PREVALENCE AND INTENSITY OF *Entamoeba histolytica* IN PATIENTS ATTENDING HEALTH CENTRES IN MATHARE SLUMS, NAIROBI COUNTY, KENYA

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School of Biological Sciences

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DECLARATION

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

Signature: …… Date: ……

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This thesis has been submitted for examination by my approval as University examiner.

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LIST OF ABBREVIATIONS

CDC ……. Center for Disease Control

DNA ..... Deoxyribonucleic Acid

ELISA ..... Enzyme Linked Immunosorbent Assay

EIA ..... Enzyme Immunoassay

GAL / GAL NAc …….. Galactose N-acetylgalactosamine

PCR ..... Polymerase Chain Reaction

PAHO ..... Pan American Health Organization

UNESCO ..... United Nations Educational, Scientific and Cultural Organization

WHO ..... World Health Organization

WORKING DEFINITION

Patient ..... persons between the ages (1 to 90 years) who attended the sampled health centres

Positive sample ..... samples infected with cysts of Entamoeba species

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DEDICATION

This research work is dedicated to my Parents and Mrs. Seklau Elizabeth Worjlor Wiles for their immense support, love and prayers.

ABSTRACT

Entamoeba histolytica being an intestinal pathogenic parasite is the causative agent of amoebiasis. It has been reported to cause acute health challenges to various individuals in developing nations, especially those living in informal settlements. This study investigated the prevalence and intensity of *Entamoeba histolytica* in patients attending health centres in Mathare slums, Nairobi County, Kenya.

Random sampling was used to collect stool samples from 800 patients in a cross- sectional study. All samples were examined by the formol-ether concentration technique and the microscopically positive samples of *Entamoeba histolytica* were further differentiated by ELISA (*E. HISTOLYTICA* II test kit). Data collected was analysed and differences in proportion were identified using logistic regression.

Based on a single stool examination, it was found that 19.5% (156/800) of the sampled population were infected with *Entamoeba histolytica,* and there was a significantly ($P < 0.05$) higher prevalence in consumers of vendor water (18.1%; 55/142) than in consumers of tap water (21.0%; 45/214). Comparison by age groups showed that 10-14 years had higher infection rates than 15 years and above (26.7% vs 18.5%; $p<0.05$).

Based on this finding that *E. histolytica* infection is present in the study area, especially amongst children, public health awareness about the disease is highly recommended, especially in schools and communities.

Key words: E. histolytica, E. dispar, Microscopy, ELISA, Kenya

CHAPTER 1: INTRODUCTION

1.1 Introduction

The genus *Entamoeba* has six protozoan species five of which are non-pathogenic and one is pathogenic. Of the six, three species including the pathogenic species are morphologically identical. The genus *Entamoeba*, comprising the commensals *Entamoeba gingivalis, Entamoeba coli, Entamoeba hartmanni, Entamoeba dispar, Entamoeba moshkovskii* and the pathogenic *Entamoeba histolytica* occur in human. Amoebae of this genus, widely distributed in both vertebrate and invertebrate animals, are characterized by possession of a vesicular nucleus with a comparatively small karyosome located at or near its centre and with varying numbers of peripheral chromatin granules attached to the nuclear membrane. Morphological differences distinguishes all species except *E. dispar, E. moshkovskii,* and *E. histolytica*. These species are morphologically identical, and of the same size range, but can be differentiated by iso-enzyme analysis, restriction fragment length polymorphism analysis, and typing with monoclonal antibodies.

To understand the true prevalence of *E. histolytica,* diagnosis of the species-complex requires methods that can distinguish the two species (*E. histolytica* and *E. dispar*) that inhabit the same region (intestine). Until recently, the species-complex referred to as *E. histolytica* was considered to infect perhaps 10% of the world's populations. Based on this complexity and the inadequacy of microscopy, the World Health Organization (WHO) in a joint statement with other organizations in 1997 stressed the need that improved methods for the specific diagnosis of *E. histolytica* infection using technologies that are appropriate for developing countries be developed (WHO, 1997). With what is now common acceptance of the genetic distinctions between the pathogenic *E. histolytica* and commensal *E. moshkovskii* and *E. dispar*, and the

finding that *E. dispar* is much more frequently encountered. The true prevalence of *E. histolytica* is perhaps closer to between 1% and 5% worldwide.

Entamoeba histolytica (Amoebiasis) occurs worldwide, however, the level of prevalence and presentation of symptoms of infection varies geographically. *Entamoeba histolytica* has been recovered worldwide, infecting approximately 50 million people annually, causing close to 100,000 deaths per year (Ravdin and Stauffer, 2005). The infection is more prevalent in the tropics and sub-tropics (area of poor sanitation and nutrition) than in colder climates. Morbidity and mortality are present in Africa, Asia, Central and South America (Petri and Singh, 2006). A study conducted in Thika District, Kenya in four public primary schools reported the prevalence of *E. histolytica* to be 19.6% in slum areas (Ngonjo *et al.*, 2012). A similar study in Bangladesh indicated that preschool children showed occurrences of *E. histolytica* associated diarrhoea each year (Benetton *et al*., 2005).

Unfortunately, the poor sanitary conditions in slum areas contribute to infection of intestinal parasites, however, very little is known about the prevalence of *Entamoeba histolytica* infection in patients attending health centres in Mathare Slums, Kenya.

1.2 Justification and significance of the study

The challenges faced by families affected by infections with intestinal protozoans especially *Entamoeba histolytica* can be related to the following important aspects: sanitation problems and the diagnostic method. Informal settlements are areas disadvantaged by broad social and health problems to children and their families due to extreme poverty and poor sanitary condition, and the prevalence, severity and risk factors associated with *Entamoeba histolytica* infection are highly associated with the level of sanitation. *Entamoeba histolytica*, the causative agent of amoebiasis is estimated to infect 50 million people annually, causing close to 100,000 death within the same year, thus making amoebiasis a global health problem. Microscopy though limited in differentiating the pathogenic *Entamoeba histolytica* from the non-pathogenic *Entamoeba dispar* remains the routine diagnostic method in developing countries rather than the additional sensitive and specific methods developed.

1.3 Main objective

To ascertain the prevalence of *E. histolytica* infection in patients attending health centres in informal settlements in Nairobi, Kenya

1.3.1 Specific objectives

- To determine the prevalence and intensity of *E. histolytica* infection in patients attending health centres in Mathare Slum, Nairobi, Kenya.
- To determine the risk factors associated with *E. histolytica* infection in this area
- To differentiate between pathogenic *E. histolytica* from non-pathogenic *E. dispar* infections.

1.3.2 Hypothesis

Entamoeba histolytica infection is widespread amongst patients attending health centres in informal settlements.

CHAPTER 2: LITERATURE REVIEW

2.1 Historical perspective

Hippocrates who may have been the first to recognize amoebiasis as a deadly disease (460 to 377 B.C.), described a patient with fever and dysentery. The *Old Testament* and Huang Ti's *Classic in Internal Medicine* (140 to 87 B.C) also made reference to dysentery (Kean, 1998). Milestones in the knowledge of *E. histolytica* and amoebiasis were (a) its description by Losch in 1873, (b) the explanation of amoebic liver abscess and colitis by Osler and his colleagues in 1890, (c) in 1961 its axenic culture was done by Diamond, and (d) differentiation of pathogenic (*E. histolytica*) from non-pathogenic (*E. dispar*) was done in 1979 (Saklatvala, 1993). In 1828 James Annesley first hinted at an association of dysentery and liver abscess. A clinical syndrome suggestive of intestinal disease was recognized in the 1800's, however the etiology was not determined then, but later a suggestion of a parasitic etiology was recorded in 1855. Fedor Losch isolated *E. histolytica* from the stool specimen of a patient with dysentery (Kean, 1988). Emetine was designated as the first effective treatment for amoebiasis in 1912 by Leonard Rogers (Rogers, 1912). Later Walker and Sellard demonstrated the infective cyst form of *E. histolytica* (Walker et al., 1913), followed by description of the life cycle by Dobell in 1925. Brumpt proposed that *E. histolytica* was pathogenic for humans. In 1978, Sargeaunt and colleagues reported that *E. histolytica* and *E. dispar* species can be differentiated using zymodeme analysis.

2.2 Life cycle and biology of *Entamoeba histolytica*

The primary hosts for *E. histolytica* are humans (Katz *et al*., 1989). Chronically infected humans are the main source of transmission. Stools infected with the cyst form of the parasite may contaminate fresh food or water. But the infection can also be transmitted by oral-anal sexual contact (Beaver *et al*., 1984). Laboratory animals including dogs, cats, and monkeys

have been experimentally infected with *E. histolytica*. These animals may also acquire human strains as a result of close contact with infected humans. Natural *E. histolytica* infections with strains morphologically similar to *E. histolytica* have been found in monkeys (Beaver *et al*., 1984). In one study, *E. histolytica* was found microscopically in stained faecal smears from six species of locally available Kenyan nonhuman primates (Muriuki *et al*., 1998). The importance of primates in zoonotic infections was studied by Jackson *et al.,* (1990) who used zymodeme analysis to investigate whether *E. histolytica* occurs as a true zoonosis. However, there are no reports of sporadic zoonotic transmission of cases between infected animals and humans, although *E. histolytica* is most commonly associated with animals.

Arthropods such as cockroaches and flies may spread the infective cysts of *E. histolytica*, indicating that these insects are capable of playing a rare but important role in transmission (Walsh**,** 1988). The life cycle of *E. histolytica* is simple. It consists of an infective cyst stage and a multiplying trophozoite stage.

2.3 Life cycle of *Entamoeba histolytica*

Cysts and trophozoites are passed in faeces. Cysts are typically found in formed stool, whereas trophozoites are typically found in diarrheal stool. Infection by *Entamoeba histolytica* occurs by ingestion of mature cysts in faecally contaminated food, water, or hands (CDC, 2010). Excystation occurs in the small intestine and trophozoites are released, which migrate to the large intestine. The trophozoites multiply by binary fission and produce cysts, and both stages are passed in the faeces. Because of the protection conferred by their walls, the cysts can survive days to weeks in the external environment and are responsible for transmission (CDC, 2010). Trophozoites passed in the stool are rapidly destroyed once outside the body, and if ingested would not survive exposure to the gastric environment. In many cases, the trophozoites remain confined to the intestinal lumen (non-invasive infection) of individuals who are asymptomatic carriers, passing cysts in their stool. In some patients, the trophozoites

invade the intestinal mucosa (intestinal disease), or, through the bloodstream, extra-intestinal sites such as the liver, brain, and lungs (extra-intestinal disease), with resultant pathologic manifestations. It has been established that the invasive and non-invasive forms represent two separate species, respectively *E. histolytica* and *E. dispar*. These two species are morphologically indistinguishable unless *E. histolytica* is observed with ingested red blood cells (erythrophagocystosis). Transmission can also arise from exposure to faecal matter during sexual contact (in which case not only cysts, but also trophozoites could prove infective (CDC, 2010).

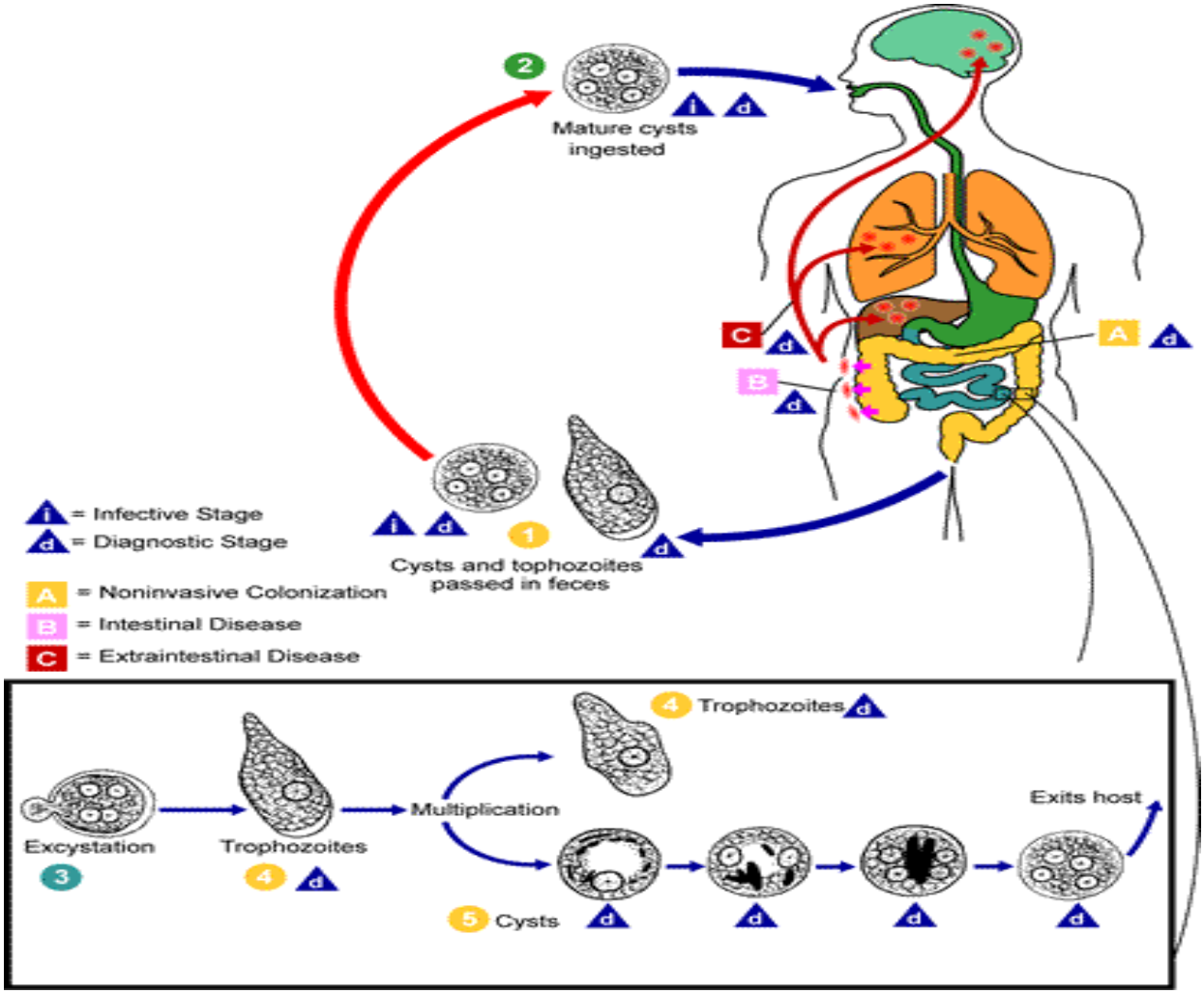


Figure 2.1: Life cycle of *E. histolytica* (CDC, 2010)

2.4 *Entamoeba histolytica* and *Entamoeba dispar* re-description

Brumpt (1925) observed that *E. histolytica* and *E. dispar* were different and proposed that they be named pathogenic and non-pathogenic species, upon which Sargeanunt *et al*., demonstrated that these amoebas could be distinguished using iso-enzyme typing and separated *E. histolytica* into pathogenic and non-pathogenic zymodemes. As such, it is possible to obtain more reliable and correct epidemiological data using the aforementioned guidelines for proper diagnosis and treatment of the disease. With microscopy alone, differentiating *E. histolytica* and *E. dispar* in stool samples is hard, but this can be achieved by using molecular tools like amoebic antigen, enzymes immunoassay (EIA) and PCR (Acuna-soto *et al*., 1993). Re-description of these species is of essence since it could enable clinicians to focus on early identification and treatment of *E. histolytica* infection in patients who are at risk and who also pose a public health problem (Reed, 2000).

2.5 Epidemiology of *Entamoeba histolytica*

The prevalence of amoebic infection, as of most enteric diseases, varies with the level of sanitation and is usually higher in the tropics and subtropics than in temperate climates. Amoebiasis is worldwide in distribution and is the third most common cause of death due to parasitic infection after malaria and schistosomiasis (Tanyuksel & Petri, 2003). *Entamoeba histolytica* is estimated to infect roughly 50 million people worldwide. An estimated 10% of the world population is infected, with higher rates occurring in developing nations where sanitation is poor (Chacon-cruz, 2009). This is expected to result in 50-100 million cases of colitis or liver abscesses per annum and up to 100,000 deaths annually resulting in a mortality rate of 1 in 500-1000 diagnosed cases (Ayeh-Kumi *et al.*, 2001).

The prevalence and presentation of both symptomatic and asymptomatic amoebiasis vary geographically and with the population of individuals affected; differing between countries and

between areas with different socio-economic conditions. The highest prevalence rates have been reported being in developing countries including Asia, Central and South America and tropical regions of Africa (Li and Stanley, 1996).

In the United States, amoebiasis is more common in immigrants and travellers from developing countries (Krogstad *et al*., 1978) and has also been reported in sexually active homosexual men (Peters *et al*., 1986). In any region, is more prevalent under crowded conditions, and may reach epidemic proportions in orphanages, prisons, and asylums. Outside such settings, in the United States, Canada, and Europe, relatively few epidemic outbreaks can usually be traced to sewage-contaminated drinking water. Most infections in these regions are due to the non-invasive species, *E. dispar*, which does not require treatment (Allason-Jones *et al*., 1986).

In Africa up to 50% of the population has been recorded to suffer from amoebiasis (Al-Harthi and Jamoom, 2007). A recent study done in Nigeria however, found 27% of school age children had *E. histolytica* infection (Reuben *et al.,* 2003). This prevalence is further supported by the study done in Kenya amongst residents of Njoro district where only 21% of patients attending Njoro district hospital tested positive for *E. histolytica* (Kinuthia et al., 2012).

In other countries of the world like Iran, a study to determine the ratio of *E. histolytica / E. dispar* infection showed prevalence of 0.7%, 3.9% and 4.6% for central, northern and southern regions, respectively (Hooshyar *et al*., 2004). Also, in a study by Aza *et al*., (2003) in Malaysia, revealed that *E. histolytica* was one of the most occurring protozoan parasites with prevalence of 21.0%. In Egypt, 38% of individuals presenting with acute diarrhoea at an outpatient clinic were found to have amoebic colitis (Stanley, 2003). Epidemiological studies have shown that low socioeconomic status and poor sanitary conditions are significant risk factors for infection of amoebiasis.

From an epidemiologic standpoint, asymptomatic patients are of utmost importance in the transmission of the disease. Cysts are relatively resistant but are killed by drying, by

temperature over 55ºC, and by super chlorination or the addition of iodine to drinking water. While contaminated water is a prime source of infection in many areas, food handlers may also play a role. The use of human faeces (night soil) for fertilizer and the contamination of foodstuffs by flies, and possibly cockroaches, may be of epidemiologic importance in some areas (Walsh**,** 1988).

A number of strains of amoebae resembling *E. histolytica* are able to survive and multiply at room temperature (unlike *E. histolytica* itself) and have been isolated from human faeces (Li and Stanley, 1996). The first such eurythermic amoeba to be isolated and grown in culture is known as the Laredo strain, now classified as *E. moshkovskii.* It has an optimum growth temperature of 25º to 30ºC and can survive at temperature from 0º to 41ºC, whereas the classic *E. histolytica* has an optimal temperature of 37ºC and can survive a range of temperatures from 20º to 43ºC (Diamond and Clark, 1993). *Entamoeba moshkovskii* is of limited pathogenicity to experimental animals and probably not pathogenic to humans. *Entamoeba moshkovskii* has been isolated from sewage plants in many parts of the world and in one study was shown to infect a substantial minority of children in Bangladesh (Benetton *et al*., 2005).

Entamoeba histolytica and *Entamoeba dispar* have been classified by various techniques such as ELISA and PCR (Pillai *et al*., 1999), which have been of great value in understanding the epidemiology of these parasites and in investigating disease outbreaks. As a result of this development, it has been reported that most of the individuals who were previously believed to have asymptomatic infection with *E. histolytica* actually carry *E. dispar,* which has never been shown to cause invasive human disease (Diamond and Clark, 1993). Furthermore, only approximately 10% of individuals who become infected with *E. histolytica* actually develop invasive disease (Gathiram and Jackson, 1987). In Sydney, Australia, a study to investigate the presence of *E. histolytica, E. dispar,* and *E. moshkovskii* in stool samples from patient population using PCR, documented a prevalence rate of 3.4% for *E. histolytica.*

2.6 Symptoms and pathogenesis of amoebiasis

The symptoms of amoebiasis has been reported in the following clinical classification (WHO, 1997).

I. Asymptomatic infections
II. Symptomatic infection
    a. Intestinal amoebiasis
        i. Dysenteric
        ii. Nondysenteric amoebiasis
    b. Extra intestinal amoebiasis
        i. Hepatic
            1. Acute nonsuppurative
            2. Liver abscess
    c. Pulmonary

Amoebic dysentery or amoebic colitis accounts for about 90% of intestinal amoebiasis. The clinical presentation of this form is mostly sub-acute and less than one month duration with symptoms ranging from mild diarrhoea to dysentery (Li and Stanley, 1996). Dysentery is characterised by an inflammatory condition of the intestine accompanied by abdominal pain and frequent stools containing both blood and mucus. Fever and systemic manifestations are usually absent and the clinical course is moderate, with symptoms disappearing with treatment. The remaining three forms are very severe and require immediate attention. Fulminating amoebic colitis consists of widespread necrotic ulcerous lesions which may perforate the peritoneum leading to peritonitis (Lucas and Upcroft, 2001).

Extra-intestinal amoebiasis brought about by haematogenous spread of trophozoites to the liver, lung, brain, skin and rarely uro-genital structures (Li and Stanley, 1996). Of these organs, the amoebic abscess is the most frequent complication and is characterised by single or multiple abscesses formed with local necrosis and liquefaction in the liver (Gene *et al*., 2004).

2.7 Treatment and prevention of amoebiasis

Leonard Rogers in 1912, prescribed emetine as the first effective treatment for amoebiasis (Rogers, 1912). If possible, a laboratory diagnosis of *E. histolytica* infection, unless confirmed by visualization of ingested red blood cells in the trophozoite, should be substantiated by serum antibody titer, presence of red blood cells in the stool, and stool *E. histolytica* antigen titer (Upcroft, 2001). Treatment varies with the clinical stage of the infection. For asymptomatic intestinal amoebiasis, treatment may not be strictly necessary, although it is perhaps imprudent to neglect such infections, which could lead to extra-intestinal disease or the infection could be transmitted to other people. Mainstays of treatment are metronidazole or the related drug tinidazole for invasive disease and paromomycin for treatment of intestinal infection. Diloxanide furoate another luminal amoebicide, is restricted to patients who only pass cysts (Stanley, 2003).

Most infections with amoebiasis are acquired through faecal contamination of food and water (Escobedo *et al*., 2003). Prevention involves measures intended to break the chain of transmission. The purity of drinking water can be achieved by disinfection through boiling. Ice cubes made with contaminated water may transmit infection, as may fruits and vegetables washed in such water. In many areas these fruits and vegetables may themselves be contaminated by the practice of using night soils for fertilizer. In most developing nations, it is best not to eat food sold by street vendors and avoid salads and fruits that you do not yourself peel.

The importance of food handlers in the spread of enteric diseases, including amoebiasis is brought to the attention of health practitioners by periodic outbreaks of hepatitis, often traced to a particular eating place and sometimes to a single employee. It stands to reason that a food handler found to have amoebiasis should not be allowed to resume that occupation until after he or she has been successfully treated.

2.8 Diagnostic methods of *Entamoeba histolytica*

Microscopy though less reliable than other specific and sensitive methods of diagnosing *E. histolytica*, remains the routine diagnostic method in developing countries. The World Health Organization (WHO) in conjunction with the Pan American Health Organization (PAHO) and the United Nations Educational, Scientific and Cultural Organization (UNESCO) in a joint statement in 1997 stressed the need to develop improved methods for the specific diagnosis of *E. histolytica* infection using technologies that are appropriate for developing nations (WHO, 1997). In diagnosis through microscopy, the presence of trophozoites that have ingested red blood cells in a stool sample strongly implies *E. histolytica* infection, but such a finding is rare (Gonzalez-Ruiz e*t al*., 1994). In the absence of haematophogous trophozoites, the sensitivity of microscopy is limited by its inability to distinguish between samples infected with *E. histolytica* and those infected with *E. dispar*, when findings have shown that *E. dispar* infection is more commonly encountered than *E. histolytica*. Despite all the issues mentioned above, the differentiation of *E. histolytica* from *E. dispar* in stool samples is the main limitation of microscopy-based diagnosis. As such, additional diagnostic methods have been developed to differentiate amoebiasis. These laboratory tests have been designed to focus on the detection of parasite antigen in the faeces by the use of monoclonal antibodies or based on the detection of parasite DNA by PCR amplification.

A stool culture method is used to diagnose the disease, which involves the culture of stool sample followed by iso-enzyme analysis (Brogstad *et al*., 1978). Obtaining results with this method may take weeks as compared to microscopy. It also requires special laboratory facilities, making it impractical for use in a developing nation. However, this method can be used to accurately distinguish *E. histolytica* from *E. dispar*.

Another method that accurately distinguishes between the species complex and which is more rapid in obtaining results is the ELISA, which works by detecting antigens in stool samples.

Several ELISA kits developed by different companies are now commercially available, including Techlab (Strachan *et al*., 1988). This test uses a monoclonal antibody against an amoebic adherence lectin that is inhabitable by Galactose N-acetylgalactosamine (Gal/Gal NAc). The lectin is conserved and highly immunogenic and because of antigenic differences between the lectins of *E. histolytica* and *E. dispar*, it can be used to identify the pathogenic species. This method is rapid and technically simple to perform, making it appropriate for use. Polymerase Chain Reaction (PCR)-based methods that amplify and detect *E. histolytica* DNA in stool samples have also been developed (Roy *et al*., 2005). The sensitivity and specificity of PCR-based methods for the diagnosis of *E. histolytica* infection both approaches those of stool culture followed by iso-enzyme analysis (Mirelman *et al*., 1997). However, field study that directly compares the PCR method with the stool culture or antigen detection methods for diagnosis of the infection suggests that these methods perform equally well.

Serological methods can also distinguish the species as well. Patients infected with the non-pathogenic strand do not develop serum anti-amoebic antibody titres, but patients with symptomatic *E. histolytica* infection develop detectable anti-amoebic antibodies, while some patients do so after recovery (Haque *et al*., 2006).This however limits the usefulness of this method for diagnostic purposes in endemic regions.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study took place in Mathare slums, Nairobi County, Kenya. Mathare is the second largest slum in Kenya, and occupies an area two miles long by one mile wide and with an estimated population of over 500,000 people (Jeffrey, 2006). Their poverty is compounded by many factors such as domestic violence, crime, drugs and alcoholism.

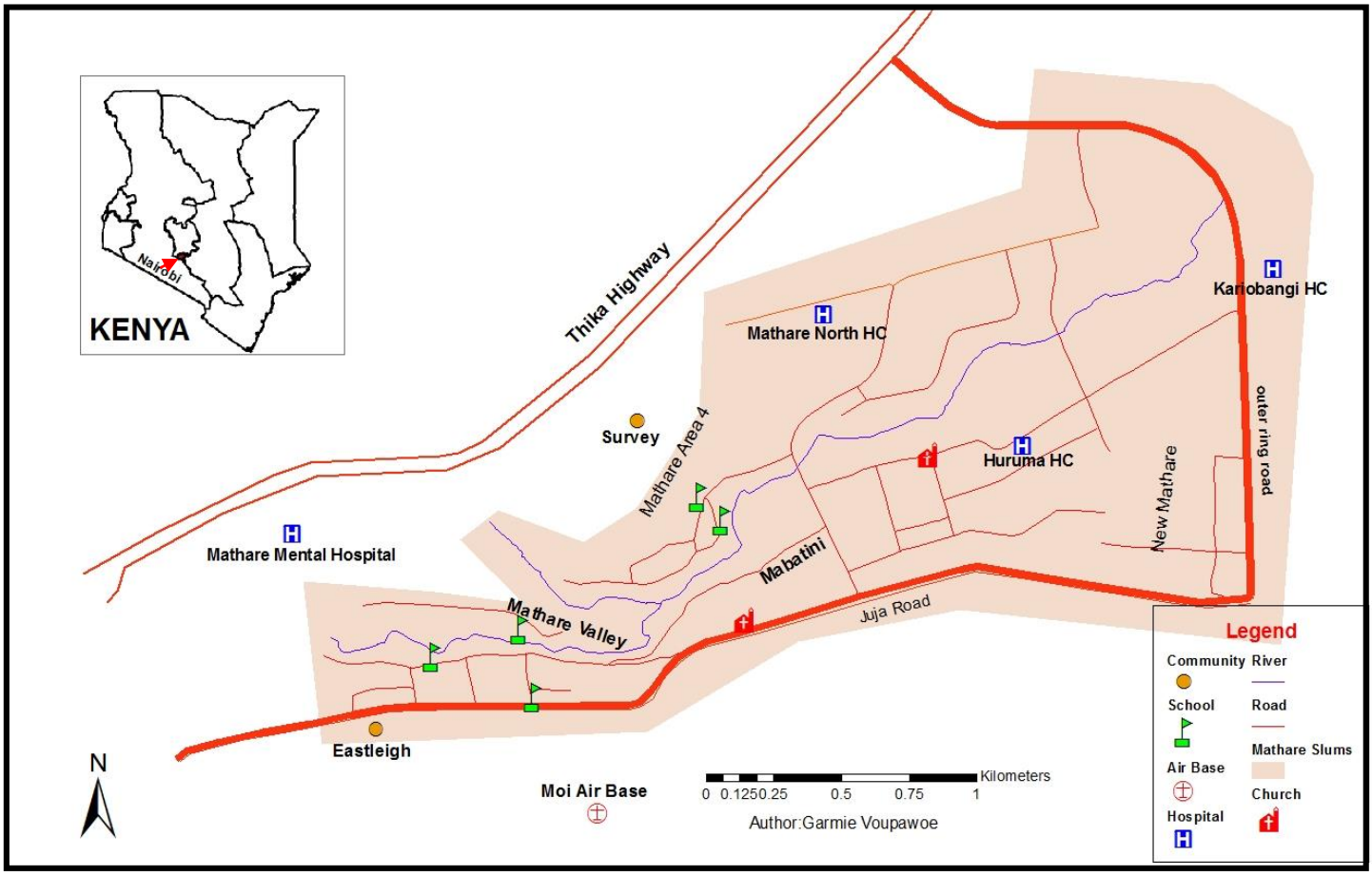


Figure 3.1: Map of the study area, Mathare slums, Kenya

3.2 Study design

The study design was cross-sectional in nature. A cross-sectional study is more appropriate to studies aimed at finding out the prevalence of a phenomenon, situation, problem, attitude or issue, by taking a cross-section of the population. However, the biggest disadvantage of this study design is, it cannot measure change. To measure change it is necessary to have at least two data collection points, that is, at least two cross-sectional studies, at two points in time, on the same population. The study designed was used to find out the prevalence and intensity of *E. histolytica* infection from a cross-section of patients visiting three health centres in Mathare slums namely: Huruma Health Centre, Kariobangi Health Centre and Mathare North Health Centre. Initial contact with the study participants was made through the health centres.

3.3 Study population

The participants in the study were from diverse ethnic backgrounds in Kenya. The study population comprises of patients between the ages of one (1) year to ninety (90) years, who visited the sampled health centres in Mathare slums, Kenya. Only consented patients were included in the study, and exclusion criteria cover patients who did not fall within the age range and did not give their consent to participate in the study. The sample size was achieved by using the formula:

$$n = \frac{P\,(100 - P)}{SE^2 + P\,(100 - P)/\,N}$$

Where:

n = sample size required
P = estimated prevalence of *E. histolytica*
SE^2 = standard error and
N = population size

Substituting the values in the formula, therefore:

$$n = \frac{20\,(100 - 20)}{2 + 20\,(100 - 20) / 500{,}000}$$

$$n = \frac{1600}{2.0032}$$

n = 798.7, approximately 800 samples

3.4 Sampling of the participants

The study was conducted in slum areas, making it difficult to use a probability sampling to select the health centres. As such, the health centres were primarily selected through quota sampling. The foremost consideration in quota sampling is the ease (convenient location) of access to the sample population. A random sampling technique was then utilized to select the eight hundred (800) study participants. This was achieved by selecting consenting patients. On a given sample collection day, patients visiting the sampled health centres were randomly approached and explained the purpose of the study and its relevance. Upon which, a verbal consent "Yes" or "No" was sought for inclusion or exclusion in the study. In cases of children, consent was given on behalf of their parents or guardians. In situations where the approached patient said "No" to participate in the study, the patient was not enlisted, and the next patient was approached in the same manner until a consent was received for inclusion in the study.

3.5 Data and specimens collections

A questionnaire was designed to capture the participant's information on age, sex and risk factors such as, source of drinking water, and accessibility toilet (Appendix-2).

Once the consented patient got enlisted in the study, only one stool sample was collected per participant presenting either with or without stomach disorder. A clean and dry screw-capped plastic bottle (polypots) for stool collection was labeled with the date and identification number matching the questionnaire of the participant. A labeled polypot and a plastic paper were given to the participants and they were allowed to take enough tissue paper for comfort. Specific instructions on how to collect the stool specimen was provided, that is, participants were told to use the plastic paper in the toilet to catch the stool. Once the stool was caught in the plastic, a portion of the stool sample (without urine in the stool) should be transferred with the plastic spoon to the polypot and screw the lid shut. They were advised to put anything used to collect the sample in a plastic bag, tie it up and put it in the bin, and afterwards wash their hands thoroughly with soap. The samples collected were transported in ice packs to the Parasitology laboratory of the School of Biological Sciences, University of Nairobi within 2-4 hours after-collection.

3.6 Processing of specimens for microscopic analysis

At the laboratory, about one gram of each stool sample was taken and processed by formol-ether concentration technique followed by iodine staining for identification of intestinal parasites through microscopy. While another portion was quickly stored in the freezer at (-20^{o}C) for ELISA analysis (Saeed and Manal, 2007).

3.7 Procedure of formal-ether concentration technique

An estimated (1 gram) of stool sample was placed in a beaker and mixed with 7 ml of 1% formal saline. A sieve was used to filter the sample into a centrifuge tube and 3 ml of diethyl ether added and shaken vigorously for 1 minute. The mixture was then centrifuged at 3,000 rpm for 3 minutes. The debris was loosened with a stick. The upper part of the test tube was cleared of fatty debris and the supernatant fluid was decanted, leaving 1 or 2 drops. The deposit

after shaking was poured on to a glass slide, a drop of Lugol iodine was then added and a coverslip was placed over it and the specimen was examined. The faecal smear was examined using the 10x and 40x objectives of the light microscope for identification and enumeration of protozoan cysts.

3.7.1 Identification of *Entamoeba* species

The description of *Entamoeba* species has depended on the parasites features such as the size of the trophozoites, cysts, the number of nuclei in the mature cyst, and the nuclear structure (Abd-Alla *et al*., 1992). *Entamoeba histolytica* is the only pathogenic *Entamoeba* species. It belongs to the subphylum Sarcodina, class Lobosea, and family *Entamoebidae*. In identification of the resistant cyst stage, trophozoites extrude all ingested material and assume a rounded form. This stage, referred to as the precyst, may be distinguished by its single rounded nucleus, absence of ingested material, and lack of a cyst wall, however, nuclear morphology is often confusing at this stage, and it is best to rely on cysts for specific identification. Mature cyst are recognized by the presence of a hyaline cyst wall. They are generally spherical but may be ovoid or irregular in shape, and they vary from about 10 to 20 um in diameter. In unstained preparations, the cyst wall is highly refractile. Cysts contain from one to four nuclei. At times the nuclei may appear as small, refractile spheres within the cytoplasm of the unstained cyst, but more often they are not visible. When stained with iodine, the cytoplasm of the cyst is a light yellowish green to yellow-brown; the nuclear membrane and karyosome are distinct and light brown. Chromatoidal bars do not stain and appear as clear spaces in the cytoplasm. If the glycogen is present in vacuoles in the cytoplasm, it stains dark yellow-brown. *Entamoeba coli* is a non-pathogenic amoeba that closely resembles *Entamoeba histolytica;* the precystics forms are seen as in *E. histolytica*, but as in that species the morphology is not very distinctive. Cysts of *E. coli* overlap the size range of *E. histolytica*, being 10 to nearly 35 um in diameter; the average diameter is definitely greater than the cysts

of the pathogenic species. The cyst wall is highly refractile and the cytoplasm granular in appearance; food vacuoles are absent. The nuclei are usually readily observed; they vary in number from one to eight. The eccentric position of the karyosome can frequently be distinguished, even in unstained amoeba. Chromatoidal bodies are less common than in *E. histolytica* but occasionally may be observed as clear, thin lines of refractile material in the cytoplasm. An iodine stain glycogen may be seen in the cysts of *E. coli*; often masses of this dark-staining material completely surround the nuclei, which are not, however, entirely obscured. From one to eight nuclei are ordinarily seen; rarely, hypernucleate forms with 16 or 32 nuclei are observed. The chromatoidals are seen to compose of splinter-shaped; heavier bodies with irregular ends are also frequently seen. The cytoplasm of *E. coli* cysts is very granular; areas occupied by glycogen before fixation are marked by empty spaces in the cytoplasm of the fixed and stained cysts.

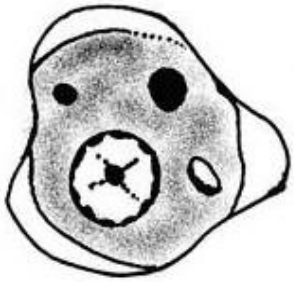
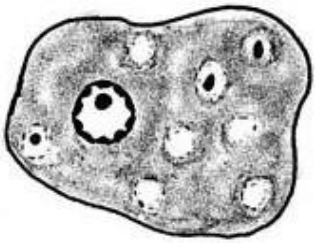
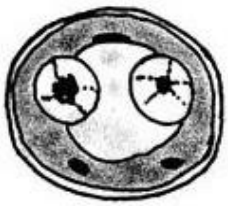
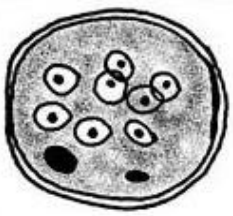
Organism	**Trophozoite**	**Precyst**	**Cyst**
Entamoeba histolytica *(Pathogenic)* *Entamoeba dispar* *Entamoeba moshkovskii*			
Entamoeba Coli			

Figure 3.2: Trophozoites and cysts of amoeba

3.8 Principle of ELISA procedure

The test uses antibodies to the adhesin. The microassay wells contain immobilized polyclonal antibody that binds adhesion of *Entamoeba histolytica/ Entamoeba dispar*. The conjugate is a monoclonal antibody-peroxidase conjugate specific for *E. histolytica.* In the assay, an aliquot of a faecal specimen is emulsified in Diluent and the diluted specimen is transferred to a microassay well. If adhesion is present in the specimen, it binds to the conjugate and immobilized polyclonal antibody during the incubation phase. Any unbound material is removed during the washing steps. Following the addition of substrate, a colour develops due to the enzyme-antibody-antigen complexes that form in the presence of adhesin.

3.8.1 Reagents

Diluent, 40 mL buffered protein solution with 0.02% thimerosal. The diluent also is to be used as the negative control solution. It has been formulated to stabilize the adhesion in fecal specimens and minimize degradation.

Conjugate, 7.0 mL mouse monoclonal antibody specific for adhesion from *E. histolytica;* coupled to horseradish peroxidase and in a buffered protein solution with 0.02%.

Substrate, 14.0 mL solution containing tetramethyalbenzidene and peroxide.

Positive Control Reagent, 3.5 mL purified adhesion from *E. histolytica* in a buffered protein solution containing 0.02% thimerosal.

20X Wash Buffer Concentrate, 50 mL 20X concentrate containing phosphate-buffered saline, detergent, and 0.02% thimerosal.

Stop Solution, 7.0 mL 0.6 N sulfuric acid. Caution: Avoid contact with skin. Flush with water immediately if contact occurs

12 Assay Well Strips, each consisting of 8 wells coated with polyclonal antibody

3.8.2 Procedure of ELISA

Specimens that were initially frozen at -20^{o}C were removed from the freezer and allowed to thaw, then all specimens were thoroughly mixed prior to performing the assay. About 0.15 to 0.02g of specimen was transferred to 400ul of diluent (buffer protein solution with 0.02% thimerosal) and thoroughly shaken to mix evenly in labelled test tubes. Three control wells which served as blank, positive and negative controls were used. Two hundred microliter of diluted specimen was transferred to the test well. The mixtures were covered with an adhesive plastic sheet and incubated for two hours at room temperature. The contents of the assay wells were then shaken out into a discard pan and washed using the diluted wash solution in a squirt bottle with a fine –tipped nozzle. The inverted plate was slapped on a dry paper towel and the washing step was repeated four times. After washing, residual liquid in the wells was completely removed. Two drops (100ul) of substrate solution were added to each well and gently tapped initially and again at 5mins to mix the substrate. This was then incubated for 10mins at room temperature. One drop (50ul) of stop solution was then added to each well. The wells were gently tapped until 2 mins before reading the addition of stop solution converted the blue colour to a yellow colour, which was quantified by measuring the optical density at 450nm (plate I) on an EIA multi-well reader.

3.8.3 Spectrophotometry

The microplate ELISA reader was set to read at 450nm and referenced/blanked against air at 630nm. The absorbance values of the positive and negative controls were determined. A sample was considered positive when the value read was higher than the negative control value (but lower than the positive control value), and negative when the value read was lower than the negative control value. A positive test result indicates that *E. histolytica* adhesion was present in the faecal specimen and was used in the analysis to determine prevalence, while a negative result indicated that *E. histolytica* was not present in the faecal specimen.

Plate 3.1: Micro wells before reading showing the blank (Bl), positive control (Pc), negative control (Nc).

3.9 Data analysis

The data collected from the study participants were keyed in Excel (Microsoft Corporation, 2010) and exported to SPSS version 20.0 for further analysis. Descriptive statistics were presented as counts, percentages, and geometric means. Intensity of cysts was computed using log (x+1) transformation, where results were expressed as geometric means of cysts per gram of faeces (cpg). Differences in proportions were determined using the Chi-squared test and logistics regression. The level of significance was established at $P < 0.05$.

3.10 Ethical consideration

Permission to conduct the study in the three health centres was obtained from the County health Services.

CHAPTER 4: RESULTS

4.1 Multiple infections of intestinal protozoa via microscopy

Amongst the participants who had protozoa infections, 11.9% (95/800) examined showed the prevalence of mixed infections as shown in *Table 4.1*. The prevalence of mixed infection in relation to health centres: Huruma Health Centre was 12.7% (48/379), followed by those attending Kariobangi Health Centre with 12.6 % (32/254) and finally those attending Mathare North Health Centre with 9.0% (15/167). Mixed infections were significantly higher in children between the age group 10-14 years old with 22.2% (30/135) infected compared to age group 15 years and above with 17.7% (106/600) and those between the ages of 5-9 years with 8.2% (5/61), ($P<0.05$). There was no significant difference in mixed infections between males and females (11.2% vs 12.7%; $P>0.05$). Those with access to toilet and those without had the prevalence rate of 11.2% (82/732) and 19.1% (13/68) respectively, the difference between the two was not significant ($P>0.05$). Consumers of vendor water showed significantly high prevalence of mixed infections with 27.9% (12/43) than those who consumed tap water with 11.0% (83/757), ($P<0.05$) as shown in *Table 4:1*

Table 4.1: The prevalence of mixed intestinal protozoa infection in the study population

		No. of tested samples	No. with no infection	No. with mixed infections		p-value
				n	%	
	Overall	800	561	95	11.9	
Health Centre	Huruma HC	379	264	48	12.7	0.784
	Kariobangi HC	254	177	32	12.6	
	Mathare North HC	167	120	15	9.0	
Age	1-4 years	4	4	-	-	0.021
	5-9 years	61	48	5	8.2	
	10-14 years	135	79	30	22.2	
	15 years and above	600	430	106	17.7	
Gender	Male	397	280	44	11.1	0.779
	Female	403	281	51	12.7	
Access to toilet	Yes	732	517	82	11.2	0.155
	No	68	44	13	19.1	
Water source	Vendored	43	27	12	27.9	0.002
	Tap	757	534	83	11.0	

Note: *$p<0.05$, significant at 95% confidence*

4.2 Prevalence of *E. dispar* and *E. histolytica*

The prevalence of *E. histolytica* and *E. coli* is shown in Figure 4.1. Two species of the genus Entamoeba were encountered in the study viz: *Entamoeba histolytica* with the overall infection rate of 19.5% (156/800) and *E. coli* with the overall infection rate of 22.3% (178/800). Across different age groups, the prevalence of both *E. histolytica* and *E. coli* were significantly high amongst participants between 10-14 years of age. The prevalence of *E. histolytica* was slightly higher in females with 20.8% (84/403) than males with 18.1% (72/397), $p>.05$. Similarly, there was no significant difference in the prevalence of *E.coli* in both males with 22.4% (89/397) and females with 22.1% (89/403), $p>.05$. Participants who had no access to toilet had higher prevalence of 29.4% (20/68) of *E. histolytica* infection than those who had access to toilet with 18.6% (136/732), $p>.05$. On the other hand, the prevalence of *E.coli* was not significantly different between those who had access to toilet with 22.0% (161/732) and those who didn't have access with 25.0% (17/68), $p>.05$. Those who consumed vendor water had higher prevalence of both *E. histolytica* and *E.coli*. Those who used vendor water had significantly higher *E. histolytica* prevalence rate of 37.2% (16/43) than those who used tap water with 18.5% (140/757), $p<0.05$.

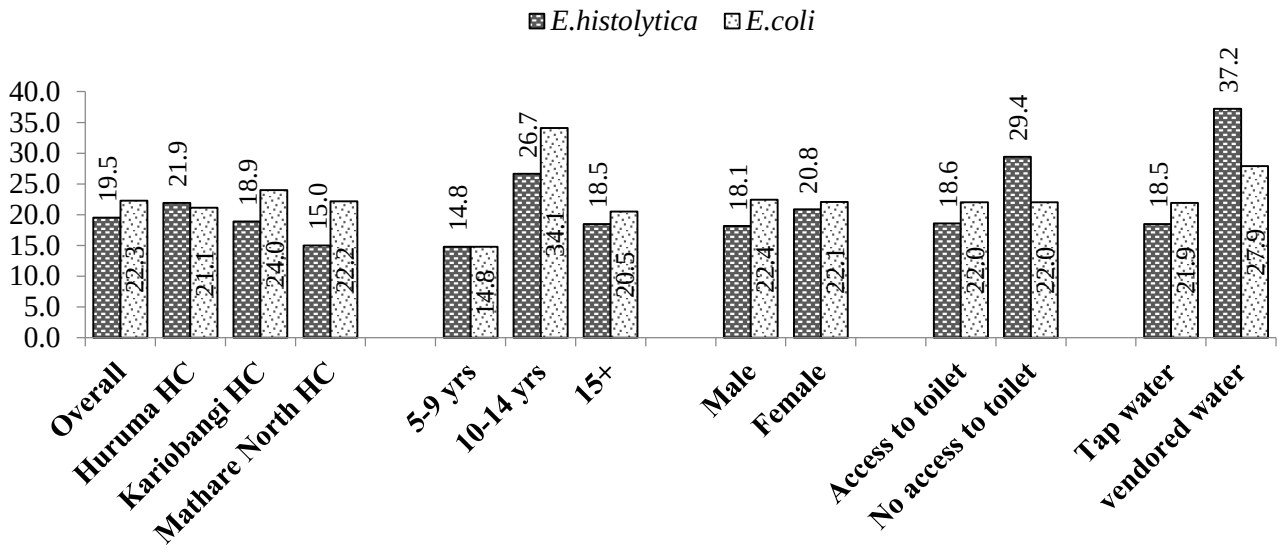


Figure 4.1: The prevalence of *E. histolytica* and *E. coli*

4.3 Intensity of *E. histolytica* in relation Health centres, age, sex, access to toilet and drinking water source via microscopy

The overall intensity for *E. histolytica* cysts was found to be 128 cyst per gram of faeces (cpg). The geometric mean parasite densities were 308 cpg, 53 cpg and 39 cpg of faeces for *E. histolytica* by patients attending Huruma, Kariobangi and Mathare North Health Centres respectively. There was no significant difference in the intensity of *E. histolytica* along age groups ($p>0.05$). However those patients who were age between 10-14 years had both high prevalence and high intensity with 137 cpg.

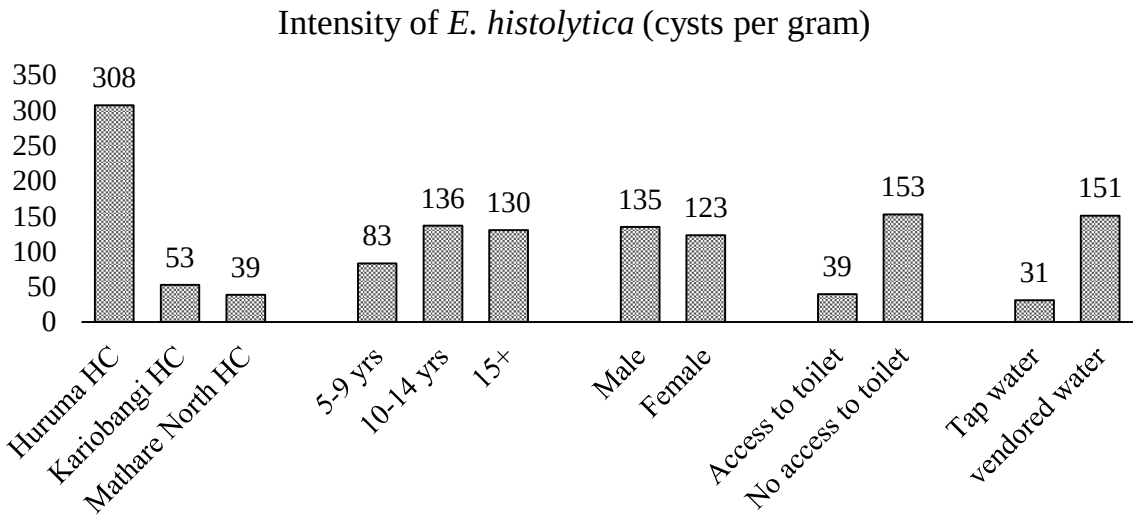


Figure 4.2: Intensity (cpg) of *E. histolytica* in relation to demographic profiles and risk factors

The intensity of the protozoan was higher in males with 135 cpg than in females with 123 cpg. The study findings indicated that those who had access to toilet had relatively low prevalence and intensity of *E. histolytica* infection. On the other hand, those who had no access to toilet showed high prevalence and high intensity with 153 cpg of the protozoan. Participants who used tap water showed low prevalence and low intensity, whereas those who consumed vendor water had high prevalence and high intensity with 151 cpg.

From the research findings, it can be concluded that there is a strong and significant positive relationship between prevalence and intensity of *E. histolytica* ($r=0.267$, $p<0.05$).This means that the higher the prevalence of *E. histolytica*, the higher the intensity of infection as measured by the number of cysts.

4.4 ELISA results for *E. dispar* and *E. histolytica*

When the 156 microscopically positive samples were subjected to ELISA, as a proportion, about 87.8% (137/156) were confirmed positive for *E. histolytica* and 12.2% (19/156) for *E. dispar*. The overall prevalence shown by the ELISA was 17.1% (137/800)

Table 4.2: showing *E. histolytica* and *E. dispar* results

Variables		Microscopically positive samples	*E. histolytica*		*E. dispar*	
			n	%	n	%
Overall		156	137	87.8	19	12.2
Health Centre	Huruma	83	72	86.7	11	13.3
	Kariobangi	48	42	87.5	6	12.5
	Mathare	25	23	92.0	2	8.0
Age	1-4 years	0	NA			
	5-9 years	9	8	88.9	1	11.1
	10-14 years	36	32	88.9	4	11.1
	15 years and above	111	97	87.4	14	12.6
Sex	Male	72	61	84.7	11	15.3
	Female	84	76	90.5	8	9.5
Access to toilet	Yes	136	125	91.9	11	8.1
	No	20	18	90.0	2	10.0
Water	Tap	140	125	89.3	15	10.7
	Vendor	16	12	75.0	4	25.0

4.5 Predisposing factors influencing the prevalence of *E. histolytica*

The occurrence of *E. histolytica* in relation to demographic profiles and risk factors was statistically evaluated using logistic regression. Children between 10-14 years of age had the highest risk levels for contacting *E. histolytica* infection as compared to those who were 15 years and above (OR=1.631; 95% CI: 1.054 – 2.524; p=0.03). However, there was no significant difference between the risk of exposure to *E. histolytica* between males and females (OR=0.816; 95% CI: 0.57 – 1.17; p>0.05). There was also no significant difference in risk of exposure to *E. histolytica* between those who had access to toilets in their area of residence than those who had no access to toilets (OR=1.273; 95% CI: 0.607 – 2.67; p>0.05). The results of the logistic regression also indicated that those who consumed vendor water had significantly higher risk of contracting *E. histolytica* than those who consumed tap water (OR=2.283; 95% CI: 0.984 – 5.294; p=0.04).

Table 4.3: Logistic regression table showing the level of prevalence of *E. histolytica* in relation to risk factors and demographic breakdown

		Odd's Ratio	95% C.I.for EXP(B)		p-value
			Lower	Upper	
Age bracket					
	5-9 yrs	0.758	0.361	1.590	0.463
	10-14 yrs	1.631	1.054	2.524	**0.028**
	15 yrs and above	1.000			
Sex	Male	0.816	0.571	1.167	0.265
	Female	1.000			
Toilet	No	1.273	0.607	2.670	0.523
	Yes	1.000			
Water source	Vendor	2.283	0.984	5.294	**0.040**
	Tap	1.000			

CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 Discussion

Results of this survey in Mathare slums indicated that the transmission of intestinal protozoan is present in the population. *Entamoeba coli, Entamoeba dispar and Entamoeba histolytica* were the intestinal protozoans observed in the study area. In this study, it was found out that prevalence rate of *E. histolytica* infection in Huruma Health Centre, Kariobangi Health Centre, and Mathare North Health Centre were 21.9%, 18.9% and 15.0% respectively. It was also found out for water source that people who consumed vendor water had higher infection rate than those who consumed tap water (37.2% vs 18.5%; $P<0.05$). At the same time it was also found out that children between the age group of 10 – 14 years had higher infection rate than those who were 15 years and older (26.7% vs 18.5%; $P<0.05$). In this study, consuming vendor water rather than piped water emerged as a risk factor that influenced *E. histolytica* infection in the population, while children between the age group of 10 -14 years were more at risk of contracting *E. histolytica* infection.

This study assessed the prevalence of *Entamoeba histolytica* in patients attending Huruma, Kariobangi and Mathare North Health Centres in Nairobi slums, Kenya. Majority of the participants were age group (15 years and above), while about 25% was age group (Less than 15 years of age). There was almost equal number of males and females which matched the population in this study.

Multiple infections were found in the study participants consisting of *Entamoeba coli, Entamoeba dispar* and *Entamoeba histolytica* which constituted about 28% of the affected subjects. Several investigations suggest that this may have far reaching effect on the health of such individuals as they may suffer from multiple morbidity associated with the disease (Booth *et. al*, 1998). This may have an effect on the type of drug to be used in the control programme.

Children experiencing heavy infections have high risk of suffering from high degrees of morbidity (Ramdath *et al*., 1995). *Entamoeba histolytica* is the most medically important species of the genius amoeba. Infection by *E. histolytica* causes amoebiasis (Okonko *et al*., 2009). In this present study, the overall prevalence of *Entamoeba histolytica* infection observed amongst the studied population was 19.5%, which was similar to results obtained in a study conducted by Omudu *et al.* (2004) who reported a prevalence of 19.1% for *E. histolytica* infection in Oguta, Imo State, Nigeria. This could be due to similarities in geographical location and socioeconomic activities of the people. However, this finding disagrees with some of the findings of previous studies done. In Nigeria, studies have had much higher prevalence rates. Okonko *et al*. (2009) reported 51.7% prevalence. Nnochiri (1965 cited in Mordi and Ngwodo, 2007) reported a value of 94.0%. The high prevalence of the protozoa can be attributed to poor sanitary practices and also the lack of safe domestic water in home. Kinuthia *et al*. (2012) identified such practices as lack of washing hands and lack of toilets as being significant to the high prevalence.

In this study, there was no significant difference in the overall prevalence of *E. histolytica* infections observed amongst the different health centres. This has been reported in other endemic communities in Busia district in Kenya (Muchiri *et al*., 2001). This could be due to the fact that the health centres are situated in the same geographical location, and apparently have similar poor sanitary and environmental challenges.

In the age-related prevalence and intensity of infections, age group (10-14 years) recorded a higher prevalence and intensity of *E. histolytica* infection compared to older age group (15 years and above) across the three health centres, the prevalence was statistically significant ($p < 0.05$). This finding is in line with Bruga *et al*. (2001) who reported that children are the most affected group with *E. histolytica* infection. It is however not surprising that the observed differences in prevalence decreases in adults. Generally children have been reported to be more

exposed to *E. histolytica* infection than adults. Reason could be that children have very active playing habits at home/school and may come into contact with food and drink that are contaminated with the infective cyst of the parasite.

In relation to sex, *E. histolytica* infection was not influenced by sex. This results agree with those from a study conducted in the central part of Turkey (Topcu and Ugurlu, 2001); that no statistically significant difference was observed between sexes and infection of *E. histolytica*. Similar findings have also been reported in the general population and school children in Nepal (Rai, 2002). This may imply that both male and female have similar predisposition to *E. histolytica* infection particularly that they share the same community and generally engage in similar activities and are hence exposed to the same hazards continually.

In this study, the prevalence of *E. histolytica* infection with respect to participants who had access and no access to toilet in their area of residence showed no significant influence on infection. This is in consonance with report of Obadiah *et al*., (2011) and Igwe (2009). The toilet hygienic and safety depends on the availability of water. Tap water is scarce in Mathare and thus most homes will tend to manage the storage. This explains why infection was common between participants irrespective of access to toilet.

The study also revealed a significant positive correlation between the prevalence and intensity of *E. histolytica* infection amongst participants who consumed tap water and vendor water, ($p<0.05$). Similar results were reported in Simbok, Cameroon (Kouontchou *et al.,* 2002) Many participants in this study claimed they used tap water for drinking. The source of some of this tap water was from broken water pipes which are highly exposed to contamination including human faeces. On the other hand, vendors of drinking water may use containers for multipurposes without periodically cleaning them. Cysts are known to persist in water for weeks or months and in the dry season, are known to withstand desiccation and survive for a

long period in the environment. Water irrespective of its source can easily be contaminated during handling, especially where sanitation and personal hygiene are generally poor.

In the current study, based on logistic regression, there was no significant difference on the risk of contracting *E. histolytica* infection between those who stated that they had access to toilets and those who didn't have the access to toilets. This may be attributed to the fact that those who had no access to toilet often used other defecation methods like flying toilets, or defecating between homes which exposed the surrounding environment to the infection. Similarly, most of the toilets had very low standards of cleanliness leading to many flies which often settle on foodstuffs and water as reported by Kinuthia *et al*., (2012). Children between the ages of 10-14 years had high risk conditions because majority of them are primary school children who share food and water in most cases. The sharing exposed them to higher risks of contracting *E. histolytica* infection.

Water source also emerged a key risk factor for *E. histolytica*. From the study, the logistic regression indicated that there was significant risk level for those who consumed vendor water than those who consumed tap water. Perhaps tap water is always treated by the Nairobi City Council making it safe for consumption. Vendor water may be liable to contamination because vendors are usually money driven and not by quality service. Due to this, they do not care about its safety to their consumers. A similar study by Mail *et al*., (2011) 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 drunk untreated water. Most often, the school going children between the ages 10-14 years are always most affected because they are very playful. They keep on sharing food and water whose cleanliness conditions are never known. As such, they create high risk conditions for contracting *E. histolytica*.

The intensity of *E. histolytica* was high (128 cpg) on average with high intensity of the protozoa realized in Huruma health centre (308 cpg) followed by Kariobangi and Mathare North health centres as seen in the results section. Again in this study, it was realized that children between the ages 10-14 years showed high prevalence of *E. histolytica* infection and high geometric mean parasite density of (137 cpg). Similar results were reported in Buea, Cameroon by Judith *et al*. (2010). This could be due to the fact that children usually eat unwashed fruits and vegetables which may be contaminated with cysts, thus facilitating their acquisition of the infections.

World Health Organization (WHO) recommends that *E. histolytica/E. dispar* should be differentiated whenever it is possible and such patients should not be treated on the basis of microscopy findings alone. Yet, regardless of symptoms, all cases presumptively diagnosed or confirmed as being caused by *E. histolytica*, should be treated to minimize the risk for progression to invasive disease. On the other hand, cases confirmed to involve only *E. dispar* should not be treated. If a patient with *E. dispar* has intestinal symptoms, a further investigative search should be made to diagnose other potential causes and in some cases treatment with drugs effective against protozoan parasites will be implemented; e.g. when no other causes are identified (WHO, 1997). A few commercial ELISA kits are available for detection of *E. histolytica,* such as the TechLab *Entamoeba* test to detect *E. histolytica/E. dispar*. *Entamoeba histolytica* and *Entamoeba dispar* have been classified by various techniques such as ELISA and PCR (Pillai et al., 1999; Verweij *et al*., 2000), which have been of great value in understanding the epidemiology of these parasites and in investigating disease outbreaks. As a result of this development, it has been reported that most of the individuals who were previously believed to have asymptomatic infection with *E. histolytica* actually carry *E. dispar*, which has never been shown to cause invasive human disease (Diamond and Clark, 1993). In this study, *E. histolytica* infection via microscopy was 19.5%, while ELISA showed 17.1%.

This however, shows that infection with *E. dispar* rather than *E. histolytica* is believed to explain, at least in part, the low rate of disease considering the high rate *E. histolytica* infection.

5.2 Conclusions

The results of this study have revealed the prevalence of *Entamoeba histolytica* to range between (17.1%) and (19.5%) among patients visiting Huruma, Kariobangi, and Mathare North Health Centres. Almost half of the children were infected with this pathogenic *E. histolytica*, making the disease a public health problem in informal settlements. This is in agreement with the hospital morbidity records. The following conclusions were therefore drawn;

i) *Entamoeba histolytica* infections are common in school children aged 10-14 years.

ii) The higher the prevalence of *E. histolytica*, the higher the intensity of infection as measured by the number of cysts

iii) Water sources is a key risk factor associated with *E. histolytica* infection

iv) Since the overall conditions of the slum areas seem similar in sanitary conditions, age and sex did not emerge to be predicting variables of *E. histolytica* infection. However, at minor situations, age played important role in influencing the prevalence of *E. histolytica.*

5.3 Recommendations

Based on the results obtained from the present study, it is recommended that public health awareness be conducted in these communities and schools on the modes of transmission, prevention and control of *E. histolytica* infection, environmental sanitations, personal hygiene and the impact of *E. histolytica*.

LIMITATIONS

Diagnosis of *E. histolytica* infection is confirmed by the recovery of the protozoan trophozoites and cysts by Parasitological techniques in the laboratory. Due to low parasites density in faeces, the specificity, sensitivity and accuracy of microscopic identification of the parasitic infection will depend on many factors including: the diagnostic technique used, the number of stools analysed, the quantity of parasites per sample and the expertise of the technician. For this study, Formol ether concentration method was used and the result was based on a single sample obtained from individual participants. Higher sensitivity of the results would have been achieved if three stool samples from each participant enlisted in the study were examined.

ELISA has very high specificity and sensitivity, but optimal results are obtained with specimens less than 24 hours old. If specimens are not assayed within this time period, they may be frozen and thawed. In this study, specimens were frozen until the entire sample collection from the field was done before ELISA examination, and by freezing and thawing multiple times, may cause specimens to lose their activity due to degradation of adhesion.

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APPENDIX1- Procedure for making 1% formal saline

Requirement:

- Lab coat
- Gloves
- Googles
- Saline
- Sodium chloride
- Distilled water
- Formaldehyde

10 millilitres of concentrated formalin plus 90ml normal saline = 0.85 sodium chloride in 100ml distilled water.

Ten percent (1%) formol saline is prepared by dissolving 8.5 g of sodium chloride in 900 ml of distilled water and mixing with 100 ml of 40 percent formaldehyde.

APPENDIX 2- Sample of the questionnaire

University of Nairobi, School of Biological Sciences

Data capture form: Prevalence and intensity of *E. histolytica* and associated risk factors amongst patients visiting health centres in Mathare slums and its environs, Kenya: Huruma, Kariobangi and Mathare North Health Centres.

Date: _____

Patient Identification number: _____

What is your current age (year)? : _____

What is your sex? F ( ) / M ( )

Do you have access to toilet in your place of residence? No ( ) / Yes ( )

What is your source of drinking water? Tap ( ) vendor ( )

APPENDIX 3- Readings from the EIA machine.

ABSORBANCE MODE

WAVELENGTHS - 450NM and 630NM

READ MODE: A to H

	A	B	C	D	E	F	G	H
1 - 1	0.060	3.150	0.091	0.061	0.055	0.049	0.104	0.051
1 - 2	0.064	0.069	0.052	0.081	**0.098**	0.054	**0.116**	0.055
1 - 3	0.056	**0.112**	0.059	**0.098**	**0.106**	**0.114**	**0.091**	**0.093**
1 - 4	0.059	0.064	**0.114**	**0.091**	0.081	0.075	0.063	**0.102**
1 - 5	**0.092**	**0.117**	**0.095**	**0.117**	0.041	**0.117**	**0.113**	0.031
1 - 6	**0.107**	**0.197**	0.062	0.054	0.049	**0.092**	**0.098**	**0.116**
1 - 7	**0.113**	**0.116**	0.065	0.061	**0.107**	0.070	**0.118**	**0.106**
1 - 8	**0.148**	**0.092**	**0.092**	**0.095**	0.086	**0.095**	**0.115**	**0.094**
1 - 9	**0.095**	**0.117**	**0.098**	**0.097**	**0.096**	0.063	**0.118**	**0.095**
1 -10	**0.098**	**0.095**	**0.095**	0.061	**0.115**	0.064	0.074	0.062
1 -11	**0.102**	**0.114**	**0.112**	**0.093**	0.042	0.050	**0.095**	**0.116**
1 -12	**0.091**	**0.117**	0.013	0.042	0.060	**0.091**	**0.113**	**0.111**

Where A : 1 -1= Blank

B; 1 — 1= Positive control

C; 1 — 1= negative control

Bold = Positive values

Ordinary = Negative values