is as high as 50% (Haque \textit{et al.}, 2006).

In the cross sectional study, 50 (19.6%) patients out of 255 patients with diarrhea or presumptive amoebiasis were positive for \textit{Entamoeba}, which compared to the retrospective study, the incidence rate was slightly lower. From 2009 to 2014 there was decline in infection generally, this can be attributed to campaigns by the government and
NGOs aimed at reducing diarrhea and other diarrhea related infections such as amoebiasis. One of such campaigns targets washing of hands at four critical times; that is before eating, after using a toilet, before preparing food and after changing the baby’s nappies.

The study found that, adult females, adult males and children under the age of five years were highly at risk of infected with *E. histolytica* (Table 4.4). Considering the percentage rate of infection, children under the age of five years were the least infected group. Amoebiasis has been reported to be rare in children below the age of five years (Heymann, 2008; CDC, 2010). In Nigeria, the highest prevalence was recorded among in children aged 6-10 years whereas the least was among those aged below five years (Reuben et al., 2013). A study that was done in Bangladesh, on *Entamoeba histolytica* infection in pre-school children and protection from subsequent amoebiasis confirmed the low prevalence of amoebiasis in children under the age of five years. This was attributed to their innate resistance due to the induced production of secretory immunoglobulin A that can diminish the adhesion between *E. histolytica* trophozoites to intestinalepithelial cells, hence reducing new infection (CDC, 2010). Children under this category were still under the care of the mothers/caregivers and are less active hence may reduce infection.

From the hospital records, females recorded a slightly higher prevalence of 15.12% compared to males, 10.20%. A similar trend was observed in the overall cross sectional study results, where there was a slightly higher rate of amoebiasis infection in females (11.76%) compared to males (7.06%). This can be attributed to the fact that in the region,
more women do domestic chores such as cooking and washing which mostly may involve contaminated water. Women also participate in tilling the land more than their male counterparts and the soil may be contaminated with the cysts of the parasite. A study done in Gambia, Western Africa revealed that, 45% of females carry the parasite versus only 25% of the male population (Bray and Harris, 1997).

5.1.2 Factors associated with amoebiasis transmission

The transmission risk factors associated with amoebiasis were found to be source of water for drinking which is prone to contamination, poor personal hygiene, type of housing and monthly earning. These factors played a significant role in acquiring the parasite. Other factors were age, level of education, occupation, place of residence and diet were also significant but not statistically different.

5.1.2.1 Age as risk factor for amoebiasis

In the current study, children under the age of five years, adult females and adult males were all at risk of *E. histolytica* infection (p<0.05). All people are believed to be susceptible to infection, but individuals with a damaged or undeveloped immunity may suffer more severe forms of the disease. The homosexuals and the citizens of industrialized countries are mainly at risk of this disease. According to the findings of Zahida *et al* (2010) people of all ages in developing countries were at risk of amoebiasis infection although prevalence among the ages varies greatly. Although cosmopolitan in
distribution, it mainly occurs in the tropics and sub tropics and other places especially in areas where there is low level of sanitation and very poor personal hygiene practices (Ibrahim, 2008). The existence of the parasite in all the age groups observed in this study could be attributed to such predisposing factors which are prevailing in the study area such as ignorance, absence of clean portable drinking water, type of housing, unhygienic methods of disposing human faeces and refuse, poverty, inadequate health care facilities as well as low standard of personal hygiene and general cleanliness.

5.1.2.2 Level of education as a risk factor for amoebiasis

Patients in all educations levels, from primary level and below, secondary level to tertiary level and above were all predisposed to amoebiasis infection (p<0.05). Higher prevalence was recorded in patients with tertiary education and above (Table 4.4). This could be attributed to the fact that most of them could be in formal employment and working away from their homes and therefore more likely to eat away from home and especially from canteens, kiosks and food joints near their work places. This concurs with a study that was conducted in Nigeria, which reported that those who eat away from home were more likely to be infected with the parasite than those who ate at their homes (Oyerinde et al., 2006).

5.1.2.3 Occupation as a risk factor for amoebiasis

Farmers, traders and patients who were in formal employment were all prone to infection (p< 0.05). In this study, more patients in formal employment were infected
with amoebiasis compared to farmers and traders (Table 4.4). The reason why those in formal employments were more likely to acquire the parasite could be that, they often eat away from home and that they may not get time to prepare their own food. To assess the prevalence of parasites transmitted from food vendors in Abeokuta, Nigeria, 100 stool samples from randomly selected food vendors who sell foods in schools and streets were examined for ova and cysts of parasites. Of the 100 examined, 72 stool samples were positive for *E. histolytica/dispar* therefore, food vendors could be the main sources of transmission of parasitic infection to consumers of their wares (Idowu and Rowland, 2006). From these results, it shows that none of the food vendors met the WHO requirement for effective hand wash which include washing of hands in hot soapy water before preparing food and after using the bathroom, after changing babies’ diapers and handling pets (WHO, 2001).

5.1.2.4 Place of residence as a risk factor for amoebiasis

The findings of this study indicated that rural residents and urban residents were both likely to be infected with amoebiasis (p<0.05). The percentage infection rate was found to be 25.3% and 6.5% in rural and urban patients respectively. The only likely explanation for this finding is that, urban residents have better social and financial conditions that may allow them to seek treatment in other health facilities including private facilities other than at Longisa County Hospital. For rural residents, Bomet County being an agricultural area, they engaged in farming hence their hands being in contact with soil which may be contaminated promoting transmission of the parasite
through oral route. This is similar to the findings of a study that was conducted to access the risk factors for *E. histolytica/E. dispar* complex in Amazon, Brazil, targeting population attending outpatient clinics, showed that place of residence had a significant influence on amoebiasis infection (Benetton *et al.*, 2005).

### 5.1.2.5 Source of water as a risk factor for amoebiasis

The source of water for domestic use was found to be a significant factor in transmission of amoebiasis. Patients who obtained water from open earth dams/ponds, rivers and well were at a greater risk of amoebiasis infection compared to those using piped water (Table 4.4). It is a common practice in the region that locals use river and open earth dams/ponds water for bathing, swimming and washing clothes. The river water also serves the domesticated and wild animals as their drinking points. The animals may defecate on water hence contaminating it. Humans may also defecate on bushes at the river banks thus being washed into water bodies. A number of studies have found that piped water in the home is safer than other sources (Ryan and Ray, 2004). A study conducted in Vietnam found that river water was an important source of *E. histolytica* infection (Blessman *et al.*, 2002).

Treating or boiling water for drinking is an effective way of controlling amoebiasis if done hygienically (Espinosa-Cantellano and Espinosa-Cantellano, 2000). In the current study, patients who treated and those who did not treat water for drinking were both at risk for amoebiasis infection (Table 4.4). Inefficient water treatment using chlorine or no water treatment at all exposes individuals to the risk of intestinal protozoan infection
(Haque et al., 2006). This implies that majority of patients interviewed in the current study could have inefficiently treated water using chlorine. For those who boiled drinking water, improper storing of the boiled water such as using uncovered containers and washing utensils using contaminated water could have exposed them to amoebiasis infection and hence the probability of infection even after treating water for drinking.

5.1.2.6 Hygiene factors
A study conducted at Nakuru County, Kenya, on selected practices among rural residents versus the prevalence of amoebiasis and giardiasis indentified poor personal hygiene as a significant risk factor for amoebiasis (Kinuthia et al., 2012). The current study established that irregular washing of hands before eating and after using the toilet predisposes patients to amoebiasis infection (Table 4.4). Washing of hands using water in a basin predisposed patients to amoebiasis infection. Ignorance (Espinosa-Cantellano and Espinosa-Cantellano, 2000) and toilet habits (Oyerinde et al., 2006) have been found as serious risk factors for amoebiasis infection. Washing hands with soap seem to reduce amoebiasis infection in the current study (Table 4.4). Washing hands with soap in warm running water for at least 15 seconds after utilizing the toilet, changing babies’ nappy and before preparing food has been established to reduce cases amoebiasis (WHO, 2001). Dirty latrines with flies located near the houses, inadequate and inappropriate use of toiletries in the latrines may increase the chance of fecal contamination (Kinuthia et al., 2012).
5.1.2.7 Diet

It was observed that, out of 255 patients interviewed, 141 (55.3%) considered a balanced diet while 114 (44.3%) did not consider a balanced diet. The two groups were both at risk of amoebiasis infection (Table 4.4). More patients who considered a balanced diet (29.1%) were infected with amoebiasis. Although considering a balanced diet enhances their immune system, proper food handling and manipulation (WHO, 2010) is important in reducing chances of infection. Ingestion of raw vegetables and raw fruits has also been identified as a risk factor for amoebiasis (Benetton et al., 2005). The type of water used to cook the food and wash utensils could have also promoted transmission of the parasite.

5.1.3 Trend of Amoebiasis

5.1.3.1 Monthly trends of amoebiasis

The results showed that, during the dry season, January to March, of almost all the years studied, an increase in amoebiasis infection rate was recorded followed by a slight decline during the long rainy season (April to August). During the dry season, water level in the dams and rivers become low and more prone to contamination. A research that was conducted to determine the prevalence of *E. histolytica/dispar* and *Giardia lambia* in primary school pupils in five villages in Kaduna and Zaria, Nigeria, revealed a higher prevalence in the dry season (January to May, with peak in April) months than the rainy seasons (Inabo et al., 2000). The Eastern part of Bomet County, Chepalungu Constituency and part of Bomet East constituency depend on dams as their main water
source for domestic use. This may explain why infection was relatively high during the months of January, February, and March.

It is worth noting that, the disease was at its peak during the month of February cumulatively for the five years of study (Table 2.2). Out of 297 stool samples collected during the month of February in the five years, 140 samples tested positive for amoebiasis translating to 47%. The region experiences high temperatures and shortage of water during this month. This is in agreement with a study that was done in Mexico, which reported that; infection rate with amoebiasis is high when there is inadequate water supply (Martha and Adolfo, 2000). Accessing clean water during this period becomes difficult leaving the residents with no choice but to use any water available that may be contaminated. Also inadequate water supply causes humans and animals to share the same water sources hence contamination by reservoir animals such as dogs and monkeys (Muruiki et al., 2002). It is during this dry period that the region also experiences outbreak of waterborne diseases such as cholera (WHO, 2011).

5.1.3.2 Yearly trends of amoebiasis

Generally, there was gradual decline in amoebiasis cases in the five years of study. The year 2013 recorded a relatively low prevalence (21.3%) of amoebiasis as compared to the other five years of the study (Figure 5.5). This may be due to the campaigns that have been done by the government and NGOs particularly through the media, targeting control of feecal-oral transmission of the parasite (WHO, 2011). This suggests a gradual decrease in prevalence of amoebiasis in the recent years.
Efforts to reduce widespread cases of amoebiasis and related diseases such diarrhea, acute respiratory infections and malaria has been done though the following activities with the aim of achieving MDGs i) Provision of emergency services particularly in rural areas through purchase of ambulances ii) Improving literacy levels among mothers as they attend antenatal and post-natal clinics. Infant feeding and weaning practices are taught during this clinics iii) Provision of sustainable clean water sources and sanitation facilities iv) Building of health centers in many parts of the County.

There still exist many challenges in Bomet County in attaining the above millennium goals such poor roads, mal-distribution of health care providers in the health centers, most of the health centers and hospitals are ill-equipped therefore unable to deal with emerging issues. Still, majority of the residents do not have access to clean water for domestic use.

5.2 Conclusions
i. The current study found that 28.7% of the patients attending Longisa County Hospital were infected with *Entamoeba histolytica* during the period, January 2009 to December 2013. The ratio for females to males to children was 7:4:1.

ii. The incidence rate was 19.6% during the period January 2014 to June 2014. The ratio for females to males to children was 15:9:1.
iii. The use of river water, open earth dams/ponds, wells, semi-permanent and grass-thatched houses, poor personal hygiene was associated with higher levels of *E. histolytica* infection.

5.3 Recommendations

i. It is recommended that, control of amoebiasis be implemented in Bomet County. This is due to the high prevalence of the disease recorded. This can be done by the health care services or the Ministry of Health.

ii. There is need for continuous stool examination for patients with diarrhea at the hospital and treatment given as the incidence rate was high to control the disease.

iii. The government should provide safe, clean water for domestic use to the residents to could reduce the use of unsafe water such as dams/ponds, wells and rivers.

iv. The use of safe water such as piped water should be promoted in the region which can be made popular through health education programs at hospital level when patients visit the hospital, open ‘Barazas’, through the media. Purifying water for drinking using solar is non-costly and effective; this can be made popular in the Bomet County.

v. Personal hygiene should to be promoted in the region through campaigns by the government and NGOs.
5.3.1 Suggestions for future research

i. There is need to establish the real prevalence of *Entamoeba histolytica* relative to *Entamoeba dispar* in Bomet County using molecular studies.
REFERENCES


CDC. (2010). Infectious diseases; epidemiology and surveillance. Amoebiasis facts Atlanta. USA.


APPENDICES

Appendix I: Checklist for the hospital record data

The checklist will guide in the collection of data from parasitological laboratory records

Year 2012 [  ]

Year 2011 [  ]

Year 2010 [  ]

Year 2009 [  ]

Year 2008 [  ]

OPD No………………

Residence……………

Age………………

Sex………………

Level of education……………………

Employment/occupation………………

Investigation……………………

Result 1……………………………

Results 2……………………………
Appendix II: Consent form

You are requested to participate in a research study conducted by Too Chebet, from School of Pure and Applied Science, Kenyatta University.

PURPOSE OF THE STUDY

The purpose of the study is to determine the prevalence of Amoebiasis among patients attending Longisa County hospital. The research is very important and your sincerity in answering the questions is highly encouraged.

Your participation in the study is voluntary, and that your non-participation would not compromise your quality of treatment in this facility or any other government health facility. In addition, confidentiality will be maintained and there will be no time when you will be required to identify yourself by name. Lastly, if you agree to participate in the study, you will be required to sign the consent form.

The research does not pose any risks whether physical or psychological that would warrant the termination of the study. Participating in the research study will not yield any direct benefits; however, the findings of the research will be shared with the Ministry of Health to help in policy making and decision making in the health facilities attended in order to correct and prevent infections in the future.

Signature of the patient ……………………………………Date……………………………………

Signature of researcher ………………………………………Date……………………………………
Appendix III: Questionnaire

I humbly request you to answer this short questionnaire which is purely designed for academic purposes. Mark (x) where applicable. Thank you in advance for your participation.

ID codes……………………

Division…………………….

1. Age

2. Sex

3. Education level

Primary [ ] Secondary [ ] Tertiary [ ] University [ ]

4. Occupation

Farmer [ ] Formal Employment [ ] Trader [ ]

5. Which of the following symptoms did you experience that made you to come to the hospital?

Loose stool [ ] Blood in stools [ ]

Abdominal cramps [ ] Nausea [ ]

Vomiting [ ] Fever [ ]

Chills [ ] Headache [ ]

Backache[ ] Muscle aches [ ]

Fatigue [ ] Other……………………..
6. Do you have existing medical problem(s) or any medical condition(s)?
   Yes [ ] No [ ]
   Describe………………………………………………………………………

7. a. Where do you get water from?
   River/stream [ ] Well [ ] Pond/dam [ ] Piped water [ ]

b. Do you treat water?
   Yes [ ] No [ ]

c. If yes, which method do you use to treat drinking water?
   Boiling [ ] Putting chemicals [ ] using solar [ ]

8. Does your household own a toilet?
   Yes [ ] No [ ]

9. If yes, what kind of toilet?
   Pit latrine [ ] flash toilet [ ]

10. a. Do you always wash hands after visiting the toilet?
    Always [ ] Sometimes [ ] Never [ ]

b. Do you wash hands before eating?
    Always [ ] Sometimes [ ] Never [ ]

c. If yes which type of water?
    Running water [ ] water in a basin [ ]

d. Do you wash hands with soap always?
    Yes [ ] No [ ]

11. What type of house do you live in?
    Permanent [ ] semi-permanent [ ] grass thatched [ ]
12. Do you eat a balanced diet in your meals?

Yes [ ] No [ ]

13. How much do you spent per month, in terms of Kenya shillings?

Less than 1000 [ ] 1001-2000 [ ] 2001-3000 [ ] 3001-4000 [ ] 4001-5000 [ ] 5001 and above [ ]
Appendix V: Authorization of collected data at Longisa County Hospital

REPUBLIC OF KENYA

COUNTY GOVERNMENT OF BOMET
MINISTRY OF HEALTH AND SANITATION

Telephone: 058-512211/5122041
Telegraph: MED311K Longisa

Email add: longishospitals@yahoo.com

Ref No. LDH/MOH/ADMIN

28\(^{th}\) December 2013

RE: AUTHORITY TO CARRY OUT RESEARCH AT LONGISA COUNTY REFERRAL HOSPITAL -

TOO CHEBET REG. NO. 156/CE/2242/10.

This is to confirm that the above named student is permitted to carry out Research work in our Laboratory, Longisa Hospital with effect from January to July 2014.

Thanks in advance.

Yours faithfully,

Fredrick K. Towett
Health Administrative Officer
LONGISA COUNTY HOSPITAL
Appendix Vi: Approval of research proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
       dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: I56/CE/22424/10

Date: 20th August, 2014

The Principal Secretary,
Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION
MS. TOO CHEBET - REG. NO. I56/CE/22424/10

I write to introduce Ms. Too Chebet who is a Postgraduate Student of this
University. She is registered for a M.Sc. degree programme in the Department
of Zoological Sciences in the School of Pure & Applied Sciences.

Ms. Too intends to conduct research for a Thesis entitled, "Prevalence of
Amebiasis and its Transmission Risk Factors in Patients Attending Longisa
District Hospital, Bomet County, Kenya."

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL