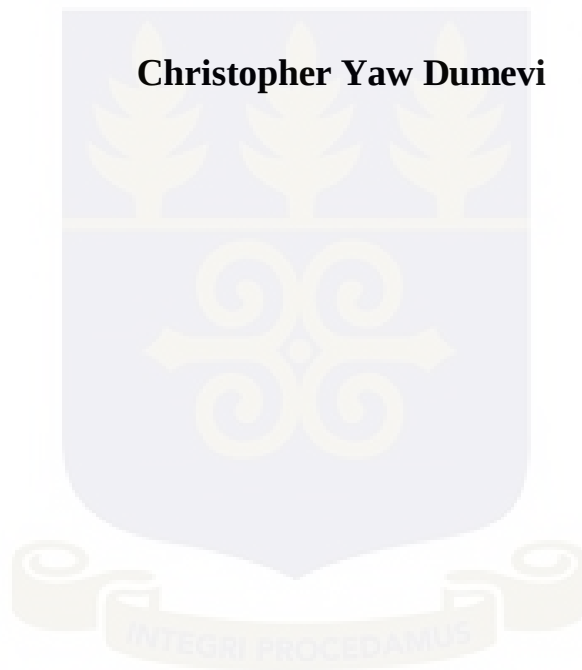


**Intestinal *Entamoeba* complex infection among School  
Children in the Ho Municipality**

**Christopher Yaw Dumevi**



**July, 2017**

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Children in the Ho Municipality**

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Ghana, Legon in partial fulfillment of the requirement for the award of  
**Master of Philosophy degree in Medical Microbiology**

**Department of Medical Microbiology**

School of Biomedical and Allied Health Sciences  
College of Health Sciences, University of Ghana, Legon

July, 2017



## DECLARATION

It is hereby declared that the work in this thesis is original and was carried out by the author under supervision and work from other authors were duly cited and acknowledged. This work has not been partially or wholly submitted to any other institution for the award of any degree.

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## ABSTRACT

Amoebiasis remains one of the commonest gastroenteritis in Ghana especially among children. The *Entamoeba* complex comprises *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii*, which are morphologically identical but genetically diverse. *Entamoeba histolytica* is the only known pathogenic protozoan *Entamoeba* species. The epidemiology of *Entamoeba* complex infections within the Ho Municipality in the South-Eastern part of Ghana has not been thoroughly investigated and the aim of this study was to determine the burden of *Entamoeba* complex infection among school children between 4-15years. This was a cross-sectional study involving 302 primary school children in six communities categorized as Urban, Peri-Urban and Rural. Single fresh stool specimens were collected and examined microscopically using the direct wet mount preparation and formal-ethyl acetate concentration technique for the identification of the protozoan cyst and trophozoites. Structured questionnaire was used to determine demographics and risk factors associated with *Entamoeba* complex infection among the school children. The overall prevalence of *Entamoeba* complex among the children was (23.8%) 72/302. The infection was highest among 8-9 years (30.4%) and 12-13year (28.8%) age groups. Children from Peri-Urban communities were more infected with *Entamoeba* complex 33/102 (32.4%), compared to Urban 25/101 (24.8%) and Rural 14/99 (14.1%). Risk factors associated with *Entamoeba* complex infection identified were source of food, fingernails biting/thumbs sucking habits and mother's level of education. The study advocates intensive epidemiological investigation and molecular characterization of *Entamoeba* complex in the Volta Region to assess the prevalence and identify the specific species/strains causing infection among school children.

## **DEDICATION**

This work is dedicated to my dearest wife Mrs. Victoria Emefa Dumevi for her support and my lovely children Deladem and Selikem who have been my source of inspiration. It is also dedicated to my lovely parents; Rev. Cephas Adehenu and Rev. (Mrs.) Christiana Adehenu. To God be the Glory.



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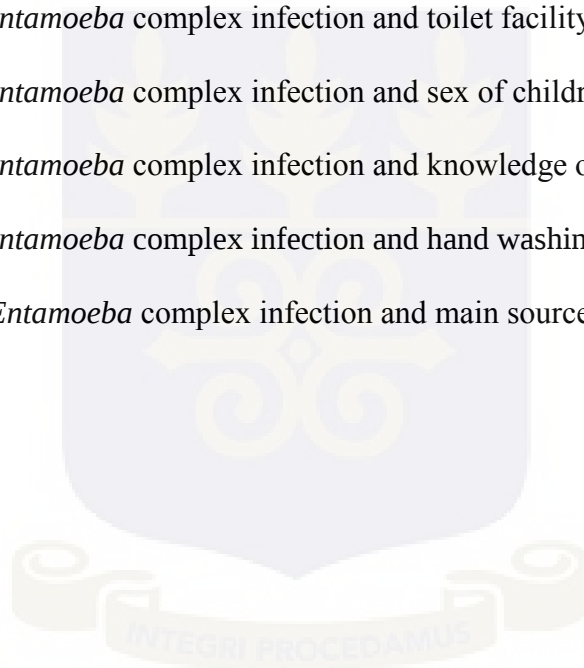
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## CHAPTER ONE

### INTRODUCTION

#### 1.1 General Introduction

Amoebiasis is a type of gastroenteritis caused by the parasite *Entamoeba histolytica* (Shrivastava *et al.*, 2011). The infection is mostly subclinical but may result in intestinal or extra-intestinal disease. An acute amoebic infection presents frequent diarrhoea or dysentery with small or bloody stools. Chronic amoebic infection may present gastrointestinal symptoms such as weight loss, fatigue and intermittent fever. Extra-intestinal amoebic infection occurs when *Entamoeba histolytica* invades the liver causing amoebic liver abscess with clinical signs such as fever and pain in the abdomen within the right upper quadrant (Ximenez *et al.*, 2010).

About 480 million people were estimated to have *Entamoeba histolytica* infection in 1981 out of which about 36 million developed colitis or extra intestinal abscesses according to Walsh, (1986). Most amoebic infections occur in developing countries due to poor sanitation (Skappak *et al.*, 2014; Chacon-Cruz, 2009). About eight (8) amoebic organisms are known to colonize the intestinal lumen of humans. These include *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba moshkovskii*, *Entamoeba polecki*, *Entamoeba hartmanni*, *Endolimax nana* and *Iodamoeba bütschlii* (Clark, 1998; Garcia *et al.*, 1999; Haque *et al.*, 1998; Leber *et al.*, 1999).

Nonetheless, they are considered generally as commensals except *Entamoeba histolytica* (Fritsche *et al.*, 2001; Garcia *et al.*, 1999; Leber *et al.*, 1999; and Petri *et*

*al.*,1999). However, *Dietamoeba fragilis*, *Entamoeba polecki* and *Iodamoeba bütschlii* occasionally are implicated in human diarrhoeal cases (Chan *et al.*, 1996; Cuffari *et al.*, 1998).

The *Entamoeba* complex comprises *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* which are all identical morphologically but are diverse genetically. Within the *Entamoeba* complex, *Entamoeba histolytica* is the only known invasive protozoan *Entamoeba* species while *Entamoeba dispar* and *Entamoeba moshkovskii* are non-invasive (Clark *et al.*, 1991; Tannich *et al.*, 1991). Amoebic infections occurs worldwide, but is common among patients seeking treatment in health facilities with diarrhoea in areas or countries with poor sanitation, particularly in the tropics while in developed countries amoebiasis is common in older patients but prevalent among sexually active homosexuals or in institutions (Samie *et al.*, 2012; Skappak *et al.*, 2014).

Statistics on prevalence of *Entamoeba* complex infection globally shows infected asymptomatic individuals make about 90% of total infected persons whereas only 10% progress to develop disease (Ayeh-Kumi *et al.*, 2001). This leads to between 50-100 million reported cases of amoebic colitis or liver abscesses yearly resulting in about 100,000 deaths. Amoebiasis is next to malaria in relation to mortality due to protozoan infection with estimated global annual prevalence of amoebic liver abscess at between 40-50 million leading to 40,000-100,000 deaths (Agha *et al.*, 2002; Ayeh-Kumi *et al.*, 2001).

Clinical presentation of amoebic infection include pain in the abdomen, diarrhoea, dysenteric stools, bloody or mucoid stools, ameboma, toxic mega colon, colonic perforations, peritonitis, amoebic cecitis and appendicitis, cutaneous amoebiasis,

rectovaginal amoebic cutis, hemorrhage among other complications (De Villiers *et al.*, 1998). Other Clinical manifestations may involve extra-intestinal organs such as the liver. The most common clinical presentation of invasive amoebic infection is amoebic liver abscess which affects organs such as the kidney, heart, brain, genitourinary tract and the pleura pulmonary (Amin, 2002).

The prevalence of *Entamoeba* complex infections among children has been varied as identified by different studies conducted in Ghana and beyond. A study conducted in Northern Iran by Sharbatkhori *et al.* (2014) revealed that the prevalence of *Entamoeba* complex in children with dysentery was 23.8% (25/105) whereas in Akure, Nigeria, the prevalence was 67.6% (188/278) according to Simon-Oke and Ogunleye, (2015). In Ghana, in Bawku District, a prevalence of 39.8% (98/246) was recorded by Verweij *et al.* (2003) while 0.21% (5/2400) was reported by Walana *et al.* (2014) among Primary school children in Kumasi. Further, a study conducted by Opintan *et al.* (2010) in the Princess Marie Louise Hospital in Accra showed a prevalence of 2.9% (5/170) among children with diarrhoea.

## 1.2 Problem Statement

Amoebic infections are global in distribution with public health importance. It is more common in developing nations than in developed nations as a result of poor sanitation (Chacon-Cruz, 2009). The protozoan parasite, *E. histolytica* causing the infection is second to *Plasmodium* (causing malaria) causing death and the third most prevalent in morbidity and mortality after malaria and Schistosomiasis combined (Shrivastava *et al.*, 2011). An Amoebic infection is transmitted when the infective cyst contaminates food or water that is consumed. It may also be spread

through direct contact with a carrier containing the cysts on the hands after defecating when proper hand washing is not done.

Infected humans may excrete about 45 million cysts of the pathogenic *Entamoeba histolytica* daily (Ryan and Ray, 2004). This is because invasive amoebiasis is the second most severe disease after malaria in terms of the distribution of the parasite (Agha *et al.*, 2002) with high prevalence in Africa, Asia, Central and South America (Petri *et al.*, 1999).

*E. histolytica* infects about 10% of the world's population in developing countries as a result of poor sanitation (Chacon-Cruz, 2009). Due to its high morbidity, an intestinal amoebic infection among children if left untreated will cause the individuals to be at higher risk of developing intestinal amoebic colitis or the extra-intestinal amoebic abscesses or death.

In Ghana, a study conducted in six (6) neighbouring communities in Kumasi by Walana *et al.* (2014) showed high intestinal protozoan prevalence of 42% with children between 5-6 years most infected compared with ages 7-8 years, 9-10 years and 11-12 years. Studies conducted by Addy *et al.* (2004) which were corroborated by Ayeh-Kumi *et al.* (2009) suggested that, younger children easily get themselves infected through contaminated water, dirt, and soil. In addition, improper personal hygiene is considered a high risk factor accounting for high prevalence in children <12 years in Ghana.

A study conducted among food vendors in Accra, Ghana revealed that poverty, inaccessible potable water and inadequate personal and environmental hygiene accounted for the carriage and increasing transmission of protozoan parasitic infection including *Entamoeba histolytica* among the food vendors (Ayeh-Kumi *et al.*, 2009). These infected food vendors become carriers and transmit the parasites to members of the public including children through contaminated foods.

The epidemiology of *Entamoeba* complex infections within the Ho Municipality in the South-Eastern part of Ghana has not been thoroughly investigated to determine the burden of *Entamoeba* complex infection among school children between 4-15years

### 1.3 Justification

Intestinal parasitic infections have serious impact on the health of children leading to iron deficiency, anaemia and other nutrient deficiencies which hinder their normal development as well as their ability to achieve their genetic growth potential (Brooker *et al.*, 2008). The species of *Entamoeba* complex are both distinct biochemically, immunologically and genetically with different phenotypes. The knowledge of epidemiology of *Entamoeba* complex infection in endemic and non-endemic areas is very critical since about 90% of infected children are asymptomatic and only about 10% show clinical signs and symptoms (Ayeh-Kumi *et al.*, 2001).

These children who have *Entamoeba* complex infection but are asymptomatic serve as carriers and can infect other children. Although *Entamoeba* complex infection is endemic in Ghana not much studies have been conducted in the Ho Municipality. This contributes to a wide knowledge gap of *Entamoeba* complex species prevalence

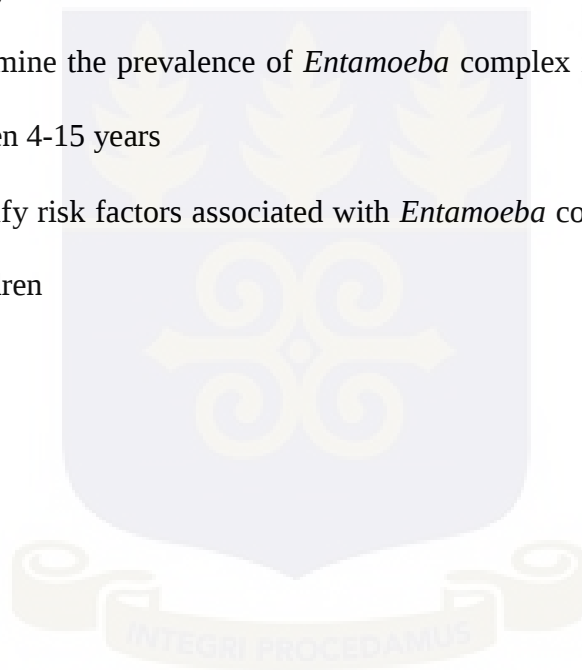
in the Ho Municipality. To overcome this challenge it is imperative to identify the prevalence of *Entamoeba* complex in the study site and its impact on public health.

#### **1.4 Aim**

To determine the burden of *Entamoeba* complex infection among school children in the Ho Municipality.

#### **1.5 Specific Objectives**

- To determine the prevalence of *Entamoeba* complex infection among school children between 4-15 years
- To identify risk factors associated with *Entamoeba* complex infection among the school children



## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1. General definition of amoebiasis

Amoebic infection or amoebiasis is a protozoan parasitic infection of the gastrointestinal tract caused by *Entamoeba histolytica* with or without clinical manifestations. Invasive amoebic infection occurs when symptoms are presented. The human body serves as the natural host while the large intestine is the main target organ (Shrivastava *et al.*, 2011). The life cycle of *Entamoeba histolytica* is simple with the cyst generally thought to be the resistant form of the parasite being the main infective form. The cysts are impervious to dryness in the soil which enables it to survive in water and in humid conditions for weeks. The natural transmitters of amoebiasis are asymptomatic cyst passers and those having amoebic colitis. Excreted cysts in stools becomes a source of food and water contamination (Ximenez *et al.*, 2010).

Transmission of amoebic infection is mainly by faeco-oral route in humans through contaminated food and water or a direct contact with symptomatic or asymptomatic carrier's faecal matter containing the cysts. This infection affects the gastrointestinal tract of humans and about 10% of people infected globally are in undeveloped countries with poor environmental hygiene (Skappak *et al.*, 2014; Vandenberg *et al.*, 2006). There is a poor understanding of amoebic infections

in Sub-Saharan Africa due to lack of species differentiation between infections caused by *Entamoeba histolytica* as against *Entamoeba dispar* (Stauffer *et al.*, 2006). *Entamoeba histolytica* is the only *Entamoeba* species associated with amoebiasis causing invasive amoebic colitis and extra-intestinal amoebiasis (Santos *et al.*, 2007; Haque *et al.*, 2006). However, mild or sub-clinical manifestations in humans may occur due to *Entamoeba histolytica* infection (Ximenez *et al.*, 2009; Leber *et al.*, 2011).

Different clinical presentations ranging from subclinical commensal colonization to a more serious invasive amoebic colitis and extra-intestinal infections are associated with amoebic infections in humans. Studies have suggested that only one in four *Entamoeba histolytica* infections present clinical signs (Santos *et al.*, 2007; Haque *et al.*, 2006; Ali *et al.*, 1993).

WHO therefore recommends the speciation between *Entamoeba histolytica* and *Entamoeba dispar* when possible and treatment of patients should not solely be based on microscopy examinations (Fotedar *et al.*, 2007). However, presumptive or confirmed diagnosis of *Entamoeba histolytica* should be treated regardless of symptoms to minimize the risk of progression to the invasive stage which may lead to death.

## 2.2 Historical background of amoebiasis

Hippocrates (460-377 BC) probably was the first to have diagnosed amoebiasis when he defined the case in a patient who had fever and dysentery. The name *Entamoeba histolytica* was first given to the protozoan parasite in 1903 by Firtz

Schaudin and by 1913, Walker and Sellards, in the Philippines identified the cysts of *Entamoeba histolytica* as the infective form (Walker *et al.*, 1913). Later reference was made to dysentery by Huang Ti and the *Old Testament in Classic in Internal Medicine* (140-87BC) (Kean, 1988). The first literature on *E. histolytica* was reviewed by Clark *et al.* (2000) and Kean (1988). Friedrich Losch was the first to have described amoebiasis and *Entamoeba histolytica* in 1873 in St. Petersburg, Russia when he observed large numbers of amoeba in the faeces of a young farmer (Losch, 1875).

The differences between amoebic colitis and amoebic liver abscess were stated by Sir William Osler. In 1961, Diamond cultured the parasite in axenic culture, and delineated the pathogenic strain (*E. histolytica sensu strictu*) and non-pathogenic *E. dispar* in 1979 (Saklatvala, 1993). James Annersley in 1828 implied a relationship between liver abscess, and dysentery asserting "...hepatic disease seems to be induced by the disorder of the bowels" (Kean, 1988). In the 1800s, the signs and symptoms indicative of intestinal infection were widespread despite the lack of proper diagnosis.

The etiology of amoebic infection was first documented in 1855 in Prague, Czech Republic where amoebic species were isolated and identified when a stool sample was analyzed from a child. Additionally, Friedrich Losch isolated *Entamoeba histolytica* in 1875 in stool samples from a patient who had dysentery (Kean 1988; Stilwell, 1955).

In 1912, Leonard Rogers prescribed emetine as the initial treatment for amoebic infection (Rogers, 1912). Walker and Sellars also established the infective form of

*Entamoeba histolytica* as the cyst Walker *et al.*, (1911). In 1925, the life cycle of *Entamoeba histolytica* was described by Dobell. Emile Brumpt proposed the presence of two parasites which are morphologically indistinguishable; the invasive *Entamoeba histolytica* and non-invasive *Entamoeba dispar* and stated that *Entamoeba histolytica* was the only human pathogenic species while *Entamoeba dispar* is a commensal (Brumpt, 1925). The axenic culture of *Entamoeba histolytica* done first by Diamond in 1961 gave insight into the cell biology and the biochemistry of *Entamoeba histolytica* (Diamond, 1961). In 1969, amoebiasis was defined by the WHO as “infection with *Entamoeba histolytica*, with or without clinical manifestations” thus suggesting all strains were potentially pathogenic but could not state why some people become asymptomatic.

Sargeant *et al.* (1978) however proved that *Entamoeba histolytica* and *Entamoeba dispar* can be distinguished by zymodeme analysis. In 1993, owing to immunological, biochemical and genetic information that supported Emile Brumpt’s description, a formal re-description of *Entamoeba histolytica* was published with *Entamoeba histolytica* named the invasive species and *Entamoeba dispar* is the non-invasive species. During a WHO meeting in 1997 at Mexico, a clear guideline to distinguish the two species was stated (WHO, 1997). In recent times, there is greater understanding of amoebic species due to the increasing knowledge in molecular based techniques.

### **2.3 Classification and etiology of *Entamoeba histolytica***

Several species under the genus *Entamoeba* which inhabit the human intestinal lumen have been identified. Six of these species include *Entamoeba dispar*,

*Entamoeba histolytica*, *Entamoeba hartmanni*, *Entamoeba coli*, *Entamoeba moshkovskii*, and *Entamoeba polecki* (Fotedar *et al.*, 2007). *Entamoeba histolytica* is the main *Entamoeba* species which causes amoebic infections and the major global protozoan death in among humans. Even though current studies showed the discovery of *Entamoeba moshkovskii* and *Entamoeba dispar* in patients with gastrointestinal symptoms, no scientific proof has established a causal connection between the existence of the two *Entamoeba* species and the clinical signs of the host (Fotedar *et al.*, 2007).

Around 1925 when discussion on speciation was rife, Emile Brumpt suggested the existence of two diverse *Entamoeba* species that could penetrate the intestine of humans. *Entamoeba histolytica* causing diarrhoea with or without dysentery and *Entamoeba dispar* excreted in stools of asymptomatic individuals. There was no molecular tool to differentiate *Entamoeba histolytica* from *Entamoeba dispar* species prior to 1990s in terms of their pathogenicity and non-pathogenicity (Clark *et al.*, 1991). These species are both genetically diverse and this diversity led to molecular studies in epidemiology in endemic geographical areas.

By the 1990s, there was sufficient proof in support of the separation of the two morphologically identical amoebic species: the pathogenic *E. histolytica* from the non-pathogenic strain *E. dispar* (WHO, 1997; PAHO, 1997; Diamond and Clark, 1993). Research has revealed that apart from *E. dispar*, two other *Entamoeba* species (*E. moshkovskii* and *E. bangladeshi*) have similar morphology in humans and diagnosis using microscopic appearance has limited their differentiation until recently (Farrar *et al.*, 2013). Certain strains of *Entamoeba histolytica* have been identified and isolated from asymptomatic individuals who excrete cyst in their

stools, those with invasive amoebiasis or with amoebic liver abscess (Ramos *et al.*, 2005; Nozaki *et al.*, 2006).

Nevertheless, *Entamoeba histolytica* isolated from stool samples have high polymorphism genetically (Ramos *et al.*, 2005) far above *Entamoeba dispar* (Ximenez *et al.*, 2009). Genetic variants of *Entamoeba dispar* have been identified in patients in endemic countries such as Brazil and Mexico. The *dispar* genetic variant ICB-ADOE was detected in Brazil from a patient with non-dysenteric colitis sustained in culture by intestinal flora showing pathology in experimental simulations of amoebic liver abscess (Shibayama *et al.*, 2007; Costa *et al.*, 2006).

In Mexico six patients had *Entamoeba dispar* after DNA sequencing when the DNA extract from their liver abscess material was analyzed (Ximenez *et al.*, 2010). *Entamoeba moshkovskii* is considered a free-living non-pathogenic species contrary to *Entamoeba dispar* and *Entamoeba histolytica*. *Entamoeba moshkovskii* has been often been isolated from individuals in developed and highly industrialized nations (Pritt *et al.*, 2008). However, its pathogenicity and the framework of its epidemiological importance in infection transmission and morbidity of amoebiasis are under study.

The *Entamoeba* Complex comprises pathogenic *Entamoeba histolytica* and the non-pathogenic *Entamoeba dispar* which are both distinct biochemically, immunologically and genetically with different phenotypes. Molecular and immunological methods led to the advancement of diagnostic tools for speciation. The knowledge of epidemiology of amoebic infection in endemic and non-endemic

areas is achieved mainly by molecular techniques (Ximenez *et al.*, 2009; Blessmann *et al.*, 2002; Haghighi *et al.*, 2003).

Furthermore, molecular epidemiological investigations have revealed amazing variability genetically (Haghighi *et al.*, 2003; Ramos *et al.*, 2005) between *E. histolytica* and *E. dispar* leading to the discovery of *E. moshkovskii* which also infects with significant frequency the human intestine. However, extensive epidemiological studies and its contributions to morbidity of *Entamoeba* infections remain unidentified (Ximenez *et al.*, 2009; Pritt *et al.*, 2008). Nonpathogenic *Entamoeba* species generally are commensals or non-invasive and are asymptomatic. However, *Entamoeba hartmanii* although morphologically similar to other *Entamoeba* species, can be differentiated based on size. *E. coli* and *E. poleckii* can also be distinguished morphologically from others using molecular techniques even though there may be an overlap of some morphologically features due to the nature of the specimen (Garcia, 2007).

Microscopy remains the main method of identifying *Entamoeba* cyst or trophozoites in stool samples although it cannot be used for speciation because the cystic and the trophozoic stages of *Entamoeba* complex (*E. histolytica*, *E. dispar*, and *E. moshkovskii*) are morphologically alike. Nonetheless, molecular method can be used to differentiate between all species of *Entamoeba* (Santos *et al.*, 2010).

Amoebic infections are classified as either symptomatic (10%) or asymptomatic (90%). Of the 10% of patients that are symptomatic, 90% of the infection is intestinal or luminal (Amoebic colitis) and 10% causing extra intestinal infection (Amoebic abscesses) (Ayeh-Kumi *et al.*, 2001). Common clinical manifestations of

invasive amoebiasis include acute colitis or acute right upper quadrant pain with fever, each having a broad differential diagnosis (Ravdin, 1994).

Untreated amoebic infection can lead to complications resulting in the development of ulcers in the caecum, appendix or adjacent portion of the ascending colon. The ulcers normally have small openings on the mucosal lining of the colon and a bigger region of damage beneath the mucosal surface (flask-shaped). Secondary infections may result when the ulcers are invaded by bacteria. The perforated ulcers on rare occasions may lead to the patient's death due to peritonitis (Petri *et al.*, 1990; Sargeant *et al.* (1978). The common pathogens isolated and identified from diarrhoeal stools are *Entamoeba histolytica* and *Entamoeba dispar*.

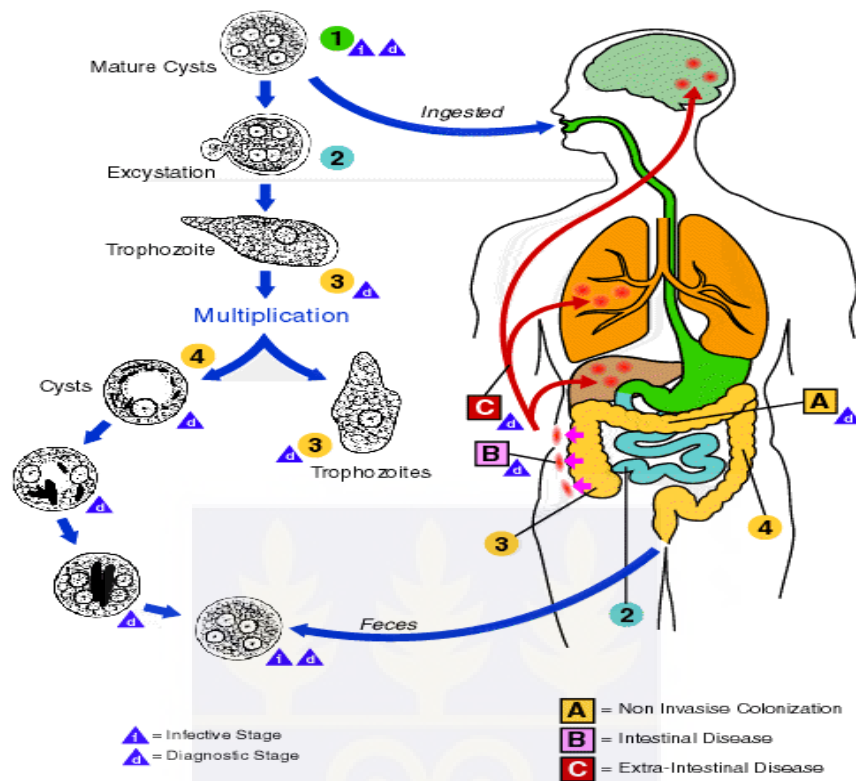
#### **2.4 Life Cycle of *Entamoeba histolytica***

Various species in the protozoan genus *Entamoeba* colonize the human body. However, disease is only associated with *Entamoeba histolytica* which is the pathogenic species while other species are relevant for diagnostic purposes. The *Entamoeba* cysts mostly are found in formed stools while the trophozoites are mostly located in watery or diarrheal stools. The ingestion of mature *Entamoeba histolytica* cysts in food or water contaminated with faecal matter leads to infection. Excystation happens in the ileum leading to the release of the trophozoites which move to the colon. The multiplication of the trophozoites by binary fission leads to the development of cysts. Cystic and the trophozoic phases are excreted in faeces. The protection conferred by the cystic walls, enables the cysts to survive up to weeks in the harsh environment externally and are the main form of transmission.

Nonetheless, the trophozoites excreted in stools quickly get destroyed outside the human body and if ingested, cannot withstand the gastric environment of the stomach. Mostly, the trophozoites are restricted to the lumen of the intestine (non-invasive amoebic infection) in asymptomatic persons, when they excrete cysts the in stool. The trophozoites in some individuals invade the mucosa of the intestine (intestinal disease), or, through the bloodstream to reach extra-intestinal organs such as the lungs, the brain and the liver (extra-intestinal amoebiasis) causing pathological signs. Research proved the existence of separate *Entamoeba* species causing invasive and non-invasive amoebiasis which include *Entamoeba histolytica* and *Entamoeba dispar*. These *Entamoeba* species are indistinguishable morphologically except when *Entamoeba histolytica* has ingested Red Blood Cells (erythrophagocytosis) (Talamás-Lara, *et al.*, 2014) as observed under the microscope. Also, infection could happen when one is exposed to faecal matter through anal sex during which both the cysts and the trophozoites may be source of infection). **Source:** (<http://www.cdc.gov/dpdx/amebiasis/> Accessed on June 30, 2016 at 9:33am)



### Life Cycle of *Entamoeba histolytica* in Humans



**Fig 1.0 Life Cycle of *Entamoeba histolytica* in Humans**

**Source:** [http://www.pharmacology2000.com/Chemotherapy/Antiparasitic/Amebiasis\\_Life\\_Cycle1.htm](http://www.pharmacology2000.com/Chemotherapy/Antiparasitic/Amebiasis_Life_Cycle1.htm) Accessed on June 30<sup>th</sup> 2016 at 9:14am

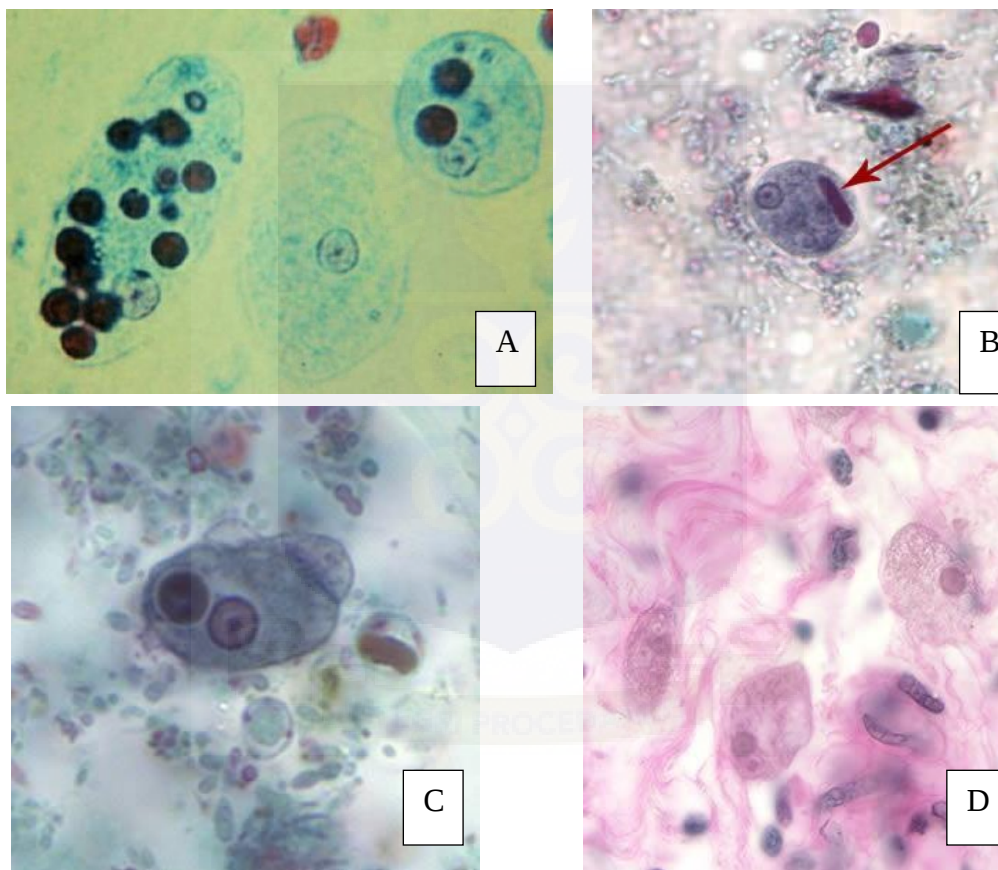
### 2.5 Morphology of the cyst and the trophozoites of *Entamoeba histolytica*

The trophozoites of *Entamoeba histolytica* is highly pleomorphic and refractive in appearance. It is the active, growing, multiplying stage and the stage that invades tissues and produces lesions. In fresh stools, it is active, fragile and motile. Usually elongated when active but may be spherical when in a resting stage. The average size is around 25  $\mu\text{m}$  (10-25  $\mu\text{m}$ ) and usually in diarrhoeric or dysenteric stools. Trophozoites can't survive the gastric environment of stomach but cyst does. In

dehydration, trophozoites forms precyst. Precyst secretes a thin but tough wall (cyst wall) around itself and it forms the cyst. Cyst unnucleated but becomes bi-nucleated then tetra-nucleated when fully matures. (Source:

<http://www.cdc.gov/dpdx/amebiasis/> Accessed on June 30, 2016 at 9:33am)

Cyst can withstand gastric acidic conditions, chlorination as well as dryness and survives in moist environment for months. When viable cysts are ingested in food or water, cysts become activated and coat ruptures and organism escapes protoplast.



**Fig1. 1A.** Trichrome stain of *E. histolytica* trophozoites with ingested erythrocytes. **Fig.1.1 B** Cyst of *E. histolytica/E. dispar* stained with trichrome. Chromatoid body with blunt ends (red arrow). **Fig. 1.1C.** Trophozoites of *E. histolytica* with ingested erythrocytes stained with trichrome. The ingested erythrocytes appear as dark inclusions. The parasite above show nuclei that have the typical small, centrally located karyosome, and thin, uniform peripheral chromatin. **Fig. 1.1D.** *Entamoeba histolytica* trophozoites in colon tissue stained with H&E. **Source:** <http://www.cdc.gov/dpdx/amebiasis/> Accessed on June 30, 2016 at 9:33am

## 2.6 Epidemiology of *Entamoeba histolytica*

Amoebic infections are common parasitic infection that occurs globally and nearly 480 million infections are recorded with *Entamoeba histolytica* resulting in between 40,000 and 110000 deaths annually (as cited in Beeching *et al.*, 2014). However, it is the most parasitic infection that results in death in tropical countries apart from Malaria and Schistosomiasis. *Entamoeba histolytica* is very endemic in areas of Africa, India, Central America and Asia. It is spread by the faecal-oral route and poor socioeconomic status and improper sanitation are predisposing factors. Other risk factors include long-term institutionalization with psychiatric illness as well as people travelling from endemic areas (Gathiram *et al.*, 1985; Ravdin *et al.*, 1990; Abd-Alla *et al.*, 1993).

*Entamoeba* species are easily transmitted among family members and infrequently in day care centres resulting in epidemics (Barwick *et al.*, 2002; Braga *et al.*, 2001). *Entamoeba histolytica* is the major cause of diarrhea among children and significantly affect their health especially in populations living in the tropics with low socio-economic status. (Stauffer and Ravdin, 2003). Other people at a higher risk of amoebic infections include: immigrants, travelers, immunosuppressed individuals, sexually active homosexuals, mental patients, and prisoners. The common species isolated and identified from diarrhoeal stools are *Entamoeba histolytica* and *Entamoeba dispar*. The incubation period of the protozoan parasite ranges from 2-4 weeks within the mucosal lining of the colon (Quinn *et al.*, 1983).

Scientific evidence has supported the fact that geographical variation of amoebiasis occurs. While in Egypt amoebic colitis is the main invasive amoebiasis (Abd-Alla *et*

*al.*, 2002), amoebic liver abscess is prevalent in South Africa. Also, a study conducted in South Africa among asymptomatic individuals using zymodeme and culture analysis showed 10% prevalence of *Entamoeba complex* while the prevalence of *E. dispar* was 90% compared with 10% *E. histolytica* infection. Females were more infected with *E. dispar* than males whereas there was equal incidence of *E. histolytica* and prevalence in both males and females (Gatharim and Jackson, 1987). However, the prevalence of *E. histolytica* was the same in all age groups, both sexes, and persons above 16 years had higher chance of invasive amoebiasis was noted (Gatharim and Jackson, 1987).

In South Korea, Japan and Taiwan, higher risk of amoebic infection was reported among homosexuals because to the oral-anal sexual activity (Watanabe *et al.*, 2011; Hung *et al.*, 2008). However in Japan, *Entamoeba histolytica* frequently occurred among people suffering from mental retardation with a prevalence and positive serology rate of 38.2% and 67.1% respectively (Nishise *et al.*, 2010). Study in Hue City (Vietnam) had estimated prevalence of amoebic liver abscess of 21 case out of 100 000 residents (Blessmann *et al.*, 2002).

In Bangladesh, impoverished population of 230 children between 2-5 years recruited for a 2-year observational study showed the following: 55% had *Entamoeba histolytica* infection within the period of the study. Out of 55% who were infected, 80% were asymptomatic with 20% having diarrhoea and 4% having symptoms of amoebic colitis. Additional 17% had further *E. histolytica* infection within the 2 years the study was conducted which was linked to a genetically diverse strain when polymerase chain reaction for the serine-rich *Entamoeba histolytica* protein

was determined. (Haque *et al.*, 2002). Additional proof in 1913 from the Philippines showed that volunteers who ingested *E. histolytica* cysts developed amoebiasis.

Among school children with poor socio-economic status in Ecuador, out of 178 children samples in cross sectional study, only 7 had asymptomatic *Entamoeba histolytica* infection. Moreover, 64% had high serologic titers implying recent infection with *Entamoeba histolytica* (Gatti *et al.*, 2002) which agrees with similar sero-survey done in Mexico (Petri and Singh, 1999). The global spread of amoebic infections is complex due to the presence of the *Entamoeba* complex which are morphologically identical but distinct genetically (Santos *et al.*, 2010). This poses a challenge to precise diagnostic tools which are lacking especially in Africa, including other less developed countries in Asia and Latin America. Studies conducted in some African countries showed that between 6% and 75% of the population carry *Entamoeba* species (Njoya *et al.*, 1999). The studies revealed the prevalence of the amoebic infection using microscopic examination. However, there must be a confirmatory test to speciate the pathogenic from the non-pathogenic organisms.

Lawson *et al.* (2004) reported 1% of *Entamoeba histolytica* infection in Aracaju, Brazil whereas 13% of the cases were *Entamoeba histolytica dispar* infections. Nonetheless in northeastern Brazil (Pernambuco state), 74.19% of the positive culture samples analyzed using PCR was *Entamoeba dispar* with no *Entamoeba histolytica* detected (Pinheiro *et al.*, 2004). In a similar endemic country like Bangladesh, the prevalence of *Entamoeba histolytica* was 4.2% in children living in slums in Dhaka using the ELISA antigen detection kits (Haque *et al.*, 2006). Among the population in a rural part of Ecuador, isoenzyme analysis showed 18.9%

*Entamoeba histolytica* infection as against 70.3% *Entamoeba dispar* infection (Gatti *et al.*, 2002).

In endemic areas such as Mexico, about 1000 to 5000 (100,000 residents annually) cases of amoebic infections were reported between 1995 and 2000 and the incidence figures of 1128.8 to 615.85 (100,000 residents annually) was reported between 2002 and 2006. As it is in most developing nations, children below 15 years remain affected the most with a higher number of children between 5 years to 9 years (Ximenez, 2009). A study conducted in Vietnam revealed that personal hygiene and socio-economic parameters determined whether one would be infected with *Entamoeba histolytica* or not instead of mere exposure to faecal matter from humans (Pham duc *et al.*, 2011).

In Africa, seroprevalence investigation in Cote d'Ivoire, Sudan and South Africa demonstrated a high prevalence of amoebic infections (Stauffer *et al.*, 2006). While amoebic colitis have been reported in Nigeria (Okeke *et al.*, 2003), amoebic liver abscess associated with invasive *Entamoeba histolytica* infection was detected in South Africa (Stauffer *et al.*, 2006).

In Ghana, the major routes of diarrhoea outbreaks are food and water with vegetables sold on open- market being a major source (Donkor *et al.*, 2010; Nkrumah *et al.*, 2011). Amoebic infections are of special interest along with other intestinal protozoan infections causing high levels of morbidity and mortality (Amoros *et al.*, 2010). Significant intestinal parasitic infection associated with vegetables is as a result of re-using waste water for agriculture in the peri-urban areas (Pham-Duc *et*

*al.*, 2013). Using just water to wash vegetables bought from open –air markets is insufficient to eliminate completely contaminating pathogens. In comparison to open-air vegetables, vegetables in supermarkets in Accra have low prevalence of parasites ten times less the prevalence in vegetables obtained in the open-markets (Duedu *et al.*, 2014).

Intestinal parasitic infection affects children the most (Brooker *et al.*, 2008). The Common effect of intestinal parasitic infection on the health of children include iron deficiency, anaemia and other nutrient deficiencies which hinder their normal development as well as their ability to achieve their genetic growth potential (Brooker *et al.*, 2008).

Apart from amoebic intestinal colitis, amoebic liver abscess is a major complication due to amoebic infection. Amoebic liver abscess and extra intestinal amoebiasis have low morbidity and occur rarely as compared to the high prevalence of asymptomatic intestinal infections (Ximenez *et al.*, 2009). Amoebic liver abscess affects people of all ages although in certain areas of high endemicity, children below 5 years and young adults between 20-45 years are the most affected. Females are less susceptible to amoebic liver abscess than males (thus 1 female: 4-6 males). (Ximenez *et al.*, 2009).

In amoebic liver abscess, about 80% of the patients show symptoms between 4-6 weeks (Ximenez *et al.*, 2009, Pritt *et al.*, 2008; Ximenez *et al.*, 2010). Common symptoms of amoebic liver abscess include fever (38°C), anorexia, diaphoresis and chills, pain in the abdomen especially in the right upper quadrant with increasing

intensity during inspiration. The pain commonly get to the shoulder and then to the back. Diarrhoea has also been occasionally reported (50% of cases) as well as nausea.



## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Study design

This study was a cross-sectional study conducted within the **Rural** (Klefe Achatime E.P. Primary and Klefe Demete E.P. Primary Schools), **Peri-Urban** (Sokode Gbogame M.A Primary and Sokode Gbogame R.C. Primary Schools), and **Urban** (Ho Bankoe E.P Primary and Ho SSNIT Flats Presby Primary Schools) communities of the Ho Municipality of the Volta Region from October 2016 – March, 2017. The study was to identify the prevalence of *Entamoeba* complex infections among school children between 4-15 years.

#### 3.2 Ethical Consideration

The ethical clearance for this study was sought and approved by the Ethical and Protocol Review Committee of the College of Health Sciences, University of Ghana, Korle-Bu. The Institutional Review Board of the Volta Regional Hospital, Ho and the Municipal Directorate of Education (Ghana Education Service), Ho also permitted that the study be carried out at the Volta Regional Hospital (Parasitology Unit) and in the selected primary schools within the Ho Municipality respectively. The inclusion of participants (Primary School Children) in the study was strictly voluntary based on the informed consent of their parents /guardians after they were made aware of the aims and objectives of the study.

### 3.3. Study Site

The study was conducted within the Ho Municipality of the Volta Region in the South-Eastern part of Ghana. Ho is the capital of the Volta Region and also the location of the Volta Regional Hospital which is currently the leading referral healthcare facility in the Region with 240 bed capacity. The hospital has an average daily OPD attendance of about 500. Single fresh stool sample was collected from each participant recruited for the study and processed in the Volta Regional Hospital Laboratory (Parasitology Unit) between October 2016 –March, 2017.

The choice of the Ho Municipality is due to the fact that not much study has been conducted on *Entamoeba* complex infection in this area. Also, considering the fast population growth and the lifestyle of some residents coupled with challenges posed by limited social amenities such as water and toilet facility informed the choice of the study site.

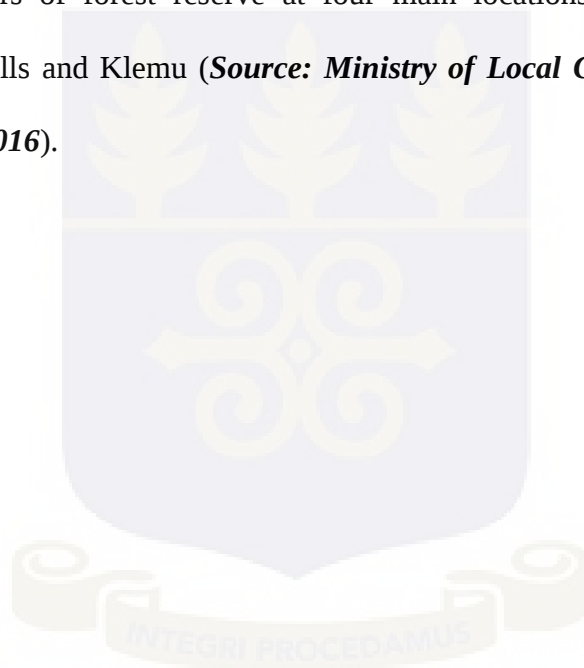
#### 3.3.1 Location, size and demographic characteristics

The Ho Municipality lies between latitudes 6° 20' N and 6° 55' N and Longitudes 0° 12' E and 0° 53' E and covers an area of 2.660 sq. km. The Municipality shares boundaries with the Adaklu District to the South, Hohoe Municipal to the North, South-Dayi District to the West and the Republic of Togo to the East. It is the home to the regional capital of the Volta Region as the largest urban centre. (Source: <http://districts.ghana-net.com> Accessed on 04-02-2017 at 9:23pm). The Ho Municipality is the most populous in the Volta Region with a total population of 271,881 with a male population of 129,180 and female population of 142,701. The

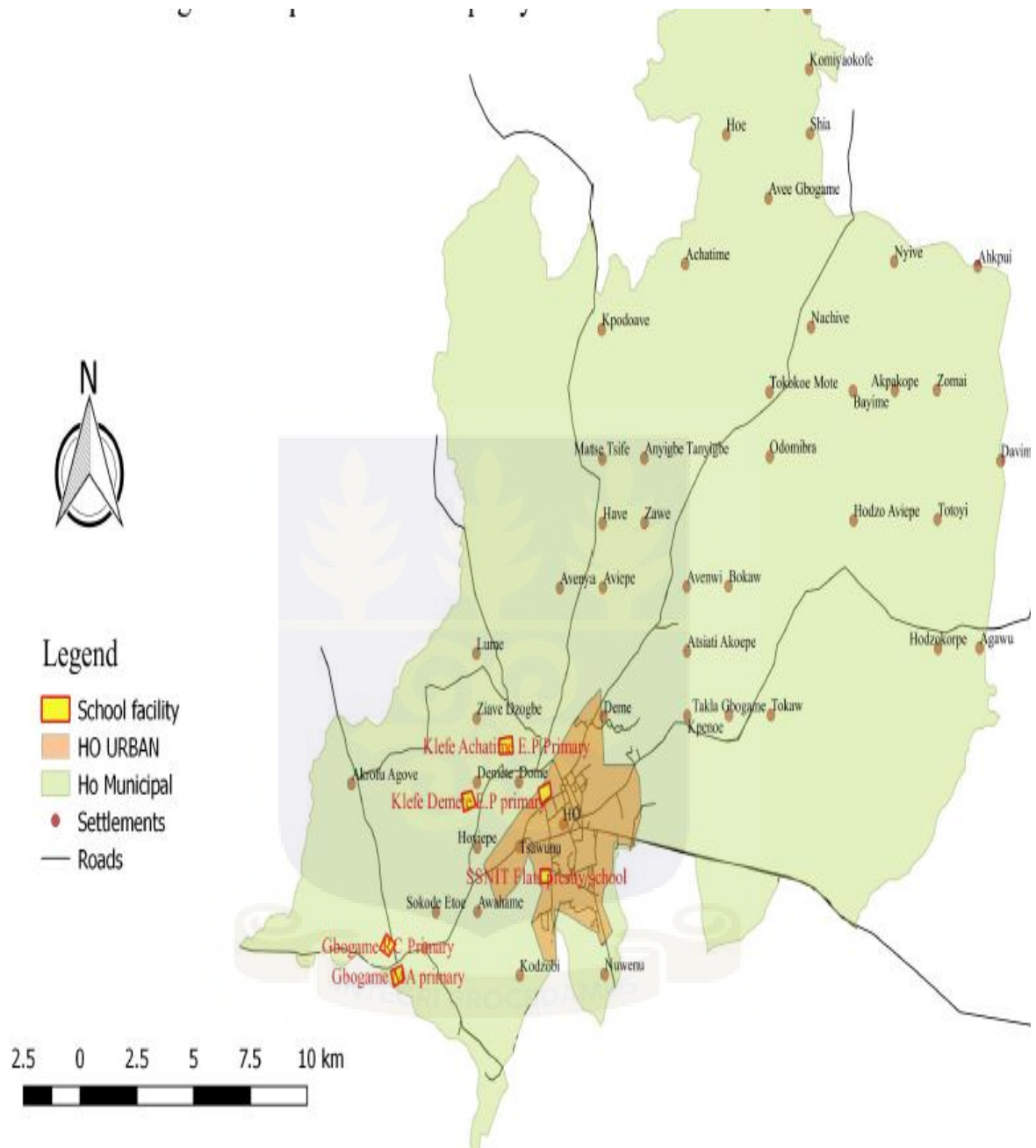
urban and rural populations are 37% and 63% respectively (*Ghana Statistical Service, 2010*). Accessed on 04-02-2017 at 9:27pm

### 3.3.2 Physical characteristics and rainfall

Generally, mean monthly temperatures range between 22°C and 32°C while annual mean temperatures range from 16.5°C to 37.8°C. Temperatures are generally high throughout the year. The two types of vegetation in the Municipality are the moist semi-deciduous forests of the hilly areas and the savannah woodland with 33.83 square kilometers of forest reserve at four main locations: Ho Hills, Kabakaba Hills, Abutia Hills and Klemu (*Source: Ministry of Local Government and Rural Development, 2016*).



## Map of Ho Municipality



**Fig. 3.1** Map extracted and modified from Town Planning Department, Ho Municipal Assembly showing the specific research sites in yellow

### **3.4. Study Subject Selection and Sampling**

The Ho Municipal Education Directorate gave approval for the study to be conducted in sixteen (16) Primary schools within the Municipality. However, due to resource constraints, six (6) primary schools were first selected by stratified random sampling for the study. The sixteen (16) primary schools were categorized into three (3) namely: Urban, Peri-Urban and Rural communities of the Municipality.

Simple Random Sampling was used to select two (2) primary schools from each category. From each category of two (2) schools, 10 pupils each were selected from Primary 1 – Primary 6 using Simple Random Sampling. The pupils were made to pick cards on which was written “Yes” or “No” from a box. All pupils who picked “Yes” were given a special code of identification were included in the study whereas those who picked “No” were excluded. About 60 pupils were selected per school and a total of 120 per category. This was replicated for the other categories and the study subjects were further grouped for analysis into ages 4-5, 6-7, 8-9, 10-11, 12-13 and 14-15 respectively.

### **3.5 Inclusion criteria and Exclusion criteria**

Children between the ages of 4-15 years who were registered in the selected schools within the Ho Municipality and whose parents and teachers have consented were recruited for the study. Pupils whose parents /guardians or teachers confirmed that they were on treatment against intestinal parasitic infection including anti-amoebic therapy were excluded.

### 3.6 Sample size determination

A minimum sample size of the population was calculated using the formula

$$n = \frac{Z^2 \times p(1-p)}{m^2}$$

*Description:* n = minimum sample size, Z = confidence level at 95% (standard value of 1.96). M = margin of error at 5% (standard value of 0.05% , P = estimated prevalence of amoebic infection in the study area.

Since the actual prevalence of *Entamoeba* complex infection in the study site could not be determined, the estimation for disease prevalence used in the sample size calculation was based on the average of the prevalence as was determined by Verweij *et al.* (2003) 39% and 2.9% Opintan *et al.* (2010) = 21% or 0.21

$$\text{Hence, } n = \frac{(1.96)^2 \times 0.21 (1- 0.21)}{(0.05)^2} = \frac{3.8416 \times 0.21 (0.79)}{0.0025} = 254.9$$

Therefore, a minimum sample size of 255 with 30% increase for contingencies was used for the study. Headteachers of the selected schools were served with approval letters from the Ho Municipal Education Directorate and were later briefed on the benefits of the study to their pupils/schools. Subsequently, most heads of schools discussed the request for the study with Parents/Guardians during their Parent-Teacher Association meetings which ended the previous school term in April, 2016. A total of **302** participants (primary school children) whose parents/guardians consented were recruited for the study. The reasons for the withdrawal of some of the participants were unclear.

### 3.7. Sample collection:

Methods of data collection from the participants were by structured questionnaire and interviews by the researcher. Additional information was obtained from Parents /

guardians of children who could not provide certain information in English Language or in the local dialect (Ewe). Information such as personal hygiene, sources of food and water, type of toilet facility at home and in school were elicited. Interviews were conducted in the local language (Ewe) for pupils who could not communicate effectively in the English Language.

With permission sought from school authorities, the entire school community was educated on the need to ensure personal hygiene and more importantly regular hand washing with soap under running water before eating and after visiting the toilet. Pupils who were Randomly Sampled were educated on how to collect the stool sample for examination. The pupils were educated on how to properly collect the stool sample without contamination. Each participant was given a well-labeled leak-proof tight-lid clean stool container with their special code written on it. A single fresh stool sample was collected from each participant without urine contaminant using the school's toilet facility. Not more than 20 stool samples were collected per school per day and processed to ensure maximum result. This was repeated in all selected schools.

To avoid contamination of other pupils or re-infection, thorough cleaning of the anus of the study subjects was ensured by the provision of tissue papers (toilet rolls) and hand washing with soap under running water was also guaranteed by the provision of soaps and water. The stool samples collected were transported to the Volta Regional Hospital Laboratory (Parasitology Unit) within 30 minutes of collection. Structured questionnaires were used to get information their demographics and risk factors associated with *Entamoeba* complex infections.

### **3.8 Laboratory Investigation**

#### **3.8.1 Macroscopy and Microscopy**

The consistency of the stool were observed and recorded as formed, semi-formed, loose, mucoid, slimy or watery in the laboratory. The colour of the stool such as dark/black, bright red, brown, green etc. was noted. A direct wet mount preparation of each fresh stool sample was done and examined followed by the Concentration of the stool by the Formol-Ethyl Acetate Sedimentation technique. Low power 10x and high power 40x objectives were used to examine the stool samples for the identification of the trophozoites or cyst of *Entamoeba* complex or any other parasite present. The microscopic identification of the morphology of the protozoan cyst or trophozoites was by bench aids.

##### **3.8.1.1 Direct Wet Smear**

This technique was purposely used to identify live motile trophozoites for protozoans, cyst (inactive dormant stage of protozoa) or egg/ova of helminthes in the stool samples. Particular species usually were *Entamoeba* complex (Tankeshwar, 2015).

**The Materials, reagents and equipment needed:** stool sample, applicator stick, slides, cover slips, plastic pipette, gloves, glass slides, small tube containing 0.5-1.0 ml of 0.85% NaCl (Normal Saline), Lugol's Iodine solution and binocular microscope with x10, x40 and x100 objectives.

##### **3.8.1.2 Procedure for Wet Smear Preparation**

Half a teaspoon (about 1g) of fresh stool was transferred into a 20 ml beaker containing about 4 ml of 0.85% NaCl and emulsified thoroughly using applicator

stick. Using a Pasteur pipette, a drop each was placed on either ends of the labeled glass slides (26x76 mm). One end was stained with Lugol's iodine but the other was unstained. Each drop of the mixture was covered with a 22 mmx22 mm cover slip. The amount of saline depended on the nature of the stool sample (less saline for watery stool). Examination of the entire slide was done with 10x objective with low light beginning from a corner of the smear thoroughly to sequential adjacent swaths. Each field was examined careful to find any motile organism. High power 40x objective was used to observe the morphological features of any motile organism (protozoan trophozoites) identified. The Lugol's Iodine diffused into the saline suspension under the coverslip. The Iodine made the organism visible but motility was lost (Melvin *et al.*,1985: Tankeshwar, 2015 ).

#### **3.8.1.3 Concentration by Formalin-Ethyl Acetate Sedimentation**

**Reagents and materials:** Ethyl-Acetate, Formalin (10%), 0.85% NaCl (Normal Saline), Lugol's Iodine, funnel, gauze, centrifuge tubes (15 ml), applicator sticks, gloves, glass slides (26x76 mm), cover slips (22 mmx22 mm), disposal glass or plastic pipettes. The equipment used included: Centrifuge, Binocular microscope with x10, x40, and x100x objectives (or approximate magnifications for low-power, high dry power and the oculars should be x10).

#### **3.8.1.4 Procedure for Formalin-Ethyl Acetate Sedimentation**

Half a teaspoon (about 1g) of fresh stool is transferred into 4 ml of 0.85% NaCl (Normal Saline) and emulsified thoroughly. After the direct wet mount preparation, the remainder of the stool- saline mixture was strained through gauze (not more than

two layers) in a funnel into a conical 15 ml centrifuge tube to 7 ml. Then, 7 ml of 10% formalin was added and mixed thoroughly. The mixture was allowed to stand for about 30 minutes for fixation. 3 mls of Ethyl-Acetate was added to make the 14 mls. Contents of the tube was mixed, stoppered and centrifuged at 3000 rpm for 3 minutes. The tubes were removed and supernatant fluid comprising Diethyl-ether, dissolved faecal debris, formol-saline decanted. The sediment at the bottom of the tube containing the parasites was re-suspended in 0.85% NaCl (Normal Saline). Using the plastic pipette, a drop of the suspension was picked and dropped on a labeled, clean grease-free glass slides and covered with cover slip for microscopic examination of cysts, trophozoites or any parasitic organisms using the x10 or x40 objective lenses (Melvin *et al.*, 1985).

### **3.9 Data handling**

All laboratory procedures and results were noted and recorded personally in the laboratory notebook. Personal details of participants were kept separate from their clinical samples by assigning identification codes to samples instead of names to ensure confidentiality. All relevant data was transferred onto Microsoft Office Excel 2010 linked software and saved in the hard disk drive. The laboratory notebook, filed sheet and other relevant information were securely kept in a lockable cabinet. Data files on the computer hard drive was protected by password. There was double entry of data to cross check errors in data capture.

### **3.10. Statistical analysis**

Data from the study sample was analyzed using Statistical Package for the Social Sciences (SPSS) software Version 20 to determine the frequency distribution of

amoebic infection among the sample population. The correlation of amoebic infection between males and females, age groups and risk factors were determined using chi-square test analysis and odds ratios at 95% confidence intervals where appropriate. Data was presented in the form of tables where appropriate.



## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 Total number of primary school children sampled

A total of 302 primary school children between the ages of 4-15 in six (6) different communities were sampled. Out of the 302 participants, 153 (50.7%) were males as against 149 (49.3%) females. This ratio indicated that, the sample population was evenly distributed in terms of gender (Table 4.1).

**Table 4.1 Total number of primary school children sampled**

| Sex          | Age group (yrs) | Number(n)  | Frequency (%) |
|--------------|-----------------|------------|---------------|
| Male         | 4-15            | 153        | 50.7          |
| Female       | 4-15            | 149        | 49.3          |
| <b>Total</b> |                 | <b>302</b> | <b>100</b>    |

#### 4.2 Macroscopic and Microscopic examination of stool samples collected

The consistencies of the stool samples were noted followed by microscopic examination. Majority 158/302 of samples were semi-formed in nature (Table 4.2). Out of a total of 77 formed stools, 5/77 (6.5%) were positive for *Entamoeba* complex, 37/158 (23.4%) were positive for *Entamoeba* complex in Semi-formed stools. From loose stool samples 22/43 (51.2%) were positive for *Entamoeba* complex, whereas 9/12 (75.0%) *Entamoeba* complex were identified in mucoid samples. A total of 1/2 (50%) each of *Entamoeba* complex was detected in greasy-formed and watery samples respectively (Table 4.2)

**Table 4.2 Stool Consistencies and *Entamoeba* Complex infection**

| Consistency        | No. examined<br>(n) | No. uninfected<br>(n) | No. infected<br>n(%) |
|--------------------|---------------------|-----------------------|----------------------|
| Formed             | 77                  | 72                    | 5(6.5)               |
| Greasy- Formed     | 2                   | 1                     | 1(50.0)              |
| Semi- Formed       | 158                 | 121                   | 37(23.4)             |
| Greasy Semi-formed | 8                   | 8                     | 0(0.0)               |
| Loose              | 43                  | 21                    | 22(51.2)             |
| Mucoid             | 12                  | 3                     | 9(75.0)              |
| Watery             | 2                   | 1                     | 1 (50.0)             |
| <b>Total</b>       | <b>302</b>          | <b>225</b>            | <b>72(23.8)</b>      |

#### 4.3 *Entamoeba* complex infection and Age of Child

The level of *Entamoeba* complex infection among the various age groups of the children are as stated below with the highest infection 21/69 (30.4%) among children between 8-9 years followed by 23/80 (28.8%) in the 12-13 years and 1/4 (25%) among 4-5 years. The infection was relatively lower 15/79 (19%) among the children between 10-11 years, 10/59 (16.9%) 6-7 years, and 2/11 (18.2%) in the 14-15 year olds. However, the level of *Entamoeba* complex infection and the age of the children was not statistically significant ( $p = 0.071$ ). The overall prevalence of *Entamoeba* complex infection among the study population was 72/302 (23.8%) Table 4.3

**Table 4.3 *Entamoeba* complex infection and Age of Child**

| Age group    | Stool examined (n) | No. infected n(%) | $\chi^2$ | P-Value | OR    | 95% CI      |
|--------------|--------------------|-------------------|----------|---------|-------|-------------|
| 4-5          | 4                  | 1(25)             | 15.770   | 0.071   | 0.264 | 0.182-2.568 |
| 6-7          | 59                 | 10 (16.9)         |          |         |       | 1.013-1.292 |
| 8-9          | 69                 | 21(30.4)          |          |         |       | 1.226-1.499 |
| 10-11        | 79                 | 15(19.0)          |          |         |       | 1.114-1.380 |
| 12-13        | 80                 | 23(28.8)          |          |         |       | 1.116-1.359 |
| 14-15        | 11                 | 2(18.2)           |          |         |       | 1.413-2.041 |
| <b>Total</b> | <b>302</b>         | <b>72(23.8)</b>   |          |         |       |             |

**4.4 Location and level of *Entamoeba* complex infection**

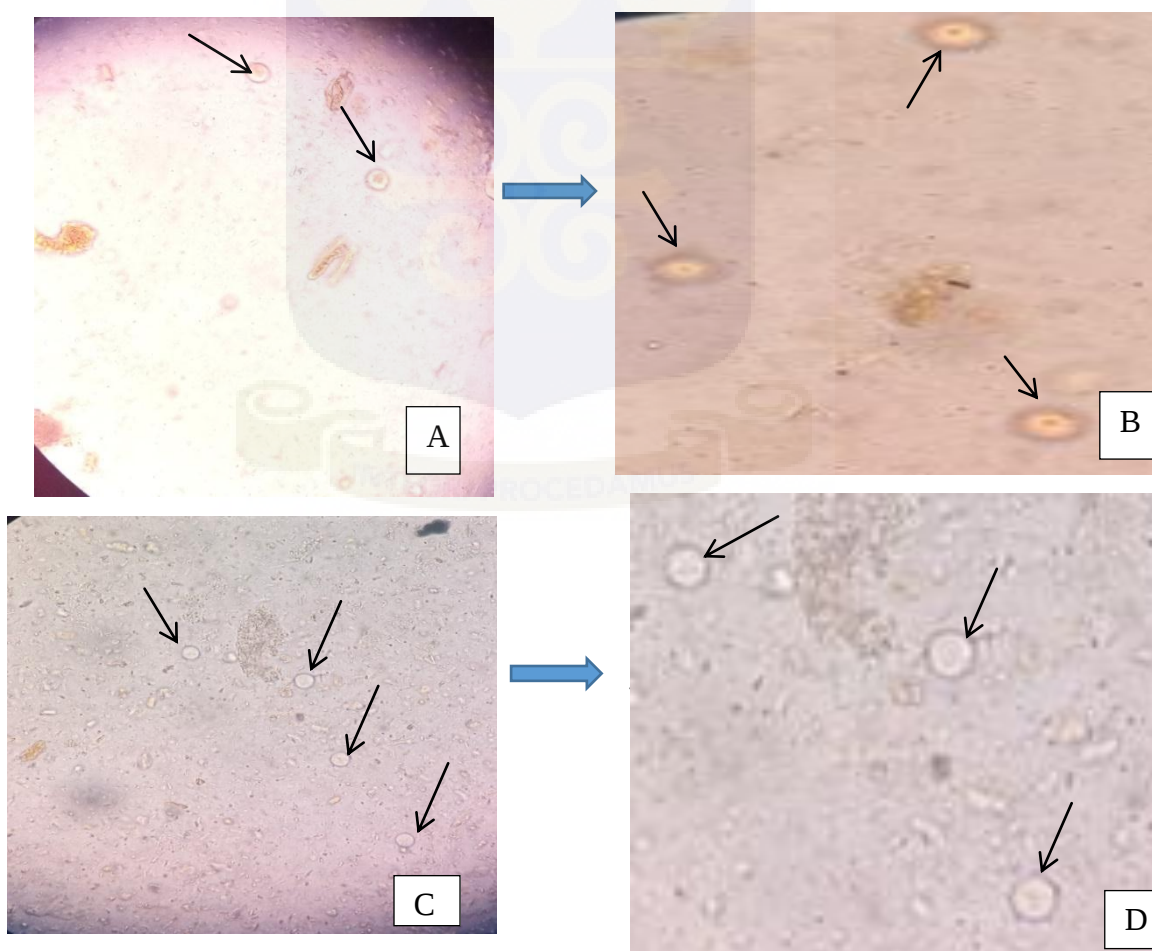
The analysis of the *Entamoeba* complex infection based on the six communities categorized as Urban, Peri-Urban and Rural are shown below. The highest rate of infection was recorded among children in the Peri-Urban community 33/102 (32.4%), followed by the Urban 25/101 (24.8%) and then children from the Rural communities 14/99 (14.1%) as shown in Table 4.4

**Table 4.4: Location and level of *Entamoeba* complex infection**

| Location          | Sex          | No. examined (n) | No. Infected n(%) |
|-------------------|--------------|------------------|-------------------|
| <b>Urban</b>      | Male         | 53               | 13(24.5)          |
|                   | Female       | 49               | 12(24.5)          |
|                   | <b>Total</b> | <b>101</b>       | <b>25(24.8)</b>   |
| <b>Peri-Urban</b> | Male         | 56               | 17(30.4)          |
|                   | Female       | 46               | 16(10.6)          |
|                   | <b>Total</b> | <b>102</b>       | <b>33(32.4)</b>   |
| <b>Rural</b>      | Male         | 45               | 5(11.1)           |
|                   | Female       | 54               | 9(16.7)           |
|                   | <b>Total</b> | <b>99</b>        | <b>14(14.1)</b>   |

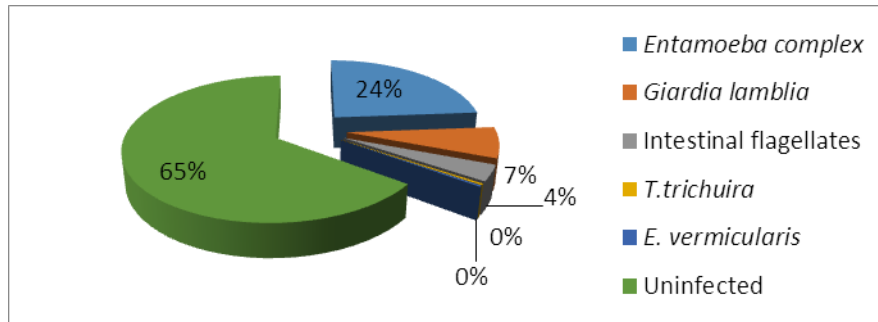
#### 4.5 Relative percentage of parasites detected by microscopy

The study detected by microscopy 72/302 (23.8%) *Entamoeba* complex infection among the school children (Fig. 4.2 and 4.3). Other parasites detected such as *Giardia lamblia* was 21/302 (6.95%) infection (Fig 4.3 and 4.4) with females having (3.9%) compared to males (2.9%). Intestinal flagellates (apart from *Giardia lamblia*) was 11/302 (3.6%) (Fig 4.3) with males having 2.3% compared to females 1.3%. Metazoans such as *Trichuris trichiura* and *Enterobius vermicularis* infection was only 1/302 (0.3%) respectively (Fig. 4.3). The prevalence of Metazoans were generally low in the study areas.

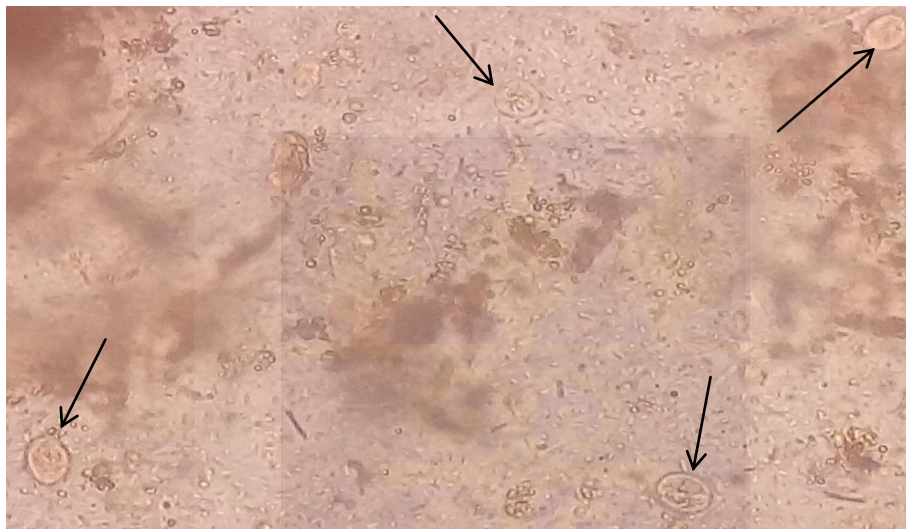


**Fig 4.2: Pictures of cysts of *Entamoeba* complex**

A & B are cysts of *Entamoeba* complex from Iodine stain, C & D are cysts of *Entamoeba* complex in wet mount.



**Fig 4.3 Pie Chart of percentage parasites detected by microscopy**



**Fig 4.4 Picture of *Giardia lamblia* cysts (other parasite) in Lugol's iodine**

#### **4.6 *Entamoeba* complex infection and source of food**

The analysis of the source of food eaten by the school children while at school revealed that *Entamoeba* complex infection was highest 54/174(31%) among school children who bought food from the street or from the school canteen compared to 18/126(14.3%) who either brought food from home to school or went home during break periods to eat. There was no infection 0/2 (0%) among 2 children who said they obtained food from their friends. There was statistical association ( $p = 0.002$ ) between source of food and *Entamoeba* complex infection among the school children (Table 4.5)

**Table 4.5 *Entamoeba* complex infection and source of food**

| Source of Food at school | No. Examined (n) | No. Infected n (%) | $\chi^2$ | P-Value | OR    | 95% CI      |
|--------------------------|------------------|--------------------|----------|---------|-------|-------------|
| Home                     | 126              | 18(14.3)           |          |         |       | 0.356-0.418 |
| Street/<br>School        | 174              | 54(31.0)           | 6.249    | 0.002   | 0.576 | 0.469-0.558 |
| Friends                  | 2                | 0(0.0)             |          |         |       |             |
| <b>Total</b>             | <b>302</b>       | <b>72(23.8)</b>    |          |         |       |             |

**4.7 *Entamoeba* complex infection and fingernails biting**

The study revealed that, *Entamoeba* complex infection was highest 25.5% (25/98) among children who either bite their fingernails or suck their fingers or thumb. Children who sometimes bite their fingernails or suck them recorded 25% (9/36) infection. The lowest rate of infection of 38/168 (22.6%) was recorded among children who do not bite their fingernails or suck their fingers/thumb. Table 4.6. There was statistical association ( $p = 0.022$ ) between the age of the children and biting of fingernails or sucking of fingers /thumb

**Table 4.6: *Entamoeba* complex infection and fingernails biting**

| Biting of fingernails | No. examined (n) | No. Infected n (%) | $\chi^2$ | P-Value | OR    | 95% CI      |
|-----------------------|------------------|--------------------|----------|---------|-------|-------------|
| Yes                   | 98               | 25(25.5)           |          |         |       | 0.375-0.483 |
| No                    | 168              | 38(22.6)           | 5.972    | 0.022   | 0.556 | 0.449-0.532 |
| Sometimes             | 36               | 9(25.0)            |          |         |       | 0.352-0.457 |
| <b>Total</b>          | <b>302</b>       | <b>72(23.8)</b>    |          |         |       |             |

**4.8: *Entamoeba* complex infection and mother's education**

Children whose mothers had Basic education (BECE) had 28/222 (12.6%) *Entamoeba* complex infection however, 23/24 (95.4%) of children who had the infection, their mothers had up to secondary education (SSCE) only. Children whose mothers had Tertiary education had 17/18 (94.4%) *Entamoeba* complex infection. Nonetheless the least infection 4/38 (10.5%) was recorded in children whose mothers had no formal education. Association existed between *Entamoeba* complex infection and mother's educational status statistically

( $p = 0.007$ ) Table 4.7

**Table 4.7: *Entamoeba* complex infection and mother's education**

| Mother's Education  | No. examined (n) | No. Infected n (%) | $\chi^2$ | P-Value | OR    | 95% CI      |
|---------------------|------------------|--------------------|----------|---------|-------|-------------|
| BECE                | 222              | 28(12.6)           |          |         |       | 0.414-0.481 |
| SSCE                | 24               | 23(95.8)           |          |         |       | 0.377-0.501 |
| Tertiary            | 18               | 17(94.4)           | 2.776    | 0.007   | 0.735 | 0.272-0.421 |
| No Formal education | 38               | 4(10.5)            |          |         |       | 0.488-0.717 |
| <b>Total</b>        | <b>302</b>       | <b>72(23.8)</b>    |          |         |       |             |

**4.9 *Entamoeba* complex infection and toilet facility at home**

The type of toilet facility used by the school children at home and its possible association with the *Entamoeba* complex infection revealed that children who used the KVIP had the highest infection 14/37 (37.8%) followed by those who used Pit latrine 15/48 (31.3%), 21/97 (21.6%) used WC, and 22/120 (18.3%) practised open defecation. There was no statistical association ( $p = 0.389$ ) between *Entamoeba* complex infection and the type of toilet facility used by the children (Table 4.8).

**Table 4. 8 *Entamoeba* complex infection and toilet facility at home**

| Toilet facility at home | No. examined (n) | No Infected n(%) | $X^2$ | P-Value | OR    | 95% CI      |
|-------------------------|------------------|------------------|-------|---------|-------|-------------|
| KVIP                    | 37               | 14(37.8)         |       |         |       | 1.062-1.452 |
| WC                      | 97               | 21(21.6)         | 4.937 | 0.389   | 0.397 | 1.252-1.480 |
| Pit latrine             | 48               | 15(31.3)         |       |         |       | 1.050-1.366 |
| Open field              | 120              | 22(18.3)         |       |         |       | 1.122-1.328 |
| <b>Total</b>            | <b>302</b>       | <b>72(23.8)</b>  |       |         |       |             |

#### 4.10 *Entamoeba* complex infection and sex of children

The total number of male children who were infected with *Entamoeba* complex was 35/153 (22.9%) compared to 37/149 (24.8) females. The p-value of 0.466 revealed no statistical association between *Entamoeba* complex infections in relation to gender (Table 4.9).

**Table 4. 9: *Entamoeba* complex infection and sex of children**

| Hand washing | No. examined (n) | No. infected n(%) | $X^2$  | P-Value | OR    | 95% CI      |
|--------------|------------------|-------------------|--------|---------|-------|-------------|
| Male         | 153              | 35(22.9)          |        |         |       | 0.387-0.468 |
| Female       | 149              | 37(24.8)          | 20.316 | 0.466   | 0.506 | 0.347-0.424 |
| <b>Total</b> | <b>302</b>       | <b>72(23.8)</b>   |        |         |       |             |

#### 4.11 *Entamoeba* complex infection and knowledge of hand washing

The knowledge of hand washing among the school children revealed that 57/226 (25.2%) had *Entamoeba* complex infection had knowledge about hand washing compared to 15/76 (19.7%) who had no knowledge about hand washing. There was

no statistical association ( $p = 0.607$ ) between the knowledge of the children regarding hand washing and *Entamoeba* complex infection (Table 4.10).

**Table 4.10** *Entamoeba* complex infection and knowledge of hand washing among children

| Knowledge of hand washing | No. examined (n) | No Infected n(%) | $X^2$ | P-Value | OR    | 95% CI      |
|---------------------------|------------------|------------------|-------|---------|-------|-------------|
| Known                     | 226              | 57(25.2)         |       |         |       | 0.412-0.477 |
| Unknown                   | 76               | 15(19.7)         | 1.958 | 0.607   | 0.748 | 0.443-0.578 |
| <b>Total</b>              | <b>302</b>       | <b>72(23.8)</b>  |       |         |       |             |

#### 4.12 *Entamoeba* complex infection and hand washing habits among school children

The study further investigated hand washing habit among the school children and the findings revealed that the least *Entamoeba* complex infection 0/9 (0.0%) was recorded among children who did not wash their hands before eating or after visiting the toilet. Children who *sometimes* use *water only* to wash their hands had the highest *Entamoeba* complex infection 20/56 (35.7%) followed by 5/17 (29.4%) who *sometimes* use *water and soap* compared to 34/158 (21.5%) who use *water and soap* to wash their hands. A total of 13/62 (21%) infected children use *only water* to wash their hands. There was no significant association ( $p = 0.434$ ) between the *Entamoeba* complex infection and the hand washing habits among the children (Table 4.11).

**Table 4.11 *Entamoeba* complex infection and hand washing habits among school children**

| Hand washing    | No. examined (n) | No. Infected n(%) | $X^2$ | P-Value | OR    | 95% CI      |
|-----------------|------------------|-------------------|-------|---------|-------|-------------|
| Water only      | 62               | 13(21)            |       |         |       | 1.173-1.472 |
| Soap + water    | 158              | 34(21.5)          |       |         |       | 1.203-1.379 |
| S(Water only)   | 56               | 20(35.7)          | 1.958 | 0.434   | 0.523 | 1.016-1.341 |
| S(Soap+ Water)  | 17               | 5(29.4)           |       |         |       | 0.961-1.510 |
| No Hand Washing | 9                | 0(0.0)            |       |         |       | 0.883-1.561 |
| <b>Total</b>    | <b>302</b>       | <b>72(23.8)</b>   |       |         |       |             |

#### 4.13 *Entamoeba* complex infection and main source of drinking water

The level of *Entamoeba* complex infection among the school children in relation to their main source of drinking water was as follows; children who drank from well/bore hole all had *Entamoeba* complex infection 2/2 (100%), 66/283 (23.3%) who drank Pipe-borne water had *Entamoeba* complex infection compared to 4/16 (25%) who drank Sachet water. No children who drank Stream/River water had *Entamoeba* complex infection (0%). There was no statistically significant association ( $p = 0.07$ ) between *Entamoeba* complex infection and the source of drinking water (Table4.12).

**Table 4.12 *Entamoeba* complex infection and main source of drinking water**

| Source of drinking water | No. examined (n) | No. Infected n(%) | $X^2$ | P-Value | OR    | 95% CI        |
|--------------------------|------------------|-------------------|-------|---------|-------|---------------|
| Pipe-borne               | 283              | 66(23.3)          |       |         |       | 0.434-0.495   |
| Well/Bore hole           | 2                | 2(100)            | 3.562 | 0.07    | 0.953 | 0.250-0.433   |
| Stream/River             | 1                | 0(0.0)            |       |         |       | -2.189-3.1805 |
| Sachet                   | 16               | 4(25.0)           |       |         |       | -0.185-1.085  |
| <b>Total</b>             | <b>302</b>       | <b>72(23.8)</b>   |       |         |       |               |



## CHAPTER FIVE

### DISCUSSION

#### 5.1 Discussion

*Entamoeba* complex infection is a common parasitic infection among children in developing nations including Ghana leading to high morbidity and mortality. However, *Entamoeba* complex comprising *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* are microscopically indistinguishable but are diverse genetically. *Entamoeba histolytica* is the medically important protozoan parasite associated with intestinal amoebic infection mostly in areas with poor sanitation and unsafe water whereas *Entamoeba dispar* and *Entamoeba moshkovskii* have not been implicated with disease.

In the present study, *Entamoeba* complex was the most common intestinal protozoan parasite identified among all the asymptomatic children sampled. The overall prevalence of *Entamoeba* complex infection was 72/302 (23.8%) among the randomly sampled school children (4-15years) in the Ho Municipality of the Volta Region, Ghana. The high prevalence of *Entamoeba* complex infection is indicative of possible high faecal consumption in either food or water among the school children as a result of poor environmental and personal hygiene. Similar studies conducted by Simon-Oke and Ogunleye (2015) recorded a prevalence of 188/278 (67.6%) among primary school children in Akure, Nigeria, whereas Walana *et al.* (2014) reported a prevalence of 5/2400 (0.21%) among Primary school children in Kumasi. Also, Verweij *et al.* (2003) reported a prevalence of 39.8% (98/246) in the Bawku District of Ghana. The significantly low rate of infection in Kumasi compared to other parts

of Ghana could be due to the low prevalence of the pathogen in the six selected communities.

Although all the study participants were asymptomatic, macroscopic and microscopic examination of their stools revealed some children had some form of diarrhoea. This is because children who produced loose and watery stools and also stool-positive for *Entamoeba* complex infection was 51.2% (22/43) and 50% (1/2) respectively. However no child produced bloody stool. The findings in the present study confirms the observations made by Opintan *et al.* (2010), who reported a prevalence of 5/170 (2.9%) among children with diarrhoea as well as Sharbatkhori *et al.* (2014) who reported a prevalence of 25/105(23.8%) among children with diarrhoea and dysentery in Northern Iran.

Due to the fact that asymptomatic children who harbour the parasite do not show any clinical signs and symptoms in order to be diagnosed and treated appropriately, they become carriers (cyst excretors) and could serve as point of transmission and infection to uninfected children (Heymann, 2008 and Stanley, 2003). These asymptomatic individuals could also develop invasive amoebiasis if left untreated. . In a bid to reduce the incidence of amoebic infection among children, periodic screening and subsequent treatment should be conducted especially in Primary schools.

In the present study, sex/gender was not statistically associated with *Entamoeba* complex infection. The difference in the level of *Entamoeba* complex infection between females (24.8%) and (22.9%) males could be as a result of socio-cultural reasons as females are more involved in household chores than their male

counterparts. In this regard, infected females become carriers/cyst passers in stools which may contaminate food, water or hands with faecal matter posing health risk to others. The level of infection among males could possibly be due to their adventurousness and playfulness since they are less restricted at home which could predispose them to the *Entamoeba* complex infection. The odd ratio 0.506 shows an equal chance of *Entamoeba* complex infection regardless of sex/gender. The present study agrees with a study conducted in North East India by Nath *et al.* (2015) which revealed that females had higher *Entamoeba* complex prevalence rate (15.2%) compared to males (11.5%).

In the present study, the source of food for children while at school was investigated and the association between source of food and *Entamoeba* complex infection among the children was statistically significant association. The results show that high incidence of *Entamoeba* complex infection 31% (54/174) was recorded among children who either buy food from the street or school canteen compared to 14.3% (18/126) who obtained their food from home. The poor personal hygiene of most food vendors, who may be naïve asymptomatic carriers pose a serious health risk to consumers especially children who patronize their foods. This is because they might know if they (food vendors) harbour infectious parasites which could easily be transmitted to consumers through food or water. Many of such food vendors do not go for routine medical check-ups to ascertain by public health standards if they are medically fit to sell food to the general public especially children.

A study conducted by Ayeh-Kumi *et al.* (2009) suggested that food vendors in Accra, Ghana, were possible source of intestinal parasitic infection including *Entamoeba*

complex as a result of lack of safe water, improper personal and environmental hygiene. Also, Stanley (2003) and the American Academy of Pediatrics (2012) further suggested that, the transmission of amoebiasis is largely through the contamination of food or water with human faeces containing the hardy infective cyst (Heymann, 2008; Ximenez *et al.* (2009) emphasizing the source of food as a possible source of *Entamoeba* complex infection

Diets obtained from the Street or School canteen are not mostly balanced hence causing malnutrition. This is attributable to the profit-driven mentality of the food vendors compared to the well-being of the consumers. Although this study has not investigated the association between the kind of food children eat and *Entamoeba* complex infection, studies proved that children not taking balanced diet could possibly be predisposed to *Entamoeba* complex infection (Petri and Haque, 2010).

Studies conducted by Duggal *et al.* (2011) and Guo *et al.* (2011) on leptin; a nutritional hormone and its relevance in *E. histolytica* immunity revealed that, low leptin levels due to malnutrition and genetic polymorphism in leptin receptor in human beings has the ability to increase susceptibility to amoebiasis (Duggal *et al.*, 2011). This study is very vital as it forms part of few researches that established that *Entamoeba* virulence and infection are determined by host factors (Morf and Singh, 2012).

Another study that used genetically engineered mice showed disparity in susceptibility to amoebic infection may be partly explained by variable leptin-receptor function. This proved that leptin is responsible for the mucosal protection,

increasing resistance to amoebiasis which corroborated earlier clinical study findings by Guo *et al.*, (2011).

The possibility that children who either bite their fingernails or suck their thumbs could have *Entamoeba* complex infection proved certain in the present study as the results revealed 25.5% *Entamoeba* complex infection in children who bite their fingernails, 25% who sometimes bite their fingernails or suck their fingers/thumbs and 22.6% who do not bite their fingernails or suck their fingers/thumbs. Due to the statistically significant association between the age of the children and biting of fingernails leading to *Entamoeba* complex infection among the children in this study, the health implications are dire for children who formed this habit.

This assertion is confirmed by the findings that *Entamoeba* complex cyst can survive days to weeks in moist external environment with the capability of causing infection (CDC, 2016). Surtiastuti and Widiastuti, (2011) also observed that fingernails biting was a possible source of *Entamoeba* complex infection since the viable cyst can survive under fingernails for up to 45 minutes. In view of the health risks, parents/guardians, and teachers and significant others must help stop this negative practise among the children through education. Regular inspection of fingernails and general personal hygiene should be intensified in the primary schools to safeguard the health of the children. Parents/guardians must use fingernail clippers to trim their ward's fingernails at home regularly.

It is assumed that the level of education of mothers should have positive impact on the health of their children because they play a crucial role in the development and

well-being of children. The results from the present study however proved otherwise. *Entamoeba* complex infection 95.8% (23/24) and 94.4% (17/18) were recorded among children whose mothers had Secondary cycle and Tertiary education respectively. Least infection was recorded among children whose mothers had no formal education 10.5% (4/38). The association between *Entamoeba* complex infection and mother's education was statistically significant.

Although educated mothers may be in a better position to reside in areas with good sanitation and safe water compared to their uneducated counterparts, the reason for the high rate of infection among children of educated mothers could possibly be that most educated mothers are career women who may not have time for their children due to the nature of their jobs and hence may not have direct control over what their children consume. Some may resort to buying food on the street for their wards or may give them money to buy food and or sachet water from school canteens which might probably have been contaminated. Some may leave their children in the care of house-helpers who may be infected and could be a possible source of intestinal parasitic infection including *Entamoeba* complex through food or water that these house-helpers may serve the children with.

There is the need for mothers especially working/career mothers to have personal time for their children regarding their food, water, and general well-being. The least infection among children whose mothers had no formal education could possibly due to their promotion of personal and environmental hygiene as well as their vigilance in matters relating to their ward's health including hygienic social amenities such as water and toilet.

Petri and Haque (2010) suggested that young children are at risk of *Entamoeba* complex infection leading to amoebiasis. The results of the present study showed that 1 out of 4 children between 4 -5 years had *Entamoeba* complex infection. This suggested a possible risk of every one child within that age bracket getting infected if proper measures are not put in place to keep them away from infection. This should be a public health concern as children within 4-5 years might have low immunity against intestinal parasitic infection including *Entamoeba* complex. However, the highest infection of 30.4% (21/69) recorded among children between 8-9years and then a relatively lower 28.8% (23/80) among the 12-13 year olds were possibly due to improper personal or environmental hygiene.

The present study also shows infection across different age groups but without any clear pattern of infection among the children relative to their age groups. In this regard, parents and relevant stake holders must safeguard the health of these children by taking them for periodic screening and treatment against the infection to prevent invasive amoebiasis. Although amoebiasis is prevalent in developing countries, the present study contradicts the findings of a study conducted by Heymann (2008), that amoebiasis is infrequent in children below 5 years.

The present study disagrees with the findings of Walana *et al.* (2014) which suggested that children 5-6years were the most infected compared to those between 7-8 years, 9-10 years and 11-12 years. The *Entamoeba* complex infection did not progress with the age of the children neither was children in lower classes more infected than those in upper classes. The rate of infection was however due to the level of exposure to the infective cyst of the *Entamoeba spp* which might have been

ingested. This contradicts the findings of Nath *et al.* (2015) which suggested that prevalence rate of *Entamoeba* complex reduced from 17.6% to 8.9% among children as they progressed from one level of the educational ladder to the other.

Lack of hygienic toilet facilities in the communities was a major risk factor in *Entamoeba* complex infection identified in the study site. Most of the children practised open defecation or ease themselves in polythene/plastic bags and throw away (“fly away”) into a nearby bush or open space. Infected children who may not clean themselves properly after defecating may contaminate their hands leading to the transmission of the pathogen to other children through sharing of foods or drinks. Others also due to poverty could not pay to use the public toilet in the community. The study also revealed that some children defecate in holes dug with simple implements (cutlass or hoe) in the backyard of their homes and covered with soil and this was also commonly practised in the peri-urban and rural communities. These shallow holes nature of these holes may be uncovered by free-range-domestic poultry birds or washed into homes or nearby water bodies by runoff when it rains posing a public risk.

Those who use the public toilets for a fee may also get themselves infected when contaminated currency notes or coins are given to them as change, the unhygienic toilet environment or contaminated toiletries used. Most public toilets also lack water and soap for patrons to wash their hands with which might be a source of infection transmission. The level of infection among those who used Water Closet may be due to lack of or inadequate water available. This may create insanitary conditions favorable for possible amoebic infection. Contaminated water closet flash handles, or

seats pose greater health risk to users. The present study agrees with the findings of Nath *et al.* (2015) which suggested that those who used unhygienic toilet facilities had higher *Entamoeba* complex infection than those who used hygienic toilets. Therefore, proper sanitary conditions including toilet facilities are critical to ensuring the health of children against *Entamoeba* complex infection.

In 2015, 2.4 billion people globally, lack access to toilet facilities which hygienically would have eliminated human excreta from human contact and 946 million people practised open defecation (UNICEF/WHO, 2015). This agrees with the study conducted by Haque *et al.* (2003) that suggested that *Entamoeba histolytica* is mainly transmitted by the faecal-oral route in areas of poor water sanitation. Also, according to UNICEF (2016), improper sanitation and hygiene behaviours pose a danger to human safety as they serve as a means of water contamination and promote the spread of diseases.

Poor sanitary and hygiene conditions in Mirpur, Bangladesh accounted for the high incidence of amoebic infection and reinfection among the preschool children. The prevalence of *Entamoeba histolytica* which is the pathogen species of *Entamoeba* complex was high and one-fifth of the children who participated in the survey was infected. (Haque *et al.* 2006). This contradicts the assertion that amoebic infection is a remote cause of diarrhoea/dysentery among children in developing countries (Black and Lanata, 2002).

Global Millennium Development Goal sanitation target has been missed by nearly 700million people as only 68% of the world's population uses improved sanitation facility (WHO/UNICEF, 2015). Approximately 82% of the global urban population

and 51% of the rural population used improved facilities. However, 7 out of 10 people are without improved sanitation facilities and 9 out of 10 people practise open defecation live in the rural areas. In Ghana, open defecation is widespread and pragmatic steps need to be taken to eliminate it so as to ensure good health and well-being of the citizenry (WHO/UNICEF, 2015). Access to safe water and sanitation would reduce the high morbidity and mortality associated with enteric-parasites including *Entamoeba histolytica* which is pathogenic. There was however no correlation between place of convenience (toilet facility) and *Entamoeba* complex infection.

Hand washing before eating is a cultural norm in many Ghanaian homes. However, proper hand washing has to be done under clean running water with soap. The knowledge of hand washing was investigated and 25.2% (57/225) of children infected with *Entamoeba* complex know they must wash their hands properly before eating as against 19.7% (15/76) who did not know. Although children were aware of the importance of hand washing before eating, this knowledge was not translated into practise as most of them did not normally wash their hands properly before eating or after defecating. The present study further investigated how the hand washing was practised among the children. The results reveal that, children who always wash their hands with water only had 21% (34/158) of the *Entamoeba* complex infection and the highest rate of 35.7% (20/56) infection was among those who sometimes wash their hands with water only.

Those who wash their hands with Water and Soap recorded 21.5% infection compared to children who sometimes wash their hands with soap and water 29.4%. It is evident that children sometimes wash their hands with water only or sometimes

use water and soap had relatively higher *Entamoeba* complex infection compared to those who practised proper hand washing with soap. This could be attitudinal problem as children have not understood why they must wash their hands. Poverty could be another possibility as proper hand washing must be done with soap under running water and the unavailability of clean water and soap could predispose the children to *Entamoeba* complex infection.

This assertion is highlighted in the UNICEF report that proper hygiene is very important particularly hand washing with soap to prevent infectious diseases (UNICEF Annual Report, 2015). The present study contradicts the suggestion by Nath *et al.* (2015) that those who do not wash their hands (18.3%) had higher infection than those who wash their hands (11.3%). The source of water determines if it is safe or unsafe for human consumption. The study showed that highest *Entamoeba* complex infection 23.3% (66/283) occurred among children who had their drinking water from Pipe-borne water.

Improper water treatment coupled with frequent pipe bursts or leakages leading to water contamination with faecal matter containing viable cysts could account for the high rate of infection among users of pipe-borne water in the study areas. Seepage of sewage containing viable *Entamoeba spp* cysts from septic tanks or soak-aways could contaminate underground water reservoirs or wells. Sachet water users also recorded 25% (4/16) of the infection. Possible Sachet water contamination could arise from the packaging, distribution, delivery, sales or handling of the water by vendors before consumption may be a cause of infection.

This study revealed 100% (2/2) *Entamoeba* complex infection was reported among children who used Well/Bore-hole as their main source of drinking water whereas no child was infected by drinking from the River/ Stream 0% (0/1). This contradicts the finding of Nath *et al.* (2015) which suggested that respondents who used well/ ponds and river water for drinking had higher infection than those who used tap/pipe borne water. Water is very critical to the survival of children and when children are made to depend on unsafe water, they are at a higher risk of deadly diseases including amoebiasis and severe malnutrition. Furthermore, unsafe water and improper sanitation are also connected to stunted growth which leads to irreversible physical and cognitive damage and seriously hinders the children's academic performance (UNICEF, 2016). Daily, over 80 children below 5 years die from diarrhoea as a result of insufficient water, improper sanitation and hygiene (Mills, 2016).

Access to safe drinking water and proper sanitation is a human right (UN, 2010) and safe water and adequate sanitation are critical for the development and well-being of humans. This is because, safe water and sanitation have serious impact on achieving adequate nutrition, gender equality, proper education and the elimination of extreme poverty (WHO/UNICEF, 2015). Although more than 90% of the world's population has access to sources of drinking water, more needs to be done to improve water quality because about half of those who use unimproved water live in Sub Saharan Africa. Provision of safe drinking water in the urban, peri-urban and especially rural areas is essential as 8 out of 10 people in the rural areas do not have access to safe drinking water but rather depends on unprotected wells / springs, and surface water (WHO/UNICEF, 2015). The

source of water however was not statistically associated with *Entamoeba* complex infection among the children.

The present study revealed that *Entamoeba* complex infection was highest 33% in children in the Peri-Urban parts of the Ho Municipality, 25% recorded among children in the Urban areas and 14% among children in the rural areas of the Municipality. Possible reason for low infection rate in the rural areas could be as a result of low level of prevalence of *Entamoeba* complex in the selected communities. The present study contradicts the findings of Nath *et al.* (2015) which showed that *Entamoeba* complex infection was higher among participants from rural communities than urban dwellers.

The present study also revealed that, the overall prevalence of *Giardia lamblia* infection among the children was 6.9% with females having 3.9% compared to 2.9% males. The overall prevalence of intestinal flagellates was 3.6% with males having the highest infection 2.3% and females having 1.3%. Metazoans such as *Trichuris trichiura* and *Enterobius vermicularis* infection was only 0.3% respectively. The prevalence of Metazoans was generally low in the study areas. This may possibly be due to the collaboration between the Ghana Health Service and the Ho Municipal Education Directorate which resulted in the mass administration of antiparasitics (Metronidazole and Albendazole in December 2015 prior to this study which commenced in October 2016) to the primary school children within the Municipality.

## CHAPTER SIX

### CONCLUSION, LIMITATION AND RECOMMENDATION

#### 6.1 Conclusion and Recommendation

Globally, many studies have been conducted on amoebiasis and the present study is an addition which addresses the causes of intestinal *Entamoeba* complex among school children within the Ho Municipality, in the South-Eastern part of Ghana. In this study, high prevalence of *Entamoeba* complex was identified among the children with females having slightly higher infection than males. Intensive public education on personal hygiene must be carried out among children to reduce or eliminate the prevalence of the infection and subsequent development of amoebiasis. The age of the children had no association with the risk of amoebic infection with lower odds (0.264). However, children between 6-13 years had the highest rate of *Entamoeba* complex infection compared to those < 6 years and > 13 years.

The source of food and *Entamoeba* complex infection was a major finding in this study as children who obtained their food from the street or school canteen had the highest *Entamoeba* complex infection. Parents /guardians must be encouraged to provide food in lunchboxes for their wards before they go to school in order to reduce or eliminate possible source infection from contaminated foods from the street or school canteen. Children who bite their fingernails or suck their fingers/thumbs frequently or infrequently all had higher *Entamoeba* complex infection. Children must also be discouraged by their parents or teachers from biting their fingernails or sucking their fingers as this could lead to possible *Entamoeba histolytica* infection.

On hand washing, most of the children did not understand the importance of hand washing with soap before eating or after defecating. In this study, most children washed their hands with only water (without soap) and this is a potential source of enteric parasitic infection including *Entamoeba* complex. Proper hand washing with soap under running water must be promoted in the schools and at homes by parents/guardians to safeguard the health of the children.

The educational level of the mothers was associated with the risk of infection as children of highly educated mothers had the highest infection compared to least educated or non-educated mothers. The level of one's education may not be the sole factor responsible for *Entamoeba* complex infection but rather the level of exposure of oneself to the source of infection. Career mothers must have time for their children's upkeep by providing them with balanced diet and inculcate in them personal hygiene virtues. Career mothers must be concerned about the kind of food or water their children consume so as to eliminate the danger of parasitic infection.

From the study, there was high prevalence of *Entamoeba* complex infection among children in the Peri-Urban and Urban areas compared to those in the Rural communities. Provision of safe water and sanitation facilities by local or central government as well as Civil Society Organization will help promote the well-being of residents especially children in communities these facilities are lacking or limited. The Ministry of Health must collaborate with the Ghana Education Service to carry out public education using the mass media on how to prevent enteric parasitic diseases such as *Entamoeba* complex infection through personal hygiene. Even though varied prevalence of *Entamoeba* complex has been reported, the

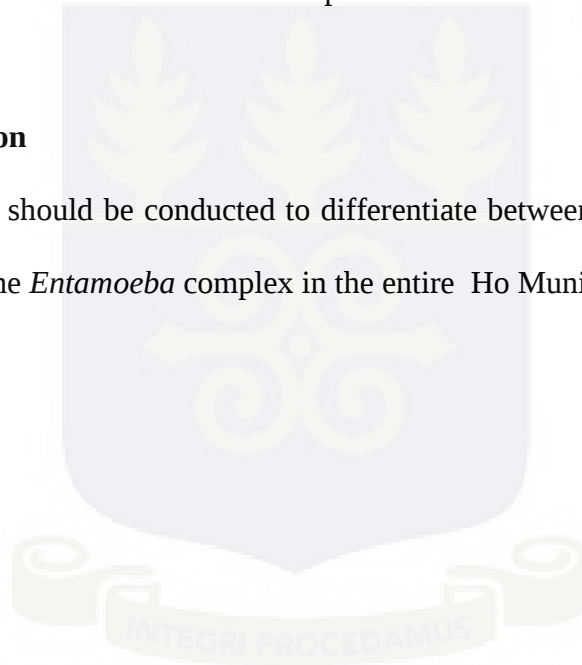
findings of the present study will serve as vital source of public health information so strategic interventions would be made to reduce the burden of *Entamoeba* complex infection in endemic areas.

#### **Limitations of the study**

- i. Collection of single stool sample, which might lead to under detection and reporting of parasites.
- ii. Inability to check for other enteric parasites such as *Cryptosporidium parvum* which may have occurred with *Entamoeba* complex infection due to the method used.

#### **Recommendation**

Molecular study should be conducted to differentiate between the species / zymodemes of the *Entamoeba* complex in the entire Ho Municipality.



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**APPENDIX A.1**

**QUESTIONNAIRE**

**Intestinal Amoebiasis among School Children in the Ho Municipality**

Dear Potential Research Participant,

This questionnaire is designed for a research work from the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, University of Ghana, Korle-Bu, Accra. Kindly read the information below carefully and provide the **CORRECT** answer as possible. All information you provide will be treated confidentially and will solely be used for this research only. It will take you about 5 minutes. Please tick [ ] or fill where appropriate.

**Participant's I D (001-200) ..... Name o f School..... Class.....**

1. Gender                      Male [    ]                      Female [    ]
2. Age group    4-5 [    ] 6-7years [    ] 8-9years [    ] 10-11 years [    ] 12-13year [    ]  
14-15[    ]
3. Place of residence, please specify .....
4. What is the main source of drinking water for your family?    Pipe-borne water [    ] Borehole/ Well [    ]    River/stream [    ]  
Other (Specify).....
5. Do you have toilet facility at home?    Yes [    ]                      No [    ]
6. If yes, where do you or your family members usually go to toilet?    KVIP [    ]  
WC [    ] Pit latrine [    ] Open field [    ]  
\* If No, where do you go to defecate? Specify.....
7. What do you do to your hands after defecating?    Clean hands on dress [    ]  
Wash hand with water only [    ]    Wash hands with water and soap [    ] Other (specify).....
8. Do you wash your hands before eating?                      Yes [    ]                      No [    ]  
Sometimes [    ]

If yes, with what? Water only [ ] Soap and water [ ] Sometimes water only [ ]  
Sometimes Soap and water [ ] No hand washing [ ]

9. Do you know why you wash your hands before eating? Yes [ ] No [ ]

10. Where do you normally get food to eat while in school? Cooked at home  
only [ ] Buy from the street / school canteen [ ] Get food from  
friends [ ] Other (specify).....

11. Do you bite your fingernails or suck your thumb/fingers? Yes [ ] No [ ]  
Sometimes [ ]

12. Does your school have toilet facility? Yes [ ] No [ ]

If yes, what type of toilet facility? WC [ ] KVIP [ ] Pit latrine [ ]

13. What is the main source of drinking water while you at school? Pipe borne  
water [ ] Well/bore hole [ ] River/ stream [ ] Sachet water [ ]  
Other (specify).....

14. Mother/guardian's educational qualification BECE [ ] SSSCE [ ]  
Tertiary [ ] Other (specify).....

15. Mother/ Guardian's occupation? Farming [ ] Artisan [ ] Trading [ ]  
Office work [ ]

Other (specify).....

16. Father/guardian's educational qualification BECE [ ] SSSCE [ ]  
Tertiary [ ]

Other (specify).....

17. Father/ Guardian's occupation? Farming [ ] Artisan [ ] Trading [ ]  
Office work [ ]

Other (specify).....

Kindly write down comments and suggestions you would like to  
bring to my  
attention.....

*Thank you for taking time to complete this questionnaire. Your participation is  
greatly appreciated.*

## APPENDIX A. 2

### SAMPLE OF INFORMED CONSENT

**Title:** Intestinal amoebiasis among school children in the Ho Municipality

**Principal Investigator:** Christopher Yaw Dumevi

**Address:** Department of Medical Microbiology, School of Biomedical and Allied

Health Sciences, P.O. Box KB 143, Korle Bu, Accra. Email:

[chrisdumevi@gmail.com](mailto:chrisdumevi@gmail.com)      **Telephone:** 0243277804 / 0208206675

1. You .....have been asked to give permission for your child/ward to **voluntarily** participate in a research study entitled “*Comparative study and characterization of intestinal amoebiasis among school children in the Ho Municipality*” the purpose of this study is to find out what makes your child/ward sick with diarrhoea. Your child’s participation will be for a few minutes. During your child’s participation, you will be asked to answer questions regarding your child’s health and you will be required to help your child to provide stool sample. If your child cannot provide stool sample, a swab from the rectum will be performed using a cotton bud on the tip of a small stick. The stool sample will be used to perform laboratory tests to determine what makes your child have diarrhoea sometimes.
2. The risk or discomfort to you or your child is minimal, and may involve mild discomfort in providing the stool samples for laboratory processing and examination.
3. The benefits that you may expect from you or your child’s participation in this study is to know what may be causing the diarrhoea in a timely manner. There are no other benefits, costs, or compensation for you or your child expected from participating in the study. But the information gained from the study will help investigate the causes of diarrhoea circulating in the country and may help the health officials plan a strategy to control or prevent it.
4. Laboratory results and the information regarding you or your child will be **strictly confidential**. Your identity during the study will be kept secret by using a research identification code.

5. A sample of your child's stool may be stored for future laboratory diagnosis to include causes of diarrhoea due to germs. In addition, any germ that is found will be stored for future studies.

6. If you have **any questions about the study, you may contact** Mr. Christopher Yaw Dumevi, the **Principal Investigator** through the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, University of Ghana. P.O.Box 143 Korle Bu. Accra. Phone: +233 24 3277804/ 0208206675. Email: [chrisdumevi@gmail.com](mailto:chrisdumevi@gmail.com) Or the **Main Supervisor**: Rev. Prof. Patrick F. Ayeh-Kumi, School of Biomedical and Allied Health Sciences, University of Ghana. P.O.Box 143 Korle Bu, Accra. Phone: +233 2440 42718. Email: [payehkumi@yahoo.com](mailto:payehkumi@yahoo.com)

7. Your child's participation in this study is **completely voluntary**. If you do not want to participate, there will be no penalty and you or your child will not lose any benefits which you or your child is otherwise entitled to.

If you want to **discontinue participating** in the study at any time, there will be no penalty and you or your child will not lose any medical care or benefits to which you or your child is otherwise entitled.

8. All answers provided by the researcher are understandable to me and are satisfactory. I understand to the best of my ability what has been explained in this consent form about my child's participation in the study. I have had enough time to consider the decision to participate or have my child participate in this study and I don't need further information to make my decision. With my signature or thumbprint below, I give my **voluntary informed consent** to have my child participate in the research as it has been explained to me.

-----  
Signature/ thumbprint parent/guardian

-----  
Date

-----  
Printed name of participant

-----  
Printed name of parent/guardian

-----  
Date

9. We certify that this form was signed/thumprinted/marked by the person above

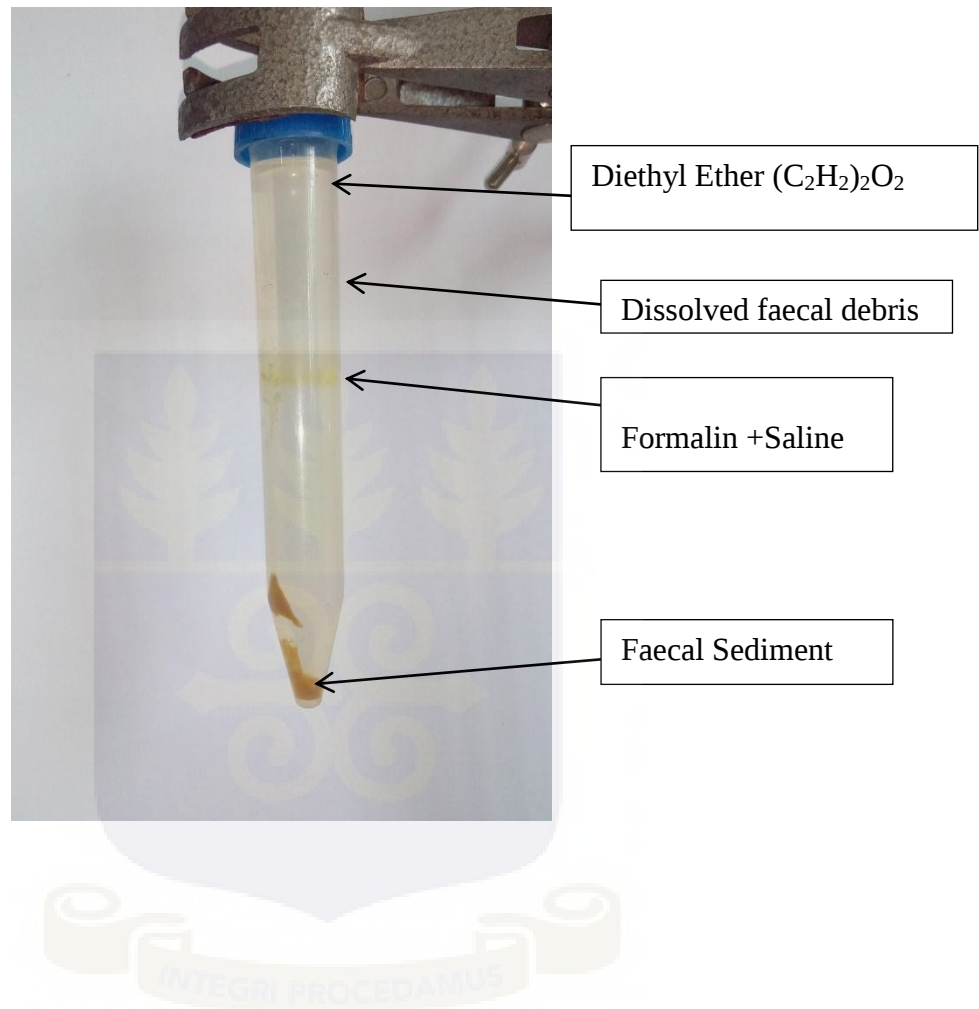
-----  
Printed name of Investigator

-----  
Signature of Investigator

-----  
Date

### APPENDIX A. 3

#### FORMOL ETHYL-ACETATE CONCENTRATION TECHNIQUE



## APPENDIX A.4

### ETHICAL CLEARANCE OF COLLEGE OF HEALTH SCIENCES



#### UNIVERSITY OF GHANA COLLEGE OF HEALTH SCIENCES

ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: .....

5<sup>th</sup> October, 2016.

**Mr. Christopher Yaw Dumevi**  
Department of Microbiology  
School of Biomedical and Allied Health Sciences  
Korle-Bu, Accra

#### ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.1 – P 4.11/2016-2017**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 5<sup>th</sup> of October, 2016 unanimously approved your research proposal.

**TITLE OF PROTOCOL: “Comparative study and characterization of intestinal amoebiasis among children in the Ho municipality”**

**PRINCIPAL INVESTIGATOR: Mr. Christopher Yaw Dumevi**

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 31<sup>st</sup> August, 2017.**

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

## APPENDIX A.5 ETHICAL CLEARANCE OF HO MUNICIPAL DIRECTORATE

**GHANA EDUCATION SERVICE  
HO MUNICIPAL DIRECTORATE**

In case of reply the Number and date of this Letter should be quoted.

My Ref: GES/VR/HM.763/59  
Your Ref No.....

  
REPUBLIC OF GHANA

P. O. Box 94, Ho, Volta Region  
Email: homedu1@yahoo.com  
Facebook: GESHoMunicipalDirectorate  
Tel.: 036 202 6489/036 202 5169

12<sup>th</sup> May, 2016

UNIVERSITY OF GHANA  
DEPARTMENT OF MEDICAL MICROBIOLOGY  
SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES  
P. O. BOX KB 143  
KORLE BU, ACCRA

(ATTN: KWAMENA W.C SAGOE PHD, THE SENIOR LECTURER & HEAD)

PROJECT ON "CHARACTERIZING AMOEBIC SPECIES CAUSING INTESTINAL AMOEBIASIS AMONG SCHOOL CHILDREN IN THE HO MUNICIPALITY"

Thank you for your letter dated 14<sup>th</sup> April, 2016 on the above activity.

Mr. Christopher Yaw Dumevi, your Mphil student is permitted to carry out the study in the following selected schools within the Ho Municipality;

|                                   |                                   |
|-----------------------------------|-----------------------------------|
| 1. Klefe Achatime E.P Primary     | 9. Sokode Gbogame M.A Prim        |
| 2. Klefe Demete E.P Primary       | 10. Sokode Gbogame R.C Prim       |
| 3. Ziavi Dzogbe Meth. Primary     | 11. Ho Poly M.A Primary           |
| 4. Ziavi Adukofe M.A Primary      | 12. Ho SSNIT Flats Presby Primary |
| 5. Akoefe Tokor M.A Primary       | 13. Ho Dome E.P Primary           |
| 6. Akoefe Kpodzi E.P Primary      | 14. Ho Dome R.C Primary           |
| 7. Matse R.C Primary              | 15. Ho Bankoe E.P Primary         |
| 8. Matse Dzorkpe/Ando E.P Primary | 16. Ho Bankoe R.C Girls' Primary  |

By this letter, you are entreated to;

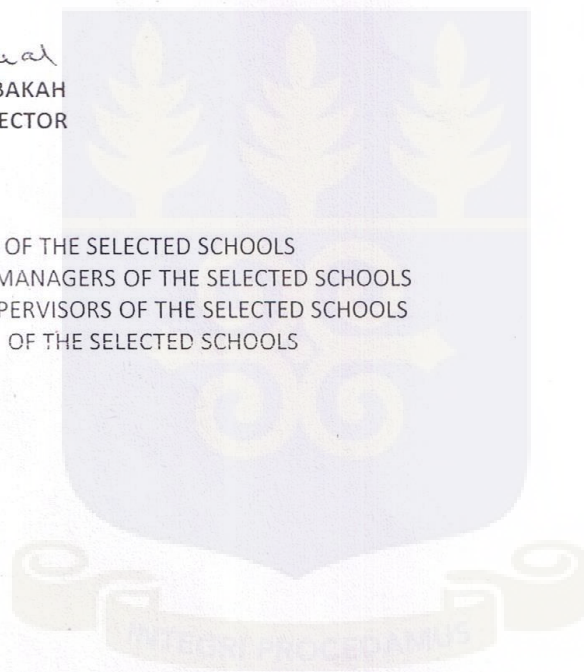
- (i) arrange with the Headteachers of the selected schools for a convenient time for the exercise in order not to disrupt academic work
- (ii) ensure that parents give their consent to their wards as noted in your introductory letter
- (iii) Submit a written report to the Municipal Director to update the directorate for the necessary action to be taken.

The Directorate is appealing to all Heads and teachers to offer Mr. Christopher Yaw Dumevi the needed assistance to achieve his vision.

Thank you.

  
MAXWELL H. GBAKAH  
MUNICIPAL DIRECTOR

CC:  
HEADTEACHERS OF THE SELECTED SCHOOLS  
THE REGIONAL MANAGERS OF THE SELECTED SCHOOLS  
THE CIRCUIT SUPERVISORS OF THE SELECTED SCHOOLS  
SMC CHAIRMEN OF THE SELECTED SCHOOLS



## APPENDIX A.6 ETHICAL CLEARANCE OF VOLTA REGIONAL HOSPITAL



### VOLTA REGIONAL HOSPITAL- HO

#### MEMO

FROM; HUMAN RESOURCE MANAGER



TO; HEADS/WARD IN-CHARGES

DATE; 18/4/2016

SUBJECT; ACADEMIC RESEARCH = MR. Christopher Yaw Dumevi

I have been directed to inform you that, the above mentioned MPhil Student from University of Ghana-Legon is conducting research into the topic,

*"Characterizing amoebic Species Causing Intestinal amoebiasis among School Children in the Ho Municipality"*

The research is for academic purposes only.  
Please give him your necessary support.