PREVALENCE OF *ENTAMOEBA HISTOLYTICA* INFECTIONS AMONG THE CHILDREN ATTENDING PRIMARY SCHOOLS IN KYUSO ZONE, KYUSO DISTRICT, KITUI COUNTY, KENYA

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REG NO 156/CE/13924/2009

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science (Applied Parasitology) in the School of Pure and Applied Sciences of Kenyatta University

AUGUST 2014
DECLARATION

I declare that this thesis is my original work and has not been presented for a degree or other awards in any other university.

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DEDICATION

I dedicate this thesis to my beloved wife Peninnah for her warm encouragement and financial assistance during the entire period of my study. “May the Almighty God reward you abundantly”.
ACKNOWLEDGMENTS

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“May God bless you all.”
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LIST OF ABBREVIATIONS

AIDS    Acquired Immunodeficiency Syndrome
ANOVA  Analysis of Variance
AMREF  Africa Medical Research Foundation
DPHO   District Public Health Officer
HIV    Human Immunodeficiency Virus
KDH    Kyuso District Hospital
NGOs   Non-Governmental Organizations
NCAPD  National Co-ordinating Agency for Population and Development
SIDA   Swedish International Development Assistance
UNICEF United Nations Children Fund
WHO    World Health Organization
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<td><strong>Amoebiasis</strong></td>
<td>A disease caused by protozoan <em>Entamoeba histolytica</em> characterized by ulceration of the large intestine.</td>
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<td><strong>Asymptomatic</strong></td>
<td>Without obvious signs and symptoms of a disease.</td>
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<td><strong>Diarrhoea</strong></td>
<td>An abnormally increased frequency of passing out liquid faecal discharge.</td>
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<td><strong>Dysentery</strong></td>
<td>An inflammatory disorder of the intestine especially of the colon that results in severe diarrhea containing mucus and/or blood in stool.</td>
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<td><strong>Prevalence</strong></td>
<td>This is the percentage of infected people in a population at a particular time.</td>
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<td><strong>Risk factors</strong></td>
<td>Factors that are positively associated with the risk of development of a disease but are not significant to cause a disease.</td>
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<td><strong>Sanitary</strong></td>
<td>Free from elements such as filth or pathogens that endanger health.</td>
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<td><strong>Zone</strong></td>
<td>An educational administrative unit made up of several schools in a district. A zone is equivalent to a location.</td>
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ABSTRACT

The occurrence of Entamoeba histolytica as a human intestinal parasite is a serious problem especially in developing countries. Entamoeba histolytica frequently infest as a commensal within the human large intestines with no overt clinical manifestations. However, trophozoites can invade the colonic epithelium and produce ulcers and dysentery, a condition known as Amoebiasis. Amoebic infections in Kyuso Zone, Kyuso District are common. The ecological aspects in the area and particularly water-related ones allow endemicity. There have been efforts to provide treatment and control of amoebic infections but the prevalence of the disease in this area has never been established. This study was aimed at determining the prevalence of E. histolytica in school children aged 6-12 years in Kyuso Zone and establish the relationship between the prevalence and ecological factors. A cross-sectional study design was used where 354 randomly selected pupils aged 6-12 years provided stool specimen which were taken to Kyuso District Hospital Laboratory for examination. They were examined microscopically for cysts/trophozoites of E. histolytica using direct wet smear and formal ether concentration technique. Questionnaires, focus group discussions and observational checklists were also used to determine risk factors. The overall prevalence of E. histolytica infections amongst the pupils was 42.1%. Chi-square (χ²) test was done to determine the relationship between prevalence and pupils’ age and gender. There was no significant association between prevalence and gender (χ²=0.9653, df=1, P>0.05) and no significant association between prevalence and age (χ²=3.5649, df=2, P>0.05). Logistic regression analysis was used to assess the significance of association between water sources and prevalence. A significant association was found between the use of dry riverbed wells and high prevalence (OR= 2.351, P=0.02) and the use of earth dam water and high prevalence (OR=2.828, P=0.0000). A significant association was found between high prevalence and use of latrines (χ²=4.7199, df=1, P=0.0298). Medical records in Kyuso District Hospital showed that 44.2% of the patients who were tested for gastro intestinal infections between April and September 2011 were found to be infected with E. histolytica. Of these patients, children aged between 6 and 12 years showed a morbidity rate of 46.9%. In this study there is clear indication that E. histolytica infections are a problem in schools in Kyuso Zone. It is therefore recommended to the ministry of education and the ministry of Public health that public health education programmes should be formulated and carried out in schools and within the community so as to inform people of good sanitation and hence reduce infections and morbidity. It is further suggested that school children be considered for chemotherapy treatment to reduce morbidity.
CHAPTER 1: INTRODUCTION

1.1 Background information

*Entamoeba histolytica* is an intestinal protozoa of humans. Several species of the genus *Entamoeba* infect humans. These include: *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba hartmani*, *Entamoeba polecki*, and *Entamoeba gingivalis* (Ash and orihel, 1980). Among these, only *E. histolytica* is considered pathogenic and the disease it causes is called amoebiasis or amoebic dysentery (Sateriale et al., 2011). *Entamoeba histolytica* is an enteric parasite that colonizes the human intestinal lumen and has the capacity to invade the epithelium. Amoebic dysentery occurs when *E. histolytica* trophozoites invade the walls of large intestines and multiply in the mucosa, forming ulcers. Most frequent manifestations of infection are dysentery, colitis, flatulent stomach, weight loss, fatigue and abdominal pain. A common outcome of invasion of the amoeba into tissues is liver abscesses which can be fatal. The pathogen secretes histolysin (Luaces, 1988), which digest the gut of the infected individual hence the Latin name, histo (tissue) lytica (destruction) (Stanley, 2003).

Ingestion of contaminated food or water containing infectious cysts leads to excystation in the intestine. Each cyst produces eight motile trophozoites which colonize the host’s colon. In those cases where the infection is not self limiting, amoebic dysentery and liver abscesses formation can occur. Ninety percent of infections with *E. histolytica* are asymptomatic and self limiting. Sateriale et al. (2011) reported that an estimated 50 million cases of invasive infection occur
annually. According to WHO (2010), amoebiasis is ranked the third most important parasitic disease and that 100,000 deaths occur annually due to the disease. The morbidity and mortality is primarily seen in the developing countries. The major cause of transmission is poor sanitation, particularly where food and water are concerned. According to UNICEF, more than 3 billion people, which translate to half of the global population, do not have access to proper sanitation (UNICEF 2009). About 3 million children die each year from diseases associated with poor sanitation worldwide. In Africa and particularly tropical Africa, over half of the population has no access to safe drinking water and are constantly faced with food insecurity and shortage, which contribute to infection with *E. histolytica* and other enteric parasites (Brooks, 2009). This, compounded with constant displacement due to civil strife and frequent floods has according to AMREF (2009), adversely contributed to high prevalence of diarrhoeal diseases.

The bulk of the population in Kenya like other developing countries lives in rural areas or in slum dwellings in towns. Sanitary facilities provision is only about 43% with 57% going without safe water and proper excreta disposal (AMREF, 2009). The semi-arid areas of Kenya to which Kyuso District belongs, have chronic shortage of domestic water. This study was aimed at establishing the extent to which *E. histolytica* infection occur among primary school children in Kyuso Zone of Kyuso District. Collection of epidemiological data is necessary to develop effective strategies against this parasite. The results should facilitate evaluation of the endemic level of amoebiasis, and consequently whether massive or focal measures of control are required.
1.2 Problem statement

The relationship between epidemiological factors and prevalence of *E. histolytica* particularly in children has not been fully investigated in Kyuso zone and organized control programs targeting transmission factors are lacking. Going by the hospital morbidity records, Kyuso Zone is an endemic area for amoebiasis; however, the prevalence of the disease in the Zone has not been established. According to Kyuso District Hospital records, the most heavily infected segment of the population is aged between 6 and 12 years (KDH, 2011). Transmission depends on sanitation related issues and the number of the asymptomatic carriers. Kyuso District has poor water and sewerage systems. Provision of safe domestic water is a big challenge in the community. A large part of the population relies on surface or sub-surface water sources exemplified by dry riverbed wells, shallow water wells, rock catchments and Earth dams. Most of these sources are highly contaminated (NCADP, 2005). In Kyuso Zone, drug administration on symptomatic victims who attend hospital is the major control strategy. This often leaves a large segment of other infected but asymptomatic cases, which easily leads to re-infection. It is likely that these factors contribute significantly towards the endemicity; this calls for an evaluation to establish the impact of epidemiological factors and current control measures on prevalence of amoebiasis.
1.3 Justification

New efforts are being made to improve understanding of the epidemiology of sanitation related intestinal infections and intensify control efforts against these parasites. To contribute to a better comprehension of the epidemiology of the intestinal protozoa, the prevalence and distribution of Entamoeba histolytica and its association with drinking water supplies was determined in primary school children in Kyuso Zone, Kyuso district of Kitui county. The investigation carried out to determine prevalence of these infections revealed the importance of this parasite in the arid and semi-arid areas to which Kyuso Zone belongs. Determination of the prevalence of this parasite will help to focus on the delivery of treatment and control measures in this area. In addition, the description of the relationship between sources of drinking water with infections with E. histolytica will contribute to the development of an integrated treatment program for this parasite in this area of Kenya.

In 2004, through SIDA funding, the DPHO carried out a survey on water and sanitation and established that the larger Kyuso District had poor water and sewerage systems, with only 15% latrine coverage. Most of the water sources are contaminated due to sharing the water points with domestic animals and also due to the fact that latrine coverage is low at 15% (NCAPD, 2005). These facts about Kyuso Zone justified the need for developing data on the prevalence of E. histolytica in the Zone. The data can be utilized to assist in evidence based decision making, and providing technical decisions for formulating integrated
strategies for prevention and control of amoebiasis and other diarrhoeal infections with a clear vision towards fulfillment of the millennium development goals (MDGs) and development of neglected diseases agenda.

1.4 Research questions

i) What is the prevalence of *E. histolytica* in school children aged 6 to 12 years in Kyuso Zone?

ii) What is the general morbidity rate due to *E. histolytica* in children aged 6-12 years amongst the patients attending Kyuso District Hospital as per the hospital records?

iii) What factors contribute to transmission of *E. histolytica* among school children in Kyuso Zone, Kyuso District?

iv) What are the control measures in place for amoebiasis in Kyuso Zone?

1.5 Null Hypotheses

i) There are no infections with *E. histolytica* in primary school children aged 6-12 years in Kyuso Zone in Kyuso District of Kenya.

ii) Environmental sanitation and poor access to safe water do not contribute to transmission of *E. histolytica* in Kyuso Zone.

1.6 General objective

To determine the prevalence of *Entamoeba histolytica* in school children aged 6-12 years in primary schools in Kyuso Zone, Kyuso District, Kitui County, Kenya.
1.6.1 Specific objectives

i) To determine the prevalence of *E. histolytica* in school going children of ages 6-12 in Kyuso Zone.

ii) To determine the general morbidity rate due to *E. histolytica* in children aged 6-12 years amongst the patients attending Kyuso District Hospital as per the hospital records.

iii) To determine the factors contributing to transmission of *E. histolytica* among school children in Kyuso Zone.

iv) To determine the control measures in place for amoebiasis in Kyuso Zone.
CHAPTER 2: LITERATURE REVIEW

2.1 Aetiology of *Entamoeba histolytica*

Amoebiasis is a gastrointestinal illness that is contracted by the eating of foods or drinking of water that has inadvertently been contaminated with the faecal amoeba known as *Entamoeba histolytica* (Botelho *et al.*, 2011). The introduction of the parasite does not necessarily develop into symptoms. A bigger percentage of infected persons are asymptomatic and they can be in this state for as long as a year but once symptoms appear, the illness can be life-threatening because it may present diarrhoea which may lead to acute dehydration (Botelho *et al.*, 2011). There are several ways by which one can contract amoebiasis; these include eating food that has been prepared by food handlers whose hands are contaminated or contamination of food with faeces either directly or indirectly.

In Malaysia, food and water-borne diseases which are closely associated with environmental and personal hygiene practices are still among the major health problems (Tengku *et al.*, 2011). The prevalence of *E. histolytica*/*E. dispar*/*E. moshkovskii* in Malaysia ranges from 1% to 61% (Tengku *et al.*, 2011). In Riyadh, Saudi Arabia, a high prevalence rate of infection amongst specific populations including food handlers (14%), school children (14.2%), expatriates (55.7%), the Abha community (13.2%) and patients attending hospitals (31.3%) was found in a study to determine the factors associated with high prevalence of *Entamaeba histolytica/dispar* infection among children (Jamila, 2014). In a study in rural southern India, the overall period prevalence of intestinal parasites was
97.4%/month (Jamila, 2014). A recent study carried out on 303 restaurant workers in 58 restaurants in Sana'a City in Yemen reported a high prevalence of *E. histolytica/dispar* (48.9%) (Al-Shibani, 2009). The infection rate of *E. histolytica/dispar* was also reported at 52%, in a similar study conducted on 132 restaurant workers in Almukala City in Yemen (Al-Haddad, 2010).

Other sources of contracting amoebiasis include drinking of contaminated water, poor sanitation in living quarters, lack of or improper use of toilets, contact with flies and items contaminated by flies, among other hygiene related issues. A study conducted to determine the prevalence and risk factors for protozoan and nematode infections among children in an Ecuadorian highland community found the prevalence of *E. histolytica* infections at 46.6% and attributed this to failure to treat drinking water and failure to wash hands before eating (Rinnes et al., 2005). A water source that may have the amoeba includes ponds, shallow wells on dry river beds, streams and earth dams. When water from these sources is consumed without proper treatment it may lead to infection (Brelet, 2000). In a study on prevalence of intestinal parasites and associated risk factors in Teda Health care North West Ethiopia, a prevalence of 4.6% for *E. histolytica* was found and was associated with absence of toilets and hand washing after toilet. (Abate et al., 2013). Contact with individuals who have amoebiasis by shaking hands can also lead to infection when one does not wash hand regularly (Brooks, 2009). Another study carried out in Malaysia to determine the prevalence and risk factors associated with *Entamoeba histolytica/dispar/moshkovskii* infection among the
Orang Asli ethnic Groups (Anuar et al., 2012), observed a prevalence of 29.5% among the Negrito ethnic group and associated this with lack of washing hands before eating.

### 2.2 Morphological and biological features of the parasite

*Entamoeba histolytica* is classified under the Sarcodina subphylum. It shows an amoeboid form and possesses pseudopodia for movement and food procurement. *Entamoeba histolytica* has cyst and trophozoite stages in its life cycle. Cysts are the infective form. They are spherical, with approximately 10 to 16 μm of diameter and present one to four nuclei (plate 1a). When mature, they have four nuclei. There is an energetic reserve (glycogen) in a distinct vacuole inside the immature cyst becoming diffuse at mature cysts, whose cell wall is composed by chitin (Botelho *et al.*, 2011). In the environment, they can survive for weeks or months, especially under favorable conditions of humidity and temperature. They are subject to degeneration under temperatures lower than 5°C and above 40°C, Tanyuksel and Petri (2003).

Trophozoites are the vegetative forms and are associated with pathology. They usually measure 20 to 40 μm, being likely to reach 60 μm in the most invasive forms. In general, it has a single nucleus (plate 1b), which is very distinguishable when stained, but rather clear in fresh preparations. Inside these preparations, the trophozoites are pleomorphic and rapidly produce thick and hyaline pseudopodis, which seem to slip over the blade’s surface, Silva and Gomes (2005). The
ingestion of food by amoeba occurs through pinocytosis (liquid particles) and phagocytosis of debris. However, in the invasive forms of amoebiasis, erythrophagocytosis and leukophagocytosis are also often verified. The process of phagocytosis starts with the ligation of the Gal lectin/GalNAc (Okada et al., 2005) and in order to promote degradation, amoebapores and cysteine proteinases are secreted to the phagosomes (Botelho et al., 2011).

2.3 Life cycle of *Entamoeba histolytica*

*Entamoeba histolytica* exhibits a typical faecal-oral life cycle consisting of infectious cysts passed in faeces and trophozoites which replicate within the large intestine. The infection is acquired through the ingestion of cysts and the risk factors mainly being contaminated food and water. The cysts pass through the stomach and excyst in the lower portion of the small intestine to produce eight immature trophozoites. Trophozoites colonize the large intestine where they feed on bacteria and cellular debris; they multiply by binary fission. Most remain in the lumen as commensals but some may invade the mucosa of the large intestine resulting into symptoms such as colitis, diarrhoea, and dysentery amongst others. Some trophozoites will be excreted in these diarrheic stools. Trophozoites may also invade the blood vessels of the large intestine and be transported to other organs such as the liver, brain and the lungs causing invasive/extra-intestinal amoebiasis which may lead to abscesses and other life threatening conditions. Encystations are triggered by the dehydration of gut contents in the colon due to absorption of water in the walls of the colon. Cysts mature by undergoing two
rounds of nuclear replication. They are passed out in faeces and may be picked by another host.

![Diagram of the life cycle of E. histolytica](image)

**Figure 2.1 Schematic diagram of the life cycle of E. histolytica**
(Stanley, 2001)

### 2.4 Pathogenesis

Although most cases of amoebiasis are asymptomatic, in some cases, the trophozoites become invasive and penetrate the intestinal mucosa, causing ulcers, dysentery and extra intestinal conditions that may be life threatening (Sateriale et al., 2011). As reported by Gamboa et al. (2011), amoebic liver abscess is the most common manifestation of invasive amoebiasis, but other organs can also be
involved including pleuropulmonary, cardiac, cerebral, renal, genitourinary and cutaneous sites. Pathogenesis depends on the invasiveness of trophozoites and is governed by three levels; chief among them are; adherence of trophozoite to the target cell, lysis of target cell, and phagocytosis of target cell (Sehgal et al., 1996). At the early stage of invasion, trophozoites inside the intestinal mucus adhere to epithelial cells through mechanisms mediated by lectins. The lectin which is the major contributive factor to the adhesion is Gal/GalNAc (Lejeune et al., 2009). Upon intimate contact, polypeptides called amoebapores are released by the parasite. Amoebapores, that are constitutively present at the cytoplasm of trophozoites (Gonzalez et al., 2008), are capable of inducing apoptosis and necrosis of eukaryotic cells and also present an antibacterial activity. Neutrophils are lysed when they come in contact with *E. histolytica* trophozoites releasing toxic products which lyse distant hepatocytes (Sehgal et al., 1996).

The phagocytic potential of trophozoites is directly linked to virulence. Factors like association of bacteria with trophozoites also influence virulence. Trophozoites from stools of many invasive patients contain ingested erythrocytes and have much higher rate of erythrophagocytosis than healthy human carrier. There are evidences of the main role of cysteine proteinases as a virulence factor for *E. histolytica*, being involved in the breach of the mucus barrier, which is crucial in the pathogenesis of amoebiasis (Lejeune et al., 2009). The proteolitic enzymes secreted by the parasite breach the mucus and the epithelial barrier, thus facilitating the penetration inside the tissue (Que and Reed, 2000). The
combination of these molecules leads to the formation of ulcers and to the subsequent migration of amoeba to the liver and other sites (Stanley, 2001).

2.5 Pathology

Amoebic infection of the human intestine ranges in spectrum from luminal colonization to mucosal invasion (Stanley, 2001). Initially trophozoites are found in the intestinal lumen and within mucosa (Sehgal et al., 1996). Following attachment to inter-glandular epithelium, the trophozoites have been found associated with the micro-ulcerations of the mucosa. Symptoms at this stage include non-specific colitis with edematous mucosa and hemorrhage (Stanley, 2001). Following attachment of amoeba, there is considerable disintegration of epithelial cell layer followed by invasion of sub-mucosa. The human inflammatory response to amoebic invasion is poor. This may be because E. histolytica can lyse inflammatory cells (Sehgal et al., 1996). With time the ulcer extends into lamina propria and further into muscularis mucosa, where progress usually stops prior to perforation. A plug of necrotic debris accumulates at the center of the ulcer.

Trophozoites are found in the leading edge at the base of the ulcer (Sehgal et al., 1996). Ulcers are typically “flask-shaped” (Botelho et al., 2011). Inflammatory response may be seen at the edges of the ulcers and involves mononuclear and giant cells with few neutrophils (Botelho et al., 2011). Ulceration of mucosa is the hallmark of invasive disease. Ulcers develop mostly in caecum and ascending colon. In about 20% of acute colitis cases, perforations occur which results in
peritonitis (Sehgal et al., 1996). Chronic ulceration results in the formation of a proliferative tuft of remaining mucosa that appears as a mass (termed amoeboma) in the lumen (Sehgal et al., 1996). Occasionally, trophozoites reach the liver by portal venules or intestinal perforation and produce abscesses. Liver abscesses, which may be up to 10 cm in diameter, occur mostly in the right lobe. Dead cells are observed in the center of the abscess whereas the trophozoites are found on the periphery. Bacteria are conspicuous by their absence in the abscesses. Ninety five per cent deaths in amoebiasis are due to liver abscess (Botelho et al., 2011).

2.6 Disease manifestation

Epidemiological data estimate that about 90% of amoebiasis cases are asymptomatic and that, among the symptomatic forms, the intestinal amoebiasis is the most frequent one (Stanley, 2003). Infections can sometimes last for years. Symptoms take from a few days to a few weeks to develop and manifest themselves, but usually it is about two to four weeks. Symptoms can range from mild diarrhoea to dysentery with blood and mucus. Patients with non-dysenteric colitis present abdominal colics and intervals of diarrhoea and asymptomatic periods of normal intestinal activity. Some cases involve a dysenteric colitis, characterized by exacerbated dyspeptic symptoms (pain, eructation, burning sensation and nausea), abdominal distension, flatulence, more than ten daily muco-bloody evacuations and constant sensation of evacuation need. The blood comes from amoebae invading the lining of the intestine. In about 10% of invasive cases the amoebae enter the bloodstream and may travel to other organs
in the body. Most commonly this means the liver, as this is where blood from the intestine reaches first, but they can end up almost anywhere. Sub-mucosa is then filled with ulcers that may cause hydro-electrolytic disturbance and malnutrition (Melo et al., 2004). Abrupt onset of nausea, vomiting, headache and mental status change should prompt rapid investigation of CNS involvement (Lacasse et al., 2009). In extra intestinal amoebiasis, trophozoites can migrate through the superior mesenteric vessel and reach the liver, where they cause inflammation, cellular degeneration and necrosis, thus forming the amoebic abscess, generally located at the right lobe. Patients present fever, intense pain at the right hypochondrium, as well as typical irradiations of biliary colic and painful hepatomegaly at palpation (Botelho et al., 2011). The most common extra intestinal manifestation, which occurs at the liver, was considered to be invariably fatal in the past. However, since the introduction of more efficient methods of diagnosis and treatment, mortality rates have fallen to 1-3% (Botelho et al., 2011).

In asymptomatic infections the amoeba lives by feeding on and digesting bacteria and food particles in the gut and a part of the gastrointestinal tract (Haque et al., 2006). Disease occurs when amoeba comes in contact with the cells lining the intestine. It then secretes amoebophores and proteolitic agents such as cysteine proteinases; these destroy cell membranes and proteins. This process can lead to penetration and digestion of human tissues, resulting first in flask-shaped ulcers in the intestine. *Entamoeba histolytica* ingests the destroyed cells by phagocytosis and is often seen with red blood cells inside when viewed in stool samples.
Especially in Latin America, a granulomatous mass (known as an amoeboma) may form in the wall of the ascending colon or rectum due to long-lasting immunological cellular response, and is sometimes confused with cancer. Theoretically, the ingestion of one viable cyst can cause an infection (Haque et al., 2006).

Amoebiasis may be aggravated by bacterial secondary infections, which are likely to cause an abscess rupture to the abdomen, the lung, the pleura or the pericardium. The hematogenic dissemination of trophozoites can injure the lung, the skin, the pericardium, the genitourinary system and the brain (Stanley, 2003). A study conducted in Vietnam reported 21 cases of hepatic amoebiasis in each group of one hundred thousand persons living in the city of Hue (Blessman et al., 2002). Hepatic lesions seem to be more incident among HIV (Human Immunodeficiency Virus) patients, as described by a study with patients from the National Hospital of Seoul, which found that 32% of patients with amoebic hepatic lesions were HIV positive (Muzaffar et al., 2006). Fulminant amoebic colitis is a severe form of the disease, mostly identified among pregnant women and immune-compromised patients and is usually reported in association with diabetes mellitus and chronic alcoholism (Stanley, 2003).

2.7 Diagnostic techniques

The most common method is direct fecal smear and staining (Appendix III) as described by Cheesbrough, (2005). Cystic forms are spherical and with up to four nuclei when mature (Plate 1a). They are found on consistent faeces, while the
trophozoitic forms which are generally pleomorphic and mono-nucleic (Plate 1b), are present in a diarrheic or pasty faecal material. This diagnostic method together with formal ether concentration technique recommended by (WHO, 2006) does not allow identification of strains. There are chances of misdiagnosis due to the intermittent elimination of *E. histolytica/E. dispers* cysts (Botelho et al., 2011) and the non-differentiation from other intestinal amoebae such as *E. coli*. Cellular debris may also jeopardize the microscopic diagnosis (Botelho et al., 2011). In a study to evaluate Selected practices among rural residents versus the prevalence of Amoebiasis and Giardiasis in Njoro district, Kenya, Kinuthia et al. (2012) proposed the need for reliable diagnostic methods other than direct microscopy for *E. histolytica* and *G. lamblia* stool tests in order to minimize the wide variation of the results that were observed. Another study carried out to determine the Prevalence and Intensity of Intestinal Parasites in School age Children in Thika District, Kenya (Ngonjo et al., 2012) observed that the formol-ether method was only 70% sensitive and hence unable to detect all the trophozoites. The Polymerase Chain Reaction (PCR) is reputed as the most specific method for the identification of *E. histolytica* infections, thus offering new perspectives for future use at the laboratorial routine. However, this method still requires optimization to become more practical and less expensive (Silva and Gomes, 2005).

In an effort to improve the diagnosis of intestinal amoebiasis, Gutierrez-Cisneros et al. (2010) showed that real-time PCR has been used for the detection and differentiation of *E. histolytica* and *E. dispers* infections. Fecal samples from 130
individuals with positive microscopic examination were analyzed by real-time PCR, which detected *E. histolytica* DNA in materials from only 10 (7.7%), while *E. dispar* DNA was found in samples from 117 individuals (90.0%).

Plate 2.1(a) Isolated plate of *E. histolytica* cyst. (Cheesbrough, 2005) Plate 2.1(b) Isolated plate of *E. histolytica* trophozoite (Cheesbrough, 2005)

2.8 Epidemiological aspects of amoebiasis

2.8.1 Distribution

*Entamoeba histolytica* has a worldwide distribution. High levels of human infection are found in India, Africa and Central and South America (Botelho *et al.*, 2011). Approximately 10% of world population is infected (Davis *et al.*, 2002). The prevalence is as high as 50% in tropical endemic areas. The rate of infection by *E. histolytica* differs among countries, socio-economic and sanitary conditions and populations as documented by Al-Harthi and Jamjoon (2007). It is
highly endemic throughout poor and socio-economically deprived communities in the tropics and subtropics.

In Egypt, 38% of individuals presenting with acute diarrhoea in out-patient clinics were found to have amoebic colitis in 2004 (Haque et al., 2006). *Entamoeba histolytica* seroprevalence studies in Mexico revealed that more than 8% of the population was positive. Another study in Yemen in 2011 on prevalence of intestinal parasites found the prevalence of *E. histolytica* to be 17.1% (Mail et al., 2011). However in Northern Ethiopia in a study to determine the prevalence of intestinal parasites and their associated risk factors in 2013 the prevalence of *E. histolytica* was observed to be as low as 4.6%. Increased cases of infections with amoebiasis are being reported globally due to co-infection with HIV/AIDS (Lacasse et al., 2009). In Kenya, the prevalence of *E. histolytica* is about 21% and although most cases go untreated due to their asymptomatic nature, this high prevalence contributes a lot to amoebiasis (WHO, 2011). A study carried out in rural parts of Thika municipality of Kenya to determine the prevalence and intensity of intestinal parasites in School Children showed an overall prevalence of intestinal protozoan parasites of 38.9% of which the most prevalent was *E. histolytica* (Ngonjo et al., 2012).

### 2.8.2 Risk factors

Environmental, socio-economic, demographic and hygiene-related behaviour is known to influence the transmission and distribution of intestinal parasitic infections (Norhayati et al., 2003). A study in Brazil identified place of residence,
age, ingestion of raw vegetables and drinking water quality as important risk factors (Benetton et al., 2005).

Prevalence of *E. histolytica* is related more to inadequate environmental sanitation and personal hygiene than to climate. Socio-economic factors as well as unpredictable factors such as food insecurity, droughts, and floods contribute to the problem (WHO, 2011). Unavailability of safe domestic water and low education on sanitation also contribute to transmission (AMREF, 2009). *Entamoeba histolytica* gains entry into the intestines through the mouth from undercooked food, vegetables, or contaminated water or hands. Poor personal hygiene, garbage disposal and poor disposal of excreta are significant for this oral-faecal infection (Blessman et al., 2002). Transmission may also be through mechanical vectors such as flies (Nyarango et al., 2008) whereby flies may carry the infective cysts from contaminated sites or dirty latrines and cause contamination of food or water.

Many tropical developing countries lack adequate supply of clean domestic water, contamination may occur at the source of water or at home due to poor sanitation (UNICEF, 2009). Another risk factor is the availability and usage of toilets. When people defecate in the open cysts of *E. histolytica* can be washed down to water bodies or may be carried by mechanical vectors such as flies and contaminate food or water sources (Cheesebrough, 2005). A study on prevalence of *E. histolytica* in a village in Côte d'Ivoire (Mamadou et al., 2010) found that
prevalence was high where toilets were lacking or were not used. The same study in, Côte d'Ivoire found that the lack of toilet use despite their existence favoured the spread of cysts. Another study on the risk of pathogenic intestinal parasites in Kisii municipality in Kenya associated the spread of *E. histolytica* with poor sanitary conditions of latrines. In Vietnam, Duc *et al.*, (2009) in a study on risk factors of *E. histolytica* observed that the type of latrine used has an association to the infection rate. In their study the users of single vault latrines showed a higher prevalence. Infection rate is higher amongst persons aged 5-40 years (WHO, 1997) but according to Tawasar *et al.* (2007) infection is higher in children than adults because children have poorer hygiene practices than adults and also their immunity is not as well developed as that of adults (Kinuthia *et al.*, 2012).

### 2.9 Amoebiasis as a public health concern

Intestinal protozoan infections are a public health concern in Kenya. Although these infections occur in all age groups, the problem is predominant among the world’s estimated 400 million school-age children. This contributes to childhood growth retardation and absenteeism from school. Amoebiasis infections occur in both rural and urban populations and can occur within the school set up especially where there is a school feeding programme or where sanitary measures are not properly observed (Ngonjo *et al.*, 2012). This necessitates regular medication especially in school going children who are more vulnerable (Kamnuvi and Situbi, 1983). The United Nations Children Fund reports that about 3 billion people lack adequate sanitation, with 1.8 million people dying every year from
diarrhoeal diseases of which amoebiasis ranks high (UNICEF, 2009). In 2004, through SIDA funding, the DPHO carried out a survey on water and sanitation and established that the larger Kyuso District had poor water and sewerage systems, with only 15% latrine coverage. Most of the water sources are contaminated due to sharing the water points with domestic animals and also due to the fact that latrine coverage is low at 15% (NCAPD, 2005). Safe drinking water and basic sanitation are of crucial importance to curb the infection rate of diarrhoeal diseases. In Kyuso zone, safe drinking water and basic sanitation is a goal which is far from being achieved. People depend on boreholes, shallow wells, dry river bed wells and earth dams for drinking water. Most of these water sources and especially the surface open ones are shared between humans and livestock and hence the water is highly contaminated with human and domestic animal faecal material exposing people to unhygienic related illnesses such as amoebic dysentery.

2.10 Control of amoebiasis
2.10.1 Improved sanitation

Improvement of sanitation, clean water supplies and health education coupled with treatment reduces amoebiasis transmission in the long term (Ngonjo et al., 2012). An analytical review of health progress, and systems performance, 1994 – 2010 by the ministry of public health and sanitation of Kenya reports that improved sanitation which includes provision of safe domestic water, availability of improved latrines and flush toilets, hand washing,
contributes greatly in reduction of diarrhoeal diseases including amoebiasis. In a study to evaluate the risk of pathogenic intestinal infections in Kisii municipality in Kenya, Nyarango et al. (2008) reported a prevalence of *E. histolytica* of 11.9% and associated this to poor sanitation amongst food handlers in the municipality. In another study to determine the prevalence of waterborne protozoan parasites in western Cameroon (Richardson et al., 2012) it was observed that due to improved sanitation in Bawa village, infections with *E. histolytica* were lower at 7.1% compared to Nloh village (15.7%) which had unimproved sanitation. Combined methods of amoebiasis control are the most appropriate approach, which will obtain dramatic reduction in prevalence and intensity of infection. Improved sanitation can result in reduced water and food contamination with human excreta. Such factors as use of toilets and latrines, improved sewage system and personal hygiene if implemented, can greatly reduce infection with *E. histolytica* (AMREF, 2009).

### 2.10.2 Health education

Health education targets encouraging personal hygiene and healthy behavior to reduce transmission and re-infection. Communities need to be educated on use of latrines, washing hands, protecting water supplies from faecal contamination, proper cooking and handling of food (Cheesebrough, 2005). Richardson et al. (2012) reported that with enhanced education programmes on sanitation and hygiene an 84% reduction in diarrhoeal diseases was realized in Bawa village in western Cameroon.
2.10.3 Food and water hygiene

Food and water contamination is a contributing factor to transmission of diarrhoeal diseases (Brooks, 2009). One can contract the amoeba by eating food that has been cooked or handled in an unsanitary manner. The food could have been handled by those who have poor hygiene or has come in contact with fecal matter either in the air or directly. A survey carried out in kisii municipality on food handlers (Nyarango et al., 2008) found that 11.9% were infected with *E. histolytica*. The infection rates *E. histolytica/dispar* was reported at 52%, respectively, in a study conducted on 132 restaurant workers in Almukala City, Yemen (Baswaid and AL-Haddad, 2008). The high prevalence of this intestinal protozoon among restaurant workers is alarming with the possible risk of food borne outbreak. Other food related practices that may contaminate food with cysts of *E. histolytica* are; Improper cooking where the food is halfway cooked, failure to wash hands thoroughly with soap and hot running water for at least 10 seconds after using the toilet or changing a baby’s diaper, and before handling food, eating raw vegetables when in endemic areas, as they may have been fertilized using human feces, eating Street Foods especially in public places where others are sharing sauces in one container (DPHS, 2010).

*Entamoeba histolytica* can also be transmitted orally by drinking infected water and it is an environmental contaminant of the water supply. Water supply is really an important risk factor for amoebiasis and several large outbreaks of Amoebic dysentery have resulted from the contamination of municipal water
supplies with human wastes (Ngonjo et al., 2012). The problem is greater in the rural areas that do not have a municipal water network and sewage system. Fecal contamination of water and food should be avoided by fundamental prophylactic policies such as, for instance, installation of basic sanitary conditions, as well as a sanitary education and strict control of food handlers especially in public eating points such as food kiosks and restaurants (Nyarango et al., 2008). Transmission may also occur within the school set up (Ngonjo et al., 2012). This can be due to unsanitary conditions in the water sources and storage of pupils’ drinking water. Where there is a school feeding programme; poor handling of food and unhygienic conditions of the kitchen may also lead to contamination. Other school factors that may be of importance in transmission of E. histolytica include; lack of washing of hands and presence, use and cleanliness of lavatories (Ngonjo et al., 2012).
CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

This study was conducted in Kyuso Zone, Kyuso District. A Zone is an Educational administrative unit comprising of several schools. Kyuso is part of the larger Mwingi District in Kitui County of Kenya. Kyuso District is located about 198 km East of Nairobi. It lies between latitude 0° 03’ and 1° 12’S and longitude 38° 47’ and 39° 57’ E and occupies an area of 804.4 km². The population density is 46 people per km² (NCAPD, 2005). It borders Mwingi District to the south, Embu and Tharaka-Nithi counties to the West, Garissa and Tana river counties to the East. It is a semi arid area and has challenges of acquisition of clean water for domestic use. Temperatures range between 26°C and 34°C.

Kyuso Zone was purposely selected for this study because it has both urban and rural settlements (NCPAD, 2005). Water sources in the Zone include; two seasonal rivers (Mughoo and Thunguthu), several bore holes, rock catchments and several Earth dams (which include Gai, Kyuso, Kaghui amongst others). The main economic activity is subsistence crop growing and livestock keeping. They grow maize, sorghum, millet, cowpeas and a few other drought-resistant crops. There is also livestock farming and they mainly keep cattle and goats. Kyuso Zone has 3 categories of health facilities; Government-run facilities include; Kyuso District Hospital, Ngaaiie dispensary and Marisi dispensary. Faith-based health facilities include Tei wa Yesu Family Care Centre and Hospital and
Kimangao Catholic mission Health Centre. Then there is The Neema hospice a private clinic.

Figure 3.1 Sketch map of Kyuso Zone, Kyuso district, Kenya (NCAPD, 2005)
3.2 Research design

This was a cross sectional study aimed at capturing the population aged between 6-12 years in Kyuso zone, Kyuso district. Cluster random sampling (Kombo and Tromp, 2006) was used to sample the schools and the pupils.

3.3 Study population

Kyuso Zone where this study was carried out has a total of 19 primary schools with approximately 5700 pupils. Of these 3610 (63%) are aged 6-12 years (Ministry of Education, 2011), and was the targeted population.

3.3.1 Criteria of inclusion

All the pupils aged 6-12 years were included in the study despite their class. These are the ages most at the risk of infection with intestinal parasites (Mamadou et al., 2010).

3.3.2 Criteria of exclusion

Those who were unwilling to participate in the study and those who were absent from school on the initial day were excluded.

3.4 Sampling techniques and Sample size determination

3.4.1 Sampling techniques

Kyuso zone has a total of 19 primary schools. A cluster random sampling was used to sample the schools. They were clustered into 4 centres (Ngaaie,
Kimangao, Kyuso and Marisi Centres) and then 2 schools picked randomly from each centre. Likewise, pupils aged 6 to 12 years from each sampled school were clustered in terms of classes and picked randomly until the calculated sample size was realized.

3.4.2 Sample size determination

The sample size was determined by the method as used by Fisher et al. (1998) and Wayne (2010) for calculating sample size.

Thus: \[
n = \frac{Z^2 p q D}{d^2} \]

Where;

n = Sample size where population <10,000 (Kyuso Zone has approximately 3610 pupils aged 6-12).

Z = Standard normal deviate (1.96) which corresponds to 95% confidence interval.

P = Proportion of the target population estimated to have a characteristic (this is approximated at 0.36 in consideration of the medical records at Kyuso District Hospital).

q=1-p; hence 1-0.36=0.64

d = Degree of accuracy desired for the study = 0.05.

D= Design effect = 1

\[n = \frac{1.96^2 \times 0.36 \times 0.64}{0.05^2} = 354\]
In this regard, 354 pupils were sampled in 8 schools out of the 19 in the Zone. The schools included Ngaaie, Itulu, Nzaalani, Gai, Mikwa, Ndathani, Kyuso and Kalole primary schools. Forty four pupils aged between 6-12 years were sampled randomly from each school except Kyuso Primary school from which 46 pupils were sampled due to a relatively higher population.

3.5 Data collection

3.5.1 Parasitological survey: Collection of stool samples

Sampling was done from all the study school children of the ages 6-12 in the sampled 8 out of the 19 schools in the Zone. The collection of stool specimens was done between September to November 2011. Cluster random sampling of the schools from the Zone’s 4 centers was done. After the children were given an explanation of the stool sample collection, they received polypots bearing serial numbers into which they were to place their samples, applicator sticks and tissue papers as apparatus for stool collection. Each school was allocated a day for the sample collection. The sample collection was done at around 8.00 a.m and it took about 30 minutes to obtain the samples. The samples were transported to the Kyuso District Hospital laboratory which is about 15 kilometers from the furthest school for examination.

3.5.2 Questionnaire survey

Questionnaires were distributed to all the sampled 8 schools where the class teachers were requested to interview their pupils of ages 6-12 years and fill the
questionnaires (Appendix I). The questionnaire took into account several aspects but only data on water supply sources, hygienic situation at home and presence and use of latrines at home were considered for this study. The teachers followed the instructions that accompanied the questionnaire and interviewed pupils separately, one after another, in an empty classroom to avoid the influence of others on their responses. Most of the questions required “Yes” or “No” responses but where elaboration was needed, the teacher gave options to the pupil to ease recollection.

3.5.3 Observational check list

Observation was done during the visits to each school to determine other factors that may be associated with transmission of amoebiosis. The factors sought included water sources, presence of toilets, and system of feeding pupils amongst others. Checklists (Appendix I) were filled.

3.5.4 Focus group discussion

A focus group discussion was held with health providers in the zone to assess the control strategies against amoebiasis in the community in general, since if the control strategies have an impact in the community they will also affect the children. The discussions also focused on the epidemiological factors that lead to infection in the community. In the discussion were the MOH (Medical Officer of Health), Kyuso District; the medical superintendent, Kyuso District Hospital; 2 nurses from Kyuso District Hospital and one from Tei Wa Yesu family care
centre and hospital; 1 medical lab technologist and the public health officer Kyuso District. Discussions were also held with teachers in the schools visited to evaluate transmission factors, health education matters and how they have impact on the school children. 3 teachers from each school were involved. A group discussion guide (Appendix I) was used to guide the discussion and the results of the discussion helped to furnish the information about the control measures of amoebiasis in the zone.

3.5.5 Hospital records
Medical records were accessed in Kyuso District Hospital for relevant data on the disease status in the general population. This was aimed at establishing the morbidity rate of amoebiasis in the general population and particularly in the children aged 6 to 12 years who were taken to the hospital.

3.6 Laboratory analysis techniques
This was done with the assistance of two qualified laboratory technicians. The collected stool specimen was first observed physically for consistency, presence of blood stains and any macroscopic parasites (Wakid, 2010). Direct smear and iodine staining methods were used to process the stool as described by Cheesbrough (2005) (Appendix III) and then observed microscopically using x10 or x40 objective for cysts or trophozoites. Formal ether concentration technique (Appendix III) was also employed for more quantitative examination.
3.7 Data analysis

Data was stratified using such variables as age, sex, number infected and number uninfected. The Children ages were divided into three age-groups (6-8, 9-10, and 11-12 years). Chi-square ($\chi^2$) tests were conducted with SPSS statistical package (Wayne, 2010) to determine the relationship between the prevalence and children's age and sex with a confidence interval (CI) of 95%. The associations between prevalence and such variables as; latrine use, food hygiene and water treatment were also examined by chi-square ($\chi^2$) tests. Associations between the parasite prevalence and water sources were examined by logistic regression conducted with SPSS statistical package (Wayne, 2010). Comparisons were made for prevalence in different age groups using one way analysis of variance (ANOVA).

3.8 Ethical approval

Permission to conduct this study was sought from the Ministry of Public Health and Sanitation, the Medical Superintendent KDH and the Ministry of Education. Participation of pupils was voluntary. All respondents included in the study were asked to give an informed consent through their parents or legal guardians before inclusion in the study (Appendix II). All work was done according to the guidelines for human experimentation in clinical research as stipulated by the Ministry of Health of Kenya. At the end of the parasitological survey, all the infected school children were treated free of charge with Metronidazol, an amoebicide (Botelho et al., 2011).
CHAPTER 4: RESULTS

4.1 Demographic characteristics of the respondents

4.1.1 Study sample

Three hundred and fifty four pupils were sampled out of the 3610 aged 6-12 years. All the sampled pupils provided stool specimen for examination and filled the questionnaire with the assistance of their teachers.

4.1.2 Distribution of respondents by age and sex

Out of the 354 pupils that participated in the study 52.5% were females and 47.5% were males. Within the 3 age groups used in the study, 32.5% were aged 6-8 years, 38.1% were aged 9-10 years and 29.4% 11-12 years (table 4.1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Respondents</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>186</td>
<td>52.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>168</td>
<td>47.5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>6-8</td>
<td>115</td>
<td>32.5</td>
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<tr>
<td></td>
<td>9-10</td>
<td>135</td>
<td>38.1</td>
</tr>
<tr>
<td></td>
<td>11-12</td>
<td>104</td>
<td>29.4</td>
</tr>
</tbody>
</table>

Data by gender indicated a higher number of females than males while by age, more of the children were within the 9-10 age group.
4.2 Prevalence of *E. histolytica* in children attending primary schools in Kyuso Zone

4.2.1 Overall prevalence
The over-all prevalence of *E. histolytica* in school children aged 6-12 years in primary schools in Kyuso Zone was 42.1% (table 4.2). Out of the 354 pupils sampled, 149 were found to be infected with *E. histolytica* while 205 were not infected.

Table 4.2: Overall prevalence, prevalence by gender and age (N=354)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>% of Uninfected</th>
<th>% of infected /prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>57.5</td>
<td>42.5</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>58.3</td>
<td>41.6</td>
</tr>
<tr>
<td>Age</td>
<td>6-8</td>
<td>64.4</td>
<td>35.6</td>
</tr>
<tr>
<td></td>
<td>9-10</td>
<td>57.1</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>11-12</td>
<td>51.9</td>
<td>48.1</td>
</tr>
<tr>
<td>Over-all prevalence</td>
<td></td>
<td></td>
<td>42.1</td>
</tr>
</tbody>
</table>

4.2.2 Prevalence by gender
Out of the 186 female pupils, 79 (42.5%) were found to be infected. One hundred and seven female pupils were uninfected. Out of 168 male pupils 70 (41.6 %) were found to be infected with *E. histolytica* (Table 4.2).
4.2.3 Prevalence by age

Out of the 115 pupils aged 6-8 years, 41 (35.6%) were found to be infected. Out of the 135 aged 9-10 years 58 (42.9%) pupils were found to be infected. Out of the 104 pupils aged 11-12 years, 50 (48.1%) were found to be infected (Table 4.2).

4.2.4 The relationship between the prevalence of E. histolytica and gender and age.

4.2.4.1 The relationship between the Prevalence of E. histolytica and gender

When Chi-square ($\chi^2$) test was done to determine the relationship between the prevalence of E. histolytica and gender (Table 4.3), a computed value of $\chi^2 = 0.9653$ was obtained against the tabulated $\chi^2$, 0.05, df=1 =3.841. Hence the E. histolytica status was observed to be independent of gender. No significant association was found between prevalence and gender ($\chi^2=0.9653$, df=1, P=0.3259).

Table 4.3: The relationship between prevalence and gender (N=354)

<table>
<thead>
<tr>
<th>Gender</th>
<th>% Positive</th>
<th>% Negative</th>
<th>Total</th>
<th>P -value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>41.6</td>
<td>58.3</td>
<td>99.9</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>Female</td>
<td>42.5</td>
<td>57.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>84.1</td>
<td>115.8</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>
4.2.4.2 The relationship between the prevalence of *E. histolytica* and children’s age

When Chi-square ($\chi^2$) test was done to determine the relationship between the prevalence of *E. histolytica* and children’s age (Table 4.4), a computed value of $\chi^2 = 1.717$ (Table 4.5a) was obtained for infected cases while a computed value of $\chi^2 = 1.26$ was obtained for uninfected cases (Table 4.5b). Hence the *E. histolytica* status was observed to be independent of age. No significant association was observed between the *E. histolytica* prevalence and Children’s age. ($\chi^2 = 3.5649$, df=2, P>0.05).

Table 4.4: The relationship between prevalence and children’s age (n=354)

<table>
<thead>
<tr>
<th>Status</th>
<th>6-8 years</th>
<th>9-10 years</th>
<th>11-12 years</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>35.6</td>
<td>42.9</td>
<td>48.1</td>
<td>P=0.424</td>
</tr>
<tr>
<td>Negative</td>
<td>64.4</td>
<td>57.1</td>
<td>51.9</td>
<td>P=0.533</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>p&gt;0.05</td>
</tr>
</tbody>
</table>
Tables 4.5 (a and b). Chi-square ($\chi^2$) test statistics on the relationship between prevalence and age

(a) Infected cases (n=354)

<table>
<thead>
<tr>
<th>Age category</th>
<th>% (infected)</th>
<th>Observed N (%)</th>
<th>Expected N (%)</th>
<th>Residual</th>
<th>Chi-square($\chi^2$)</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8</td>
<td>35.6</td>
<td>36</td>
<td>42.3</td>
<td>-6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-10</td>
<td>42.9</td>
<td>43</td>
<td>42.3</td>
<td>0.7</td>
<td>1.717</td>
<td>2</td>
<td>P=0.424</td>
</tr>
<tr>
<td>11-12</td>
<td>48.1</td>
<td>48</td>
<td>42.3</td>
<td>5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>127</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Uninfected cases

<table>
<thead>
<tr>
<th>Age category</th>
<th>% (uninfected)</th>
<th>Observed N (%)</th>
<th>Expected N (%)</th>
<th>Residual</th>
<th>Chi-square($\chi^2$)</th>
<th>df</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-8</td>
<td>51.9</td>
<td>52</td>
<td>57.7</td>
<td>-5.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-10</td>
<td>57.1</td>
<td>57</td>
<td>57.7</td>
<td>-0.7</td>
<td>1.260</td>
<td>2</td>
<td>P=0.533</td>
</tr>
<tr>
<td>11-12</td>
<td>64.4</td>
<td>64</td>
<td>57.7</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>173</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2.4.3 Prevalence in different age groups by Analysis of Variance

When the prevalence in different age groups was analyzed using Analysis of Variance (ANOVA), there was no significant association found between prevalence and age ($F=1.128$, $P=0.346$) (Table 4.6).
Table 4.6: Prevalence in different age groups (ANOVA)

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>1.651</td>
<td>6</td>
<td>0.275</td>
<td>1.128</td>
<td>0.346</td>
</tr>
<tr>
<td>Within Groups</td>
<td>84.635</td>
<td>347</td>
<td>0.244</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>86.285</td>
<td>353</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.2.5 General Morbidity rate due to *E. histolytica* among patients attending Kyuso District Hospital by Hospital records.

4.2.5.1 Overall morbidity rate

Medical records in the Kyuso District Hospital laboratory between April and September 2011 were studied. Within this period, a total of 337 patients across all ages from Kyuso Zone had been tested for gastrointestinal infections using stool as a test specimen during the routine clinical tests. Of the total number of patients, 19.6% were aged between 6 and 12 years. Out of the 337 patients 149 (44.2%) were found to be infected with *E. histolytica* while the uninfected were 188 (55.8%). Amongst the children aged 6-12 years, 31 (46.9%) were found to be infected while 53% were uninfected (Appendix IV). Hence the overall morbidity rate due to *E. histolytica* among those aged between 6 and 12 years was 46.9%.

Table 4.7: Overall morbidity rate, prevalence by gender (n=377)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>% of Uninfected</th>
<th>% of infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>56.9</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>54.1</td>
<td>45.8</td>
</tr>
<tr>
<td>Overall morbidity rate</td>
<td></td>
<td></td>
<td><strong>44.2</strong></td>
</tr>
</tbody>
</table>
4.2.5.2 Morbidity rate by gender

Of the 337 patients, of various ages 60.5% were female while 39.5% were male. Of the 204 females, 88 (43.1%) were found to be infected while 116 (56.9%) were uninfected. Of the 133 males, 61 (45.8%) were found to be infected with *E. histolytica* (table 4.7). Out of the 149 infected cases, 88 (59.1%) were females while 61 (40.9%) were males. Of the 31 infected children aged 6-12 years, 17 (54.8%) were females while 14 (45.2%) were males (table 4.7).

4.2.5.3 Morbidity rate by age

The largest proportion of the patients was aged between 1-20 years 53.1% (Table 4.8). They showed a morbidity rate of 45.9%. Patients aged above 40 years showed relatively lower infection rates.

Table 4.8: Morbidity rate by age (N=337).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total no.</th>
<th>% Uninfected</th>
<th>% of Infected</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>55</td>
<td>72.7</td>
<td>27.2</td>
<td>P=0.000</td>
</tr>
<tr>
<td>6-10</td>
<td>46</td>
<td>56.5</td>
<td>43.4</td>
<td>P=0.703</td>
</tr>
<tr>
<td>11-15</td>
<td>34</td>
<td>41.2</td>
<td>58.8</td>
<td>P=0.072</td>
</tr>
<tr>
<td>16-20</td>
<td>44</td>
<td>45.5</td>
<td>54.5</td>
<td>P=0.371</td>
</tr>
<tr>
<td>21-25</td>
<td>27</td>
<td>51.9</td>
<td>48.1</td>
<td>P=0.681</td>
</tr>
<tr>
<td>26-30</td>
<td>27</td>
<td>59.3</td>
<td>40.7</td>
<td>P=0.072</td>
</tr>
<tr>
<td>31-35</td>
<td>21</td>
<td>38.1</td>
<td>61.9</td>
<td>P=0.016</td>
</tr>
<tr>
<td>36-40</td>
<td>21</td>
<td>71.4</td>
<td>28.5</td>
<td>P=0.000</td>
</tr>
<tr>
<td>Above 40</td>
<td>62</td>
<td>56.5</td>
<td>43.5</td>
<td>P=0.0162</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>337</strong></td>
<td><strong>55.8</strong></td>
<td><strong>45.1</strong></td>
<td><strong>P=0.274</strong></td>
</tr>
</tbody>
</table>

Chi-square tests ($\chi^2$) conducted to determine the relationships between the patients age and morbidity rate found that the association was insignificant ($\chi^2 = 1.198$, df=1, P>0.05).
4.2.5.4 The relationship between morbidity rate and patients’ gender

Chi-square test ($\chi^2$) done to determine the relationship between the morbidity rate due to *E. histolytica* and the patients gender (Table 4.9) revealed a computed value of $\chi^2 = 0.1448$ against the tabulated $\chi^2$, 0.05, df=1, =3.841. (Hence a conclusion that the status of *E. histolytica* was independent of gender was made).

No significant association between morbidity rate and gender was found ($\chi^2 = 0.1448$, df =1, $P>0.05$).

<table>
<thead>
<tr>
<th>Gender</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>88 (43.1)</td>
<td>116 (56.9)</td>
<td>P=0.162</td>
</tr>
<tr>
<td>Male</td>
<td>61 (45.8)</td>
<td>72 (54.1)</td>
<td>P=0.424</td>
</tr>
<tr>
<td>Total</td>
<td>149 (44.2)</td>
<td>188 (55.8)</td>
<td></td>
</tr>
</tbody>
</table>
4.3 Factors contributing to transmission of *E. histolytica* amongst school children in Kyuso Zone.

Table 4.10: Demographic factors of the respondents and the risk factors for *E. histolytica* (n=354)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>No of respondents</th>
<th>% infected</th>
<th>Odds ratio(OR)</th>
<th>Chi-square ($\chi^2$)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6-8</td>
<td>115</td>
<td>35.6</td>
<td>0.593</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9-10</td>
<td>135</td>
<td>42.9</td>
<td>0.286</td>
<td>3.5649</td>
<td>0.424</td>
</tr>
<tr>
<td></td>
<td>11-12</td>
<td>104</td>
<td>48.1</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>186</td>
<td>42.5</td>
<td></td>
<td>1.26</td>
<td>0.9653</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>168</td>
<td>41.6</td>
<td></td>
<td></td>
<td>0.3259</td>
</tr>
<tr>
<td>Source of water</td>
<td>Riverbed wells</td>
<td>159</td>
<td>29.5</td>
<td>2.351</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Bore hole</td>
<td>24</td>
<td>16.6</td>
<td>1.609</td>
<td></td>
<td>0.076</td>
</tr>
<tr>
<td></td>
<td>Tap</td>
<td>42</td>
<td>9.5</td>
<td>0.968</td>
<td></td>
<td>0.278</td>
</tr>
<tr>
<td></td>
<td>Rock catchment</td>
<td>20</td>
<td>15</td>
<td>1.484</td>
<td></td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>Earth dam</td>
<td>57</td>
<td>40.3</td>
<td>2.828</td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>52</td>
<td>3.8</td>
<td></td>
<td></td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Distance to water source</td>
<td>&lt;1km</td>
<td>123</td>
<td>41</td>
<td>0.364</td>
<td>3.24</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>1-5km</td>
<td>157</td>
<td>13</td>
<td>1.901</td>
<td>54.76</td>
<td>0.0000</td>
</tr>
<tr>
<td></td>
<td>&gt;5km</td>
<td>74</td>
<td>9</td>
<td>2.314</td>
<td>67.24</td>
<td>0.0000</td>
</tr>
<tr>
<td>Water source protected</td>
<td>Yes</td>
<td>232</td>
<td>17</td>
<td>0.435</td>
<td>1.286</td>
<td>0.257</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>122</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment of water at home</td>
<td>Yes</td>
<td>223</td>
<td>6</td>
<td>1.427</td>
<td>11.645</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>131</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of latrine at home</td>
<td>Yes</td>
<td>218</td>
<td>14.7</td>
<td>0.54</td>
<td>4.7199</td>
<td>0.0298</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>136</td>
<td>24.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garbage/Refuse disposal</td>
<td>Garbage pit/dustbin</td>
<td>194</td>
<td>10</td>
<td>0.24</td>
<td>11.524</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Outside compound</td>
<td>161</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.1 Association between *E. histolytica* prevalence and water sources
Dry river bed wells and Earth dams had the highest prevalence rates amongst the water sources at 29.5% and 40.3% respectively (Table 4.10). A significant association was found between the use of Dry riverbed wells and high prevalence of *E. histolytica* OR (odd ratio) =2.351, CI= 95%; \((\chi^2 =10.042, \text{ df}=1, \text{ P}=0.02)\) (Table 4.10). A significant association was also found between use of Earth dam water and prevalence (OR=2.828, CI= 95%). \((\chi^2 =13.489, \text{ df}=1, \text{ P}=0.000)\) (Table 4.11).

**Table 4.11: Logistic regression analysis on significance of the association between water sources and prevalence.**

<table>
<thead>
<tr>
<th>Water sources</th>
<th>OR</th>
<th>df</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riverbed well</td>
<td>2.351</td>
<td>1</td>
<td>0.002</td>
<td>2.452 - 44.896</td>
</tr>
<tr>
<td>Bore holes</td>
<td>1.609</td>
<td>1</td>
<td>0.076</td>
<td>0.848 - 29.496</td>
</tr>
<tr>
<td>Tap</td>
<td>0.968</td>
<td>1</td>
<td>0.278</td>
<td>0.458 - 15.129</td>
</tr>
<tr>
<td>Rock catchment</td>
<td>1.484</td>
<td>1</td>
<td>0.120</td>
<td>0.679 - 28.679</td>
</tr>
<tr>
<td>Earth dam</td>
<td>2.828</td>
<td>1</td>
<td>0.000</td>
<td>3.739 - 76.491</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.3.2 Association between *E. histolytica* prevalence and other risk factors

4.3.2.1 Latrines

Of the 354 respondents 136 (38.4%) did not have latrines in their homes, preferring to relieve themselves in the bushes while 61.6% had latrines at home. Through observational checklists it was observed that 70% of the latrines observed had very low standards of cleanliness. When Chi-square test was done to determine the association between latrine use and the status of *E. histolytica* (Table 4.10), a computed value of $\chi^2 = 4.7199$ was obtained against the tabulated $\chi^2$ 0.05, df=1, = 3.841. Hence there was a significant association between the use of latrines and *E. histolytica* status ($\chi^2 = 4.7199$, df=1, P=0.0298).

4.3.2.2 Water treatment

Of the 354 respondents 223 (62.9%) had their drinking water treated in their homes (Table 10). Of these 6% were found to be infected. Of the 37.0% who did not have their drinking water treated at home, 25% were found to be infected (Table 4.10). A significant association was found between treatment of drinking water and high prevalence (OR=1.427, CI=95%), ($\chi^2 = 11.645$, df=1, P=0.002).

4.3.2.3 Protection of water sources

Of the total respondents, 232 (65.5%) had their water sources protected either by fencing or covering. Of these, 17% were found to be infected with *E. histolytica*. Of the 34.5% whose water sources were not protected 11% were found to be infected (Table 4.10). No significant association was observed between protection of water sources and prevalence (OR=0.435, CI=95%) ($\chi^2 = 1.286$, df=1, P=0.257).
4.3.2.4 Distance from the water sources

Of the total respondents, 34.7% had their water sources less than one Kilometer away, 41% of these were found to be infected. Some other 44.3% had their water sources 1 to 5km away, of these 13% were infected. Those who had their water sources more than 5km away were 20.9%. Of these, 9% were infected (Table 4.10). A significant association was observed between prevalence and the distance to water source, (OR=0.364, 1.901, 2.314, CI=95%) ($\chi^2=3.24, 54.76, 67.24$, df=2, P<0.05).

4.4 Control measures in place for amoebiasis in Kyuso Zone

4.4.1 Community Education

Through focus group discussions, it was established that several NGOs and Health organizations are collaborating with government agencies to educate residents on control of hygiene-based diseases including amoebiasis. Their aim is improving health standards which may help reduce infections with hygiene-based diseases. Some of the organizations are also offering sanitation improvement programmes such as construction of latrines in schools and in the households of community based organization (CBOs) members. An example of such organization is the Action Aid Kenya. Others are organizing hand washing shows at chief’s barazas. These groups were stratified according to their level of participation in community Education based on their frequency of meetings with the community members (Table 4.12). Table 4.12 shows that the participation of the various groups in community education was significantly less likely to impact
on the community sanitation improvement since the odds ratio (OR) >1, chi-square (χ²) >1, and 95% confidence interval (CI) was inclusive of 1.

Table 4.12: Participants in community Education (n=15)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total no of contact days per month</th>
<th>Level of participation (%)</th>
<th>OR (CI=95%)</th>
<th>χ²</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tei wa yesu mission</td>
<td>4</td>
<td>26</td>
<td>1.046</td>
<td>23.04</td>
<td>0.0000</td>
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<tr>
<td>Redcross society</td>
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<td>13</td>
<td>1.901</td>
<td>54.76</td>
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<tr>
<td>Action Aid</td>
<td>1</td>
<td>7</td>
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<td>73.96</td>
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<td>13</td>
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</tr>
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<td>Ministry of Livestock dev.</td>
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<td>7</td>
<td>2.587</td>
<td>73.96</td>
<td>0.0000</td>
</tr>
<tr>
<td>Ministry of Agriculture</td>
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<td>7</td>
<td>2.587</td>
<td>73.96</td>
<td>0.0000</td>
</tr>
<tr>
<td>Researchers</td>
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<td>20</td>
<td>1.386</td>
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<td>Chief’s baraza</td>
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<td>7</td>
<td>2.587</td>
<td>73.96</td>
<td>0.0000</td>
</tr>
</tbody>
</table>
4.4.2 Provision of safe water

Some NGOs in partnership with government ministries are providing safe water within Kyuso Zone. This is by; provision of water tanks in schools and hospitals, sinking bore holes, construction of Earth dams, and the development of Kiambere piped water project amongst others. The success level of these projects in provision of safe domestic water in the Zone was assessed based on the total numbers provided; accessibility and usability of this water at home by the pupils’ house hold (Table 4.13).

**Table 4.13: Provision of safe domestic water (n=60)**

<table>
<thead>
<tr>
<th>Water project</th>
<th>Total numbers</th>
<th>(%)</th>
<th>OR (CI=95%)</th>
<th>$\chi^2$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanks</td>
<td>42</td>
<td>70</td>
<td>-0.847</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earth dams</td>
<td>6</td>
<td>10</td>
<td>2.197</td>
<td>$\chi^2=0.00083$</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>Piped water</td>
<td>1</td>
<td>2</td>
<td>3.892</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bore holes</td>
<td>10</td>
<td>16</td>
<td>1.658</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water buzzers</td>
<td>1</td>
<td>2</td>
<td>3.892</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 13 shows the main methods that NGOs and the government are using to provide safe domestic water in Kyuso zone. It shows that the methods of provision of safe domestic water was less non-significantly likely to impact on
improvement of sanitation in the zone; Chi-square ($\chi^2$) <1, Odds ratio (OR) >1 and 95% confidence interval (CI) was inclusive of 1.
CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Socio-demographic information

This study is the first to document the prevalence of *Entamoeba histolytica* in school children in Kyuso Zone, Kyuso District. The study was carried out among school children because they are one of the high risk groups to get infected with intestinal parasites and was generally accessible. Kyuso is a semi-arid area with erratic rainfall, consequently the poverty index is high (NCAPD, 2005). The distance from these pupils’ homes to water sources was generally observed to be long with some families having to walk 10 km to get to water sources (Table 10). The most common sources of water were the dry river bed-wells whose water is usually contaminated due to animal and human faeces (Mamandou *et al.*, 2010).

Latrine coverage in the Zone was observed to be 38.6% and NCAPD (2005) reported that it could be as low as 15% in the larger Kyuso District. This low coverage makes people to defecate in the bushes, a factor that leads to transmission of *E. histolytica*.

5.1.2 Prevalence of *E. histolytica*

This study showed an overall prevalence of 42.1% among the school children aged 6-12 years in Kyuso Zone. This high infection rate in the zone confirms the high morbidity rate of 46.9% for amoebiasis observed in the hospital records. The prevalence was slightly below the prevalence of 44.6% observed by Otula
(unpublished) in a study to determine the factors associated with prevalence of parasites causing intestinal infection among primary school children aged 6-15 years in Bondo District Kenya. However, the prevalence was way up above the prevalence of 20.83% reported by Kinuthia et al. (2012) in a study on selected practices among rural residents versus the prevalence of amoebiasis and Giardiasis in Njoro District, Kenya. Ngonjo et al. (2012) found the prevalence of *E. histolytica* amongst school children in Thika District, Kenya to be 14.6% while Mamandou et al. (2010) reported an 18.8% prevalence of *E. histolytica* in school children in Agboville area in Côte d’Ivoire. Duc et al. (2009) in a study in an Agricultural community in Vietnam reported a 34.8% prevalence of *E. histolytica* amongst primary school children. This relatively high prevalence in Kyuso Zone was attributed to poor sanitary conditions within these rural areas and majorly to lack of safe domestic water.

Female pupils were more infected (42.5%) than the male pupils (41.6%) but with no significant difference. This was attributed to the fact that due to socio-cultural life style of the area, girls are more likely to interact with contaminated environment (food, water, among others) than boys (Brelet, 2000). It was also observed that the older pupils (11-12 year bracket) had a higher prevalence rate (48.1%) than the younger ones. This was attributed to the fact that being older, they are more likely to be involved in chores involving contaminants; for example, manual activities such as collecting fire wood in nearby bushes. Comparatively they also have relatively poorer hygiene measures since their
younger counterparts are washed but these ones wash themselves (Mamandou et al., 2010).

5.1.3 Morbidity rate due to *E. histolytica* in children aged 6 to 12 years amongst the patients attending Kyuso district hospital by hospital records

Children aged 6 to 12 years who were taken to Kyuso District Hospital for diarrhoeal complaints showed a morbidity rate of 46.9% for *E. histolytica*. This confirms the observed prevalence in the schools (42.1%). The high morbidity rate compared to the prevalence was probably because as was observed, many residents of Kyuso zone don’t often seek treatment for what they consider minor ailments such as stomach aches and hence those who did had higher chances of testing positive for *E. histolytica* since they were already symptomatic. The morbidity rate was noted to be higher in these ages than in adults; this was attributed to the fact that this age group has a relatively poorer hygiene and engages more in risky practices (Mamandou et al., 2010).

A study carried out by Ibrahim (2012) amongst children aged less than 12 years in an hospital in Iraq observed a prevalence of 9.8% and associated this with factors such as; low immunity against various pathogens as these age groups are comparatively less resistant to diseases, poor hygiene and toilet training, overcrowding, low socioeconomic status. Additionally, the children feel free to play anywhere irrespective of the cleanliness or dustiness of play grounds (Ibrahim, 2012). Moreover, the sale of adulterated and impure edibles also
adversely affects the health of young ones as they are likely to buy edibles on their way to and from school and they have least observance of hygiene measures on the edibles (Ibrahim, 2012).

An overall morbidity rate of 44.3% among the patients of all ages was observed in the patients who visited Kyuso district hospital with stomach complains. This was way up above the 20.32% prevalence reported by Kinuthia et al. (2012) in a study at Njoro PCEA Health Centre in Njoro District, Kenya, and 11.9% prevalence reported by Nyarango et al. (2008) in a study conducted at Kisii District Hospital in Kisii Municipality, Kenya. The high prevalence as noted is attributed to poor sanitary practices and also to lack of safe domestic water in Kyuso Zone. This was also observed by Kinuthia et al. (2012) where he identified such practices as lack of washing hands and lack of toilets as being significant to high prevalence. Dhanabal et al (2014) in a study on the prevalence of intestinal parasites in low socioeconomic areas from south Chennai, India, reported that good hand washing which ordinarily should interrupt the transmission of some of the parasites is expectedly inadequate in situations where water supply takes a lot of manual effort and the tendency is to use water sparingly. This eventually results in further transmission by direct and indirect contact.

In Kyuso Zone, the Morbidity rate by gender was 43.1% in females and 45.8% in males. Al-Malki (2014) in a study of prevalence of E. histolytica in Jeddah Saudi Arabia, reports that a higher morbidity rate in males compared to females may be
associated with the higher risks for infection in males due to daily activities carried out. It was observed that in Kyuso zone most men do manual jobs far from their homesteads where they are most likely to eat and drink water from outlets such as road side kiosks hence increasing their chances of infection. Dhanabal et al. (2014) also observed a higher prevalence in males than females but also pointed out that the infections are likely to be linked to the everyday activities of the individuals rather than gender.

5.1.4 Factors contributing to transmission of E. histolytica amongst school children in Kyuso Zone

Adoption of practices that may predispose an individual to infectious agents can promote the spread of parasitic infections amongst humans (Kinuthia et al., 2012). These include but are not limited to consumption of contaminated water and general sanitation negligence. In Kyuso zone it was observed that such practices are common among the residents and corresponded to increased prevalence amongst school children.

Use of water that is likely to be contaminated was one of such factors. Dry river bed wells and Earth dams are the main water sources in Kyuso Zone. There was a significant association between use of dry riverbed wells and earth dams and prevalence an indication that the water sources were more contaminated compared to the lower prevalence in those who used other sources such as bore holes and tap water. Since dry river bed wells and earth dam water sources are
both surface-open, they are often likely to be contaminated by human and animal excreta, unhygienic practices such as bathing and washing clothes in the water sources among other practices. Mail et al. (2011) in a study to determine the factors associated with high prevalence of intestinal parasites in Yemen identified the use of well water as a predictor of *E. histolytica* infections. Other sources where water is usually enclosed such as bore holes and tap water; there was no significant association between the use of the water and prevalence.

In Kyuso zone, significant association was found between the use of latrines and prevalence of *E. histolytica* (*P*=0.0298). This was attributed to the fact that those who had no toilets often defecated in the bushes and this led to contamination of water sources and foodstuffs such as vegetables. Most of the toilets had very low standards of cleanliness leading to many flies which occasionally settle on foodstuffs and water as reported by Kinuthia et al. (2012). Water contamination could also occur at home because of the 37.0 % who did not have their drinking water treated at home, 25% were found to be infected. A significant association was found between treatment of drinking water and high prevalence (*χ²*=11.645, df=1, *P*=0.002). Mail et al. (2011) in a study to determine the factors associated with high prevalence of intestinal parasites in Yemen reported that drinking untreated water was significantly associated with high prevalence. Another study in Malaysia to determine the prevalence of *E. histolytica* indicated a prevalence of 22.9% amongst members of an ethnic group who drank untreated water.
No significant association was observed between protection of water sources and prevalence in Kyuso Zone ($\chi^2=1.286$, df=1, $P=0.257$). However as reported by Al-Malik (2014) protection of wells and other water sources reduces chances of contamination and hence reducing infection rates. Mamandou et al. (2010) also reported in a study in Côte d’Ivoire that the unprotected water sources are usually highly polluted by human and animal excreta due to rainfall run off making them carry a population of parasitic cysts. The distance to water source was observed to be another factor that was significantly associated with prevalence ($\chi^2=3.24, 54.76, 67.24$, df=2, $P<0.05$), with 20.9% of the respondents’ households obtaining their domestic water more than 5km away. However this as reported by Mamandou et al (2010) may largely be due to the containers used and the methods of water transportation and not on the actual distance.

5.1.5 Control measures against amoebiasis in Kyuso Zone

The participation of the various groups in community education was significantly less likely to impact on the community sanitation improvement since the odds ratio (OR) >1, chi-square ($\chi^2$) >1, and 95% confidence interval (CI) was inclusive of 1. Community education was not observed to be effective because the frequency of meetings by education groups was low; they targeted the adult members of the community and were more Agriculture-based than Health-based. Health education in rural areas is necessary in order to enhance the adoption of hygienic practices and appropriate lifestyles. A study carried out in the villages of Panipat of Haryana State, India on a sample of 60 rural
school going children aged 8-10 years concluded that if a need based school health education program is developed for different age groups, it leads to improvement and practices regarding personal hygiene (Meena, 2009). Richardson et al. (2012) reported that with enhanced education programmes on sanitation and hygiene an 84% reduction in diarrhoeal diseases was realized in Bawa village in western Cameroon.

Provision of safe water to homes was going on at very low rate since tap water connection served only a small section of the population in Kyuso Town while the rest of the population in the zone relied on the other relatively unsafe sources of water. The methods of provision of safe domestic water were less non-significantly likely to impact on improvement of sanitation in the zone; Chi-square ($\chi^2$) <1, Odds ratio (OR) >1 and 95% confidence interval (CI) was inclusive of 1. Water supply is really an important risk factor for amoebiasis and several large outbreaks of Amoebic dysentery have resulted from the contamination of municipal water supplies with human wastes (Ngonjo et al., 2012). The problem is greater in the rural areas that do not have a municipal water network and sewage system such as Kyuso Zone.

The findings of this study show that infections with E. histolytica among school children aged 6 to 12 years in Kyuso Zone are high. They also show that environmental sanitation and poor access to safe water contribute to transmission of E. histolytica in Kyuso Zone; therefore the null hypothesis is rejected.
5.2 Conclusions

The results of this study are an indication that *Entamoeba histolytica* is a public health concern in Kyuso Zone. Indeed almost half of the children were infected with this pathogenic intestinal protozoon. This agrees with the hospital morbidity records. The following conclusions were therefore drawn;

i) *Entamoeba histolytica* infections are common in school children aged 6-12 years in Kyuso Zone. This is indicated by the high prevalence (42.1%) observed. Prevalence is also high in the general population as indicated by hospital morbidity records.

ii) A significant association was observed between water sources and prevalence where particularly the use of dry river bed wells and Earth dams were significant. The parasite’s prevalence decreases when tap water is used and increases when surface water is used.

iii) Age and gender variables are not relevant for prevalence of *E. histolytica*. Where some minor variations occurred in the prevalence amongst the different gender and age groups, it was associated to socio-economic behaviours.

iv) Control measures in place for amoebiasis in Kyuso Zone are not effective.

5.3 Recommendations

i) The impact of control measures would be maximized if health education programs could be formulated and directed to school children in particular and to the community in general.
ii) Urgent chemotherapy treatments should be delivered to the most infected segment of the population especially school children.

iii) There is urgent need for provision of safe domestic water in Kyuso Zone and suggestively to broaden the Kiambere piped water project to reach all the homesteads. There should also be efforts by the government agencies to fund community based sanitation projects like public toilets in all shopping centres and other public places in order to improve the level of sanitation in Kyuso Zone and environs. The general population should be encouraged to construct and use toilets. Proper use of the toilets should also be encouraged.

5.4 Suggestions for future studies

i) Whereas this study was focused on prevalence in School Children, further work is needed to establish the prevalence in the general population without reliance on Hospital records alone.

ii) Further work is necessary to establish the extend of morbidity in School Children due to amoebiasis.

iii) Studies should be carried out on the prevalence of other intestinal parasitic diseases within Kyuso Zone so as to help in laying strategies of reducing morbidity in the populations.
REFERENCES


Tengku, S. and Norhayati, M. (2011) Public health and clinical importance of


APPENDICES

Appendix I: Research tools

(a) Questionnaire

Sex:  (1) Male [  ]    (2) Female [  ]

Age:  (1) 6-8 [  ]   (2) 9-10 [  ]   (3) 11-12 [  ]

Class:  (1) 1 [  ]      (2) 2 [  ]      (3) 3 [  ]      (4) 4 [  ]      (5) 5 [  ]      (6) 6 [  ]     (7) other [  ]

(a) Where is your main source of water?  (1) River [  ]   (2) borehole [  ]   (3) tap [  ]   (4) rock catchment [  ]   (5) earth dam [  ]     (6) other [  ], specify-------

(b) How far is the water source   (1) <1km [  ]   (2) 2km [  ]   (3) 3km [  ]   (4) 4km [  ]   (5) >5km [  ]

(c) Is the water source protected?    (1) Yes [  ]    (2) No [  ]

If yes, how?    (1) Fencing [  ]    (2) other [  ], specify-------

(d)How is water at home stored?  (1) Tanks [  ] (2) jericans [  ]   (3) drums [  ]   (4) clay pots [  ] (5) other [  ], specify-----

(e) Is this water storage container usually covered?   (1) Yes [  ]   (2) No [  ]

(f) Do you treat drinking water at home?  (1) Yes [  ]      (2) No [  ]

If yes, how?  (1) Boiling [  ]   (2) Chlorine [  ]   (3) other [  ], specify---------

(g) Is there a latrine at your home? (1) Yes [  ]       (2) No [  ]

If yes, who uses the latrine?  (1) Every one [  ] (2) Adults only [  ]     (3) other [  ], specify--------

(h) How do you dispose off refuse? (1) Garbage pit [  ] (2) outside the compound [  ] (3) Dust bin [  ] (4) other [  ], specify----------
(b) Observational checklists in the Schools visited on possible factors of transmission

SCHOOL______________________________________________________________

ENROLMENT_________________________

(a) Water source (1) River [ ] (2) Bore hole [ ] (3) Earth dam [ ] (4) Tap [ ]
(5) other [ ] specify_____

(b) Water storage (1) Tanks[ ] (2) Drums [ ] (3) Jericans [ ] (4) other [ ], specify_____ 

(c) Presence of water point for washing hands for pupils (1) Present [ ] (2) Absent [ ]

(d) (i) Presence of Pupils Lavatories (1) Present [ ] (2) Absent [ ]

(ii) Number of lavatories if present (1) ≤ 4 [ ] (2) ≥ 5[ ]

(iii) Type of lavatories (1) Pit latrine [ ] (2) Flush toilet [ ] (3) Other [ ], specify_____

(iv) Cleanliness of the Lavatories (1) Very clean [ ] (2) Clean [ ] (3) Dirty [ ]

(e) System of Pupil feeding (1)School feeding [ ] (2)Pupils carry food to school [ ] (3) Other [ ], specify_________

(f) Cleanliness of the kitchen, if School feeding (1) Very clean [ ] (2) Clean [ ] (3) Dirty [ ]

(g) Pupils food storage, if they carry food from home (1) Room [ ] (2) under tree [ ] (3) Other [ ], specify______
(c) Focus group discussion guide

1. Prevalence and epidemiology in the community in general;
   - What are the common symptoms of amoebiasis?
   - How would you rank the incidents of amoebisis in the community compared to other diseases?
   - What ecological/environmental conditions lead to outbreaks of amoebiasis?
   - What risk factors are associated with transmission of amoebiasis in this community?
   - Are there education programmes targeting pupils aged 6 to 12 years in the zone?
   - Are there education programmes in the zone targeting the general population?

2. Common water sources.

3. Contamination of Water sources.

4. Methods of excreta disposal in the community/schools.

5. Control measures for amoebiasis in the Zone.
Appendix II: Consent Note

I----------------------------------- (Name of parent or legal guardian).

Having full capacity to consent for------------------------- (Name of the pupil) who is under 18 years, do hereby volunteer to participate in the research study entitled ‘Prevalence of *Entamoeba histolytica* in school children attending schools in Kyuso Zone, Kyuso District, Kitui county, Kenya’. I understand that my child will be;

- Asked questions related to knowledge and practice of personal hygiene and sanitation.
- Required to provide stool specimen for investigation of *Entamoeba histolytica*.

I have also been informed that;

- Participation is entirely voluntary and the pupil can withdraw at will at any time.
- The findings will not be given to any unauthorized person.
- There will be no financial gain or loss to me for participating in the study.

Name---------------------------------- (Pupil’s parent or legal guardian)

Signature----------------------------------

Date----------------------------------
Appendix III: Laboratory Analysis Techniques

(a) Direct faecal smear technique

Reagents and equipment: Normal saline, applicator stick, slides, microscope, Lugol’s iodine solution.

Procedure:

1. Place a drop of Lugol’s iodine on a clean slide. Emulsify a small sample of stool on the iodine using an applicator stick. Place a cover slip.

2. Place the slide on the microscope slide and examine for cysts systematically using x10 or x40 objective.

3. To examine for trophozoites apply the above procedure using normal saline in place of Lugol’s iodine.

(b) Formal-ether concentration technique

Reagents and equipment: Formal saline, centrifuge, centrifuge tubes, timer, ether, gauze, funnel, microscope, slides, applicator stick.

Procedure:

1. Emulsify a small sample of stool with 5% normal saline. Strain it through the layers of Gauze in a funnel to a centrifuge tube until 7mls are reached.

2. Add 3mls of ether to make 10mls. Mix and stopper the tube and centrifuge at 500 rpm for 2 minutes. Remove the tube and discard the supernatant.
3. Transfer a drop of the residue to a clean slide and place a cover slip. Place the slide on a microscope slide.

4. Examine the residue for trophozoites and cysts using x10 and x40 objective.
Appendix IV: A summary of *E. histolytica* cases as per Hospital records at Kyuso district hospital between April and September 2011.

<table>
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<th>Gender</th>
<th>Positive</th>
<th>Negative</th>
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</tr>
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<td>22</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9</td>
<td>24</td>
<td>33</td>
</tr>
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<td>6-12</td>
<td>M</td>
<td>11</td>
<td>16</td>
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</tr>
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<td>F</td>
<td>20</td>
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<td>39</td>
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<td>9</td>
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<td>149</td>
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</tbody>
</table>
Appendix V: Ethical Approvals

(a) Ministry of Public Health and Sanitation

MINISTRY OF PUBLIC HEALTH AND SANITATION

TO WHOM IT MAY CONCERN.

RE: DAVID MULI KAVII ID NO. 21370666

The above named is a student at Kenyatta University undertaking a research on prevalence of *Entamoeba histolytica* in Primary schools in Kyuso District.

This office is aware of his field research here and all the necessary assistance will be accorded to him from this office.

We hope the same will be extended to all the schools he visits.

Yours faithfully

THE MEDICAL OFFICER OF HEALTH

JOSEPH MUTUA
FOR DMOH - KYUSO
(b) Ministry of Education

MINISTRY OF EDUCATION

DISTRICT EDUCATION OFFICE
P.O BOX 35- 90401
KYUSO
6/7/2011

RE: PERMISSION TO UNDERTAKE RESEARCH

The bearer of this letter namely David Muli Kavili is hereby permitted to undertake research as per the attached letter.

WANDURUA G. K
FOR DISTRICT EDUCATION OFFICER
KYUSO
THE DISTRICT EDUCATION OFFICER
KYUSO DISTRICT

THROUGH
THE PRINCIPAL
KALONZO SEC SCHOOL

Dear madam,

RE: REQUEST FOR PERMISSION TO CONDUCT A RESEARCH IN KYUSO SCHOOLS

I am a Msc student at Kenyatta university taking Applied Medical Parasitology course. I request to conduct a research entitled "Prevalence of Entamoeba histolytica in school children attending Primary schools in Kyuso Zone, Kyuso District".

The study will run from June to December 2011. I promise that I will abide by all ethics and health considerations and that the results of the study will help in improving health standards in the locality.

Thanks in advance.

Yours Faithfully,

David Muli Kavili
(c) Graduate school, Kenyatta University

KENYATTA UNIVERSITY
GRADUATE SCHOOL

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Our Ref: I56/CE/13924/2009

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION DAVID M. KAVILI—REG. NO.
I56/CE/13924/2009

I write to introduce Mr. David M. Kavili who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the Department of Zoological Sciences.

Mr. Kavili intends to conduct research for a M.Sc proposal entitled, “Prevalence of Entamoeba Histolytica in School Children Attending Primary Schools in Kyuso Zone, Kyuso District, Kitui County, Kenya.”

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR—DEAN, GRADUATE SCHOOL
Appendix VI: Pictorials

(a) Water sources in Kyuso Zone

(i) Gai Earth dam near Gai primary school

(ii) A borehole near Ngaaie primary school

(iii) Water from Thunguthu seasonal river near Itulu Primary School.
(b) Microscopic observation of *E. histolytica* at Kyuso District Hospital Laboratory