Entamoeba histolytica infection in children in Accra: prevalence and risk factors

By

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This thesis is submitted to the University Of Ghana, Legon in partial fulfillment of the requirement for the award of MPHIL Medical Microbiology degree

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Dorcas Coffie under supervision towards the award of Master of Philosophy in Medical Microbiology in the Department of Medical Microbiology, University of Ghana.

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ABSTRACT

Background: *Entamoeba histolytica* causes amoebiasis in humans which is a major cause of morbidity and mortality in children in developing countries like Ghana where there are problems with sanitation. Studies conducted in Ghana have not placed much emphasis on the prevalence of this infection in both symptomatic and asymptomatic children in Accra. Thus, the aim of this study was to determine the prevalence of *Entamoeba histolytica* among children in Accra, and to identify some risk factors associated with infection.

Method: A cross sectional study was conducted involving two hundred and thirty-one (231) children of ages 1 day to 15 years in Accra. These included children with diarrhoea/dysentery presenting at Princess Marie Louise Children’s Hospital (PML); and supposedly healthy (asymptomatic) children from Wesley Methodist Basic School, De Youngsters International School and Agbogbloshie community. All the 231 samples collected were screened by microscopy. Ninety-two (92) out of the 231 samples were selected for ELISA analysis using Techlab *E. histolytica* II antigenic detection kit and 46 samples selected for PCR analysis.

Results: Two samples were positive for *Entamoeba* complex by microscopy. A prevalence of 0.9% was recorded. Another two samples were positive for *E. histolytica* by ELISA, giving a prevalence of 2.2%. For *E. histolytica*-specific PCR, amplification was achieved in four samples. A prevalence of 8.7% was obtained. Risk factors like age less than 5 years, the type of water drank and type of toilet facility were related to infection, although not statistically significant. Yeast cells and other enteric parasites like *Entamoeba coli, Giardia lamblia, Hymenelopsis nana, Schistosoma mansoni* and *Taenia* species were also identified by microscopy.

Conclusion: *E. histolytica* was detected among children in Accra, especially those under 5 years, using microscopy, ELISA and PCR.
DEDICATION

I dedicate this work to my mum, Mrs. Rosemary Yaa Adutwumwaa Koufie. You have been a great and loving mentor to me, my inspiration to attain higher heights in life. Thank you mummy for your provision, encouragement, love and support which has enabled me to come this far. God richly bless you and give you more years to live to enjoy the fruits of your labour.

I love you very much.
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“All the way my Saviour leads me; what have I to ask beside? Can I doubt His tender mercy, who through life has been my guide? Heavenly peace, divinest comfort; here by faith in Him to dwell. For I know whate’er be fall me, Jesus doeth all things well” (SDAH 516). Thank You so much my Heavenly Father for Your love, care, provision and blessings upon me. I could never have come this far if You were not by my side. I love you dearly and will always.

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LIST OF ABBREVIATIONS

ODH - Ohio Department of Health

WHO - World Health Organization

GSS - Ghana Statistical Service

MDPH - Massachusetts Department of Public Health

AMREF - Africa Medical and Research Foundation

UNICEF - United Nations International Children’s Emergency Fund

PML - Princess Marie Louise Childrens’ Hospital

JWMBS - John Wesley Methodist Basic School

DYIS - De Youngsters International School

AAP - America Academy of Pediatrics
CHAPTER ONE
INTRODUCTION

1.1 Background

The human intestinal lumen harbours pathogenic and non-pathogenic amoebic organisms. Non-pathogenic amoebae include *Entamoeba dispar*, *Entamoeba moshkovskii*, *Chilomastix mesnili*, *Entamoeba hartmanni*, *Entamoeba coli*, and *Entamoeba polecki*. Although *Endolimax nana*, *Dientamoeba fragilis* and *Iodamoeba butschlii* have been implicated in some diseases in humans, *Entamoeba histolytica* is considered the most pathogenic and invasive intestinal amoeba (Sateriale & Huston, 2011; Ohio Department of Health [ODH], 2014). Literally, the term ‘histolytica’ means “tissue dissolving” (Stanley, 2003) which refers to the carnivorous habit of the organism in that it is able to hydrolyse host tissue (Aribodor *et al.*, 2012) and induce programmed cell death (Knoll *et al.*, 2011). Amoebic organisms move using pseudopodia. Reproduction is by asexual binary division (Petri & Haque, 2010).

*Entamoeba* complex comprises the three *Entamoebae* with identical morphology; *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* (Regan *et al.*, 2014; Joyobrato *et al.*, 2015). Apart from trophozoites of *E. histolytica* ingesting red blood cells in an invasive disease, cysts and trophozoites of pathogenic *E. histolytica* are indistinguishable morphologically by microscopy from that of non-pathogenic *E. dispar* and *E. moshkovskii* (Ali *et al.*, 2003; Petri & Haque, 2010). This led to the recommendation given by World Health Organization (WHO) in the 1990s to develop and apply specific diagnostic methods which will enable distinguishing between *E. histolytica* and *E. dispar* (WHO, 1997). Since all diseases were known to be caused by *E. histolytica*, this reclassification did not greatly affect the existent morbidity and mortality data on the cases of invasive disease. However, the true incidence and prevalence data of *E. histolytica* in
asymptomatic infections were affected. This is due to the fact that cysts of *E. dispar* were detected in the stool of majority of asymptomatic individuals (Heckendorn *et al.*, 2002).

*Entamoeba moshkovskii* was recently identified in people living in endemic areas (Fotedar *et al.*, 2007). It was thought to be a free-living amoeba mostly found in sediments of anoxic and clean rivers, and pools at the coast of sewage and brackish water. Reports of *E. moshkovskii* human infection have come from Bangladesh, South Africa, Australia, Italy, Iran, and North America (Haque *et al.*, 1998). Thus, the epidemiology of amoebiasis has been made more complex due to the three genetically different but morphologically indistinct species by microscopy (Petri & Haque, 2010); the technique mostly used in tropical countries with limited resources (Tanyuksel & Petri, 2003). Some characteristics that distinguish *E. moshkovskii* from *E. histolytica* and *E. dispar* include its ability to grow at room temperature, resistance to emetine, and its osmotolerance. It has become necessary to investigate further, the pathogenic ability of *E. dispar* and *E. moshkovskii* since some studies in India and Bangladesh have attributed gastrointestinal symptoms with it (Fotedar *et al.*, 2007).

About 10% of the population worldwide is infected with *E. histolytica* (WHO, 2013). Those who are at high risk of being infected are immigrants, travelers, residents of institutions (Haque *et al.*, 2006; Zibaei *et al.*, 2012) and homosexuals who engage in oral-anal sex (Hung *et al.*, 2012). Asymptomatic infections (*E. histolytica* present in stool although no colitis or extra intestinal disease is present) account for about 90% of all infections (Haque *et al.*, 2006; Ximenez *et al.*, 2011). *Entamoeba histolytica* causes amoebiasis which is an invasive disease (Stanley, 2003; Sateriale & Huston, 2011) of the large intestine but sometimes can affect other viscera such as the liver, lungs, pleura, pericardium, spleen, and more rarely, to the genitor-urinary tract, brain, and
the skin (Pearson, 2009; 2012). Among the three leading parasitic cause of death worldwide, the disease is second to malaria and schistosomiasis (Pham et al., 2011).

Gastrointestinal diseases caused by *Entamoeba histolitica* are among the most important factors that contribute to childhood mortality (estimated 2.5 million deaths annually) and morbidity (Kosek et al., 2003). They create negative effects on cognitive function and growth in children (Rossignol et al., 2012) in tropical Africa where majority of the population are not privy to safe drinking water and are faced with food insecurity (Brooks, 2009). Amoebiasis also dominates especially within schools in rural and urban populations where there is a school feeding programme or where sanitary measures are not observed properly within the school set up (Ngonjo et al., 2012). Although the rate of mortality is low in developed nations, *E. histolytica* and other pathogens cause diarrhoeal diseases that lead to about 9% of all hospital admissions of children below 5 years (Brooks, 2009).

1.2 Problem statement

*Entamoeba histolytica* has serious effects on infected children, causing higher death rates among infected babies and infants (Delialioglu et al., 2004). Although this is an issue of concern, the prevalence of this infection in children in Accra is not known. Meanwhile, conditions in this part of the country are likely to foster and sustain transmission of the parasite. Having a density of 1,236 persons per square kilometer approximately, Greater Accra is more populated than any of the other regions in Ghana. Majority of the population (90.5%) inhabit the urban areas as a result of the concentration of industries and commercial activities (Ghana Statistical service [GSS], 2010). Sanitation is very poor in most of the areas, with solid wastes and faecal matter filling
drains. Where there are no drains, wastes are sometimes gathered behind or in front of houses. There is constant water shortage although the communities are serviced with pipe borne water. Sachet water which has become the city’s most consumed drinking water has also proven not to be microbiologically safe (Obiri-Danso et al., 2003; Kwakye-Nuako et al., 2007; Osei et al., 2013).

Due to the fact that amoebiasis is not often suspected in children, when a child is presented with gastrointestinal disease, *E. histolytica* is often one of the last pathogens suspected by most clinicians. Thus, by the time all other options have been exhausted and treatment has failed, the parasite would have been able to cause more harm to the child. The wrong treatment given in such instances can go a long way to causing drug resistance (Morris et al., 1996), rendering the correct treatment useless when finally given. Wrong treatment and its harmful effects on the child is also seen in the case where *E. dispar* and *E. moshkovskii* infections which account for majority of all infections and do not need treatment are mostly treated since they are mistaken for an *E. histolytica* infection.

### 1.3 Justification

Although this is not the first study to be conducted on *Entamoeba histolytica* infection in Ghana, the study is necessary to determine the prevalence of *E. histolytica* in children in Accra. The study will also serve as a baseline for studies to be conducted in other parts of the country in order to obtain the true epidemiological picture of the disease in Ghanaian children. It will also draw the attention of clinicians to incorporate as part of the initial diagnosis of childhood gastrointestinal infections, *E. histolytica* infection even among new-borns. It will also suggest to the Ministry of Health how necessary it is to bring on board specific diagnostic tools for the detection of *E. histolytica* in children with gastrointestinal infections. This is to prevent wrong treatment which
can lead to drug resistance. Individuals and communities will also be educated on the importance of maintaining good personal and environmental sanitation to prevent such infections.

1.4 Aim

- To determine the prevalence of *Entamoeba histolytica* infection and associated risk factors in children in Accra.

1.5 Specific Objectives

- To determine the carriage of *Entamoeba* complex among children.

- To identify *Entamoeba histolytica* and risk factors associated with infection.
CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology

*Entamoeba histolytica* is prevalent in parts of Africa, Indian subcontinent, the Far East, and Central and South America (WHO & UNICEF, 2013). This is not because of the tropical high temperatures and humidities of these countries; for these factors are known to destroy *E. histolytica* (Nnochiri, 1975). Rather, the higher prevalence and incidence reflect the poor personal hygiene and environmental sanitation (Hegazi *et al*., 2013) practiced by endemic dwellers, compounded by limited resources, illiteracy, and public ignorance (Ibrahim, 2008). Epidemics are rare and few clusters are reported from households and institutions in developed countries like the United States where there is good sanitation (ODH, 2014).

Although prevalent in adult (Khan *et al*., 2005), the parasite has also been reported among children (Guven, 2003). The disease was known to be rare in children under the age of five (Massachusetts Department of Public Health [MDPH], 2006), especially under a year old infants (Ajero *et al*., 2008). However, the prevalence of amoebiasis is increasing so much that, even newborns are reported to be infected (Guven, 2003). Thus, breast-feeding and different socioeconomic status might not protect infants from infection with *E. histolytica* in endemic areas (Ilikkan *et al*., 2005).
2.2 Prevalence of *Entamoeba histolytica*

Prevalence rate of *E. histolytica* infection in children has been reported to differ in many studies conducted at different locations, globally (Hamit *et al*., 2008). Differences in prevalence rates are mostly related to behavioural, physiological, nutritional and ecological factors such as low level of health facilities, sanitary (or hygienic) conditions, lack of education, low economic (income) status, and ingestion of contaminated food and water (Hamit *et al*., 2008). According to WHO (2011), although unpredictable climatic factors such as food insecurity, droughts, and floods contribute to the problem, prevalence of *E. histolytica* often relates to inadequate personal hygiene and environmental sanitation. A similar report was given earlier by the Africa Medical and Research Foundation (AMREF) in 2009 that the unavailability of safe domestic water and low education on sanitation contribute to constant transmission of the parasite amidst civil strife and rampant floods. Below are some information on the prevalence rates of *E. histolytica* recorded in Ghana and other parts of the world.

2.2.1 Ghana

Attention has not been placed on finding the prevalence of *E. histolytica* in children although some studies have been conducted in Ghana. In 2003, Verweij and his colleagues conducted a study on the prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in a rural area in Northern Ghana. Two-hundred and forty-six (246) human faecal samples were obtained from 12 villages in Bawku District. A high prevalence (98/246; 39.8%) of the *Entamoeba* complex by microscopy was recorded. Using Real-time PCR, prevalence of *E. dispar* was 82.8%. However, only one *E. histolytica* case was detected.
Another study conducted by Ayeh-Kumi et al. (unpublished data) at the Korle-Bu Teaching Hospital examined 550 stool samples from patients who visited the Central laboratory. Prevalence rates of 9.1% and 7.4% were recorded for *E. histolytica* and *E. dispar* infections respectively. At the Fevers Unit of Korle-Bu Teaching Hospital, Accra, Ayi et al. (2006) also recorded a prevalence of 13.7% among HIV/AIDS patients between the ages of 18 and 60 years. The INSTANT™ CHECK was used for the detection of anti *E. histolytica* antibodies in the serum of patients.

Haddock & Awadzi in 1970 reported for the first time in Ghana, the use of metronidazole in the treatment of invasive amoebiasis. Thirty (30) patients who attended hospitals in Accra, Secondi and Takoradi were recruited into the study. Their criteria for cure were the absence of diarrhoea or macroscopic blood in the stools and failure to find trophozoites or cysts of *E. histolytica* in saline preparations of two stool specimens at the end of treatment. Two failures recorded were both chronic cases; one with a rectal stricture who was later cured with emetine and the other, a 12-year old boy who disappeared before he could be treated further but was however seen after 6 months with no diarrhoea and *E. histolytica* in stool. These two cyst passers were asymptomatic. Undoubtedly, there is a gap in the prevalence of *E. histolytica* infection among children in Ghana which needs to be filled.

2.2.2 Other parts of the world

Mexico, Brazil, and Ecuador represent countries in Latin and Central America where *Entamoeba histolytica* is endemic. In Mexico, incidence rate of intestinal amoebiasis reported annually between 1995 and 2000 was 1000 to 5000 cases per 100,000 inhabitants and 1128.8 to 615.85 per 100,000 inhabitants from 2002 to 2006. The group mostly affected was those under 15 years of age, with increase in children aged 5 to 9 years (Ximenez, 2011). Out of the 178 children of low
socioeconomic status included in a cross-sectional study conducted in Ecuador, only 7 were found to be asymptotically infected with *E. histolytica*. However, serologic titers implying recent infection were shown in more than 64% of children (Gatti *et al*., 2002). Conditions that favoured infection included crowded living, particularly when preschool children defaecate anywhere in the living area of the family (MDPH, 2006).

Amoebiasis is widespread in Nigeria (Ajero *et al*., 2008) and has especially become an important health problem among children of school-going age (Ogbe & Isichei, 2002; Ukpai & Ugwu, 2003; Agbolade *et al*., 2004). The higher prevalence is a reflection of the poor personal and environmental sanitation, public ignorance and illiteracy of endemic villagers (Asaolu *et al*., 1992). Occupation and standard of living within the population also facilitates the acquisition of *E. histolytica* in Nigeria (Oyerinde, 1999). Several studies have been conducted among primary school children; Oke and Ogunleye (2015) found a prevalence of 67.63% (188/278) for *Entamoeba* complex in Akure, Ondo State. Amaechi and colleagues (2014) also obtained a prevalence of 16.0% (48/300) for *Entamoeba histolytica* specifically in Ukwa West Local Government Area, Abia State. A similar study conducted by Gimba *et al.* (2014) recorded an *Entamoeba histolytica* infection as 18.6% (65/350) in Abuja.

A number of studies have also been conducted in the Asian continent. Between November 1996 and March 1997, single stool samples collected from 680 children in Bangladesh and examined for *E. histolytica* and *E. dispar* infections revealed an overall prevalence of asymptomatic *Entamoeba* complex colonization as 17.3%, 4.8% and 10.4% respectively for antigen detection, microscopy and culture. A prevalence of 4.7% was recorded for *E. histolytica* infection (Haque *et al*., 2006). In an earlier conducted study, 230 children between 2 and 5 years of age were enrolled in a 2-year observational study that investigated innate and acquired resistance to amoebiasis in
Bangladesh. Within this period, 55% of children acquired *E. histolytica* infection, out of which 80% remained asymptomatic and 20% had associated diarrhoea. Also, an additional 17% acquired *E. histolytica* infections which were possibly due to a second genetically distinct strain for the serine-rich *E. histolytica* protein as was revealed by polymerase chain reaction (Haque *et al.*, 2002).

2.3 **Differentiation of amoebic organisms**

Features like the size of cysts and trophozoites, the structure of the nucleus, and the number of nuclei in the matured cyst differentiate the various amoebae (Figure 2.1). Cyst of *Entamoeba hartmanni* is round, less than 10µm in diameter and contains two nuclei. It has several chromatoidal bars with square or round ends after staining. When unstained, the cyst cannot be differentiated from cysts of other *Entamoeba* species. The trophozoites are the smallest of the *Entamoeba* trophozoites (less than 12µm in diameter) and are not round. *Entamoeba hartmanni* was formerly known as “smallrace” *E. histolytica* or a synonym of *E. histolytica* (Tanyuksel & Petri, 2003).

*Entamoeba coli* has spherical cyst with eight nuclei, peripheral chromatin and irregular karyosomes. Trophozoites have karyosomes which are large and irregular and nuclei with irregular clumps of chromatin at the periphery. Trophozoites of *Entamoeba polecki* are 15-20µm and their motility resembles that of *Entamoeba coli*. The nucleus has small karyosome at the centre and fine chromatin granules at the periphery. The cyst has one nucleus which in rare cases are binucleate or quadrinucleate (Tanyuksel & Petri, 2003). Cysts and trophozoites of *E. histolytica*, *E. dispar* and *E. moshkovkii* cannot be differentiated morphologically (Regan *et al.*, 2014).
**Figure 2.1** Comparative morphology of amoebae showing stages of cyst, trophozoite and their enlarged nuclear structure (Adapted from Paniker, 2007)
2.4 Morphological structure of *E. histolytica*

The cyst is spherical, between 10 and 15 µm in diameter, and surrounded by a double membrane with a refractile wall likely to contain chitin. At maturity the cyst contains four nuclei, chromatoid bodies (ribosomal assemblies) and glycogen. These chromatoid bodies are refringent rods which stain black with haematoxylin and are visible in fresh specimen. The cyst is resistant to environmental conditions as well as the acidic nature of the stomach.

The trophozoite is pleomorphic in shape and has a diameter ranging from 10 to 50 µm. It has an outer coating composed partly of mucopolysaccharide, undetermined anionic groups, and the lectin A concanavalin that forms an antibody complex which spreads out over the cell to conceal the protozoan. The endoplasm is dark and granular and contains many large food vacuoles, lysosomes and a thin endoplasmic reticulum but lacks mitochondria, Golgi bodies and true ribosomes. The nucleus has a two-layered membrane and is difficult to be seen in a fresh trophozoite. Chromatin is arranged in a regular fashion on the membranes and is linked like the spokes of a wheel to the central karyosome. Within the nuclear membrane are very regularly spaced pores. Intranuclear vesicles with acid phosphatase activity are typical of *E. histolytica* (John & Petri, 2006).

The trophozoite is highly motile and metabolic (MDPH, 2006). Motility is mostly progressive and directional, and seen in only freshly passed specimen. The amoeba moves by pushing out broad, rounded pseudopodia that can reach 100µ in length from the refringent, clear, peripheral ectoplasm (John & Petri, 2006). This system helps to attach the amoebae and serve the endocytosis, which is essential for the pathogenic forms. Their constant motion is fuelled by converting glucose and pyruvate anaerobically to ethanol (Reeves, 1903). It is believed that many of the metabolic enzymes of the amoeba originate from prokaryotes by the lateral transfer of bacterial genes.
Due to its inability to neither survive in the environment nor transit through the acidic condition of the stomach, it is known as the non-infectious form (Stanley 2003).

Figure 2.2   Labelled drawing of *E. histolytica*

A. Cyst; B. Trophozoite (Adapted from Ryan *et al.*, 2009)

2.5 Transmission

*Entamoeba histolytica* is transmitted when a person ingests cysts in faecally contaminated food or water (Stanley, 2003). The commonest sources of infection are licking or sucking faecally contaminated hands and fingernails (Aribodor *et al.*, 2012). The species can also be transmitted via anal sexual practices (Salit *et al.*, 2009) or through colonic irrigation devices by direct rectal inoculation (American Academy of Pediatrics, 2012). Indirect infection that occurs as a result of handling objects like rectal probes, bedding, and basins that are contaminated with cysts also occurs. Flies, cockroaches and other insects may act as vectors of cystladen faeces (Graczyk *et al.*, 2005). Patients and individuals who have relapsed or have reached the chronic phase excrete the cysts, posing high danger to other individuals (Vreden *et al.*, 2000). However, infection does not
always lead to the production of disease due to the fragility of trophozoites and the absence of cysts in dysenteric stools (Heymann, 2008).

2.6 Life cycle

Infection begins when one ingests cyst (with 4 nuclei) of *E. histolytica* in faecally contaminated water or food (Barwick *et al.*, 2002). Usually, the period of incubation in host is 2 to 4 weeks. However, it may vary from few days to months or even years (ODH, 2014). Eight trophozoites (or amoebulae) exhibiting amoeboid motility are produced as excystation occurs in the lumen of the intestines. The trophozoites colonize the large intestine by using the galactose and *N*- acetyld-galactosamine (Gal/GalNAc)–specific lectin to adhere to colonic mucins (Petri *et al.*, 2002). This is the most important phase of infection. Trophozoites feed on food particles and bacteria (Stauffer & Ravdin, 2003).

There is no sexual cycle in the reproduction of trophozoites (Ghosh *et al.*, 2000); the trophozoites grow and multiply by direct cell division (binary fission). After a certain number of divisions the amoebae lose their motility and become encysted. Encystation is likely to be triggered by amoebae aggregating in the mucosal lining and this occurs by means of the Gal/Gal- NAc-specific lectin. Cysts are shed in stool and the life cycle continues as individuals ingest faecally contaminated water or food. The cysts may be passed for periods of up to four years and can remain infective for 24 to 48 hours in a dry environment, but up to 2 or 3 weeks in moist or aquatic environments (deltas, river overspill area, lake shores, harbours, etc.). Trophozoites may also be shed in the stool but they cannot survive outside the human host (Eichinger, 2001).
2.7 Pathogenesis

The function of the intestinal mucous layer is to prevent *E. histolytica* from adhering to the underlying epithelium and to slow down the motility of trophozoites. For invasion to occur, trophozoites destroy the epithelial cells, lymphocytes and neutrophils. This become possible when the parasite lectin binds host *N*-acetylgalactosamine on O-linked cell-surface oligosaccharides (Petri *et al.*, 2002). The lectin interaction with glycoconjugates is multivalent and stereospecific (Yi *et al.*, 1998). When trophozoites penetrate the intestinal mucosal layer which functions as a barrier to invasion, colitis occurs. Immediately after the amoebic contact, a distal effector molecule in the apoptotic pathway called human caspase 3 is activated. This is necessary for destruction of cells in vitro and for the in vivo formation of amoebic liver abscess (Yan & Stanley, 2001). The amoebae resident in the intestine can enter the liver via the portal vein and lymphatic vessels to cause amoebic liver abscesses and abscesses in the brain, lungs, spleen and pleura (Stanley, 2003). According to the intensity of the damage of tissues, inflammation around well-established colonic ulcers and liver abscesses is minimal, although early invasive amoebiasis triggers intense inflammatory response (Brandt & Tamayo, 1970).
2.8 Disease presentation

Amoebiasis has been grouped into two types; intestinal and extra-intestinal (Tasawar et al., 2013).

2.8.1 Intestinal amoebiasis

This involves dysenteric and non-dysenteric amoebic colitis. Patients present with dysentery, abdominal pain and tenderness (Stanley, 2003) and weight loss. Usually, the disease manifests as diarrhoea without dysentery (presence of blood and mucus) (Lourenszen et al., 2010) which last for about three days (Petri & Haque, 2010). Diarrhoea is defined as “increased volume, fluidity or frequency of faecal discharges compared with the patient's normal stools” (Niehaus et al., 2002). Necrotizing colitis, intussusceptions, peritonitis or perforation may rapidly develop in young children with amoebic dysentery (Petri & Haque, 2010). Symptoms may be similar to those of inflammatory bowel disease. Amoebomas mostly occur in the caecum, and are usually mistaken for colonic carcinoma (America Academy of Pediatrics, 2012).

2.8.2 Extra-intestinal amoebiasis

Extra-intestinal amoebiasis which commonly involve abscess of the liver, brain or lung (Heyman, 2008) due to the haematogenous dissemination of *E. histolytica* occurs in small proportion of patients (America Academy of Pediatrics, 2012). The most common form is amoebic liver abscess usually occurring in men than in women. Some symptoms include cough, fever, and a constant, dull, aching abdominal pain in the upper quadrant or epigastrium (Petri & Haque, 2010). The liver can be infected many months or even years before diagnosing abscesses (Santi-Rocca et al., 2009). Amoebic liver abscess (ALA) may extend and/or rupture into the abdomen, chest, or brain to cause abscess (Alavi, 2007). Signs indicative of systemic infection involve fever, polymorphonuclear leukocytes and rigors (Huston et al. 1999).
Cutaneous amoebiasis is a rare kind of amoebiasis which occurs as a result of an *E. histolytica* infecting previously damaged human skin (Nagai & Frazier, 1933). Although rare, this condition has been reported as sexually transmitted amoebiasis in literature (Monhanty *et al.*, 2010).

**Figure 2.3** Clinical presentation of amoebiasis

A. Intestinal specimen from a patient with acute amoebic colitis, showing many nodular lesions; B. Macroscopic lesions in patient with cutaneous amoebiasis; C. Amoebic liver abscess (ALA) (Adapted from Espinosa-Cantellano & Martínez-Palomo, 2000; Moran *et al.*, 2013).
2.9 Laboratory diagnosis of amoebiasis and problems associated

Techniques that are currently used in diagnosing *Entamoeba histolytica* infection include microscopy, polymerase chain reaction (PCR), immunofluorescence (IFA), and serological methods like enzyme-linked immunosorbent assay (ELISA), latex agglutination and indirect haemagglutination assay (IHA) (Petri *et al*., 2000).

Diagnosis of amoebiasis for many years in Africa is mostly dependent on the microscopic (wet mount, fixed or stained smear examination) identification of trophozoites or cyst of *E. histolytica/E. dispar/E. moshkovskii*, usually reported as *E. histolytica* (Ackers, 2002) in the patient’s stool or colonic mucousa rather than molecular techniques. When haematophagous trophozoites are not present, it becomes difficult to distinguish samples infected with *E. histolytica* from that infected with *E. dispar* and *E. moshkovskii* (Ali *et al*., 2008; Heymann, 2008). Again, some studies have also reported of *E. dispar* containing RBCs in stool of some patients (Fotedar *et al*., 2007). Amoebiasis is sometimes overdiagnosed due to the presence of white blood cells (polymorphonuclear cells and macrophages) and other nonpathogenic amoeba. Dysentery caused by campylobacter or shigellosis may also be misdiagnosed as amoebic colitis by microscopy in an area where *E. dispar* is endemic (Stanley, 2003). Sensitivity of microscopy therefore becomes limited. Aside being non-specific, about half to two-thirds of *E. histolytica* colonic infections that are detected by culture is missed in microscopic stool ova and parasite examination.

For several years, the “gold standard” for diagnosis has been stool culture technique followed by isoenzyme analysis (Clark & Diamond, 2002). Faecal specimens, rectal biopsy or liver abscess aspirates are samples from which *E. histolytica* can be cultured. Culture of *E. histolytica* has been shown to be 50-70% successful in reference laboratories. Although very useful, the process is not fit for routine diagnosis in developing countries where *Entamoeba histolytica* is endemic as a result
of its sophisticated equipment and the time taken (1-14 weeks) to perform. Isoenzyme (zymodeme) analysis is costly, labour intensive, and mostly gives false-negative results for many microscopy positive stool specimens (Strachan et al., 1988).

In the developed countries where infections are less common, *E. histolytica* can be diagnosed using serological methods (Ohnishi et al., 1997). However, it is difficult to distinguish past from present infections in the developing world since people are exposed to the parasites constantly (Caballero et al., 1994). In an endemic area of Vietnam, a study conducted on asymptomatic individuals revealed that 83% of those infected had detectable anti-amoebic antibodies (Blessmann et al., 2002). Many different assays have been developed to detect antibodies to *E. histolytica* infections. With the exception of ELISA, all the other assays are either time-consuming (immunodiffusion), less sensitive and nonspecific (IHA and Latex agglutination test), requires skills in culture and antigen preparation (IFA) or costly to perform (Complement fixation) (Fotedar et al., 2007).

Molecular techniques like the *E. histolytica* antigen-specific ELISA or real-time PCR (Othman et al., 2010) are necessary to adequately diagnose *E. histolytica* in clinical samples and to provide in endemic countries, the correct epidemiology of amoebiasis (Blessmann et al., 2003; Haque et al., 2010). This also helps to avoid unnecessary treatment. Among the various techniques, the only test specific for *E. histolytica* is the TechLab (Blacksburg, VA) *E. histolytica* stool antigen detection test. Compared with culture, the TechLab *E. histolytica* stool antigen detection test has shown specificity of 90% and sensitivity of 87%. Currently, TechLab *E. histolytica* II kit (second generation *E. histolytica* II kit) which is a newer version, more sensitive and specific than the TechLab *E. histolytica* has been produced due to some disadvantages observed with the former; using PCR, samples that may be positive for *E. dispar* may sometimes give false-positive outcomes (Furrows et al., 2004) due to cross-reactivity (Roy et al., 2005; Visser et al., 2006).
TechLab *E. histolytica* II kit is more sensitive and specific than real-time PCR (Roy *et al.* 2005). Development of *E. dispar* and *E. moshkovskii* antigen detection kits are in progress (Samie *et al*., 2012).

Colonoscopy is more effective for the diagnosis of amoebic colitis than sigmoidoscopy as the disease is mostly restricted to the ascending colon or caecum. There may be difficulty in identifying *E. histolytica* when enemas or cathartics are used in the preparation of the patient. Diagnosis of amoebic abscesses involves the use of MRI, CT and ultrasound liver studies but is not able to differentiate amoebic from pyogenic abscess. When an infection is suspected to have spread beyond the intestine, a blood test is recommended (Visser *et al*., 2006).

### 2.10 Treatment and Management

Treatment of amoebiasis is based on eliminating from the lumen of the intestines and tissues, cysts and trophozoites of *E. histolytica*. The drug of choice is the nitroimidazole derivatives which are metronidazole, ornidazole and tinidazole (Stanley, 2003). Metronidazole is a 1-(2-hydroxyethyl)-2-methyl-5- nitroimidazole drug which stops the growth of parasitic and anaerobic bacteria infections (Micromedex, 2006). It is the most effective drug used in treating *E. histolytica* infections; severe, moderate, mild intestinal symptoms or liver abscesses. The drug is administered orally. However in severe cases, it is given intravenously. The toxicity is low (Tanyuksel *et al*., 2005) and it is ineffective against cysts (American Academy of Pediatrics, 2012). Metronidazole (or tinidazole) is followed by diloxanide furoate, iodoquinol or paromomycin (luminal agents) (Gonzales *et al.* 2009). Since diarrhoea is a usual side effect when paromomycin is taken, it is not advisable to administer metronidazole and paromomycin at the same time so that assessment of the patient’s response will not be impaired (Petri & Haque, 2010). Adequate consultation must be
done before treating pregnant women. In the first trimester, while there is no evidence for teratogenicity, metronidazole is generally contraindicated (Heymann, 2008). Alcohol consumption must be forbidden during treatment because of the drug’s disulfiram (Antabuse) effect. Alternatively, a patient with invasive disease whose treatment failed or is not tolerated is given a luminal amoebicide after dehydroemetine.

Another drug effective for treating liver abscess is chloroquine phosphate administered alongside metronidazole (or tinidazole). Luminal amoebicide can also be given after dehydroemetine if need be (American Academy of Pediatrics, 2006). There is complete and rapid healing of the bowel and usually between 8 months and 2 years, hepatic abscesses disappear (Ximenez et al., 2011). Luminal amoebicides like iodoquinol, paromomycin, or diloxanide are used in treating asymptomatic carriers (cyst excreters) (American Academy of Pediatrics, 2012). Thus, patients are prevented from developing invasive disease and as well transmitting the infection to other people (Heymann, 2008). Tetracyclines, neomycin, spiramycin, and other antibiotics are useful only in serious forms of the disease like fulminant amoebic colitis requiring direct action on the intestinal flora. If amoebae are not completely eradicated, recurrence is common (Ximenez et al., 2011).
2.11 Control and Prevention

Good sanitation both at the environmental and personal level is necessary in preventing and controlling amoebiasis. The general public must be educated on personal hygiene especially washing of hands thoroughly with warm water and soap before eating or handling food, after visiting the toilet and after changing diapers. Proper disposal of sewage and regular cleaning of bathrooms and toilet is also important. The food and water taken in must be safe as well; raw vegetables must be properly washed and cooked before eating (Pearson, 2009).

Infection is usually difficult to control in institutions like the mental home and daycare setting. Cases should be carefully followed to avoid transmission of the parasite to other people. A child who develops acute diarrhoea should be separated from the other children in order to avoid contact. It is necessary to examine a child with bloody stools and antimicrobial therapy given before admission. Since children may not show symptoms but continue to excrete cysts in stool, the frequency in hand washing must be increased. Any child attendant who becomes infected must not be allowed to care for the children until there is no more diarrhoea and tests for *Entamoeba histolytica* infection in three follow up stools is negative (Heymann, 2004).

No vaccine has been developed yet but with the advancement in knowledge, it is hoped that a vaccine will be developed in few years to come (Parija, 2011) to prevent mortality and decrease morbidity in developing nations.
2.12 Mortality rate

Deaths due to amoebiasis vary; rate of mortality in complicated disease (like fulminant amoebic colitis, cerebral amoebiasis or chest involvement) is much higher than in uncomplicated disease (which is less than 1%). Liver abscess records 1% mortality rate when it is uncomplicated but greater than 15% when complicated. There is about less than 20% in pleurisy, near 40% in pericarditis, greater than 50% in fulminant amoebic colitis and when the brain is infected, greater than 90% (Stanley, 2003). There is high mortality recorded in untreated cases of invasive amoebiasis (Nesbitt et al., 2004). Among infected babies and infants, higher death rates are common (Delialioglu et al., 2004). Those who take adrenocortical hormones and show bad prognoses are patients with malnutrition, pregnancy and malignant tumours (Stenson et al., 2001).

2.13 Risk factors associated with E. histolytica infection in children

A number of risk factors have been associated with E. histolytica infection in children although the role each plays has not been fully established. Disposing off wastes illegally close to human settlement, improper hand washing, use of open type latrines, unsafe drinking water, ingestion of raw vegetables, residence, age less than one year and male gender has been reported to contribute massively in increased diarrhoeal and amoebic dysentery cases (Benetton et al., 2005; Ademiluyi & Odugbesan, 2013; Moustafa et al., 2013).

Age plays an important role in the prevalence rate of E. histolytica infection (Zahida et al., 2013). In 1961, Chandler and Read reported that infection in infants (under a year old) is rare; there is gradual increase in incidence from childhood to young adulthood. The rate of infection is highest between the ages 5years and 40years (WHO, 1997). However, Kinuthia and his colleagues in 2012
reported that children are often infected than adults as a result of their less developed immunity and poorer hygiene practices.

Some studies conducted found no association between sex and infection. Any differences observed were probably related to exposure rather than susceptibility due to sex (Rivera, 1972; Simon-Oke & Ogunleye, 2015). However, some studies report that males are more infected than females (Gimba et al., 2014; Amaechi et al., 2014). Some reasons given for the high prevalence in males include the fact that they engage in outdoor activities like farming, hunting, fishing, etc. which increase their exposure to *E. histolytica* infection. Other researchers have also reported of high prevalence in females than in males. Although females are usually more immune to parasitic infections, various stages of female reproductive cycle give room for opportunistic parasites to establish themselves as hormonal fluctuations occur and sometimes do affect immunity (Mazigo et al., 2010). Immunity in the expectant mother is also decreased, increasing the risk of parasitic diseases (Roberts et al., 2001). Women are also usually exposed to water-borne diseases due to the fact that they do most of the cooking and washing of clothes in households (Jamieson et al., 2006).

Bottle feeding as well as inadequate exclusive breastfeeding displays a significant risk factor for acute gastroenteritis (GE) among breastfed babies (Moustafa et al., 2013; Amal et al., 2015). Jamila (2014) reported a high prevalence of 52.6% for non-breast-fed babies and low prevalence of 9.1% for babies who were breastfed. Low prevalence in breast-fed babies was attributed to the fact that colostrum and breastmilk have lethal effect on *E. histolytica*, thereby preventing disease (Heird, 2010).
The source and type of water used domestically and for drinking plays a major role in *E. histolytica* transmission. As a result of poor sanitation, contamination is likely to occur at the source of water or at home causing many tropical developing countries to lack adequate supply of clean domestic water (United Nations Children Fund [UNICEF], 2009). The availability and usage of toilet facility is also key in transmitting *E. histolytica*. Defecating openly can cause the washing down of *E. histolytica* cysts into water bodies or contaminate food and sources of water when carried mechanically by vectors like flies (Cheesebrough, 2005). In a village in Côte d'Ivoire, a prevalence of 21% was recorded in places where toilets were lacking or were not used despite their existence. Cyst spread was favored by the inhabitants’ refusal to use their available toilet facilities (Mamadou *et al.*, 2010). In relation to type of toilet used, pit toilet users recorded the highest infection (88.89%) in the work conducted by Simon-Oke and Ogunleye (2015). Bush users had the second highest prevalence (77.78%) and the least (55.29%) among users of water closet. Likewise in Vietnam, a higher prevalence was found among users of single vault latrines (Duc *et al.*, 2009).

With respect to prevalence and the type of community sampled, Gimba *et al.* (2014) as well as Amaechi *et al.* (2014) in their reports stated that there was no association (P>0.05). However, Amal and his colleagues (2015) identified *E. histolytica* and other enteropathogens in their study which were significantly prevalent in the low socio-economic group, with high prevalence (50%) among patients coming from the north.
2.14 Case Studies

A 4-month old baby boy was sent to the Lorestan University of Medical Sciences Hospital in a remote district in Khorramabad, Southwest Iran. The baby could not feed properly, his stool texture had changed, he was vomiting, had hyperactive bowel sound, the abdomen had distended mildly and had episodes of retching. The mother had earlier on suffered diarrhoea and abdominal pain. By using Lugol’s iodine solution to directly examine fresh faecal samples, *E. histolytica* cysts as well as trophozoites with erythrocytes and several white blood cells were identified. Metronidazole syrups (35–50 mg/kg/BW/day) and oral rehydration salt (ORS) were given to the infant for 5 days. There was an improvement in his health afterwards and he started feeding normally with breast milk. No parasite was seen after a repeat wet mount and iron haematoxylin technique were performed. After seven days, his condition stabilized and he was discharged (Zibaei *et al.*, 2012).

In India, a 14-day old full term girl was born to a primigravida in an endemic region. The baby was brought to the hospital with history of mucoid stools although the mother delivered normally through the vagina. After evaluation of the possible source of infection, the kind of water supply was found to be the cause since the child was given pipe-borne water soon after she was born. She weighed 2.7 kg and her vital signs, chest, skin and central nervous system, urine and chest radiographs were normal upon physical examination. However, she had distended abdomen with decreased bowel sounds but no sign of organ enlargement. Abdominal radiographs showed distended loop gas in the colon. Fresh stools were collected and sent immediately to the laboratory for examination. *E. histolytica* was identified by concentration technique. Occult blood was positive with many white blood cells and *E. histolytica* trophozoites but blood culture was negative. The baby was not given anything orally due to the mucoid stools, decreased bowel sounds, abdominal distension and stool occult blood. She was given fluids with vitamin K and
antibiotics. From the fifth day of hospital stay, tinidazole administered intravenously was used for treatment. On the 10th day, the contour of her abdomen became normal and stool examination repeated was negative for occult blood, white blood cells and *E. histolytica*. On the 25th day, the patient was discharged. There were no complications and relapse when she was followed up for 3 months (Pushpendra, 2010).

A one-day old female who weighed 2.9kg, born at 38 weeks of gestation by cesarean as a result of rupturing of the amniotic membrane prematurely was presented with dysentery and currant jelly-like vomiting after birth in Korea. The baby seemed to be infected before being born. The mother suffered abdominal pain and diarrhoea seven days before delivery. She ate swine intestines stuffed with rice and vegetables. Although the symptoms persisted before delivery, she had not being treated. The baby had soft and flat abdomen with no evidence of organomegaly. Her vital signs as well as urine test conducted were also normal. Apart from gas in the stomach and colon, radiographs of the abdomen did not detect any other sign. Occult blood with several white blood cells was seen upon examination of her stool. Antibiotic infusion and vitamin K was administered to her but 6 hours after being born, the bloody mucoid stool and vomiting still persisted. Trophozoites of *E. histolytica* were identified three days after birth when the stool was examined. Trophozoites were again seen when gastric juice was aspirated and examined 6 days after birth. Treatment with metronidazole was successful; her stool became normal after 9 days and result was negative for occult blood, *E. histolytica* and leukocytes. Within 24 days, the patient was discharged (Kahng & Kim, 2007).
2.15 Genetic characteristics of infecting strains in pathogenesis

The population structure of *E. histolytica* strains as well as virulence and disease outcome of the parasite have been studied; only few polymorphic genetic loci (genetic markers) have been targeted and identified (Clark, 2006; Paul *et al*., 2007). Among these are protein coding genes [serine – rich *E. histolytica* protein (SREHP) and chitinase] and non-coding DNA [strain specific gene and tRNA gene linked short tandem repeats (STR)] of PCR-amplified genes (Haghighi *et al*., 2003; Samie *et al*., 2008). The SREHP gene contains repetition of sequence motifs (Stanley *et al*., 1990) with isolates showing differences in their genes (Clark & Diamond, 1993). In a study conducted by Ayeh-Kumi *et al*., 2001, 54 isolates from children in Dhaka, Bangladesh were identified in 34 distinct SREHP polymorphisms. Eighty-six percent (86%) of individuals develop mucosal IgA response against the GalNAc, although such genetic variation exist (Haque *et al*., 2001; 2002). Some studies have also indicated that the SREHP marker must have caused symptoms of the intestine (Ayeh-Kumi *et al*., 2001; Samie *et al*., 2008). However, all studies conducted on the SREHP marker reported of differences among isolates of *E. histolytica* with respect to the geographic location (Ayeh-Kumi *et al*., 2001; Samie *et al*., 2008; Simonishvili *et al*., 2005; Tanyuksel *et al*., 2008).

Another cell surface protein, the galactose (Gal) and Nacetyl-D-galactosamine (GalNAc) lectin is a heterodimer of 170 kDa (heavy) and 31/ 35kDa (light) subunits which are noncovalently associated with a 150kDa (intermediate) subunit (Petri *et al*., 2002). The Gal/GalNAc lectin is an important virulence factor for the parasite adherence and invasion into host. The Carbohydrate Recognition Domain (CRD) is located within the lectin heavy subunit cysteine-rich region (Dodson *et al*., 1999) which upon vaccination, prevent amoebiasis in animal models. In humans, acquired immunity is associated with secretory IgA responses (Haque *et al*., 2001). Ali *et al*.
(2007) in their study in Bangladesh observed that the parasite genome influences the outcome of infection. This is because the tRNA-linked STR genotyping caused the differences between parasite genotypes in the intestine and the liver abscess of same patients.

2.16 Host immune response to \textit{E. histolytica}

To maintain intestinal homeostasis, mucosal immunoglobulins (Ig) play important role in defending the gut (Lamm, 1997). Among the Ig that the plasma cells within the lamina propria produce is the secretory IgA (sIgA) which prevents the adherence and breaching of mucosal barrier by pathogens (Lamm, 1998). Thus, responses of the anti-Gal-lectin IgA in the mucous are very essential in preventing colonization and invasion of the \textit{Entamoeba} (Abd-Alla \textit{et al}., 2006).

Although IgA titres serve a protective role, some studies also suggest that IgG plays the opposite role (Kaur \textit{et al}., 2004). In a two-year study in Bangladesh, children with anti-amoeba IgG antibodies in their serum developed 37\% more current and severe \textit{E. histolytica} infections when compared with children who did not have anti-amoeba IgG (Haque \textit{et al}., 2002).

Another important protective response is the cell-mediated immune response (Guo \textit{et al}., 2011). Asymptomatic carriers of \textit{E. histolytica} have increased levels of interferongamma (IFN-\(\gamma\)) which is the response of a T helper (Th) 1. However, a higher level of of IL4 which resembles response of a Th2 is displayed by patients having invasive amoebiasis (Sánchez-Guillén \textit{et al}., 2002). When macrophages were treated with IFN-\(\gamma\), a high amoebicidal activity in an in vitro study was induced (Denis & Chadee, 1989).

By the time it gets to the chronic phase, the host immune response would have been evaded by \textit{E. histolytica} in many ways. The Gal/GalNAc-specific lectin is similar in sequence and cross-reacts
antigenically to a human leukocyte antigen (CD59), which is responsible for the prevention of the complement C5b–C9 membrane attack complex assembly (Braga et al., 1992). The complement anaphylatoxins C3a and C5a are frequently destroyed by the cysteine proteinases of E. histolytica (Reed et al., 1995). Secretory IgA and IgG in the serum are also degraded by the cysteine proteinases which may prevent the opsonization of the amoebae. The respiratory burst of macrophage and presentation of antigen by class II major-histocompatibilitycomplex (MHC) molecules are also suppressed by E. histolytica.
2.17 Pathogenic organisms associated with *E. histolytica* infection

Some enteric pathogens have been identified as associated with *E. histolytica* infection in some studies conducted. Among these are viruses, bacteria, and parasites (Table 2.1).

<table>
<thead>
<tr>
<th>RESEARCHER</th>
<th>PATHOGENS</th>
<th>LOCATION</th>
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<td>Nyantekyi <em>et al.</em>, 2010</td>
<td><em>Schistosoma mansoni, Trichurus trichiura, Ascaris lumbricoide, hookworm, Hymenolepis nana</em></td>
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<td>Ayalew, 2006</td>
<td><em>Giadia lamblia</em> and <em>Cryptosporidium parvum</em></td>
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<td>Amjed <em>et al.</em>, 2012</td>
<td><em>G. lamblia</em></td>
<td>Iran</td>
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<td>Petri &amp; Singh, 1999</td>
<td><em>Shigella dysenteriae</em> and <em>Shigella flexneri</em></td>
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<td>Moustafa <em>et al.</em>, 2013</td>
<td><em>Adenovirus, Shigella, Salmonella</em>, Campylobacter and <em>E. coli, G. lamblia, Ascaris lumbricoide, Trichinella spiralis and Strongyloides stercoralis</em></td>
<td>Jeddah, Saudi Arabia</td>
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<td>Amal <em>et al.</em>, 2012</td>
<td><em>Salmonella species, enteropathogenic Escherichia coli and Shigella</em></td>
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<td>Samie, 2012</td>
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<td>Nwanguma &amp; Alumanah, 2008</td>
<td><em>Plasmodium falciparum</em> and <em>G. lamblia</em></td>
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CHAPTER THREE
MATERIALS AND METHODS

3.1 Study design

Between May and August, 2017, a cross-sectional (prevalence) study was conducted. Stool samples were collected from children of ages 1 day to 15 years.

3.2 Study site description

This study was conducted within two districts; Ashiedu Keteke and Ayawaso Central in the Greater Accra region of Ghana.

3.2.1 Ashiedu Keteke district

The Ashiedu Keteke sub metro district is the smallest in size of the ten sub Metropolitan districts of Accra. It is an indigenous Ga community. The people of this district are mostly traders, although other forms of occupation like fishing, fish mongering and food vending also exist. The district has a total population of about 117,525 individuals, forming 3.5% of the total population of Greater Accra region (GSS, 2012). In the Ashiedu Keteke sub metro, Princess Marie Louise Children’s Hospital (PML), Wesley Methodist Basic School (WMBS) and Agbogbloshie community were the sites selected for sample collection.

Princess Marie Louise Children's Hospital is a Ghana Health Service institution. The facility provides medical care services, pursues diseases control and offers reproductive and child health (RCH), family planning (FP) and nutrition services (Raymond, personal communication, May 16, 2016). PML was selected because it is a point of call for most children from all walks of Accra.
who suffer various infections, and therefore where most gastrointestinal infections in children are likely to be reported.

Wesley Methodist Cathedral Basic School is located at Palladium, a suburb of Accra. Currently, the population of pupils at the KG, primary and JHS are 141, 633 and 148 respectively (Elsy, Assistant headmistress, personal communication, May 17).

Agbobloshie is often known as “Africa’s largest e-waste dump”. The area is densely populated due to migration from rural parts of Ghana to Accra (Koranteng & Darko, 2011; Brigden et al., 2008; Stein, 2013). Most people use public toilet facilities which include water closets and pit latrines for commercial use.

![Figure 3.1](http://ugspace.ug.edu.gh)

**Figure 3.1** Map of Ashiedu Keteke district (Google map data 2017)
3.2.2 Ayawaso Central district

The Ayawaso Central district forms a major part of the Accra Metropolis. The vast health problems recorded in this district is as a result of the high/dense population of people despite the small area covered. The total population is about 142,322, forming 4.6% of the total population of Greater Accra region (GSS, 2012). In the Ayawaso Central district, De Youngsters International School (DYIS) was the only site for sample collection. Although hospitals like Maamobi Government hospital and Mallam Atta Market hospital were initially included, it was difficult to obtain diarrhoea samples from children due to the scarcity of such cases and the fact that the facilities see mostly adults.

De Younsters International School is located at Kokomlemle. It was established in 1980 and has about five (5) branches in Accra with KG, primary and JHS facilities and over 3000 student population (Donkor, Assistant headmaster, personal communication, May 19).

![Map of Ayawaso district](http://ugspace.ug.edu.gh)

**Figure 3.2** Map of Ayawaso district (Google map data 2017)
The two schools were selected based on the fact that one (De Younsters International School) is an international school where most pupils hail from moderate to high economic homes whereas the other (Wesley Methodist Basic School) is a government institution where most pupils are from moderate to poor economic homes. This was to help compare the prevalence of *E. histolytica* with respect to the economic status in the country.

### 3.3 Study population

Children of ages 1 day to 15 years were recruited into the study. Research subjects were divided into 3 groups based on their ages; 0-59 months (<5 years), 60-119 months (5-<10 years) and 120-180 months (10-15 years).

#### 3.3.1 Inclusion criteria

- Children with diarrhoea (≥3 unformed stools per day) or dysentery who were hospitalized or attended outpatient clinics.
- Asymptomatic children (supposedly healthy) who attended selected schools (JWMBS and DYIS) or lived within the Agbogbloshie community.

#### 3.3.2 Exclusion criteria

- Children on anti-diarrhoea treatment at the time of sample collection.
3.4 Approval

Ethical approval was obtained from the Ethical and Protocol Review Committee of the College of Health Sciences (CHS), University of Ghana. Permission to access the various sites was obtained from the Accra Metro Education office and the Regional Health Directorate, as well as the Head and teachers of the various schools. Informed consent was obtained from parents/guardians before their children were recruited into the study. For parents and guardians who could not read or understand English, details of the study was explained to them verbally in a local Ghanaian language (Twi or Ga) they could understand.

3.5 Sample size determination

Prevalence rate of *Entamoeba histolytica* infection in Accra has been previously recorded as 13.7% when serum samples were taken from the Korle-Bu Teaching Hospital (Ayi et al., 2006). Thus, this prevalence was used as the estimated prevalence rate.

Minimum sample size was calculated using the formula, \( n = \frac{Z^2 \times P \times (1-P)}{m^2} \)

Where \( n \) = minimum sample size

\( Z \) = standard value at 95% confidence level = 1.96

\( P \) = estimated prevalence = 13.7% = 0.137 (Ayi et al., 2006)

\( m \) = margin of error = 0.05

\[ n = \frac{(1.96)^2 \times 0.137 \times (1- 0.137)}{(0.05)^2} = 181.68 \sim 182 \]

The minimum sample size was 182. However, this size was increased by 25% for contingencies, making the minimum sample size 228.
3.6 Sampling technique

At PML, all children who presented with diarrhoeal disease as indicated by the clinician and whose parents/guardians signed the consent form were recruited into the study. In the schools, all pupils from the various classes were invited to be part of the study. Consent forms were given to the pupils to be signed by parents/guardians. Again, pupils whose parents signed the consent form were recruited into the study. At Agbogbloshie, the community was divided into four clusters. One cluster was selected randomly. All households within the cluster were invited to partake in the study. However, only children whose parents gave their approval were recruited into the study.

3.7 Data and sample collection

By administering questionnaires, data on the name, age, gender, school, nationality, diarrhoeal status, finger-sucking habit, residence, source and type of drinking water, type of toilet facility used at home, handwashing behaviour, residence, the occupation and educational level of parents/guardians, etc. of the subjects were obtained.

At PML hospital and Agbogbloshie community, stool containers were given out to parents/guardians for samples to be collected. With the assistance of teachers, freshly passed stools were collected in clean stool containers in the schools.

Samples when collected, were sent immediately (within 20 minutes) to the Department of Medical Microbiology laboratory to be examined. This was to avoid disintegration of trophozoites. Samples were divided into two; one part was used for microscopy, and the other part stored in -20°C freezer to be used later for ELISA and PCR analysis.
3.8 Laboratory procedures (Analysis)

3.8.1 Microscopy

All the stool samples collected were first observed physically for consistency, presence of blood stains and macroscopic parasites (Wakid, 2010). Samples were processed using direct smear (saline wet mount), Lugol’s iodine wet mount (staining) and formol-ether concentration to assist in the detection of any parasitic trophozoites, cysts, worm eggs, larvae, red blood cells and white blood cells that may be present in stool. Specimens were observed microscopically using x10 and x40 objectives. Identification of *E. histolytica* species complex was dependent on the characteristic morphology of trophozoites and cysts. There was no attempt made to detect bacteria or viruses from the stool samples. However, other microorganisms identified by microscopy were reported.

3.8.1.1 Direct faecal smear and iodine staining technique

A drop of 0.9% saline was placed on a clean microscopic slide and about 2mg of each stool sample was emulsified on it using an applicator stick. A cover slip was placed on it and the slide was examined systematically under a low power objective and low light intensity for the identification of cysts or trophozoites. The slide was passed over a lighted flame to enhance the motility and visibility of trophozoites which usually appear transparent and shows slow directional movement (Ayeh-Kumi et al., 2001). Morphological features of the trophozoites/ cysts, and difficult-to-see nuclear elements in wet mounts were further made visible when a drop of Lugol’s iodine was applied to the stool samples. Cysts stained with iodine have a golden brown appearance and creates a better contrast between the cytoplasm, nuclei and glycogen (Cheesborough, 1998).
3.8.1.2 Formol-ether concentration technique

Low parasitic load can cause cysts of *E. histolytica/ E. dispar/ E. moshkovskii* to be missed during wet preparations. Thus, formol-ether concentration method is necessary for their recovery. Ten milliliters (10ml) of 10% formalin was added to 4g of fresh stool in a 15ml tube and thoroughly mixed. The mixture was left for 30 min on the bench at room temperature for fixation and was strained into a 15ml conical centrifuge tube through a piece of gauze. Saline (0.9%) was added to about two-thirds of the volume of the tube. The mixture was then centrifuged at 500x g for 10 minutes. The supernatant fluid was poured out and saline was again added to the sediment as above and was centrifuged for 10 min at 500x g. Again, the supernatant fluid was decanted and the sediment was re-suspended in 10% formalin. Ethyl acetate (5ml) was added to the mixture and for a minimum of 30 seconds, the tube was shaken vigorously after being capped. The supernatant fluid was then decanted after the suspension was re-spun for 10 min at 500x g. After stirring the sediments at the base of the tube, a drop was placed on a microscope slide and examined systematically under the microscope. This was to identify cysts of *Entamoeba* present. Morphology of cysts was further enhanced when iodine was added to the preparation.
3.8.2 ELISA for *Entamoeba histolytica* detection

Antigens of *E. histolytica* in stool samples were detected using the Techlab *E. histolytica* II kit (Blacksburg, VA). Ninety-two samples (92) were selected from the 231 samples; 47 from the hospital (PML) and 45 from the community (14 from JWMBS, 14 from DYIS and 17 from Agbogbloshie community). Samples were selected based on the consistency of stool; loose and mucoid stool samples were selected randomly in other to maximize the chance of identifying an *E. histolytica* infection.

The *E. histolytica* II antigen detection kit (TechLab, Blacksburg, VA) was used following the manufacturer’s instructions. The kit is an enzyme immunoassay which detects rapidly, the adhesion of *E. histolytica* in stools of humans. Constituents of the kits include diluents (40ml of buffered protein solution with 0.02% thimerosal) which serve as an emulsifier and the negative control; Conjugate (7.0ml of mouse monoclonal antibody for adhesion of *E. histolytica* specifically and horseradish peroxidase in a protein solution buffered with 0.025 thimerosal); Positive control (3.5 ml of purified adhesion from *E. histolytica* in a buffered saline, detergent and 0.2% thimerosal); Substrate (14.0 ml tetramethylbenzidine and peroxidase solution); 20X wash buffer concentrate (50 ml of a 20X concentrate containing phosphate-buffered saline, 0.2% thimerosal and detergent); and Stop solution (7.0 l); 0.6 N sulfuric acid; and micro titre wells containing immobilized polyclonal antibodies.

The diluents were used to emulsify small quantities of stool specimen. The diluted specimen was transferred to a microtitre well and was incubated for two hours at 29°C; any adhesions in the specimen will be bound to the immobilized polyclonal antibody. After washing, unbound materials were removed and the conjugate was then added for 5 minutes. Addition of a substrate caused the
development of a colour, which occurs as a result of the complexes formed by the enzyme, antibody and antigen binding when the adhesion is present.

3.8.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction was performed for the amplification of specific genes of *E. histolytica* and *E. dispar*. Forty-six (46) samples were randomly selected and subjected to PCR analysis. These included the two positive samples for *Entamoeba* complex by microscopy, ELISA-positive samples, as well as negative samples by both techniques.

3.8.3.1 Isolation (Extraction) of DNA

QIAamp DNA Stool Mini Kit (QIAGEN GmbH, Germany) designed for total DNA purification from stools (both fresh and frozen samples) was used for isolating DNA. The extraction was done using the instructions in the manual. This involved lysis of stool specimen in Buffer ASL at 70°C; adsorption of substances that inhibit PCR and damage DNA in the stool to inhibitEX; centrifugation and pelleting of the inhibitEX reagent; and purification of DNA in the supernatant fluid on QIAamp spin columns.

For DNA to be purified on the QIAamp spin columns, proteins were digested with Proteinase K at 70°C incubation period, DNA was bound to the QIAamp silica-gel membrane and samples were spun for the impurities to be washed away. Using buffers AW1 and AW2 (wash buffers) the DNA bound to the membrane was washed twice. This was to ensure that all impurities were removed. Pure DNA was eluted from the spin columns in low salt buffer (buffer AE) equal to room temperature. Yield of DNA is in the range of 5-100ug and suitable for use in PCR reactions (Ayeh-Kumi *et al*., 2001).
Table 3.1    Primers used in this study

<table>
<thead>
<tr>
<th>PRIMERS</th>
<th>SEQUENCE</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>TTT GTA TTA GTA CAA</td>
<td>Haque et al., 1998</td>
</tr>
<tr>
<td>E2</td>
<td>A GTA [A/G] TA TTG ATA TAC T</td>
<td>Haque et al., 1998</td>
</tr>
<tr>
<td>Eh1</td>
<td>5’-AAG CAT TGT TTC TAG ATC TGA G-3’</td>
<td>Troll et al., 1997; Khairnar &amp; Parija, 2007</td>
</tr>
<tr>
<td>Eh2</td>
<td>5’-AAG AGG TCT AAC CGA AAT TAG-3’</td>
<td>Troll et al., 1997; Khairnar &amp; Parija, 2007</td>
</tr>
<tr>
<td>SREHP5-</td>
<td>GCT AGT CCT GAA AAG CTT GAA GAA GCT G</td>
<td>Clark &amp; Diamond, 1993</td>
</tr>
<tr>
<td>SREHP3-</td>
<td>GGA CTT GAT GCA GCA TCA AGG T</td>
<td>Clark &amp; Diamond, 1993</td>
</tr>
<tr>
<td>nSREHP5-</td>
<td>TGA AGA TAA TGA AGA TGA TGA AGA TG</td>
<td>Ayeh-Kumi et al., 2001</td>
</tr>
<tr>
<td>nSREHP3-</td>
<td>TAT TAT TAT CGT TAT CTG AAC TAC TTC CTG</td>
<td>Ayeh-Kumi et al., 2001</td>
</tr>
</tbody>
</table>

3.8.3.2 Small subunit rRNA PCR (with primers E1 and E2)

Primers E1 and E2 amplify a 0.9kb fragment of both *E. histolytica* and *E. dispar* rRNA genes. Primer E2 is constructed two-fold degenerately; it is a mixture with half corresponding to the *E. histolytica* sequence and the other half corresponding to the *E. dispar* sequence. Hot-start technique was used for all the PCR reactions.
Table 3.2 Amplification conditions for small subunit rRNA PCR (with primers E1 and E2)

<table>
<thead>
<tr>
<th></th>
<th>TEMPERATURE</th>
<th>TIME</th>
<th>NUMBER OF CYCLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq polymerase activation</td>
<td>95°C</td>
<td>5mins</td>
<td>1</td>
</tr>
<tr>
<td>Denaturing</td>
<td>92°C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>56 °C</td>
<td>30sec</td>
<td>45</td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72 °C</td>
<td>10mins</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4 °C</td>
<td>∞</td>
<td></td>
</tr>
</tbody>
</table>

3.8.3.3 16S-like ribosomal RNA gene PCR (with primers Eh1 and Eh2)

Primers Eh1 and Eh2 amplify a 439 bp of the 16S-like ribosomal RNA of *E. histolytica*.

Table 3.3 Amplification conditions for 16S-like ribosomal RNA PCR

<table>
<thead>
<tr>
<th></th>
<th>TEMPERATURE</th>
<th>TIME</th>
<th>NUMBER OF CYCLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq polymerase activation</td>
<td>95°C</td>
<td>5mins</td>
<td>1</td>
</tr>
<tr>
<td>Denaturing</td>
<td>94°C</td>
<td>30 sec</td>
<td>45</td>
</tr>
<tr>
<td>Annealing</td>
<td>58 °C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72 °C</td>
<td>10mins</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4 °C</td>
<td>∞</td>
<td></td>
</tr>
</tbody>
</table>
3.8.3.4 Nested *E. histolytica* SREPH gene PCR

Nested PCR involved two sets of primers. The first set (SREPH-5 and SREPH-3) amplifies a 549bp fragment of the SREPH gene (Diamond & Clark, 1993) in the initial PCR. The second set of primers (nSREPH-5 and nSREPH-3) found within the fragment amplified by SREPH-5 and SREPH-3, results in the amplification of a 450bp fragment of the SREPH gene of *E. histolytica*. Both PCR reactions used a hot-start technique and followed the same protocol except for the differences in primers and annealing temperatures. The template DNA in the nested PCR was obtained by taking 1µl of a 1:50 dilution of the initial PCR product (Ayeh-Kumi *et al.*, 2001).

**Table 3.4 Amplification conditions for SREPH PCR reaction**

<table>
<thead>
<tr>
<th></th>
<th>TEMPERATURE</th>
<th>TIME</th>
<th>NUMBER OF CYCLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq polymerase activation</td>
<td>95°C</td>
<td>15mins</td>
<td>1</td>
</tr>
<tr>
<td>Denaturing</td>
<td>94°C</td>
<td>1min</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>50°C</td>
<td>1.5min</td>
<td>30</td>
</tr>
<tr>
<td>Extension</td>
<td>72°C</td>
<td>2min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72°C</td>
<td>4min</td>
<td>1</td>
</tr>
</tbody>
</table>

3.8.3.5 Agarose gel electrophoresis of PCR products

Agarose gel (1.5%) electrophoresis (in 1x Tris-Borate-EDTA buffer) with ethidium bromide staining was used to visualize the PCR products under UV light.
3.9  Statistical analysis

Data was analyzed using statistical package for social science (SPSS) program version 16. Qualitative data was presented in numbers and percentages. Chi-square test was used to evaluate the level of association between the different parameters studied. Significance was considered at \( p \) value less than 0.05.
CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of research subjects

Out of the 231 children recruited into the study, 116 (50.2%) were males and 115 (49.8%) were females. Majority of the children were in the age category 0-59 months (<5 years), followed by the 120-180 months (10-15 years) and lastly, the 60-119 months (5-<10 years). The mean age of the children was 73.2±54.7 months. The difference in age groups at the various study locations was statistically significant (P<0.001). Table 4.1 represents the age distribution of children in each study site.

Table 4.1 Age group distribution (in months)

<table>
<thead>
<tr>
<th>Research site</th>
<th>Age group (months)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-59</td>
<td>60-119</td>
<td>120-180</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>PML</td>
<td>64 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>64 (27.7)</td>
<td></td>
</tr>
<tr>
<td>JWMBS</td>
<td>7 (13.2)</td>
<td>36 (67.9)</td>
<td>10 (18.9)</td>
<td>53 (22.9)</td>
<td></td>
</tr>
<tr>
<td>DYIS</td>
<td>9 (14.8)</td>
<td>6 (9.8)</td>
<td>46 (75.4)</td>
<td>61 (26.5)</td>
<td></td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>28 (52.8)</td>
<td>14 (26.4)</td>
<td>11 (20.8)</td>
<td>53 (22.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Total, n(%)</strong></td>
<td><strong>108 (46.8)</strong></td>
<td><strong>56 (24.2)</strong></td>
<td><strong>67 (29.0)</strong></td>
<td><strong>231 (100)</strong></td>
<td></td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants.
Type of drinking water. Sachet water was the most consumed water among the three types of drinking water used by study participants in this study. A relatively small number drank bottled water and pipe-borne water (Table 4.2). The difference in the type of water consumed by participants was statistically significant (P<0.001).

Table 4.2 Distribution of the type of drinking water used by participants

<table>
<thead>
<tr>
<th>Research site</th>
<th>Type of drinking water</th>
<th></th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sachet water</td>
<td>Pipe-borne water</td>
<td>Bottled water</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>PML</td>
<td>63 (98.4)</td>
<td>1(1.6)</td>
<td>0(0)</td>
<td>64 (27.7)</td>
<td></td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>50 (94.3%)</td>
<td>1 (1.9%)</td>
<td>2 (3.8%)</td>
<td>53 (22.9)</td>
<td></td>
</tr>
<tr>
<td>JWMBS</td>
<td>48 (90.6%)</td>
<td>5(9.4%)</td>
<td>0(0%)</td>
<td>61 (26.5)</td>
<td></td>
</tr>
<tr>
<td>DYIS</td>
<td>51 (83.6%)</td>
<td>0(0%)</td>
<td>10(16.4%)</td>
<td>53 (22.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>213 (92.0%)</strong></td>
<td><strong>7 (3.0%)</strong></td>
<td><strong>12 (5.0%)</strong></td>
<td><strong>231 (100)</strong></td>
<td></td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants.

Availability of toilet facility. All participants from Agbogbloshie (53) and majority of pupils from JWMBS (40) had no access to toilet facility at home. However, almost all pupils from DYIS (54) and quite a good number of children from PML (40) had toilet in their homes (Table 4.3). The difference was statistically significant (P<0.001).
Table 4.3  Availability of toilet facility in participants’ home

<table>
<thead>
<tr>
<th>Research site</th>
<th>No, n (%)</th>
<th>Yes, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML</td>
<td>24 (37.5)</td>
<td>40 (62.5)</td>
<td>64 (27.7)</td>
</tr>
<tr>
<td>JWMBS</td>
<td>40 (75.5)</td>
<td>13 (24.5)</td>
<td>53 (22.9)</td>
</tr>
<tr>
<td>DYIS</td>
<td>7 (11.5)</td>
<td>54 (88.5)</td>
<td>61 (26.5)</td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>53 (100)</td>
<td>0 (0)</td>
<td>53 (22.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>124 (53.7)</strong></td>
<td><strong>107 (46.3)</strong></td>
<td><strong>231 (100)</strong></td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants.

Type of toilet facility used. While majority of the study participants used pit latrine for their relief, a close number also used water closet either at home or in the community, with relatively few using the bush (Table 4.4). Again, the association between research site and type of toilet facility used by participants was statistically significant (P<0.05).

Table 4.4  Type of toilet facility used by study participants

<table>
<thead>
<tr>
<th>Research site</th>
<th>Water closet, n (%)</th>
<th>Pit latrine, n (%)</th>
<th>Bush, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML</td>
<td>38 (59.4)</td>
<td>24 (37.5)</td>
<td>2 (3.1)</td>
<td>64 (27.7)</td>
</tr>
<tr>
<td>JWMBS</td>
<td>15 (28.3)</td>
<td>38 (71.7)</td>
<td>0 (0)</td>
<td>53 (22.9)</td>
</tr>
<tr>
<td>DYIS</td>
<td>53 (86.9)</td>
<td>8 (13.1)</td>
<td>0 (0)</td>
<td>61 (26.5)</td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>3 (5.7)</td>
<td>42 (79.2)</td>
<td>8 (15.1)</td>
<td>53 (22.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>109 (47.2)</strong></td>
<td><strong>112 (48.5)</strong></td>
<td><strong>10 (4.3)</strong></td>
<td><strong>231 (100)</strong></td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants.
**Finger-sucking behavior.** Although majority of the children did not suck their finger, relatively few were engaged in finger-sucking (Table 4.5). There was statistically significant association between study site and finger-sucking habit of children (P<0.05).

<table>
<thead>
<tr>
<th>Research site</th>
<th>Does not suck finger, n (%)</th>
<th>Sucks finger, n (%)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML</td>
<td>57 (89.1)</td>
<td>7 (10.9)</td>
<td>64 (27.7)</td>
</tr>
<tr>
<td>JWMBS</td>
<td>36 (67.9)</td>
<td>17 (32.1)</td>
<td>53 (22.9)</td>
</tr>
<tr>
<td>DYIS</td>
<td>55 (90.2)</td>
<td>6 (9.8)</td>
<td>61 (26.5)</td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>48 (90.6)</td>
<td>5 (9.4)</td>
<td>53 (22.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>196 (84.8)</td>
<td>35 (15.2)</td>
<td>231 (100)</td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants.

**Educational level of parents/guardians.** Parents (both mothers and fathers) of children from DYIS were the most educated, with none having no formal education. However, Agbogbloshie community recorded the least educated parents/ guardians (Table 4.6). The difference in the educational level of parents/guardians at the various study sites was statistically significant (P<0.001).
Table 4.6   Educational level of mothers and fathers of participants at the research sites (n=231)

<table>
<thead>
<tr>
<th>Level</th>
<th>Research site</th>
<th>PML</th>
<th>JWMBS</th>
<th>DYIS</th>
<th>Agbogbloshie</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>NFE</td>
<td></td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>29</td>
<td>48</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>16</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td>JHS</td>
<td></td>
<td>28</td>
<td>19</td>
<td>14</td>
<td>13.9</td>
<td>74</td>
</tr>
<tr>
<td>SHS</td>
<td></td>
<td>4</td>
<td>4</td>
<td>21</td>
<td>7</td>
<td>36</td>
</tr>
<tr>
<td>Tertiary</td>
<td></td>
<td>6</td>
<td>1</td>
<td>22</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>64</td>
<td>53</td>
<td>61</td>
<td>53</td>
<td>231</td>
</tr>
</tbody>
</table>

Mothers

<table>
<thead>
<tr>
<th>Level</th>
<th>Research site</th>
<th>PML</th>
<th>JWMBS</th>
<th>DYIS</th>
<th>Agbogbloshie</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>NFE</td>
<td></td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>JHS</td>
<td></td>
<td>21</td>
<td>28</td>
<td>4</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>SHS</td>
<td></td>
<td>19</td>
<td>13</td>
<td>20</td>
<td>16</td>
<td>68</td>
</tr>
<tr>
<td>Tertiary</td>
<td></td>
<td>6</td>
<td>1</td>
<td>34</td>
<td>3</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>64</td>
<td>53</td>
<td>61</td>
<td>53</td>
<td>231</td>
</tr>
</tbody>
</table>

Fathers

<table>
<thead>
<tr>
<th>Level</th>
<th>Research site</th>
<th>PML</th>
<th>JWMBS</th>
<th>DYIS</th>
<th>Agbogbloshie</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>NFE</td>
<td></td>
<td>11</td>
<td>6</td>
<td>1</td>
<td>21</td>
<td>39</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>JHS</td>
<td></td>
<td>21</td>
<td>28</td>
<td>4</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>SHS</td>
<td></td>
<td>19</td>
<td>13</td>
<td>20</td>
<td>16</td>
<td>68</td>
</tr>
<tr>
<td>Tertiary</td>
<td></td>
<td>6</td>
<td>1</td>
<td>34</td>
<td>3</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>64</td>
<td>53</td>
<td>61</td>
<td>53</td>
<td>231</td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants, NFE: No formal education, JHS: Junior High School, SHS: Senior High School.
4.2 Findings from macroscopy

With the unaided eye, no parasite was seen in any of the samples collected from the various study sites. Stain of blood was seen in only one (0.43%) mucoid sample from a one-year old patient who was presented at PML hospital with diarrhoea. Most of the samples were loose and semi-formed, with quite a number being mucoid and formed. Very few of the samples were either mucoid-formed or mucoid-semi-formed (Table 4.7).

<table>
<thead>
<tr>
<th>Research site</th>
<th>Mucoid n (%)</th>
<th>Loose n (%)</th>
<th>Semi-formed n (%)</th>
<th>Formed n (%)</th>
<th>Mucoid+ formed n (%)</th>
<th>Mucoid+ semi-formed n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PML</td>
<td>26(40.6)</td>
<td>33(51.6)</td>
<td>5(7.8)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>JWMBS</td>
<td>1(1.9)</td>
<td>9(17.0)</td>
<td>22(41.5)</td>
<td>20(37.7)</td>
<td>1(1.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>DYIS</td>
<td>3(4.9)</td>
<td>18(29.5)</td>
<td>25(41.0)</td>
<td>12(19.7)</td>
<td>1(1.6)</td>
<td>2 (3.3)</td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>12(22.6)</td>
<td>12(22.6)</td>
<td>19(35.8)</td>
<td>9(17.0)</td>
<td>1(1.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Total, n (%)</strong></td>
<td><strong>42(18.2)</strong></td>
<td><strong>72(31.2)</strong></td>
<td><strong>71(30.7)</strong></td>
<td><strong>41(17.7)</strong></td>
<td><strong>3(1.3)</strong></td>
<td><strong>2 (0.9)</strong></td>
</tr>
</tbody>
</table>

PML: Princess Marie Louise Childrens’ Hospital, JWMBS: John Wesley Methodist Basic School, DYIS: De Youngsters International School, n: number of study participants
Statistically, there was significant difference in stool consistencies of participants from the various research sites (P<0.001). The age group 0-59 months recorded the highest of the mucoid and loose stools. Samples of children in the category 60-119 months were mostly semi-formed and formed. Children of the 120-180 age-group had more of loose and semi-formed stools (Table 4.8). The association between the age category of participants and consistency of stools was statistically significant (P<0.001).

**Table 4.8  Distribution of stool consistency according to age groups**

<table>
<thead>
<tr>
<th>Age group (months)</th>
<th>Mucoid n (%)</th>
<th>Loose n (%)</th>
<th>Semi-formed n (%)</th>
<th>Formed n (%)</th>
<th>Mucoid+ formed n (%)</th>
<th>Mucoid+semi-formed n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-59</td>
<td>2(34.3)</td>
<td>39(36.1)</td>
<td>27(25.0)</td>
<td>5(4.6)</td>
<td>0(0)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>60-119</td>
<td>3(5.4)</td>
<td>13(23.2)</td>
<td>17(30.4)</td>
<td>19(33.9)</td>
<td>2(7.1)</td>
<td>2(0%)</td>
</tr>
<tr>
<td>120-180</td>
<td>3(4.5)</td>
<td>20(29.9)</td>
<td>27(40.3)</td>
<td>17(25.4)</td>
<td>1(1.47)</td>
<td>0(0%)</td>
</tr>
<tr>
<td><strong>Total n (%)</strong></td>
<td><strong>42(20.3)</strong></td>
<td><strong>72(31.2)</strong></td>
<td><strong>71(30.7)</strong></td>
<td><strong>41(17.7)</strong></td>
<td><strong>3(0%)</strong></td>
<td><strong>2(0%)</strong></td>
</tr>
</tbody>
</table>
4.3 Microscopy

Two (2) out of the 231 samples examined by microscopy were positive for *Entamoeba* complex (*E. histolytica*/*E. dispar*/*E. moshkovskii*). Cysts and trophozoites of *E. histolytica*/*E. dispar*/*E. moshkovskii* were detected in wet mount and iodine preparations of stool samples (figure 4.1). Children who were positive were a 4-month old female baby who was presented at Princess Marie Louise Children Hospital (PML) with loose stools; and a 6-year old female pupil from John Wesley Methodist Basic School (JWMBS) having formed mixed with mucoid stools. Prevalence obtained for each research site was 1.6%, 1.9%, 0% and 0% for PML, JWMBS, DYIS and the Agbogbloshie community respectively. The overall prevalence of *E. histolytica* species complex infection by microscopy was 0.9%.

![Microscopic images of *Entamoeba* species complex](A,B,C,D)

**Figure 4.1** *E. histolytica E. dispar E. moshkovskii* observed under the microscope
A & B. Trophozoites moving in wet mount preparations; C. Cyst in wet mount; D. Iodine stain of cyst (×40)
4.4 **Antigen Detection Test (ELISA)**

Using the Techlab *E. histolytica* II ELISA kit (Blacksburg, VA) specific for the detection of *Entamoeba histolytica* antigen in stool, two samples were positive out of the 92 samples that were screened (Figure 4.2). Positive children were a 2-month old baby boy and a 3-year old boy all presented at PML with loose stools. A prevalence of 2.2% for *Entamoeba histolytica* was obtained by ELISA. None of the two samples that were positive for *E. histolytica* species complex by microscopy was positive for *E. histolytica* by ELISA.

![Image of ELISA test results](image)

**Figure 4.2** Determination of *E. histolytica* prevalence using Techlab *E. histolytica* II kit

*P.* Positive control; *N.* Negative control; Arrows showing the two positive samples.
4.5 Polymerase Chain Reaction (PCR)

Using Primers E1 and E2, amplification was not achieved in any of the 46 DNA samples analysed by PCR. However, amplification was achieved in 3 samples at the 200bp when primers Eh1 and Eh2 were used (Figure 4.7). Amplification of the SREPH gene at the 450bp was also successful in one sample (Figure 4.8). Thus, 4 DNA samples were successfully amplified by PCR. The prevalence of *E. histolytica* for the 4 positive samples was recorded as 8.7%. Positive samples were from the 6-year old pupil from JWMBS who was also positive for *E. histolytica* species complex by microscopy; the 2-month old baby boy and the 3-year old boy from PML who were positive for *E. histolytica* by ELISA; and lastly, a 6-month old male child also from PML who was neither *E. histolytica* species complex-positive by microscopy nor *E. histolytica*-positive by ELISA.

![Figure 4.3](image_url)

**Figure 4.3** Gel of amplified genes of *E. histolytica* DNA from stool samples

A. 16s-like rRNA PCR products; B. Nested SREPH PCR products; LA. 200 bp ladder (Bioline); LB. 100bp ladder (Bioline); P. Positive *E. histolytica* control; N. Negative control
4.6 Risk factors associated with *E. histolytica* infection

Some factors relating to *E. histolytica* infection in children in this study were age, sex, type of drinking water, source of food, finger-sucking behavior, parents’ educational level and type of parent occupation. These were however, not statistically significant (Table 4.9).

**Table 4.9 Factors associated with *Entamoeba histolytica* infection**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Number of <em>E. histolytica</em> positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
</tr>
<tr>
<td>0-59months (&lt;5years)</td>
<td>3</td>
</tr>
<tr>
<td>60-&lt;119months (5-&lt;10years)</td>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>1</td>
</tr>
<tr>
<td>Males</td>
<td>3</td>
</tr>
<tr>
<td>Source of drinking water</td>
<td></td>
</tr>
<tr>
<td>Sachet water</td>
<td>3</td>
</tr>
<tr>
<td>Tap water</td>
<td>1</td>
</tr>
<tr>
<td>Source of food</td>
<td></td>
</tr>
<tr>
<td>From home</td>
<td>3</td>
</tr>
<tr>
<td>Food vendor</td>
<td>1</td>
</tr>
<tr>
<td>Finger sucking?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
</tr>
<tr>
<td>Parents’ educational level</td>
<td></td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>2</td>
</tr>
<tr>
<td>No formal education</td>
<td>2</td>
</tr>
<tr>
<td>Fathers</td>
<td></td>
</tr>
<tr>
<td>Junior High School (JHS)</td>
<td>3</td>
</tr>
<tr>
<td>Primary</td>
<td>1</td>
</tr>
<tr>
<td>Parent occupation</td>
<td></td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
</tr>
<tr>
<td>Traders</td>
<td>3</td>
</tr>
<tr>
<td>Unemployed</td>
<td>1</td>
</tr>
<tr>
<td>Fathers</td>
<td></td>
</tr>
<tr>
<td>Traders</td>
<td>3</td>
</tr>
<tr>
<td>Security</td>
<td>1</td>
</tr>
</tbody>
</table>
4.7  Intestinal parasites identified by microscopy

Other parasites such as *Giadia lamblia*, *Entamoeba coli*, *Schistosoma mansoni*, *Hymenolepsis nana* and *Taenia species* were also detected under the microscope (Figure 4.4). *Giadia lamblia* and *Hymenolepsis nana* were more frequent. Table 4.9 shows the distribution of the parasites according to the study site, age, sex, and stool consistency.

![Figure 4.4 Other enteric parasites identified by microscopy](http://ugspace.ug.edu.gh)
Table 4.10  Summary of other parasites identified

<table>
<thead>
<tr>
<th>Type of parasite, n</th>
<th>E. coli</th>
<th>G. lamblia</th>
<th>H. nana</th>
<th>S. mansoni</th>
<th>Taenia spp</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PML</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>JWMBS</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td></td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>DYIS</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>Agbogbloshie</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>4 (28.6)</td>
</tr>
<tr>
<td><strong>Total, n (%)</strong></td>
<td>2 (14.3)</td>
<td>5 (35.7)</td>
<td>5 (35.7)</td>
<td>1 (7.1)</td>
<td>1 (7.1)</td>
<td>14 (100.0)</td>
</tr>
</tbody>
</table>

| **Age group**      |         |            |         |            |            |           |
| (months)           |         |            |         |            |            |           |
| 0-59               | 1       | 3          | 2       | 1          |            | 7 (50.0)  |
| 60-119             | 1       | 2          | 3       | 1          |            | 7 (50.0)  |
| **Total, n (%)**   | 14 (100.0)|            |         |            |            |           |

| **Sex**            |         |            |         |            |            |           |
| Males              | 1       | 1          | 1       |            |            | 3 (21.4)  |
| Females            | 1       | 4          | 4       | 1          | 1          | 11 (78.6) |
| **Total, n (%)**   | 14 (100.0)|            |         |            |            |           |

| **Stool consistency** |         |            |         |            |            |           |
| Mucoid              | 2       | 1          | 1       |            |            | 4 (28.6)  |
| Loose               | 2       | 1          | 2       |            |            | 5 (35.7)  |
| Semi-formed         | 1       | 2          |         |            |            | 3 (21.4)  |
| Formed              | 1       |            | 1       |            |            | 2 (14.3)  |
| **Total, n (%)**    | 14 (100.0)|            |         |            |            |           |
4.8 Yeast cells identified

Yeast cells (Figure 4.5) were seen in 25 out of the 231 stool samples examined by microscopy; 18 (72%) from PML, 1 (4%) from JWMBS, 2 (8%) from DYIS and 4 (16%) from Agbogbloshie. Almost all the samples which had yeast cells (23/25, 92%) were from children below 5 years of age, with one each from the 60-119 months (4%) and 120-180 months (4%) age group. Loose stools recorded the most of the yeast cells than the other stool types (Figure 4.5).

![Yeast cells identified in a stool specimen under the microscope, some showing characteristic budding.](image)

Figure 4.5 Yeast cells identified in a stool specimen under the microscope, some showing characteristic budding.

Yeast cells were found mostly in loose and mucoid stools, with few in semi-formed but none in formed stools (Figure 4.6).

![Distribution of yeast cells by stool consistency](image)

Figure 4.6 Distribution of yeast cells by stool consistency
CHAPTER FIVE

DISCUSSION

5.1 Prevalence of *Entamoeba* complex

A prevalence of 0.9% recorded for *Entamoeba* complex by microscopy can be considered low. Although microscopy is known to have suboptimal sensitivity, the technique was performed with much expertise. Thus, the low prevalence recorded may be due to the fact that the parasite was not endemic in the research areas selected for the study; or samples contained mostly trophozoites which could have degenerated with time due to their fragility and inability to survive outside the host (Heyman, 2008).

Low prevalence of *Entamoeba* complex has also been recorded in some studies elsewhere. By using direct smear and formalin-ether concentration methods, a prevalence of 0.6% was recorded in Nigeria (Agbike, 2009), 0.78% in Iran (Hooshyar *et al*., 2004) and 0-1.9% in the Philippines with an overall prevalence of 1.1% (Salazer *et al*., 1990). Other studies have also reported low but relatively higher prevalence rates by microscopy; 8.51% in Zaria and Kaduna in Nigeria (Inabo *et al*. 2000), and 11.7% in Thailand (Sirima *et al*., 2008).

Relative to this current study, a high prevalence (39.1%) of *Entamoeba* complex was recorded in the Northern sector of Ghana (Verweij *et al*., 2003). In 1991, Polderman and his colleagues also recorded high numbers of amoebic infections and other intestinal parasites in this same sector. According to Verweij *et al*. (2003), these intestinal parasites present indicated a serious faeco-oral transmission; high prevalence of enteric parasites most likely dwelt on sanitation issues.
5.2  **Prevalence of *E. histolytica***

*Entamoeba histolytica* is pathogenic, causing severe diseases in children (Ilikkan *et al.*, 2005). Thus, a prevalence of 8.7% (4/46) recorded by PCR in children, most of which were below 5 years of age in this study, is of public health concern. In the Northern sector of Ghana, one *E. histolytica* case was recorded by Verweij and his colleagues (2003) using Real-Time PCR. Throughout the country, including Greater Accra region, there have been reports of inadequate sanitation infrastructure and poor personal and environmental hygiene practiced by inhabitants, coupled with overcrowding (GSS, 2012). These are conditions that foster the continual existence of *Entamoeba histolytica* infection (Ajero *et al.*, 2008).

5.3  **Techniques used in detection of *E. histolytica***

Whereas microscopy indicates current infection, ELISA and PCR can detect both past and recent infections when antigens and genetic material of *E. histolytica* are present in stool. Thus, the inability to identify cysts or trophozoites in stool samples does not necessarily mean lack of infection. Microscopy revealed two *Entamoeba* complex infections; one confirmed as *E. histolytica* infection by PCR, whereas amplification was not achieved in the other. Although this could be an *Entamoeba dispar* or *Entamoeba moshkovskii* infection, it could also be an *Entamoeba histolytica* which could not be amplified. This finding is in conformity with that of Fotedar *et al.* (2007) who also identified *Entamoeba* species by microscopy which could not be amplified by PCR. Majority (14/21) of their samples contained only trophozoites which could have degenerated with time. Thus, PCR most likely produces better results with *Entamoeba* cysts-containing samples than with trophozoites-containing samples (Aguirre *et al.*, 1997).
The two ELISA-positive samples were amplified using the *E. histolytica*-specific primers Eh1, Eh2, as well as the SREPH primers. Thus, PCR confirmed all the *E. histolytica*-positive samples by ELISA. However, inability to achieve amplification in any of the samples using the *Entamoeba* genus primers, E1 and E2 (for both *E. histolytica* and *E. dispar*) could be due to the amplification conditions used or the non-specific nature of the genus primers.

### 5.4 Other pathogens

The WHO estimates about 3.5 billion people affected by intestinal parasitic infection, majority being children (WHO, 2007). The pathogenic parasites like *Hymenolepsis nana*, *Schistosoma mansoni*, *Taenia spp*, and *Giadia lamblia* revealed in this study by microscopy could contribute to the gastrointestinal diseases in children.

Detection of *Giardia lamblia* (5) and *Hymenolepsis nana* (5) in more children suggests that these parasites are more predominant. *G. lamblia* was the most prevalent globally (10.8%), followed by *Entamoeba* (4.3%) and *Cryptosporidium* (4.0%) when the World Health Organisation Task Force investigated parasites that could be transmitted to humans (WHO 2007). Giardiasis has also been reported as one of the commonest gastroenteritis among children in Accra (Opintan *et al.*, 2010; Nkrumah & Nguah, 2011). Like *E. histolytica*, *G. lamblia* infection is by consumption of water contaminated with cysts of the parasite, causing symptoms such as diarrhoea, weight loss, epigastric pain, nausea and vomiting. Thus, poor sanitation as well as lack of adequate supply of treated water poses higher risk of infection. Anim-Baidoo *et al.* (2013) recorded an overall prevalence of 8.4% among symptomatic (16.7%) and asymptomatic (7.5%) children at Korle Gonno, Accra. Al-Harthi and Jamjoom in 2009 also found *E. histolytica/E. dispar/E. moshkovskii* and *Giardia lamblia* as the most common parasites in Makkah, Saudi Arabia.
In another research conducted at the Osu orphanage home in Accra, Ghana (Duedu et al., 2015), *Hymenolepis nana* was also found to be one of the most prevalent parasites identified among others like *Giardia duodenalis, Schistosoma mansoni, Ascaris lumbricoides, Taenia species, hookworm*, etc. The study revealed that the inhabitants of the orphanage relied on pipe-borne water for their daily chores and sachet water for drinking. Water was supplied by tankers during shortages whose source could not be verified. Thus, parasites could be transmitted through these means (Duedu et al., 2015).

Among the four study sites, John Wesley Methodist Basic School (JWMBS) and Agbogbloshie community which recorded the highest percentage of parasites (7/14, 50% and 4/14, 28.6% respectively) reflect lack of adequate sanitation and sanitary infrustracture in the communities. Majority of pupils from JWMBS and all the children from Agbogbloshie recruited did not have access to toilet facility at home. Most parents from Agbogbloshie did not have apartments of their own and therefore slept outside buildings, in uncompleted structures, and in people’s kiosk where there is less sanitation and no toilet facility. Therefore most of them visited public pit latrines and the rest used public water closet and the bush.

Yeast cells and parasites seen in most of the clinical samples of children with gastrointestinal infections could be the cause of the high diarrhoeal/dysenteric cases recorded especially among the under five years in this study.
5.5  **Risk factors associated with *E. histolytica* infection**

Due to the numbers recorded, it is difficult to draw a definite conclusion on the association between risk factors and *E. histolytica* infection; the association was statistically insignificant. However, an attempt was made to relate *E. histolytica* infection in this study and the factors likely to foster transmission of the parasite.

5.5.1  Age-related infection

Categorization of participants in this study into the three age groups [0-59 months (<5 years), 60-119 months (5 to <10 years) and 120-180 months (10 to 15 years)] made it easier for the large number of participants recruited to be analysed and compared. Three out of the four (3/4) *E. histolytica*-infected children who were below 5 years of age suggest that children under 5 years have higher risk of being infected with *E. histolytica* since they have lower immune competence as compared to the older age groups. Out of these 3 children below 5 years, two (2/3) were less than a year old. Although prevalent and severe in this group, *E. histolytica* infection in infants is not often suspected (Pushpendra, 2010) since colostrum and mature human milk have been known to have a remarkable destructive effect on *E. histolytica* (achieved by bile salt-stimulated lipase) (Akisu *et al.*, 2004; Heird, 2010). Ilikkan *et al.* (2005) stated that breastfeeding in endemic areas do not protect infants from *E. histolytica* infection. Thus, the less than 1 year old are equally exposed to infection. Moustafa and his colleagues (2013) also reported a high prevalence rate of *E. histolytica* (44/120, 36.7%) among infants. According to their study, inadequate breastfeeding recorded higher percentage among the *E. histolytica* cases.

The 1/4 of *E. histolytica* infection recorded for the age group 60-119 months (5-<10 years) in this study may be attributed to the fact that children in this group have less knowledge in the importance
of cleanliness and thereby play freely regardless of how clean or dusty the environment is (Ibrahim, 2012). Meanwhile, zero prevalence rate was recorded for the age group 10-15 years who are more matured and conscious of health and infection. This is supported by the claims of Iqbal and his colleagues (2009) who recorded the lowest prevalence rate in the 10-12 years group and attributed it to maturity and consciousness of hygiene and infection. Rheuben et al. (2013) also stated that, due to the matured nature of the 11-15 years and the >16 years group, less prevalence was recorded as compared to the younger age categories.

5.5.2 Sex-related E. histolytica infection

Since the prevalence of E. histolytica in males (12.5%) was higher than that recorded in females (4.5%) but statistically insignificant, infection may be as a result of exposure rather than susceptibility due to the sex of the children involved.

5.5.3 Domestic- and drinking water-related infection

Pipe-borne water used by all the E. histolytica-positive children as their source of domestic water suggests that, contamination of the pipe-borne water could be a possible source of E. histolytica infection, as also reported by Wurtz (1994). E. histolytica is one of the commonest protozoans found in water (Koneman et al., 1992; Steiner et al., 1997) which is indicative of environmental contamination and handling of water (LeChevaillier et al., 1991). A study conducted in Accra identified protozoans in two of the five samples of pipe-borne water analysed, with heterotrophic plate count bacteria (HPC) which is used to assess the quality of water, above the recommended limits of 500 cfu/mL (Osei et al., 2013).
The type of water storage used at home can also foster the transmission of infection, because an association between storage of household water supplies and *E. histolytica* infection has been reported in some studies (Oyerinde *et al*., 1999; Dawet *et al*., 2012). Again, lack of regular water treatment (Rukmanee *et al*., 2008) and resistance of the parasite to water purification system (leChevaillier *et al*., 1991) plays an important role. In Jos (Nigeria), Lawan *et al*. (2004) reported a statistically significant relationship between gastro-intestinal infection and treatment of water among the less than five years.

In this study, almost all (75%) of the *E. histolytica*-positive children drank sachet water, with few (25%) drinking pipe-borne water. According to WHO (2004), packaged water is the fastest growing beverage category globally. It has become the most consumed and easily accessible source of drinking water in Accra. This is because more consumers perceive this type of water as safer and healthier alternatives to tapwater (Kwakye-Nuako *et al*., 2007; Osei *et al*., 2013), although several studies have revealed that the microbiological and chemical qualities of sachet water, like tap water, is also not in conformity to the National standards (Obiri-Danso *et al*., 2003; Kean *et al*., 2004; Kwakye-Nuako *et al*., 2007).

### 5.5.4 Type of toilet facility

Users of both pit latrines and water closets in this study were equally infected. Lack of adequate supply of water and poor sanitation around these two types of toilet facilities is likely to predispose a child and relatives to infection when flies which carry cystyladen faeces contaminate food and water sources (Cheesebrough, 2005; Reuben *et al*., 2013).
CHAPTER SIX

CONCLUSION, RECOMMENDATION AND LIMITATION

6.1 Conclusion

Prevalence rates of 0.9% and 8.7% were obtained for *Entamoeba* complex and *Entamoeba histolytica* infections respectively in this study. A prevalence of 8.7% for an infectious parasite like *Entamoeba histolytica* especially in children below 5 years as recorded in this study is an issue of concern. Although not statistically proven, the type of domestic (pipe-borne water) and drinking water (sachet water), and access to toilet facilities were associated with *E. histolytica* infection. Relatively high number of other parasites was recorded in the basic school (JWMBS) and Agbogbloshie community. Like *E. histolytica*, these parasites have also been associated with faeco-oral transmission as a result of bad sanitation practiced by individuals and communities, and could contribute to the diarrhoeal or dysenteric cases recorded in children in this study.

6.2 Recommendations

- Further studies by researchers must be carried out to obtain the total epidemiological picture of amoebiasis in Ghanaian children.
- Through health programmes, the Ministry of Health can intensify public awareness on the source and adverse effects of amoebiasis and other intestinal infections on the wellbeing of children, especially those below 5 years.
6.3 Limitation

ELISA and PCR analysis were performed only on some selected samples. Thus, a higher prevalence of *E. histolytica* could have been obtained if all the samples had been screened. With this limitation notwithstanding, enough care was applied to ensure that results obtained were credible.
REFERENCES


southern Ghana: etiology and association with intestinal inflammation and malnutrition. 


APPENDIX

A.1 Questionnaire

Name of child............................................................................................................................

Age of the child ....................... Sex of the child (Male/ Female)

Occupation of Parent/Guardian

Mother .............................................. Father .................................................................

Educational level of mother (Primary/JHS/SHS/Tertiary)

Educational level of father (Primary/JHS/SHS/Tertiary)

Residential address ....................................................................................................................

Source of drinking water: Sachet water ( ) Bottled water ( ) Tap water ( )

Source of domestic water: Tap ( ) Well ( ) Stream ( ) Borehole ( ) Others.........................

Does your child attend school? Yes/ No

Do you breastfeed your child? Yes/ No

Do you or your child wash hands before eating? Yes/ No/ Sometimes

If yes, what do you wash with? Water only/ Water and soap

Do you have toilet facility at home? Yes/ No If Yes, which type? .................................

Type of toilet visited if No: Water closet ( ) Pit latrine ( ) Bush ( ) Others.........................

Do you/ or your child wash hands after visiting the toilet? Yes/ No/ Sometimes

If yes, what do you wash with? Water only/ Water and soap

Does the child suck finger? Yes ( ) No ( )
A.2  Consent note

University of Ghana

School of Biomedical and Allied Health Sciences

Department of Medical Microbiology

P.O.BOX 4236

Korle-Bu, Accra.

Dear Parent/ Guardian,

INFORMED CONSENT FORM

A study on amoebiasis is being conducted among Ghanaian children between the ages of 1 day and 15 years. I will like to seek your permission to include your ward in this study by allowing him/her to present faecal samples to be analysed at the laboratory. The decision to participate is purely voluntary and you will not be penalized in any way if you decide not to allow your ward to participate. Please feel at ease to ask questions in case things are not very clear. The results of this study will be coded and strictly kept confidential. Below is a brief description of the study.

The study: Entamoeba histolytica infection in children in Accra: prevalence and risk factors

Entamoeba histolytica is a parasite that causes amoebiasis, a disease which presents as diarrhoea, dysentery, and sometimes, extra intestinal disease of the liver, lung and brain. Each year, about 50 million individuals become infected leading to about 100,000 deaths. Children especially become easily infected as they eat or drink contaminated food and water, and have contact with dirty hands or objects. The disease has serious effects on their cognitive and physical development. High prevalence of this disease has been attributed to poor environmental sanitation, poor personal hygienic practices and lack of portable water, especially in developing countries like Ghana. Even
in clean environments, amoebiasis is still known to infect children. There is no risk involved in this type of study. It will be beneficial to parents after the study has been conducted, to know the status of their wards with regards to *Entamoeba* infection so that the needed medical attention can be sought.

**Contact**

Please direct questions and queries regarding this study to Miss Dorcas Coffie (0543041058), an Mphil student at the Department of Medical Microbiology, University of Ghana (School of Biomedical and Applied Sciences, Korle-Bu).

*Supervisors:* Rev. Prof. Patrick Ayeh Kumi (0244042718) and Dr. Patience Borkor Tetteh Quarcoo (0244633251).

**Participation**

Upon reading and understanding the above, I give my full consent to my ward participating in this study.

__________________________  ______________________  __________________
Name of Parent/ Guardian     Signature/ Thumbprint     Date
### Table A.1  Small subunit rRNA PCR reaction (with primers E1 and E2)

<table>
<thead>
<tr>
<th>REAGENT</th>
<th>AMOUNT (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>5.0</td>
</tr>
<tr>
<td>Primer E1</td>
<td>1.25</td>
</tr>
<tr>
<td>Primer E2</td>
<td>1.25</td>
</tr>
<tr>
<td>2× Gotaq</td>
<td>12.5</td>
</tr>
<tr>
<td>DNA</td>
<td>5.0</td>
</tr>
<tr>
<td>Total volume</td>
<td>25.0</td>
</tr>
</tbody>
</table>

GoTaq Green Master Mix (Promega) = Taq DNA polymerase, dNTPs, MgCl2, reaction buffers, and loading dye.

### Table A.2  16S-like ribosomal RNA PCR reaction

<table>
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<th>REAGENT</th>
<th>AMOUNT (µl)</th>
</tr>
</thead>
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<tr>
<td>Distilled water</td>
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</tr>
<tr>
<td>Primer Eh1</td>
<td>1.25</td>
</tr>
<tr>
<td>Primer Eh2</td>
<td>1.25</td>
</tr>
<tr>
<td>2× Gotaq</td>
<td>12.5</td>
</tr>
<tr>
<td>DNA</td>
<td>5.0</td>
</tr>
<tr>
<td>Total volume</td>
<td>25.0</td>
</tr>
</tbody>
</table>
### Table A.3  SREPH gene PCR reaction

<table>
<thead>
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<th>REAGENT</th>
<th>AMOUNT (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>1.5</td>
</tr>
<tr>
<td>Primer F- [SREPH-5 and nSREPH-5] (6pmol)</td>
<td>5.0</td>
</tr>
<tr>
<td>Primer R - [SREPH-3 and nSREPH-3] (6pmol)</td>
<td>5.0</td>
</tr>
<tr>
<td>2x Gotaq</td>
<td>12.5</td>
</tr>
<tr>
<td>DNA</td>
<td>5.0</td>
</tr>
<tr>
<td>Total volume</td>
<td>25 µl</td>
</tr>
</tbody>
</table>
A.1 Ethical approval

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

3rd March, 2016.

Dorcas Coffie
Department of Medical Microbiology
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu, Accra

ETHICAL CLEARANCE


The Ethical and Protocol Review Committee of the College of Health Sciences on the 2nd of March, 2016 unanimously approved your research proposal.

TITLE OF PROTOCOL: “Prevalence and Speciation of Entamoeba species among children in Accra, Ghana”

PRINCIPAL INVESTIGATOR: Dorcas Coffie

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee’s duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 30th September, 2016.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: ..........................................................
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS
Head of Department
GHANA EDUCATION SERVICE

My ref. No. GES/ACD/PJ73

Our Ref. No...............................

THE CIRCUIT SUPERVISOR
USSHER CIRCUIT (C13)

REPUBLIC OF GHANA

November 18, 2015.

LETTER OF INTRODUCTION: MS. DORCAS COFFIE

I wish to write to introduce Ms. Dorcas Coffie an MPhil; student in the Department of Microbiology, School of Biomedical and Allied Health Science, College of Health Sciences, Korle Bu. She has been granted permission to collect faecal samples from the students at John Wesley Methodist Basic School in the Ashiedu Keteke Sub-Metro in the Accra Metropolis to enable her to undertake her project work.

The School Health Education Programme (SHEP) collaborates with Ministries, Departments and Agencies (MDA), Non Governmental Organizations (NGOs) as well as individuals for effective implementation of programmes. This is one such collaboration between the Ghana Education Service and Ms. Dorcas Coffie.

By this letter, I entreat the Headteachers and Staff especially the school based School Health Education Programme (SHEP) Coordinators to give them the necessary support needed for effective implementation of the programme.

However, these activities should not affect the regular contact hours.

I am counting on your usual cooperation.

ANGELA TENA MENS AH
DIRECTOR OF EDUCATION
ACCRA METROPOLIS

CC.
MS. DORCAS COFFIE
THE HEAD OF SCHOOL
MEMO

PRINCESS MARIE LOUISE CHILDREN’S HOSPITAL

TO: HEAD OF CLINICAL SERVICES
FROM: HEALTH SERVICES ADMINISTRATOR
CC: OPD IN-CHARGE, WARD IN-CHARGES AND HEAD OF NURSING ADMINISTRATION
DATE: MAY 16, 2016
SUBJECT: INTRODUCTION: DORCAS COFFIE (MS).

The aforementioned student from School of Biomedical and College of Allied Health Science, Korle-Bu has been granted permission to undertake research at this Hospital.

She is researching into the topic “Prevalence Entamoeba Species Among Children in the Accra”.

Kindly give her the necessary assistance to enable her complete the research successfully.

Thank you.