PREVALENCE OF *ENTAMOEBA HISTOLYTICA* INFECTION IN CHILDREN AGED 1-5 YEARS IN ZARIA, NIGERIA

BY

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DECLARATION

I, AGBIKE, Happiness Igwe hereby declare that the work in the thesis entitled ''Prevalence of *Entamoeba histolytica* Infection in Children Aged 1-5 Years in Zaria, Nigeria'' has been performed by me in the Department of Biological Sciences under the supervision of Prof. I.H.Nock, Dr. I.S.Ndams and Dr. E. Kogi.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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CERTIFICATION

This thesis entitled ‘‘Prevalence of *Entamoeba histolytica* Infection in Children Aged 1-5 Years in Zaria, Nigeria’’ by Agbike, Happiness I. meets the regulations governing the award of the degree of Master of Science (Zoology) of Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research is dedicated to the Almighty God the creator of life and to my late father Mr. Johnson E. Agbike whose love and care I miss so much.

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ABSTRACT

To elucidate on the current state of amoebiasis in children in Zaria, the prevalence of the disease in pre-school children was determined by a more specific/sensitive ELISA. Stool samples were collected from children with diarrhoea cases (129), from two hospitals which covered Zaria city and its environs those without diarrhoea (54) served as control. Microscopy and the TechLab *E. histolytica II* ELISA kit were used to carry out the test. Out of 183 stool samples screened, 42.62% prevalence of *Entamoeba histolytica* infection was obtained by ELISA, while microscopy showed 0.6% infection with *Entamoeba histolytica*, 5.46% with *Entamoeba coli*, 6.01% with *Isospora hominis*, 3.83% with *Ascaris lumbricoides*, 5.46% with Hookworm and 1.64% with *Trichuris trichiura*. Significant association ($p<0.05$), was established between infection and diarrhoea, and the home location of the children. Age, sex, socio-economic status, type of toilet, source of drinking water and finger sucking habit ($p>0.05$) showed no significant association with amoebiasis. Results obtained using ELISA test show that *E. histolytica* is endemic in Zaria, therefore, more public awareness programmes should be intensified towards promoting understanding of the adverse effects of amoebiasis and the need to effect control. Also *E. histolytica* should be specifically identified, to enhance specific and effective treatment.

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LIST OF ABBREVIATIONS

1. WHO – World Health Organisation
2. PAHO - Pan American Health Organisation
3. UNESCO - United Nations Educational Scientific and Cultural Organisation
4. ELISA - Enzyme–Linked Immunosorbent Assay
5. PCR - Polymerase Chain Reaction
6. DNA – Deoxyribonucleic Acid
7. EIA – Enzyme Immunoassay
8. NCCL - National Committee for clinical laboratory

CHAPTER ONE

1.0 INTRODUCTION

Amoebiasis is an infection caused by a protozoan parasite called *Entamoeba histolytica* (WHO, 1997). Several species of *Entamoeba*, such as: *E. coli, E. dispar*, *E. moshkovskii, E. hartmanni* and *E. polecki* infect humans, but not all of them are associated with the disease (Kagan, 1970; Mehmet and Petri, 2003). *Entamoeba histolytica* is well recognized as a pathogenic amoeba associated with intestinal (amoebic colitis) and extra-intestinal (liver abscess) infections (WHO, 1997).

Intestinal amoebiasis is frequently asymptomatic. Symptomatic cases vary from dysentery with fever, chills and bloody or mucoid diarrhoea alternating with periods of constipation. Also, invasive infection can cause severe amoebic dysentery (Lauren, 2004). Extra-intestinal amoebiasis occurs when the parasite invades other organs such as liver, lung or brain causing abscesses (Lauren, 2004). Only approximately 10% of *E. histolytica* infected individuals show clinical symptoms with intestinal and/or extra-intestinal pathology (Gatti *et al.,* 2002).

Amoebiasis occurs worldwide, with approximately 50 million people infected annually, causing close to 100,000 deaths per year (WHO, 1997; Ravdin and Stauffer, 2005). Amoebiasis is more common in areas of poor sanitation and nutrition particularly in the tropics (Ravdin and Stauffer, 2005). Morbidity and mortality occurs in Africa, Asia and Central and South America (Petri and Singh, 1999; 2006). The prevalence and presentation of symptomatic *E. histolytica* infection varies geographically. For instance, a study in Bangladesh indicated that preschool children

showed episodes of *E. histolytica* associated diarrhoea each year (Stanley, 2003). A comparative study in Egypt and South Africa also showed that amoebic colitis was predominant in Egypt, whereas amoebic liver abscess is predominant in South Africa (Stauffer *et al.*, 2006). In Ilesha, Nigeria, childhood dysentery associated with *E. histolytica* has been reported by Ogunlesi *et al*. (2005).

Infection by the parasite can be acquired by the faecal-oral route either directly by person-to-person contact or indirectly by eating or drinking faecally contaminated food or water (Petri and Singh, 1999; 2006). Studies have identified inadequately treated drinking water and ingestion of raw vegetables as risk factors for infection in addition to failure to wash hands before eating and low socio-economic status (Benetton *et al.*, 2005; Rinne *et al*., 2005). Generally, the risk of infection is highest in areas of poverty and in settings with poor sanitation where barriers between human faeces, food and water are inadequate (Stanley, 2003).

Entamoeba histolytica is the pathogenic species of *Entamoeba* that causes amoebic dysentery or amoebic colitis and other invasive forms of the disease (Lucas and Upcroft, 2001). For several years researchers have been searching for methods that will allow accurate and reliable diagnosis of amoebiasis. The diagnosis of intestinal amoebiasis primarily relies on the detection of parasites in the stool, but the epidemiological implications of *E. histolytica* versus *E. dispar* infection can be fully realized only if there are methods to distinguish the two species when dealing with large number of samples. The World Health Organisation (WHO), the Pan American Health Organisation (PAHO) and the United Nations Educational Scientific and

Cultural Organisation (UNESCO) published a joint statement in 1997 and stressed the urgent need to develop improved methods for the specific diagnosis of *E. histolytica* infection using techniques that are appropriate for developing countries (WHO, 1997).

In the past, diagnosis of the disease was based on identifying *E. histolytica* cysts and trophozoites in stool specimen using light microscopy (Gonzalez-Ruiz, *et al*., 1994). Recently, with the discovery of the two morphologically identical but genetically distinct human parasites; pathogenic *E. histolytica* and non-pathogenic *E. dispar* living in the same region (intestine), microscopy is no longer reliable (Huston *et al*., 1999). Sensitive and specific molecular techniques have been developed to differentiate these two species. These include the detection of *E. histolytica* antigen using Enzyme–Linked Immunosorbent Assay (ELISA), the use of the Polymerase Chain Reaction (PCR) to amplify amoebic DNA and the culture of stool samples followed by iso-enzyme analysis. Of these three tests methods, only antigen detection using ELISA can be performed rapidly and easily, making it the diagnostic test method of choice for clinical use in the developing world (Huston *et al*., 1999). In the light of clinical and public health importance of amoebiasis in Nigeria, this study was carried out to determine the prevalence of amoebiasis in preschool children using the antigen detection (ELISA) method.

1. 1 Statement of the Problem

In Zaria, sanitation is poor and safe water is not easy to come by. On these and similar accounts, diarrhoea in children is high with corresponding morbidities and deaths. (Yakubu and Sathiakumar, 1985).

Amoebiasis, caused by *Entamoeba histolytica*, is well associated with diarrhoea, especially in children, in most parts of Nigeria. However, the significance of this parasite in diarrhoea in children, especially in the light of modern and more sensitive methods of diagnosis are not known.

1.2 Justification

With the discovery of pathogenic *E. histolytica* and non-pathogenic *E. Dispar,* which are morphologically identical but genetically different, microscopy is no longer reliable in the diagnosis of amoebiasis (Huston *et al*., 1999). Indeed, WHO has suggested that *E. histolytica* should be specifically identified (WHO, 1997).

The Enzyme-Linked Immunosorbent Assay employed in this study is sensitive and specific for *E. histolytica,* it is rapid and technically simple to perform thus, more reliable to define the status of child amoebiasis and its accompanied health consequences, especially in Zaria.

1.3 Aim

To establish the significance of *Entamoeba histolytica* in diarrhoeal conditions in preschool children in Zaria.

1.4 Objectives

The objectives of this study are:

1. To determine the prevalence of *Entamoeba histolytica* infection in preschool children in Zaria.
2. To determine the risk factors associated with *Entamoeba histolytica* infection in preschool children in Zaria.

1.5 Hhypothesis

Entamoeba histolytica infection is not a public health problem in preschool children in Zaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspective

Amoebiasis may have been first recognised as a deadly disease by Hippocrates (460 to 377 B.C), who described a patient with fever and dysentery. Milestones in the study of *E. histolytica* and amoebiasis were: its descriptions by Losch in 1873; the delineation of amoebic liver abscess and colitis by Osler and his colleague in 1890; its axenic culture by Diamond in 1961 and differentiation of pathogenic *E. histolytica* from non-pathogenic *E. dispar* in 1979 (Saklatvala, 1993).

Suggestion of *E. histolytica* as etiology of diarrhoea was first recorded in 1855 from a case where amoeba was observed in a stool sample from a child with dysentery in Prague (Kean, 1988). In 1875, Fedor Losch isolated what he believed to be a causative agent which he named *Amoeba coli* from the stool sample of a patient with dysentery (Stilwel, 1955; Kean, 1988). In the Philippines, Walker and Sellards documented the cyst form of *E. histolytica* as the infective form of the parasite (Walker and Sellards, 1913). In 1925, Dobell further described the life cycle of *E. histolytica*. Brumpt proposed that *E. histolytica* and *E. dispar* were identical morphologically, but that only *E. histolytica* was pathogenic for humans (Brumpt, 1925). Sargeaunt *et al.* (1978) reported that *E. histolytica* and *E. dispar* species can be differentiated using zymodeme analysis.

With the application of new molecular biology-based techniques, tremendous advances have been made in the knowledge of the diagnosis, natural history and epidemiology of amoebiasis. As more about the molecular and cell biology of *E. histolytica* is been discovered, there is great potential for further understanding of the pathogenesis of the disease (Huston *et al.*, 1999).

2.2 Classification of *Entamoeba histolytica*

Description of *Entamoeba* species depend on the features of the parasites, such as the size of the trophozoites and cysts, the number of nuclei in the mature cyst and the number of structures in the trophozoites (Abd-alla, 1992). *E. histolytica* is the only pathogenic species and its classification has been proposed by Levine *et al.* (1980) as shown below:

Kingdom	Animalia
Phylum	Sarcomastigophora
Sub-phylum	Sarcodina
Class	Lobosea
Family	Entamoebidae
Genus	*Entamoeba*

2.3 Life cycle of *Entamoeba histolytica*

The life cycle of *E. histolytica* has been considered by many researchers as simple and humans and perhaps some non-human primates are the only natural hosts (Stanley, 2003). It consists of an infective cyst stage and a multiplying and invasive trophozoite stage (Mehmet and Petri, 2003; Huston *et al.,* 1999). Infection usually begins with the ingestion of cysts in food or water that has been contaminated by

infected human faeces. The cysts travel through the gut lumen to the small intestine (terminal ileum), where excystation occurs. After exycstation, nuclear division is followed by cytosplasmic division (reproduction by binary fission) to give rise to eight daughter trophoziotes (Huston *et al*., 1999).

The trophozoites are the motile forms which reside in the lumen of the large intestine (cecum and colon), where they adhere to the colonic mucus and epithelial layers. Adherence is due to the interaction of a D-galactose or N-acetyl- D-galactosamine-inhibitable amoebic lectin with host-derived glycol-conjugates (McCoy *et al.,* 1994). Re-encystation of the trophozoites occur within the lumen of the colon, resulting in the excretion of cysts in the faeces and the life cycle continues (Huston *et al*., 1999).

2.4 Morphological forms of *Entamoeba histolytica*

Two forms of *E. histolytica* exist, these are: the motile and invasive trophozoite and an infective cyst. The *E. histolytica* cysts are round in shape, usually 10-15µm in diameter, with four nuclei and are surrounded by a retractile wall that may include chitin (Stanley, 2003). The chromatoid bodies and glycogen vacuoles though present cannot be seen at cyst stage (Clark *et al.,* 2000). It is the mature cyst when consumed in contaminated food or water that is infectious. Cysts can remain alive outside the host for weeks or months, especially under damp conditions (Ivey, 1980), but are rapidly destroyed at temperatures under -5^{o}C and over 40^{o}C (Ivey, 1980). Cysts are not invasive; they survive the acidic environment of the stomach and travel through the small intestine to terminate at the ileum, which after excystation to form trophozoite also terminate at the colon (Clark *et al.,* 2000).

The *E. histolytica* trophozoites unlike the cysts are highly motile and have a pleomorphic shape with the diameter varying from 10 to 50µm (Reeves, 1984). Trophozoites move by extending creeping projections of cytoplasm, called *pseudopodia*, which pull them along. They also use these projections to surround and engulf food particles. The energy for this constant motion comes from the anaerobic conversion of glucose and pyruvate to ethanol (Reeves, 1984; Melmet and Petri, 2003). The cytoplasm frequently contains many red blood cells that have been ingested. They have a single nucleus and no mitochondria, but many of their metabolic enzymes seem to be of prokaryotic origin; possibly acquired from the lateral transfer of genes from bacteria (Clark and Roger, 1995).

The trophozoite of *E. histolytica* can be converted to a pre-cyst form. This form matures into a tetra-nucleated cyst as it does migrate down and out of the colon. The pre-cyst contains a nucleus and aggregates of ribosomes called chromatoid bodies, as well as food vacuoles that are extruded as the cell shrinks to become a mature cyst (Huber *et al.,* 1988; Clark *et al.,* 2000). Trophozoites are easily destroyed in the outside environment, degenerating within minutes, but while in the body can penetrate the gastrointestinal mucosa and migrate to other organs, causing extra-intestinal infections (Clark *et al*., 2000).

2.5 Mode of Transmission

Human beings are the primary known reservoir for *E. histolytica* (Katz *et al.,* 1989). The main source of transmission is the chronically infected human who pass stools infected with cysts of *E. histolytica* that may contaminate fresh food or water through

poor personal hygiene (Ravdin, 2002). Another common source of transmission is oral-anal sexual contact (Philips *et al.,* 1981; Quinn *et al.,* 1981).

Zoonotic transmission of *E. histolytica* has been suggested by Jackson *et al.* (1990), but this is not clear. Experimental infections with *E. histolytica* have been produced in dogs, cats, rats, monkeys and other laboratory animals. These animals may acquire human strains as a result of close contact with humans. Natural infections with strains morphologically similar to *E. histolytica* have been found in monkeys (Beaver et al., 1984). The importance of wildlife (primates) in zoonotic infections was studied by Jackson *et al.* (1990), who used zymodeme analysis to investigate whether *E. histolytica* occurs as a true zoonosis. In Nigeria, Mbaya and Nwosu (2005) reported clinical amoebiasis among captive chimpanzees at the Sandal kyarimi park, Maiduguri. They concluded that the animals must have acquired *E. histolytica* infection from one of the human attendants that was shedding the cysts in his faeces at the time of the outbreak.

According to Walsh (1988), the infective cysts of *E. histolytica* may be spread by arthropods such as cockroaches and flies, suggesting that these insects are able to play a rare but important role in transmission of this disease.

2.6 Pathogenicity

About 90% of the people who become infected with *E. histolytica* are asymptomatically colonized (Gathiram and Jackson, 1987). There are possible virulence factors of *E. histolytica* such as cysteine proteinases, Gal/Gal NAc-inhibitable lectin and amoebapore (Gilchrist and Petri, 1999). *E. histolytica* contains

proteolytic enzymes (collagenase and neutral proteases) and cysteine proteases, which facilitate its tissue invasion. The parasite also elaborates a range of enzymes on the amoebic surface which induces membrane-associated neuraminidase and β-glucosaminidase (Werries *et al.,* 1983). Other components potentially necessary for pathogenesis of *E. histolytica* include a Ca^{2+} -binding protein and calmodulin (Yadava *et al.,* 1997).

Infection occur by ingestion of *E. histolytica* matured cysts and after a certain period of excystment, trophozoites colonize the large intestine (cecum and colon) and adhere to the intestinal epithelium by interaction of the parasite Gal/Gal NAc-inhibitable lectin with host-derived glycoproteins, which are high affinity ligands for amoebic lectin (Pillai and Kain, 2000). The Gal/Gal NAc-binding lectin facilitates target cell adherence, complement resistance and cytotoxicity (McCoy *et al.,* 1994).

Monoclonal antibodies recognizing the lectin can strikingly affect both *in vitro* adherence and cytotoxicity (Petri *et al.,* 1989). The lectin of *E. dipar* shows decreased adherence, binding and contact–dependent (Dodson *et al*., 1997; Pillai, 1997). Extracellular killing of neutrophils, macrophages and erythrocytes by *E. histolytica* has been demonstrated (Guerrant *et al.,* 1981). The human colonic mucin layer may prevent the host cell from undergoing cytolytic activity by neutralizing the binding agents on the lectin during attachment (Ravdin and Guerrant, 1981). In addition, galactose blocks the cytopathic effect on host tissue (Guerrant *et al.,* 1981).

2.7 Epidemiology

Amoebiasis is worldwide in distribution and is the third most common cause of death due to parasitic infection after malaria and schistosomiasis. An estimated 40,000 to 100,000 deaths from amoebiasis occurs worldwide annualy (WHO, 1997). The best estimate as at 1986 is that *E. histolytica* causes between 34 million and 50 million symptomatic infections, that is, amoebic colitis or liver abscess each year (Walsh, 1986). The occurrence of amoebic liver abscess is 5-50 times less common than that of diarrhoea (Walsh, 1986).

The prevalence and presentation of both symptomatic and asymptomatic amoebiasis vary geographically and with the population of individuals affected; differing between countries and between areas with different socio-economic conditions. The highest prevalence rates have been reported to be in developing countries and regions such as the Indian subcontinent, parts of central and South America, and tropical regions of Africa (Li and Stanley, 1996).

In industrialized countries such as the United States, amoebiasis has been reported in sexually active homosexual men (Peters *et al.,* 1986). Most infections in these regions are due to the non-invasive species, *E. dispar,* which does not require treatment (Allason-Jones *et al*., 1986). Infection in developed nations have also been observed in immigrants, tourists who travel to areas of endemic infection (Krogstad *et al.,* 1978), institutionalised persons (Cheng and Wang, 1999), human immunodeficiency virus HIV-positive individuals (Lowther *et al.,* 2000) and among the handicapped (Lee *et al.,* 2000).

In other countries of the world like Philippines, Cross and Basacca-Sevilla (1980) reported 5-7% prevalence rate for *E. histolytica,* while Salazar *et al*. (1990) reported a lower prevalence of 1.1% in six areas presumed to be "hyper-endemic". A field study in some regions of Iran to determine the ratio of *E. histolytica / E. dispar* showed prevalence of 0.7%, 3.9% and 4.6% for central, northern and southern regions respectively (Hooshyar *et al.,* 2004). Further study was carried out in Gonbad city of Iran in 2006 on differential diagnosis of the two protozoa *(E. histolytica / E. dispar)* using PCR method and high prevalence of *E. dispar* was observed (Mojarad *et al.,* 2006). In Makkah, Saudi Arabia, *E. histolytica* infection is one of the significantly common pathogenic protozoa encountered; a prevalence rate of 2.6% was reported by Saeed and Manal (2007). Also, in a study by Aza *et al.* (2003) in Malaysia, *E. histolytica* is one of the most occurring protozoan parasites; prevalence of 21.0% was reported.

Reports from parts of Nigeria also show endemicity of amoebiasis due to *E. histolytica*. In Ahmadu Bello University Teaching Hospital Zaria, Kaduna state, report shows 23% *E. histolytica* as an etiology of chronic diarrhoea among children (Yakubu and Sathiakumar, 1985), another study reports 8.51% in five rural villages in Kaduna and Zaria (Inabo *et al*., 2000), in Abeokuta, 5.7% was reported (Okodua *et al*., 2003), while in Ilesa, 80.5% was also reported (Ogunlesi *et al.,* 2005). Epidemiological studies have shown that low socioeconomic status and bad sanitary conditions are significant independent risk factors for infection. In addition, people living in developing countries have a higher risk and earlier age of infection than those in developed regions (Fuchs *et al.,* 1988).

E. histolytica and *E. dispar* have been classified by various techniques such as ELISA and PCR (Pillai *et al.,* 1999; Verweij *et al.,* 2000), which have been of great value in understanding the epidemiology of these parasites and in investigating disease outbreaks. As a result of this development, it has been reported that most of the individuals who were previously believed to have asymptomatic infection with *E. histolytica* actually carry *E. dispar,* which has never been shown to cause invasive human disease (Diamond and Clark, 1993). Furthermore, only approximately 10% of individuals who become infected with *E. histolytica* actually develop invasive disease (Gathiram and Jackson, 1987). In Sydney, Australia, a study to investigate the presence of *E. histolytica, E. dispar,* and *E. moshkovskii* in stool samples from patient population using PCR, documented a prevalence rate of 3.4% for *E. histolytica*.

2.8 Prevalence in Children

Human infection with *E. histolytica* is prevalent worldwide and common among children in the developing world (Wanke *et al.,* 1988). Diarrhoea is estimated to cause up to 19% of the annual 10 million under-five deaths in the developing world (WHO 1997; Kosek *et al*., 2003). Amoebiasis is common in children and is associated with diarrhoea/ dysentery in the endemic countries of the world (Wanke *et al.,* 1988). The rate of mortality from diarrhoeal diseases in the world can be decreased by better therapy, interventions that promote sanitary conditions and also education of the inhabitants to take part in primary health care activities (WHO, 1995 and Aksoy *et al.,* 2007).

Acute diarrhoeal diseases continue to be one of the major causes of morbidity and mortality in the developing countries such as Bangladesh, where one in ten children die before their fifth birthday (Petri *et al*., 2002). In Dhaka, Bangladesh, where diarrheal disease is the leading cause of death in children younger than six years of age, approximately 50% have been reported to have been exposed to the disease (Haque *et al.,* 2001; Haque *et al.,* 2006).

In endemic countries such as Bangladesh, amoebiasis in children has been reported (Haque *et al.,* 2003; Haque *et al*., 2006). A recent study in Bangladesh has highlighted the prevalence of *E. mohkovskii* (21.1%) which has been known over the years to be non-pathogenic, suggesting that this infection is common among children aged 2-5 years (Ali *et al*., 2003). A report from Mexico in paediatric patients showed that much of the diagnoses of amoebiasis made were of non-pathogenic *E .dispar* in infant (Redondo *et al*., 2006).

Sero-prevalent investigations of amoebiasis in some tropical areas indicated that prevalence could be high during epidemic (Haque *et al.,* 2001). Sero-prevalence of infections with *E. histolytica* and *G. lamblia* was reported in Southern India (Shetty *et al.,* 1992). A very low prevalence of 0.19% of *E. histolytica* has been reported among other intestinal parasites in Turkey (Aksoy *et al*., 2007).

Reports from parts of Nigeria also show prevalence of amoebiasis in children (Yakubu and Sathiakumar, 1985; Inabo *et al.,* 2000 and Ogunlesi *et al.,* 2005).

Amoebic colitis and liver abscess were also reported in Egypt and South Africa (Stauffer *et al.,* 2006).

Low standards of hygiene and sanitation, particularly those related to crowding, contamination of food and water and inadequate disposal of faeces are all high risk factors for infection with *E. histolytica* (Martinez and Espinosa, 1998). Sometimes up to 50% of the population is affected in regions with poor sanitary conditions and during epidemics (Caballero *et al.,* 1994).

2.9 Pathology and Clinical Signs

E. histolytica causes disease by invading the intestinal epithelium and producing intestinal lesions which may later spread to extra-intestinal sites. Four forms of intestinal amoebiasis have been described by Lucas and Upcroft (2001), these are: Amoebic dysentery or amoebic colitis, fulminating amoebic colitis, amoebic appendicitis and amoeboma.

Amoebic dysentery or amoebic colitis accounts for close to 90% of intestinal amoebiasis. The clinical presentation of this form is usually sub-acute and less than one month duration with symptoms ranging from mild diarrhoea to classic dysentery (Li and Stanley, 1996). Dysentery is characterised by inflammatory condition of the intestine accompanied by abdominal pain and frequent stools containing both blood and mucus (Martinez-Palomon and Espinosa-Cantellano, 1998). Fever and systemic manifestations are generally absent and the clinical course is moderate, with symptoms disappearing with treatment.

The remaining three forms are very severe and require immediate medical care. Fulminating amoebic colitis consists of widespread of necrotic ulcerous lesions which may perforate the peritoneum leading to peritonitis (Lucas and Upcroft, 2001). Amoebic appendicitis is similar to its bacterial counterpart, while amoeboma is a lesion associated with necrosis, inflammation and oedema of both mucosa and sub-mucosa of the colon (Martinez-Palomon and Espinosa-Cantellano, 1998).

Extra-intestinal amoebiasis is brought about by haematogenous spread of trophozoites to the liver, brain, lung, skin and rarely uro-genital structures (Li and Standley, 1996). Among these organs, the amoebic liver abscess is by far the most common complication and is characterised by single or multiple abscesses formed with local necrosis and liquefaction in the liver (Panosian, 1988; Seeto, 1999 and Genel *et al*., 2004).

2.10 Redescription of *Entamoeba histolytica* and *Entamoeba dispar*

E. histolytica and *E. dispar* are currently recognised as distinct species (Diamond and Clark, 1993) based on genetic, biochemical and immunologic studies (Sargeaurt *et al*., 1978; Strachan *et al*., 1988). Therefore, it is possible to obtain more reliable and correct epidemiological data using the aforementioned studies for better diagnosis and treatment of the disease.

Differentiation of *E. histolytica* and *E. dispar* in stool samples is not easy on the basis of microscopy alone. But this can be done using molecular tools like amoebic antigen, DNA detection, enzymes immunoassay (EIA) and PCR (Acuna-soto *et al*.,

1993; Haque *et al*., 1998; Zama *et al*., 2000). Redescription of these organisms is of great importance because it allows the clinician to focus on early identification and treatment of *E. histolytica* infection in the minority of patients who are at risk and pose a major public health problem (Reed, 2000). Lucas *et al* (2001) observed that redefinition of *E. dispar* (non-pathogenic) and *E. histolytica* (pathogenic) means that only the patients carrying *E. histolytica* should be considered for treatment.

2.11 Diagnosis

The World health organisation (WHO), the Pan American Health Organisation (PAHO) and the United Nations Educational Scientific and Cultural Organisation (UNESCO) published a joint statement in 1997 in which they stressed the urgent need to develop improved methods for the specific diagnosis of *E. histolytica* infection using technologies that are appropriate for developing countries (WHO. 1997).

In the past, light microscopy has been the method of choice to diagnose amoebiasis. With microscopy, the presence of trophozoites that have ingested red blood cells in a stool sample strongly suggests *E. histolytica* infection, though such a finding is rare (Gonzalez-Ruiz *et al.,* 1994). In the absence of haematophagous trophozoites, the sensitivity of microscopy is limited by its inability to distinguish between samples infected with *E. histolytica* and those infected with *E. dispar.* The *E. dispar* infection is more common than *E. histolytica* (Redondo *et al*., 2006).

A stool culture method is another method used for the diagnosis of the disease, which involves the culture of stool samples followed by isoenzyme analysis (Krogstad *et al*., 1978). This method can accurately distinguish *E. histolytica* from *E. dispar*. However, this method takes between one and several weeks to carry out and requires special laboratory facilities, making it impractical for use in the developing world, where most cases of amoebiasis occur (Strachan, 1988).

Methods that involve ELISAs to detect antigens in stool samples are able to distinguish accurately between infection with *E. histolytica* and *E. dispar.* These are replacing microscopy and stool culture for both clinical and research purposes (Mirelman *et al.,* 1997). Several ELISA kits developed by different companies are now commercially available. For example, *E. histolytica* 11 test kit developed by Techlab (Strachan *et al.,* 1988; Haque *et al.,* 1997) can distinguish between *E. histolytica* and *E. dispar* infections (Mirelman *et al.,* 1997). This test uses a monoclonal antibody against an amoebic adherence lectin that is inhibitable by Gal or Gal NAc. The lectin is conserved and highly immunogenic and because of antigenic differences between the lectins of *E. histolytica* and *E. dispar*, it can be used to identify the pathogenic species. The sensitivity of this method is >85% and its specificity when compared with that of stool culture methods is > 90% (Haque *et al*., 1998). Only antigen detection using ELISAs are rapid and technically simple to perform, making it appropriate for use in the developing world (Haque *et al.,* 1998).

Several PCR-based methods that amplify and detect *E. histolytica* DNA in stool samples have also been developed (Katzwinkel-Wladarsh *et al.*, 1994; Troll *et al.,*

1997; Roy *et al.,* 2005). The sensitivity and specificity of PCR-based methods for the diagnosis of *E. histolytica* infection both approach those of stool culture followed by isoenzyme analysis (Mirelman *et al.,* 1997). However, field studies that directly compare the PCR method with the stool culture or antigen detection methods for diagnosis of the *E. histolytica* infection suggests that these methods perform equally well.

Serological methods can also be used to distinguish accurately between prior infection with *E. histolytica* and *E. dispar* (Krupp, 1970; Cross and Basaca-Sevilla, 1980). Patients infected with *E. dispa*r do not develop serum anti-amoebic antibody titres, but patients with symptomatic *E. histolytica* infection develop detectable anti-amoebic antibodies and some patients develop antibodies after they recover from infection (Krupp, 1970; Haque *et al*., 2006). Levels of anti-amoebic antibodies remain elevated in the serum for years; this however limits the usefulness of this method for diagnosis in endemic regions where the frequency of prior amoebic infection can be as high as 50% (Krupp, 1970; Shetty *et al*., 1988).

Future studies using highly purified amoebic antigens rather than crude amoebic extracts as the target antigen, might provide more accurate information on the sero-prevalence of the disease in endemic regions (Huston *et al*., 1999).

2.12 Treatment and Control

Historically, in 1992, Leonard Rogers prescribed emetine as the first effective treatment for amoebiasis (Rogers 1912). Symptomatic patients with bloody stools

containing motile trophozoites with ingested erythrocytes were advised to be treated according to the severity of the disease (Lucas and Upcroft, 2001). Severe cases may require surgery but the corner-stone of treatment for amoebiasis remains the nitromidazole derivatives (metronidazole, tinidazole, ornidazole), out of which only metronidazole is available (Stanley, 2003).

Amoebic colitis is treated with metronidazole, followed by a luminal agent, e.g iodoquinol to eradicate colonisation (Pehrson and Bengtsson, 1984), usually taken thus; adults: Metronidazole 750mg tid for 7-10 days, in conjuction with iodoquinol 650 tid for 20 days; and children: metronidazole 35-40mg/kg/24hours in conjunction with iodoquinol 40 mg/kg/24hours in four divided doses for 7-10 days (Lucas and Upcroft, 2001). When possible, fulminant amoebic colitis, even with perforation is managed with the addition of antibiotics to deal with bowel flora (Monga *et al.,* 1976).

Asymptomatic individuals with *E. histolytica* infection should be treated with a luminant agent (iodoquinol or diloxanide furoate) to eradicate infection, so as to be free from the risk of developing invasive disease, and the fact that such individuals are a risk to public health (Gathiram and Jackson, 1987). *E. dispar* infection does not require treatment, but should alert the physician that the infected person has been exposed to faecally contaminated food or water (Stanley, 2003).

2.13 Prevention

Contamination of food and water with human faeces must be prevented for these are usually complicated by the high incidence of asymptomatic cyst passers (Escobedo *et*

al., 2003). Boiling water kills *E. histolytica* cysts and should be encouraged, especially in endemic developing nations. Uncooked foods, including salads and vegetables should be properly washed and treated with vinegar before consumption.

Potable filters used for water filtrations provide various degrees of protection. In addition, appropriate water and sewage systems should be ensured, hygiene habits should also be improved (Escobedo *et al*., 2003).

More public awareness programmes to promote a better understating on the adverse impacts of amoebiasis and other health problems among children are necessary (Aza *et al.,* 2003). Such awareness will bring about positive changes and adaptation in both social and cultural practices that can help in reducing the infection rate through proper use of water supply and practice of modern sanitation (Aza *et al.,* 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Zaria (11° 3' N; 7° 42' E) Nigeria, which is located about 83km north of Kaduna, along the Kaduna-Kano highway (Mortimore, 1970). Zaria is comprised of two Local Government Areas namely; Zaria and Sabon Gari. By the existing pattern of settlement, Zaria urban is composed mainly of four districts namely; Zaria City, Tudun Wada, Sabon Gari and Samaru.

Zaria is made up of a natural and stable ecosystem in the northern Guinea Savannah zone, with a discontinuous layer of sparsely distributed short trees followed by relatively continuous layers of tall, medium and short grasses; rainfall is below 125mm (Mortimore, 1970). Zaria is an old commercial, administrative and academic town in Northern Nigeria, but most parts have poor environmental hygiene as evident by heaps of refuse dumps coupled with indiscriminate disposal of human and animal wastes, even by the side of streets (Aina, 2002).

3.2 Ethical clearance

Two hospitals in Zaria were selected for the study; the Institute of Child Health Banzazau and the Paediatric Unit of Hajia Gambo Sawaba Memorial Hospital Kofan Gayan. Ethical approval was obtained to undertake the study from the Chairman, Ethical Committee, Ahmadu Bello University Teaching Hospital Shika, Zaria and permission was granted by the Medical Director of Hajia Gambo Sawaba Memorial Hospital, Kofan Gayan, Zaria.

3.3 Collection of samples

Children between 3months to 60 months were recruited into the study. Stool specimens were collected systematically from children presenting with diarrhoea and from children presenting with other diseases like malaria and typhoid (non-diarrhoea), the later served as the control group. Such children were referred by the medical personnel to the researcher. Samples were collected into disinfected, plastic specimen bottles at the various hospitals, with specific instructions given to the parents/guardians of the children who collected the samples and submitted to the researcher. The samples were transported in ice packs to the Parasitology laboratory of the Department of Biological Sciences, Ahmadu Bello University Zaria within 45mins, where they were assayed. One to two grams (1-2g) of each stool sample was fixed in two millilitres (2mls) of 10% formal saline for examination by microscopy. While another portion was quickly stored in the deep freezer (-20^{o}C) for ELISA (Saeed and Manal, 2007).

3.4 Administration of Questionnaire

A structured questionnaire was administered to the parents/guardians of the one hundred and eighty-three (183) children recruited into the study. Respondents who could not read or write in the English language were interviewed in Hausa by the researcher. The questionnaire was designed to provide information such as age, sex, home location of the child, socio-economic status of the parents/guardians and risk factors such as, source of drinking water, type of toilet and finger sucking habit (Appendix 1).

3.5 Sample size determination

Due to unavailability of data on the true prevalence of amoebiasis in children below six (6) years in Zaria, a prevalence of 8.51% based on the previous study of Inabo *et al*. (2000) was used to determine the sample size required for the study, using the formula;

$$n = \frac{Z^2 Pq}{L^2}$$

Where;

n = sample size

Z = for standard normal distribution at 95% confidence interval = 1.96

P = prevalence of 8.51% (0.0851) from Inabo *et al*. (2000)

q = l – p = 1 – 0.0851= 0.915

L = allowable error = 5% or 0.05

Substituting the values in the formula therefore:

$$n = \frac{1.96^2 \times 0.0851 \times 0.915}{0.05^2}$$

$$n = \frac{3.84 \times 0.0779}{0.0025}$$

n = 119.60 Approximately 120

3.6 Laboratory Techniques

3.6.1 Microscopy: Formol-Ether Concentration Test

The one to two grams (1-2g) of each specimen initially fixed with 2mls of 10% formal saline was emulsified in about 6mls of 10% formal saline by stirring with a clean glass rod in test tube. The suspension was filtered over two layers of surgical

gauze into a 10mls-capacity centrifuge tubes. 2mls of diethyl-ether was then added into each tube and shaken vigorously. This was centrifuged using a centrifuge machine (GF-6 Model, England-FJ1179) at increasing speed to a maximum of 1500 rpm for about 5mins. The supernatant was discarded and a drop of lugol's iodine was added to the residue. After tapping each tube, smears were made on clean slides, after which they were examined using a binocular microscope at magnifications of ×100 and ×400 for the presence of *E. histolytica/E. dispar* complex cysts, cysts, oocysts and eggs of other intestinal parasites (NCCL, 1997).

3.6.2 ELISA TEST

Sensitivity and Specificity

The TechLab (*E. histolytica* II kit) is considered as one of the ELISA test used to distinguish between *E. histolytica* from *E. dispar* infections (Mirelman, *et al.*, 1997). The *E. histolytica* II kit is highly sensitive. It is capable of detecting approximately 0.2-0.4ng per well of adhesin from *E. histolytica* in stool samples. Indeed, it has been shown to be as sensitive and specific as culture with isoenzyme analysis. When compared, sensitivity has been shown to be 96.9% and specificity 100% (Redondo *et al*., 2006), hence making it ideal for use.

Principle of the Procedure

The *E. Histolytica* II test uses antibodies to the adhesin. The micro-assay wells contain immobilized polyclonal antibody that binds adhesin of *E. Histolytica*. The conjugate is a monoclonal antibody-peroxidase conjugate specific for *E. Histolytica* adhesin. In the assay, if adhesin is present in the specimen, it binds to the conjugate

and immobilised polyclonal antibody during the incubation phase. All unbound materials are removed during the washing step. On addition of substrate, a colour develops due to the enzyme-antigen complexes that form in the presence of adhesin which is read at a specific wavelength (450/630nm) on the ELISA reader (TECHLAB® Blacksburg, VA 24060 U.S).

Procedure

The manufacturer's guide was carefully followed as summarised below:

1. Specimens that were initially frozen at □20°C were removed from the freezer and allowed to thaw at room temperature.
2. All specimens were thoroughly mixed prior to performing the assay.
3. Approximately 0.15 to 0.20g of specimen (about the size of a pea) was transferred to 400µl of diluent and thoroughly shaken to mix evenly in labelled test tubes. For formed specimens, a swab was coated completely and transferred to the diluent. The swab was mixed in diluent to remove as much sample as possible and squeezed against the side of the tube to express any residual liquid. This procedure resulted in the transfer of approximately 0.15 to 0.20g of specimen.
4. Three control wells were used each time the test was performed. These wells served as blank, positive and negative controls. One well was used for each patient's sample, (additional control wells are not needed to run a greater number of samples)
5. A drop (50 µl) of conjugate was added to a positive control well, negative control and patient sample well. The blank was left empty.

6. A drop (50 µl) of the positive control reagent (provided in the kit) was added to the positive control well and two drops (100µl) of the negative control i.e. diluents to the negative control well. 200µl of diluted specimen was transferred to the test well. The mixtures were covered with a plastic adhesive sheet and incubated for two hours at room temperature.
7. The contents of the assay wells were then shaken out into a discard pan and washed using the diluted wash solution in a squirt bottle with a fine-tipped nozzle, directing the wash solution to the bottom of the well with force. The inverted plate was slapped on a dry paper towel and the washing step was repeated four times.
8. After washing, residual liquid in the wells was completely removed by striking the plate once again unto a dry paper towel until no liquid came out.
9. Two drops (100µl) of substrate solution were added to each well and gently tapped initially and again at 5mins to mix the substrate. This was then incubated for 10min at room temperature.
10. One drop (50µl) of stop solution was then added to each well. The wells were gently tapped until 2mins before reading. The addition of stop solution converted the blue colour to a yellow colour, which was quantitated by measuring the optical density at 450nm (Plate I) on an EIA multi-well reader (Model no. 2101), produced by Sigma Diagnostic (St Louis, MO 63103, USA), (Saeed and Manal 2007).

Spectrophotometry

The microplate ELISA reader was set to read at 450nm and referenced/blanked against air at 630nm. The absorbance values for the positive and negative controls were determined (Appendix 2A-2C). A sample was considered positive when value read was higher than the negative control value (but lower than the positive control value), and negative when the value read was lower than the negative control value. A positive test result indicates that *E. histolytica* adhesin was present in the faecal specimen and was used in the analysis to determine prevalence and association, while a negative result indicated that *E. histolytica* was not present in the faecal specimen.

3.7 Data Analysis

- Prevalence was calculated and expressed as percentages (%).
- Data was tested for significance using chi-square analysis.
- Odds ratio was calculated for association between risk factors and infection.
- Significance was determined at $P < 0.05$ at 95% Confidence Interval.

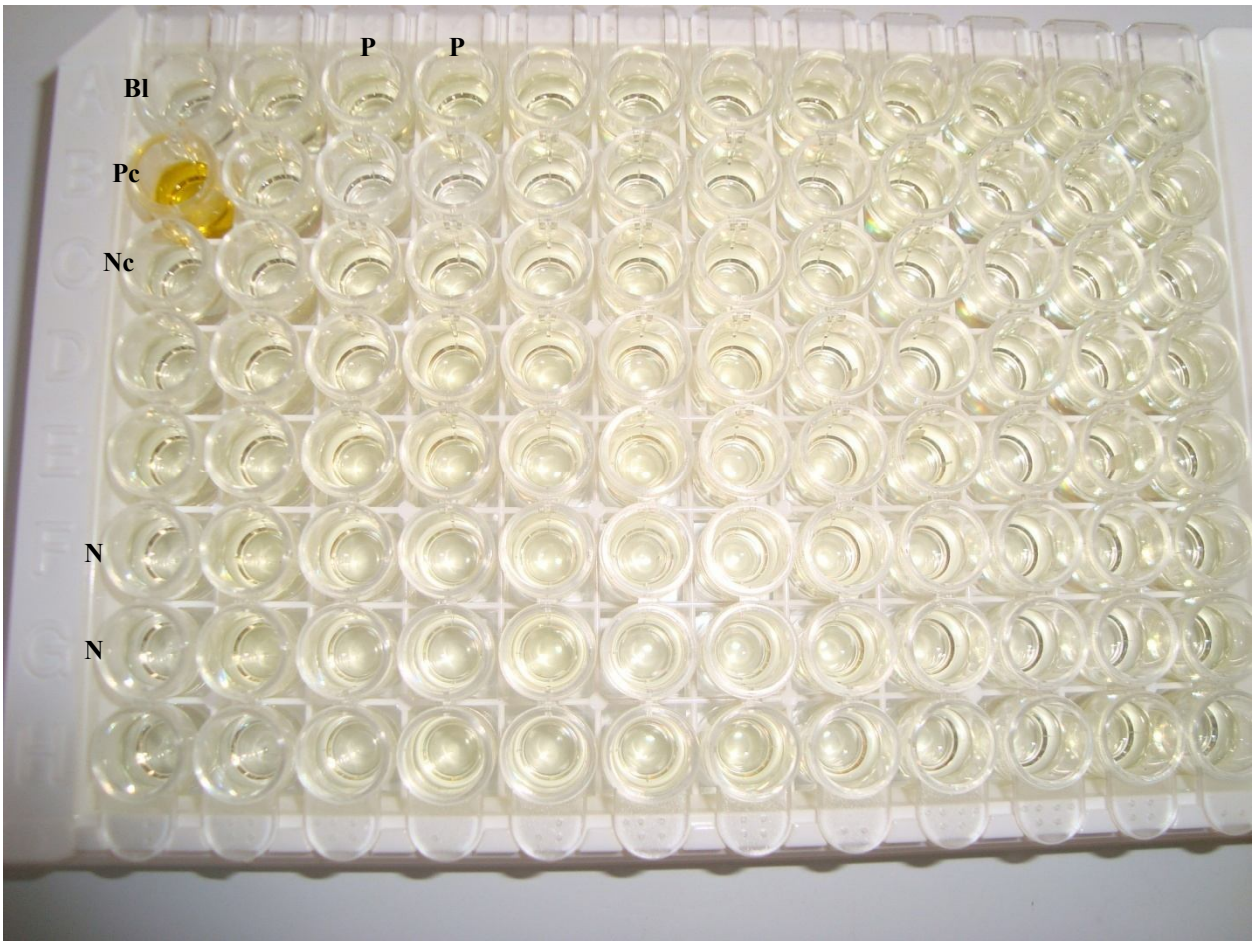


Plate I. Micro wells before reading showing the blank (Bl), positive control (Pc), negative control (Nc) and test wells of positive (P) and negative (N) reactions.

4.0 RESULTS

4.1 Microscopy

Table 1 shows the prevalence of intestinal parasites observed in this study. Out of 183 samples, 10 (5.46%) were positive for *Entamoeba coli* and Hookworm. A prevalence of 6.01% (11) was observed for *Isospora hominis,* while *Trichuris trichiura and Entamoeba histolytica* were the lowest with 1.64% (3) and 0.55 % (1) respectively.

Table 1: Prevalence of intestinal parasites in stool of children below six (6) years in Zaria

Intestinal Parasites	**Number infected (n=183)**	**Prevalence (%)**
Entamoeba coli	10	5.46
Isospora hominis	11	6.01
Ascaris lumbricoides	7	3.83
Hookworm	10	5.46
Trichuris trichiura	3	1.64
**Entamoeba histolytica*	1	0.55

* *E. histolytica* confirmed by ELISA; n, sample size; %, percentage

4.2 ELISA

4.2.1 Prevalence of *E. histolytica* infection in children according to sex

The result in Table 2 shows the prevalence of *E. histolytica* infection in children. The result shows that of the 183 samples collected, 78 were found to be infected giving an overall prevalence of 42.62%. Of the one hundred and four (104) samples collected from male children, 50(48.08%) were positive for *E. histolytica*. The prevalence in female children was 34.44%, that is, 28 out of 79. There was no significant difference ($p>0.05$) in prevalence with regards to sex.

Table 2: Prevalence of *E. histolytica* infection in children in relation to sex

Sex	**Number examined (%)**	**Number positive (+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**P-value**
Male	104 (56.83)	50	48.08	2.93	1.69	0.93; 3.07	0.06
Female	79 (43.17)	28	35.44				
Total	**183**	**78**	**42.62**				

4.2.2 Prevalence of *E. histolytica* infection in Children according to Age

The prevalence of *E. histolytica* infection according to age is presented in Table 3. The result shows that infection was prevalent in all age groups. The highest prevalence was recorded in children between 12-23months (47.83%) while the lowest was in 48-59months (27.27%). There was no significant difference ($p>0.05$) in *E. histolytica* infection and age. Correlation analysis ($r=-0.57$) shows that *E. histolytica* infection decreased with increase in age.

Table 3: Prevalence of *E. histolytica* infection in children in relation to age

Age (Months)	**Number examined (%)**	**Number positive(+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**p-value**
3-11	43 (23.50)	18	41.86	0.00	0.96	0.45;2.03	0.95
12-23	46 (25.14)	22	47.83	0.43	1.33	0.64;2.74	0.51
24-35	28 (15.30)	12	42.86	0.03	1.01	0.42;2.45	0.86
36-47	20 (10.93)	9	45.0	0.00	1.11	0.40;3.10	O.99
48-59	11(6.01)	3	27.27	0.56	0.49	0.10;2.10	0.45
60	35 (19.13)	14	40.0	0.03	0.88	0.39;1.97	0.87
Total	**183**	**78**	**42.62**				

r= - 0.57

4.2.3 Prevalence of *E. histolytica* infection in children in relation to source

of drinking water

Prevalence of *E. histolytica* infection in relation to source of drinking water is presented in Table 4. Of the 183 respondents (parents/guardians), the highest prevalence was observed in packaged water (54.55%) while he lowest was in Borehole (16.67%). However, there was no significant difference ($p>0.05$) in the sources of drinking water in this study.

Table 4: Prevalence of *E. histolytica* infection in children in relation to source of drinking water

Water source	**Number Examined (%)**	**Number positive (+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**P-value**
Tap	84(45.90)	37	44.05	0.04	1.11	0.59; 2.09	0.83
Well	76(41.53)	33	43.42	0.00	1.06	0.56; 2.00	0.97
Borehole	12(6.56)	2	16.67	2.49	0.25	0.04; 1.27	0.11
Packaged water	11(6.01)	6	54.55	0.26	1.67	0.43; 6.60	0.61
Total	**183**	**78**	**42.62**				

4.2.4 Prevalence of *E. histolytica* infection in children in relation to type of toilet

Of the 183 respondents (parents/guardians), 15 (48.39%) of children from homes where water closet is used were positive for amoebiasis. For pit latrine, the prevalence was 41.45% (63). The highest prevalence (48.39%) was observed in homes where water closet was used. However there was no significant difference (P>0.05) in the type of toilet used in the children's homes.

Table 5: Prevalence of *E. histolytica* infection in children in relation to type of toilet

Type of toilet	**Number examined(%)**	**Number positive(+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**P-value**
Water system	31 (16.94)	15	48.39	0.26	1.32	0.57; 3.07	0.61
Pit latrine	152 (83.06)	63	41.45	0.26	0.76	0.33; 1.75	0.61
Total	**183**	**78**	**42.62**				

4.2.5 Prevalence of *E. histolytica* infection in children in relation to finger sucking habit

Prevalence of *E. histolytica* infection in children in relation to finger sucking habit is presented in Table 6. Of the 183 respondents (parents/guardians), 14(7.65%) reported that their children were sucking a finger, out of which 6(42.86%) were positive for amoebiasis, while 169(92.35%) reported their children do not suck a finger, out of which 72(42.60%) were positive. However, there was no significant difference ($p>0.05$) between finger sucking habit and amoebiasis (OR=1.01).

Table 6: Prevalence of *E. histolytica* infection in children in relation to finger sucking habit

Sucking finger	**Number examined (%)**	**Number positive(+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**P-value**
Yes	14(7.65)	6	42.86	0.07	1.01	0.30; 3.39	0.80
No	169(92.35)	72	42.60				
Total	**183**	**78**	**42.62**				

4.2.6 Prevalence of *E. Histolytica* infection according to children's home location

Table 7 shows that out of the 183 respondents (parents/guardians). The highest prevalence (63.89%) was observed in children living outside Zaria city (Tudun/Wada, Sabon/Gari, Gyellesu, Gaskiya, Wusasa and Samaru), while 37.41% for Zaria city (Zaria city and Banzazau) was observed. There was significant difference ($P<0.05$) in the children's home location and *E. histolytica* infection.

Table 7: Prevalence of *E. histolytica* infection in relation to children's home location

Location	**Number examined (%)**	**Number positive(+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**P-value**
Zaria city	147 (80.33)	55	37.41	8.27	0.34	0.15; 0.77	0.004
Zaria environs	36 (19.67)	23	63.89				
Total	**183**	**78**	**42.62**				

4.2.7 Prevalence of *E. histolytica* infection in children in relation to Diarrhoeal status

On Table 8, out of 183 children that were tested, 129(70.49%) had diarrhoea while 54(29.51%) were down with other diseases such as malaria and typhoid other than diarrhoea. 61(47.28%) of the children with diarrhoea were positive for *E. histolytica* infection and had the highest prevalence. Those with no diarrhoea, had a prevalence of 31.48 %(17). There was a significant association ($p<0.05$, OR=1.95) between diarrhoea and *E. histolytica* infection.

Table 8: Prevalence of *E. histolytica* infection in children in relation to diarrhoeal status

Diarrhoeal status	**Number Examined (%)**	**Number positive (+)**	**Prevalence (%)**	χ^2	**OR**	**CI**	**P-value**
Diarrhoea	129 (70.49)	61	47.28	3.88	1.95	1.00; 3.82	0.034
Non-diarrhoea	54 (29.51)	17	31.48				
Total	**183**	**78**	**42.62**				

4.2.8 The prevalence of *E. histolytica* infection in children in relation to the occupation of their parents/guardians

Table 9 shows that out of the 183 respondents (parents/guardians), the highest prevalence was observed in children whose parents are traders (52.62%) while the lowest was in children whose parents were farmers. There was no significant difference ($p>0.05$) in parents'/guardians' occupation.

Table 9: Prevalence of *E. histolytica* infection in children in relation to occupation of Parents/guardians

Occupation	Number examined (%)	Number positive (+)	Prevalence (%)	χ^2	OR	CI	P-value
Civil servants	42 (22.95)	21	50.00	0.85	1.47	0.71; 3.11	0.36
Trader	57 (31.15)	30	52.62	2.82	1.81	0.92; 3.57	0.09
Farmer	11 (6.01)	2	18.18	1.89	0.28	0.04; 1.45	0.17
Labourers	11 (6.01)	4	36.36	0.01	0.76	0.18; 3.02	0.91
Transporters	24 (13.11)	9	37.50	0.10	0.78	0.30; 2.04	0.75
Professionals	38 (20.77)	12	31.58	2.39	0.55	0.24; 1.25	0.17
Total	**183**	**78**	**42.6**				

CHAPTER FIVE

5.0 DISCUSSION

In this study, the prevalence of *E. histolytica* by microscopy was very low (0.6%). Similar studies elsewhere have also reported low prevalence. For example, a prevalence of 0-1.9% was observed in the Philippines (Salazer *et al*., 1990), 0.78%, 3.9% and 4.6% for central, northern and southern parts of Iran, respectively (Hooshyar *et al*., 2004). However, moderate prevalence have also been reported from microscopic studies; 8.51% in Zaria and Kaduna, Nigeria (Inabo *et al*., 2000), 21.0% in Malaysia (Aza *et al*., 2003) and 11.7% in Thailand (Sirima *et al*., 2008). Microscopy requires well trained microscopist and samples be observed within 20-30 minutes. Furthermore, interfering substances such as antibiotics, laxatives, antacid, cathartics (magnesium sulphate) and anti-diarrhoeal drugs (kaolin or bismuth) can significantly reduce the population of the parasite to low levels not easy to detect by microscopy.

The results of the study also showed the occurrence of several intestinal parasites of public health importance besides *Entamoeba histolytica* among the study group (Table 1). This corroborates the previous report made by Legesse and Erko (2004) where several parasites of varying magnitude were observed in stool samples among school children in Ethiopia. This calls for concern since the children were found to be already exposed to such infections so early in life.

A prevalence of 42.62% of *E. histolytica* infection reported in this study using ELISA underscores the importance of this method over microscopy. This study shows that the disease is endemic in Zaria. The result agrees with previous studies published by Choudhuri *et al.* (1991) who reported that *E. histolytica* infection is endemic in most temperate and tropical climates in developing countries. Serological study is regarded a better method of revealing true prevalence, which reflects both the present and past infections and rates can be up to about 50% (Choudhuri *et al.,* 1991). In Nigeria, Yakubu and Sathiakumar (1985) documented 23% in Zaria and Ogunlesi *et al*. (2005) reported 11.0% in under-five children with dysentery in Ilesha. The prevalence of amoebiasis varies with population of individuals affected and with the level of sanitation. It is generally higher in the tropics and sub-tropics than in temperate climates. Up to 50 % of the population can be affected in regions with poor sanitary conditions and during an epidemic (Caballero-Salcedo *et al*., 1994). In the same vein, Li and Stanley (1994) observed that *E. histolytica* is found worldwide, but the highest prevalence are in developing countries and regions such as the Indian sub-continent, parts of Central and South America and tropical regions of Africa, including Nigeria as revealed in this study.

The prevalence of amoebiasis in male children (48.08%) was not significantly different from that in female children (34.44%), suggesting that both the male and female children in the study area have the same predisposition to *E. histolytica* infection. This agrees with the report of Bruga *et al*. (2001). However, higher prevalence in female children than male children due to large sample size has been reported by Hooshar *et al*. (2004) and Sirima *et al*. (2008).

Children of five years and below are the most susceptible group to amoebiasis. It is not surprising therefore that the observed differences in prevalence with increase in age was not significant, confirms the susceptibility of this group of children to amoebiasis. Generally, children have been reported to be more exposed to infection with *E. histolytica* than adults. They are more susceptible to water-borne and food-borne infections, because their playing and hygiene practices predispose them to infection than older age groups; more so, their immune systems are not fully developed and their level of health education is not sufficient to distinguish the dangers of contamination. This is in agreement with Bruga *et al.* (2001) who reported a prevalence of 14.9 % (110/735) and 25.4 % (187/735) for *E. dispar / E. histolytica* complex, the most affected group in their study was 1-5 years. UNICEF (1998) documented that *E. histolytica* accounts for 19% of the 10 million under-five deaths in the developing world. In Dhaka, Bangladesh diarrhoeal disease is the leading cause of death in children younger than 6 years of age, with an approximately 50% of children having serological evidence of exposure to *E. histolytica* by 5 years of age (Haque *et al*., 2003).

Breast-fed children are generally known to be more resistant to protozoan infections, though isolated cases are not infrequent (Molbak *et al.*, 1994) and were observed in this study. It is worth noting that children less than one year who are still breast-feeding had a high prevalence of 41.86%. The said children probably acquired the infection at home from contaminated food and water given to them by caregivers who in turn are poor in personal hygiene.

Water is a major source through which amoebiasis is contracted, especially by children. In this study, it was observed that there was no significant difference in the sources of drinking water. However, the highest infection was found among children from homes where the source of drinking water was packaged water (54.5%) followed by tap (44.0%) and well (43.4%). Many rural communities in northern Nigeria depend on well water for drinking and other uses; many of these wells are usually left uncovered and are subject to contamination with cysts of *E. histolytica* which are the infective stage, from various types of wastes including human and animal faeces. Cysts are known to persist in water for weeks or months and in the dry season, are known to withstand desiccation and survive for a long period in the environment (Inabo *et al*., 2000). In this study, the source of drinking water did not significantly influence the prevalence of amoebiasis suggesting that there is an underlying factor influencing prevalence. Water, irrespective of its source can easily be contaminated during handling, especially where sanitation and personal hygiene of caregivers are generally poor. It is easy to contaminate water and food by the use of contaminated hands and utensils. Furthermore, safety of the booming business of packaged water (pure water) has been questioned for long. This study suggests the need to investigate this further. All these explain why neither tap nor packaged water conferred significant protection to the children against amoebiasis.

The type of toilet facility used in homes of the children in this study area similarly showed no significant influence on infection. The relative safety of the water closet system depends on the availability of water. Tap water is increasingly becoming a scarce commodity in Zaria and thus most homes do not actually have the luxury and

safety of water closet system. This explains why infection found residence in homes irrespective of the toilet facility in use.

Insignificant high prevalence of infection among children that suck finger (42.86%) and those that do not suck their finger (42.60%) was observed in this study. The habit of sucking finger is formed by children; it is a reflex action and is done without the consciousness that the finger may be dirty. Finger sucking has the potential to aid direct transmission, although it did not in this study. In other words, whether a child does or does not suck his/her finger, he/she was vulnerable to contract amoebiasis in the study area, suggesting that an underlying factor rather than sucking fingers was at play.

The results from this study showed that the children's environment influenced infection with *E. histolytica.* The significant higher prevalence was observed in children living outside Zaria city (63.9%) than those within Zaria city (37.41%). The significant difference observed with respect to study site is not easy to explain in the light of the similar features that characterises both environs. Generally, Zaria city and its environs are characterised by poor environmental hygiene as evidenced by heaps of refuse dump coupled with indiscriminate disposal of human wastes, even by the road side as reported by Aina (2002). Children are often found playing and picking objects from such heaps of refuse dump thereby being exposed to infection with pathogens including *E. histolytica*. Defective plumbing (which is commonly observed in Zaria) can permit sewage to contaminate drinking water by close running of water pipes and sewage lines. The most dramatic incidence of this kind was reported in

Chicago, USA, in 1993, where 1000 cases (with 58 deaths) from the outbreak of *E. histolytica* infection as a result of defective plumbing system (Ozlem and Hakan, 2005). Another source of infection with *E. histolytica* is the use of sewage effluent for irrigation purposes, which is also practiced in Zaria. Faecal contamination of vegetables/fruits from such places and consumed without proper washing places children at risk of infection.

Entamoeba histolytica is well associated with diarrhoea and was observed in this study. The prevalence for children with diarrhoea (47.3%) was significantly higher than in children without diarrhoea (31.5%). Diarrhoea has been considered to be the major contributor to childhood mortality and morbidity in the developing world. It causes an estimated 2.5million death each year (Murray and Lopez, 1997; Kosek *et al*., 2003). One of the etiologies of diarrhoea is *E. histolytica* and is endemic in the developing world (Kosek *et al*., 2003) even Zaria, (Inabo *et al*., 2000). The study of the etiology of chronic childhood diarrhoea among Nigerian children was conducted by Yakubu and Salthiamur (1985) using microscopy method in Ahmadu Bello University Teaching Hospital Zaria. The results showed that *E. histolytica* (23%) was one of the common parasites detected of all the parasitic pathogens. In Elsie Gashes village, the National Centre for the Mentally Retarded Metro Manila, Salazar and Pasay (1991) used stool and serological methods to screen 15 patients with diarrhoea, stomach pain and other symptoms and 13 (86.6%) were reported positive for *E. histolytica*. Our study and related works show that *E. histolytica* is a major cause of diarrhoea in children. On the other hand, children who had no diarrhoea (54) and yet tested positive on ELISA (31.48%) are asymptomatic carriers and considered

potential cyst-passers and such are of public health importance being capable of spreading the parasite.

The prevalence of infection among the different occupational groups of the parents/guardians of the children in this study varied insignificantly, the highest being in children whose parents/guardians were traders (52.63%) followed by civil servants (50%). This implies that, the prevalence of *E. histolytica* infection in children cut across the socioeconomic background of their parents/guardians. This is contrary to epidemiological studies which show that low socioeconomic status is a risk factor for infection and that infection; particularly the parasitic ones, are seen in regions with low socioeconomic status (Aksoy e*t al*., 2007). The observation made here is important; it suggests, as we have always held, that sanitation and the personal hygiene of caregivers was poor such that irrespective of the source of water, type of toilet etc, the children were generally vulnerable to contract infection on account of contamination facilitated by caregivers.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

To our knowledge, this is the first study in Zaria that the ELISA technique was used to determine the prevalence of *E. histolytica* infection among preschool children. Infection in the children was not influenced by age, sex, source of drinking water, nature of home toilet and occupation of the parents/guardians. Importantly, diarrhoea was found to be well associated with *Entamoeba histolytica*, so also was amoebiasis associated with the environment of the children. Infection was attributed to caregivers who take care of the children on account of their poor hygiene habits as deduced by the fact that infection was not influenced by the occupation of parents/guardians.

This study concludes that amoebiasis is prevalent in children in Zaria with potential health consequences than earlier estimated. It also suggests that all cases of diarrhoea in children reported in hospitals should be considered for specific tests and treatment.

Accordingly, the followings are recommended:

- More public health awareness programmes be promoted towards a better understanding of the source and adverse impacts of amoebiasis among children.
- Improvement of sanitation, provision of safe water and good toiletry habits.

- Proper and effective diagnostic techniques, such as, the use of sensitive and specific methods like ELISA and treatment of infected individuals should be encouraged and made available in hospitals and rural health centres.
- Further studies involving a larger sample size and older age groups be carried out.

REFERENCES

Abd-Alla, M. D., el-Hawey, A. M. and Ravdin, J. I. (1992). Use of an enzyme-linked immunosorbent assay to detect anti-adherence protein antibodies in sera of patients with invasive amebiasis in Cairo, Egypt. *American Journal of Tropical Medicine and Hygiene,* 47: 800-804

Acuna- Soto, R., Samuelson, J., De Girolami, P., Zarate, L., Millan-Velasco, F., Schoolnick, G. and Wirth, D. (1993). Application of the polymerase chain reaction to the epidemiology of pathogenic and non-pathogenic *Entamoeba histolytica. American Journal of Tropical Medicine and Hygiene, 48:58-70.*

Aina, O. E. (2002). Solid waste management in Zaria. Unpublished B.Sc. (project) of Department of Geography, A.B.U. Zaria, pp.2-7.

Aksoy, U., Akisu, C., Bayram-Delibas, S., Ozkoc, S., Sahin, S. and Usluca, S. (2007).Demographic status and prevalence of intestinal parasitic infections in school children in Izmir, Turkey. *The Turkish Journal of Paediatrics*, 49:278-282.

Ali, I. K. M., Hossain, M. B., Roy, S., Ayeh-Kumi, P. F., Petri, W. A. Jr., Haque, R. and Clark,C. G. (2003). *Entamoeba moshkovskii* Infections in Children in Bangladesh. *Emerging Infectious Diseases*, 9(5): 580-584.

Allason- Jones, E., Mindel, A., Sargeaunt, S. and Williams, P. (1986). *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. *New England Journal of Medicine*, 315:353-356.

Aza, N., Ashley, S. and Albert, J. (2003). Parasitic Infections in Human Communities Living on the Fringes of the Crocker range park, Sabah, Malaysia. *ASEAN Review of Biodiversity and Environmental Conservation (ARBEC), http://hwww.arbec.com.my/pdf/art II*

Benetton, M.L., Gonclaves, A.V., Meneghini, M.E., Silva, E.F. and Carneiro, M. (2005). Risk Factors for infection by *Entamoeba histolytica/E. dispar* complex: an epidemiological study conducted in outpatient clinics in the city of Manaus, Amazon Region, Brazil. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 99:*532-540.

Brumpt, E. (1925). Etude Sommaire de 1 "*Entamoeba dispar*" n. Sp. Amibe a kystes quadrinuclees, Parasite de L" homme. *Bulletin Academy of Medicine (*Paris), 94**:**943-952.

Caballero-Salcedo, A., Viveros-Rogel, M., Salvatierra, B., Tapia-Conyer, R., Sepulveda–Amor, J., Gutierrez, G. and Ortiz-Ortiz, L. (1994). Seroepidemiology of amebiasis in Mexico. *American Journal of Tropical Medicine and Hygiene,* 50: 412-419.

Cheng, H.S. and Wang, L.C. (1999). Amoebiasis among institutionalized psychiatric patients in Taiwan. *Epidemiology and Infection,* 122:317-322.

Choudhuri, G., Prakash, V., Kumar, A., Shahi, S.K. and Sharma, M. (1991). Protective immunity to *Entamoeba histolytica* infection in subjects with anti-amoebic antibodies residing in a hyperendemic zone. *Scandinavian Journal of Infectious Diseases,* 23:771-776.

Clark, C. G. and Roger, A. J. (1995). Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*. *Proceedings of the National Academy of Sciences of United States of America,* 92:6518-6521.

Clark, C. G., Espinosa, C.M and Bhattacharya, A. (2000). *Entamoeba histolytica*: an overview of the Biology of the Organism, In: Ravdin, J.I. (Ed), Amoebiasis. Imperial College Press, London; United Kingdom, pp 1-14.

Clark, C.G., Zaki, M. and Ali, I.K.M. (2002). Genetic diversity in *Entamoeba histolytica*. *Journal of Biosciences,* 2 (**6**)**:**603-607.

Cross, J.H. and Basaca-Sevilla, V. (1980). Seroepidemiology of amebiasis in the Philippines. *Philippine Journal of Microbiology and Infectious Diseases*, 9(**1**):21-26.

Diamond, L.S. and Clark, C.G. (1993). A redescription of *Entamoeba histolytica,* In: Schaudinn, 1903 (Emended Walker, 1911) Separating it from *Entamoeba dispar.* Brumpt, 1925. *Journal of Eukaryotic Microbiology,* 40:340-344.

Dodson, J. M., Clark, C. G., Lockhart, L.A., Leo, B. M, Schroeder, J. W. and Mann, B. J. (1997). Comparison of adherence, Cytotoxicity and Gal/Gal NAC lectin gene structure in *Entamoeba histolytica* and *Entamoeba dispar, Parasitological International,* 46: 225-235.

Escobedo, M.A., Homedes, N., Aldana, V., Alt, K.V., Serrano, B.N. and Garcia, R. (2003). Assessment of parasitic diseases in children in five communities in the border region of Far West Texas. Texas Department of Health Office of border health (TDH), pp 1-20.

Fuchs, G., Ruiz-Palacios, G. and Pickering, L.K (1988). Amebiasis in the pediatric population, In: J.I. Ravdin (Ed.), Amebiasis: Human infection by *Entamoeba histolytica.* John Wiley and sons, New York, N.V., pp 594-613.

Gathiram, V. and Jackson, T.F.H.G. (1987). A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica. South African Medical Journal*, 72**:**669-672.

Gatti, S., Swierczynski, G.I., Robinson, F., Anselmi, M., Corrales, J., Moreira, J, *et al.* (2002). Amebic Infection due to the *Entamoeba histolytica/ dispar* complex: a study of the incidence in a remote rural area of Ecuador. *American Journal of Tropical Medicine and Hygiene,* 67(**1**):123-127.

Gilchrist, C.A. and Petri, W.A.Jr. (1999). Virulence factors of *Entamoeba histolytica. Current Opinion in Microbiology,* 2:433-437.

Gonzalez-Ruiz, A., Haque, R., Aguirre, A., Castanon, G., Hall, A., Guhl, F. (1994). Value of Microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica. Journal of Clinical Pathology,* 47:236-239.

Gonin, P. and Trudel, L. (2003). Detection and differentiation of *E. histolytica* and *E.dispar* isolates in clinical samples by PCR and Enzyme-Linked Immunosorbent Assay. *Journal of Clinical Microbiology*, 41:237-241.

Gottlieb, B., Reyes, H., Trivino, X.,Vega, J., Arriagada, L. and Heernandez, J.(1983). Intestinal parasites in children at Kindergatens: Comparative study of socio-economic levels. *Review of Medical Chil*., 111(**10**) 1035-1038.

Guerrant, R.L., Brush, J., Ravdin, J.I., Sullivan, J.A and Mandell, G.L. (1981). Interaction between *Entamoeba histolytica* and human polymorphonuclear neutrophils. *Journal of infectious Diseases,* 143:83-93.

Haque, R., Neville, L.M., Hahn, P. and Petri, W. A. Jr. (1995). Rapid diagnosis of *Entamoeba* infection by using *Entamoeba histolytica* stool antigen detection kits. *Journal of Clinical Microbiology*, 33: 2558-2561.

Haque, R., Faruque, A.S. and Petri, W.A., Jr. (1997). *Entamoeba histolytica* and *Entamoeba dispar* infection in children in Bangladesh. *Archives of Medical Research,* 28:317-318.

Haque, R., Ali, I.K.M., Akther, S. and Petri, W.A.Jr. (1998). Comparison of PCR, isoenzyme analysis and antigen detection for diagnosis of *Entamoeba histolytica* infection. *Journal of Clinical Microbiology*, 36: 449-452.

Haque, R., Ali, I.K.M., Sack, R.B., Farr, B.M., Ramakrishnan, G. and Petri, W.A.Jr. (2001). Amebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *Journal of Infectious Diseases,* 183:1787-1793.

Haque, R., Mondal,D., Kirkpatrick, B. D., Akther, S., Farr, B. M., Sack, B. R. and Petri, W. A. Jr. (2003). Epidemiologic and clinical characteristics of acute diarrhoea with emphasis on *Entamoeba histolytica* infections in preschool children in an urban slum of Dhaka, Bangladesh. *American Journal of Tropical Medicine and Hygiene*, 69 (**4**):398-405.

Haque, R., Mondal, D., Duggal, P., Kabir, M., Roy, S., Farr, B.M. Sack, B. and Petri, W.A.Jr. (2006). *Entamoeba histolytica* infection in children and protection from subsequent amoebiasis. *Infection and Immunity*, 74 (**2**): 904-909.

Hooshyar, H., Rezaian, M., Mahmoodi, M., Parnia, S. and Mohammadi, S.S. (2004). A field study of the distribution of *Entamoeba histolytica/dispar* cyst passers in Northern, Central and Southern Iran. *Iranian Journal of Public Health,* 33 (**2**)**:**28-32.

Huber, M., Garfinkel, L., Gitler, C., Mirelman, D., Revel, M. and Rozenblatt, S. (1988). Nucleotide sequence analysis of an *Entamoeba histolytica* ferredoxin gene. *Molecular Biochemical Parasitolology,* 31:27-33.

Huston, C.D., Haque, R. and Petri, W.A.Jr. (1999). Molecular-based diagnosis of *Entamoeba histolytica* infection. *Expert Reviews in Molecular Medicine*. *http://www.expertreviews.org/99000599h.htm*.

Inabo, H., Galadima, M., Ogbadu, L.J. and Okuofu, C.A. (2000). Prevalence of *Entamoeba histolytica* and *Giardia lamblia* in primary school pupils in five rural villages around Kaduna and Zaria, Nigeria. *The Nigerian Journal of Parasitology*, 21**:**61-67.

Ivey, M. (1980). Phylum Protozoa. In: Sonnenwirth, A. C. and Jarrett, L, (ed.). Gradwohl's Clinical Laboratory Methods and Diagnosis. The C. V. Mosby Co., St Louis, Mo. pp 2081-2093.

Jackson, T. F., Sargeaunt, P. G., Visser, P. S., Gathiram, V., Suparsad, S. and Anderson, C. B. (1990). *Entamoeba histolytica*: naturally occurring infections in baboons. Archives of Investigative Medicine, 1: 153-156.

Kagan, I. G. (1970). Serologic diagnosis of parasitic disease. The *New England Journal of Medicine,* 282(**12**):685-686.

Katz, M., Despommier, D.D. and Gwadz, R. (1989). *Entamoeba histolytica,* In: Parasitic Diseases (2nd ED) Springer-Verlag. pp 136-143.

Kean, B.H. (1988). A history of Amebiasis, In: Ravdin, J.I. (ed) Amebiasis: human infection by *Entamoeba histolytica,* John Wiley and Sons, Inc., New York, N.Y. pp 1-10.

Kosek, M., Bern, C. and Guerrant, R.L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992-2000. *Bulletin of World Health Organisation,* 81:197-204.

Krogstad, D.J., Spencer, H.C.Jr., Healy, G.R., Gleason, N.N., Sexton, D.J. and Herron, C.A. (1978) Amebiasis: epidemiologic studies in the United States, 1971-1974. *Annals of Internal Medicine,* 88:89-97.

Krupp, T.M. (1970). Antibody response in intestinal and extra-intestinal amebiasis. *American Journal of Tropical Medicine and Hygiene,* 19:57-62.

Lauren, D. (2004): Distinguishing between Pathogenic and Non-Pathogenic Species of *Entamoeba. Science,* 35(**10**):613-615.

Lee, J., Park, G., Lee, D., Park, S. and Yong, T. (2000). Intestinal parasite infections at an institution for the handicapped in Korea. *The Korean Journal of Parasitology*, 38**:**179-181.

Legesse, M. And Erko, B. (2004). Prevalence of intestinal parasites among schoolchildren in a rural area close to the southeast of Lake Langano, Ethiopia. *Ethiopia Journal of Health and Development*, 18(**2**):116-120.

Levine, N. D., Corliss, J. O. and Cox F. E. G. (1980). A newly revised classification of the protozoa. *Journal of Protozoology*, 27:37-58.

Li, E. and Stanley, S.L. Jr. (1996). Protozoa; Amebiasis. In: Parasitic Disease of the Liver and Intestines. *Gastroenterology Clinics of North America,* 25:471-492.

Lowther, S.A., Dworkin, M.S. and Hanson, D.L. (2000). *Entamoeba histolytica/ Entamoeba dispar* infections in human immunodeficiency virus-infected patients in the United States. *Clinical Infectious Diseases,* 30:955-959.

Lucas, R. and Upcroft, J.A. (2001). Clinical significance of the redefinition of the agent of amoebiasis. *Revista Latinoamericana de Microbiologica,* 43(**4**):183-187.

Martinez-Palomo, A. and Espinosa – Cantellano, M. (1998). Intestinal amoebae. In: Cox FEG, Kreier, J.P. Wake line, D., (Eds.) Topley and Wilsons's Microbiology and Microbial Infections. (9th Ed), 5:157-177.

Mbaya, A.W. and Nwosu, C.O. (2005). Outbreak of clinical amoebiasis among captive chimpanzees (*Pan troglodytes*) at the Sanda kyarimi Park, Maiduguri, Nigeria. *Nigerian Veterinary Journal,* 1:68-73.

Mc Coy, J.J., Mann, B.J. and Petri, W.A. Jr. (1994). Adherence and Cytotoxicity of *Entamoeba histolytica,* or how lectins let parasites stick around. *Infection and Immunity,* 62:3045-3050.

Mehmet, T. and Petri, W.A. Jr. (2003). Laboratory diagnosis of Amebiasis. *Clinical Microbiology Reviews*, 16(4): 713-729.

Mirelman, D., Nuchamowitz, V. and Stolarky, T. (1997). Comparison of use of Enzyme-Linked Immunosorbent Assay-based kits and PCR amplification of rRNA genes for simultaneous detection *of E. histolytica and E.dispar. Journal of clinical Microbiology,* 35: 2405- 2407.

Mojarad, E.N., Haghighi, A., Rad, M.A., Mesgarian, F., Nejad, M.R. and Zal, M.R. (2006). Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* in Gonbad city, Iran. *Iranian Journal of Parasitology,* 2(2):48-52.

Monga, N.K., Sood, S., Kaushik, S.P., et al (1976). Amebic Peritonitis. *American Journal of Gastroenterology,* 66:366-373.

Mortimore, M. J. (1970). Zaria and its regions. *Annals of the Association of America Geographers*, 60(**1**):73-80.

Murray, C.J.L and Lopez, A.D. (1997). Alternative projections of mortality and disability by cause 1990-2020: Global burden of disease study. *Lancet*, 349: 1498-1504.

National Committee for clinical laboratory (NCCLS). Procedures for the recovery and identification of parasites from the intestinal tract. Approved guideline. Pennsylvania 1997.

Ogunlesi, T.A., Okeniyi, J.A.O., Oyedeji, O.A., Oseni, S.B.A., Oyelami, O.A., Njokanma, O.F (2005). Childhood dysentery in Ilesa, Nigeria. The unusual role of *Entamoeba histolytica*. *The Internet Journal of Tropical Medicine,* 2**(2**) 1-4.

Okodua, M., Adeyeba, O. A., Jaffeng, Y.M. and Okpala, H.O. (2003). Age and Sex distribution of intestinal parasitic infection among HIV infected subjects in Abeokuta, Nigeria. *Online Journal of Health and Allied Society,* 2 (**4**). *http://www.ojhas.org/issue8/2003-4-3.htm*.

Ozlem, O. and Hakan, S. (2005). The contamination of various fruits and vegetables with *Enterobius vermicularis*, *Ascaris* eggs, *E. histolytica* cysts and *Giadia* cysts. *Science Direct*, 16(**6**):557-560.

Panosian, C.B. (1988). Parasitic diarrhea. *Infectious Disease in Clinics of North America,* 2:685-703.

Pehrson, P.O. and Bengtsson, E. (1984). A long-term follow up study of amebiasis treated with metronidazole. *Scandinavian Journal of Infectious Diseases,* 16**:**195-198.

Peters, C.S., Sable R., Janda, W.M., Chitton, A.L. and Kocka, F.E. (1986). Prevalence of enteric parasites in homosexual patients attending an outpatient clinic. *Journal of Clinical Microbiology,* 24:684-685.

Petri, W. A. Jr., Chapman, M. D., Snodgrass, T., Mann, B. J. B. And Ravdin, J. I. (1989). Subunit structure of the galactose and N-acetyl-D-galactosamine-inhibitable adherence lectin of *E. histolytica*. *Journal of Biological Chemistry*, 264:3007-3012.

Petri, W.A.Jr., Snodgrass, T.L., Jackson, T.F., Gathiram, V., Simjee, A.E., Chadee, K. and Chapman, M.D. (1990). Monoclonal antibodies directed against the galactose-binding lectin of *Entamoeba histolytica* enhance adherence. *Journal of Immunology*, 144: 4803-4809.

Petri, W.A.Jr. and singh, U. (1999). Diagnosis and management of amoebiasis. *Clinical Infectious Diseases*, 29:1117-1125.

Petri, W.A.Jr., Haque, R., Lyerly, D. and Vines, R.R. (2002) Estimating the Impact of amebiasis on health. *Parasitology Today*, 16:320-321.

Petri., W.A.Jr. and Singh, U. (2006). Enteric Amoebiasis, In: Guerrant, R.L., Walker, D.H., Weller, P.F., eds. Tropical Infections diseases. Principles, pathogens and practice. 2nd Ed. Philadelphia, P.A: Elesier Churchill Livingstone, pp 967-983.

Philips, S.C., Mildvan, D., William, D.C., Gelb, A.M. and White, M.C. (1981). Sexual Transmission of enteric protozoa and helminths in a veneral–disease-clinic population. *New England Journal of Medicine,* 305:603- 606.

Pillai, D. R., Britten, D., Ackers, J. P., Ravdin, J. I. and Kain, K. C. (1997). A gene homologous to hg12 of *Entamoeba histolytica* is present and expressed in *Entamoeba dispar*. *Molecular Biochemical Parasitology*, 87: 101-105.

Pillai, D.R., Keystone. J.S., Sheppard, S.C., Maclean, J.D., Macpherson, D.W. and kain.C.(1999). *Entamoeba histolytica* and *Entamoeba dispar* epidemiology and comparison of diagnostic methods in a setting of none endemicity. *Clinical Infectious Diseases*, 29:1315-1318.

Pillai, R. and Kain, K.C. (2000). Recent developments in amoebiasis: the Gal/Gal NAc lectins of *Entamoeb*a *histolytica* and *Entamoeba dispar. Microbes Infection,* 2:1775-1783.

Quinn, T.C., Corey, L., Chaffee, R.G., Schuffler, M.D. Brancata, F.P. and Holmes, K.K. (1981). The etiology of anorectal infections in homosexual men. *American Journal of Medicine,* 71:395-406.

Ravdin, J.I. and Guerrant, R.L. (1981). Role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*. Study with mammalian tissue culture cells and human erythrocytes. *Journal of Clinical Investigations*, 68:1305-1313.

Ravdin, J.I. (2002). *Entamoeba histolytica* (Amebiasis), In: Mandell: *Principles and Practice of Infectious Diseases*. 5th ed. Philadelphia Living Stone, pp 2798-2807.

Ravdin, J.I and Stauffer, W.M. (2005). *Entamoeba histolytica* (Amoebiasis). In: Mandel, G.L., Benneth, J.E., Dolin, E. eds. Mandell, Douglas and Bennett's. *Principles and Practice of Infectious Diseases.* 6th ed. Philadelphia, P.A.: Churchill, Livingstone, pp. 3097-3111.

Redondo, R.B., Mendez, L.G.M. and Baer, G. (2006). *Entamoeba histolytica* and *Entamoeba dispar.* Differentiation by Enzyme-Linked Immunosorbent Assay (ELISA) and its Clinical correlation in pediatric patients. *Parasitologia Latinoamericana,* 61:37-42.

Reed, S. L. (2000). Clinical manifestations and diagnosis in: J. I. (ed.), Amebiasis. Imperial College Press, London, United Kingdom, pp 113-126.

Reeves, S.E. (1984). Metabolism of *Entamoeba histolytica.* Schaudinn 1903. *Advanced Parasitology*, 23:105-142.

Rezaian, M. (2000). Amoebiasis, in: The epidemiology of Common Diseases in Iran. Ed, F. Azizi Eshtiagh press, 2nd ed, Tehran, pp 377-386.

Rinne, S., Rodas, E.J., Galer-Unti, R., Glickman, N., Glickman, L.T. (2005). Prevalence and risk factor for protozoan and nematode infections among children in an Ecuadorian highland community. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 99**:**585-592.

Roger, A.J. (1995). Direct evidence for Secondary loss of Mitochondria in *Entamoeba histolytica*. *Proceedings of the National Academy of Sciences*, U.S.A., 92: 6518-6521.

Rogers, L. (1912). The rapid cure of amoebic dysentery and hepatitis by hypodermic injection of soluble salts of emetine. *British Medical Journal*, 1**:**1424.

Roy, S., Kabir, M., Mondal, D., Ali, I.K.M., Petri, W.A.Jr. And Haque, R. (2005). Real-Time-PCR Assay for diagnosis of Entamoeba histolytica infection. *Journal of Clinical Microbiology*, 43**(5)**:2168-2172.

Saeed, A.A. and Manal, B.J. (2007). Diagnosis and differentiation of *Entamoeba* infection in Makkah AlMukarramah using microscopy and stool antigen detection kits. *World Journal of Medical Science*, 2(**1**)**:**15-20.

Saklatvala, T. (1993). Milestones in parasitology. *Parasitology Today*, 9:347-348.

Salas, S. D., Heifetz, R. and Barret-Connor, E. (1990). Intestinal parasites in Central American Immigrants in the United States. *Archives of International Medicine*, 150(**7**):1514-1516.

Salazar, N.P., Pasay, C.J., Avenido, A.O., Macapasir, S.R., Lena, M.J., Maguinsay, V.M., Angus, P.R. and Bandalan. J.M. (1990). Detection of *Entamoeba histolytica* in routine stool examination. *Philippine Journal of Microbiology and Infectious Diseases,* 19**(23)**:57-60.

Sanuki, J., Asai, T., Okuzawa, E., Kobayashi, S. and Takeuchi, T. (1997). Identification of *Entamoeba histolytica and E. dispar* cysts in stool by Polymerase Chain Reaction. *Parasitology Resolution,* 83:96-98.

Sargeaunt, P.G., Williams. J.E. and Grene, J.D. (1978). The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 72: 519-521.

Sargeaunt, P.G., Williams. J.E. (1979). Electrophoretic isoenzyme, patterns of the pathogenic and non-pathogenic intestinal amoebae of man. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73: 225-227.

Seeto, R. K. (1999). Amebic Liver abscess: epidemiology, Clinic features and outcome. *Western Journal of Medicine,* 170:104-109.

Shetty, N., Das, P., Pal, S.C and Prabhu, T. (1988). Observations on the interpretation of amoebic serology in endemic areas. *Journal of Tropical Medicine and Hygiene,* 91: 222-227.

Shetty, N. Narasimha, M., Elliott, E., Raj, I.S. and Macaden, R. (1992). Age-specific sero-prevalence of amoebiasis and Giardiasis in southern Indian Infants and children. *Journal of Tropical Paediatrics,* 38:57-63.

Sirima, K., Siriphan, B. And Suphatra, W. (2008). Intestinal parasitic infections in Srimum Suburban area of Nakhon Ratchasima Province, Thailand. *Tropical Biomedicine*, 25(**3**):237-242.

Stanley, L.S.Jr. (2003). Amoebiasis. *Lancet*, 361:1025-1034.

Stauffer, W., Abd-Alla, M. and Ravdin, J.I. (2006). Prevalence and incidence of *Entamoeba histolytica* infection in South Africa and Egypt. *Archives of Medical Research,* 37:266-269.

Stilwell, G.G. (1955). Amebiasis: its early history. *Gastroenterology*, 28: 606.

Strachan, W. D., Chiodini, P.L., Spice, W.M., Moody, A.H. and Ackers, J.P. (1988). Immunological differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica. Lancet*, 1**:**561-563.

Troll, H., Marti, H. and Weiss, N. (1997). Simple differential detection of *Entamoeba histolytica* and *Entamoeba dispar* in fresh stool specimens by sodium acetate-acetic acid-formalin concentration and PCR. *Journal of clinicial Microbiology,* 35:1701-1705.

Tannich, E., Horstmann, R. D., Knobloch, J. And Arnold, H. H. (1989. Genomic DNA differences between pathogenic and non-pathogenic *entamoeba histolytica. Proceedings of the National Academy of Science in United States of America,* 86:5118-5122.

UNICEF (1998). State of the World's Children.

Verweij, J.J., Blotkamp, J., Brienen, E.A.T., Aguirre, A. and Polderman, A.M. (2000). Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* cysts using Polymerase Chain Reaction on DNA isolated from faeces with spin columns. *European Journal of Clinical Microbiology and Infectios Diseases*, 19:358-361.

Walker, E.L. and Sellards, A.W. (1913). Experimental entamoebic dysentery. *Philipine Journal of Science and Tropical Medicine,* 8**:**253.

Walsh, J.A (1986). Problems in recognition and diagnosis of Amoebiasis: Estimation of the global magnitude of morbidity and mortality. *Review of Infectious Diseases*, 8:228-238.

Walsh, J.A. (1988). Transmission of *Entamoeba histolytica* infection, In: Ravdin, J.I. (ed.), amebiasis: human infection by *Entamoeba histolytica.* John Wiley and Sons, Inc., New York, N.Y. pp.106-119.

Wanke, C., Butler, T. and Islam, M. (1988). Epidemiological and clinical features of invasive amebiasis in Bangladesh: a case-control comparison with other diarrheal disease and post mortem findings. *American Journal of Tropical medicine and Hygiene*, 38:335-341.

Werries, E., Nebinger, P. and Franz, A. (1983). Degradation of biogenic Oligosaccharides by beta-N-acety glucosaminidase secreted by *Entamoeba histolytica. Molecular Biochemical Parasitology,* 7**:**127-40.

World Health Organisation (1995). Division of diarrhoeal and acute respiratory disease control. Integrated management of the sick child. *Bulletin of world Health Organisation,* 73:735-740.

World Health Organization (1997): Amoebiasis. *WHO Weekly Epidemiological Records*, 72:97-100.

Yadava, N., Chandok, M. R., Prasad, J., Bhattacharya, S., Sopory, S. K. and Bhattacharya, A. (1997). Characterization of EhCaBP, a calcium binding protein of *Entamoeba histolytica* and its binding proteins. *Molecular Biochemical Parasitology*, 84: 69-82.

Yakubu, A.M. and Sathiakumar, N. (1985). The etiology of chronic childhood diarrhoea among Nigerian children. *Journal of Diarrhoeal Diseases Research*, 3(**3**):145-148.

Yilmaz, H., Akman, N. and Goz, Y. (1999). Distribution of intestinal parasites in two societies with different socio-economic status in Van. *Eastern Journal of Medicine*, 4(**1**):16-19.

Zaman, S., Khoo, J., Ng, S.W., Ahmed, R., Khan, M.A., Hussain, R. and Zaman, V. (2000). Direct amplification of *Entamoeba histolytica* DNA from amoebic liver abscess pus using polymerase chain reaction. *Parasitology research*, 86:724-728.

Appendix 1- The Questionnaire Sample

Department of Biological Sciences,
Faculty of Science,
Ahmadu Bello University,
Zaria.

Dear Sir/Madam.

QUESTIONNAIRE

I am a postgraduate student of Ahmadu Bello University, Zaria. This is a Postgraduate research on the topic: Prevalence of Amoebiasis in Preschool Children in Zaria, Kaduna State, Nigeria. Kindly fill the Questionnaire as honest as you can. Every information you give will be treated confidentially and used for the purpose of this research work only. Thank you for your co-operation.
Agbike Happiness I.

1. Age of the child----------
2. Sex of the child----------
3. Occupation of the Parent/Guardian----------
4. Source of drinking water (a) Tap( ) (b) Well( ) (c) Steam( ) (d) Borehole()
5. Type of toilet (a) Water closet( ) (b) Pit latrine( ) (c) Bush( )
6. Does the child suck finger? Yes( ) No( )
7. Type of food eaten by the child before onset of----------
8. Residential address----------

Appendix 2a- Readings from the EIA machine. Plate IA

ABSORBANCE MODE

WAVELENGTHS - 450NM and 630NM

READ MODE: A to H

	A	B	C	D	E	F	G	H
1 – 1	0.099	2.087	0.074	0.073	0.065	0.073	0.070	**0.087**
1 – 2	0.057	0.071	0.060	0.064	0.070	0.070	0.069	0.065

Where A; 1 -1= Blank

B; 1 – 1= Positive control

C; 1 – 1= negative control

Bold = Positive value

Ordinary = Negative values

Appendix 2b- Readings from the EIA machine. Plate IB

ABSORBANCE MODE

WAVELENGTHS - 450NM and 630NM

READ MODE: A to H

	A	B	C	D	E	F	G	H
1 – 1	0.060	3.150	0.090	0.061	0.075	0.045	0.068	0.071
1 – 2	0.064	0.069	0.057	0.081	0.051	0.049	0.050	0.055
1 – 3	0.056	0.066	0.059	0.059	0.090	0.085	0.074	0.071
1 – 4	0.059	0.064	0.062	0.076	0.085	0.071	0.063	0.063
1 – 5	0.070	0.053	0.065	0.068	0.076	0.076	0.88	0.053
1 – 6	0.045	0.055	0.067	0.054	0.042	0.064	0.046	0.068
1 – 7	0.078	0.065	0.084	0.061	0.076	**0.093**	0.073	0.069
1 – 8	**0.092**	0.071	**0.098**	0.062	**0.107**	**0.117**	**0.098**	0.080
1 – 9	0.061	0.068	0.064	0.064	0.086	0.070	0.080	0.073
1 – 10	0.068	0.065	**0.095**	**0.095**	**0.117**	**0.091**	**0.114**	0.087

Where A; 1 -1= Blank

B; 1 – 1= Positive control

C; 1 – 1= negative control

Bold = Positive values

Ordinary = Negative values

Appendix 2c- Readings from the EIA machine. Plate II

ABSORBANCE MODE

WAVELENGTHS - 450NM and 630NM

READ MODE: A to H

	A	B	C	D	E	F	G	H
1 – 1	0.072	3.172	0.117	**0.128**	**0.147**	O.104	0.089	0.105
1 – 2	**0.133**	**0.132**	0.108	**0.134**	**0.133**	**0.166**	**0.144**	0.091
1 – 3	**0.145**	0.078	**0.150**	**0.130**	**0.155**	**0.131**	**0.126**	0.115
1 – 4	**0.172**	0.067	**0.131**	**0.152**	**0.145**	0.117	0.110	**0.142**
1 – 5	**0.148**	0.117	**0.121**	**0.134**	**0.127**	**0.143**	**0.147**	**0.125**
1 – 6	**0.136**	0.111	**0.146**	**0.120**	**0.133**	**0.120**	0.111	**0.121**
1 – 7	**0.131**	**0.127**	**0.132**	**0.147**	0.112	0.114	0.116	**0.135**
1 – 8	**0.148**	0.114	**0.142**	0.111	**0.126**	**0.128**	**0.122**	0.111
1 – 9	**0.150**	**0.159**	**0.121**	**0.120**	**0.138**	**0.118**	**0.118**	0.107
1 – 10	**0.146**	**0.128**	0.101	**0.125**	0.111	**0.124**	0.106	0.102
1 – 11	**0.134**	**0.145**	**0.140**	**0.140**	**0.137**	**0.125**	0.100	**0.145**
1 – 12	**0.129**	**0.144**	0.114	**0.135**	**0.132**	**0.128**	**0.145**	0.113

Where A; 1 -1= Blank

B; 1 – 1= Positive control

C; 1 – 1= negative control

Bold = Positive values

Ordinary = Negative values

Appendix 3-Ethical Approval

Appendix 4: Prevalence of *Entamoeba histolytica* infection in children living in Zaria city and its environs

Location	Number Examined (%)	Number positive(+)	Prevalence (%)	χ^2	P- value
Zaria city	115 (62.84)	49	42.61	24.48	0.01
Banzazau	32 (17.49)	6	18.75		
Tudun wada	16 (8.74)	9	56.25		
Sabongari	6 (3.28)	4	66.67		
Gyellesu	3 (1.64)	0	0.0		
Gaskiya	6 (3.28)	6	100.0		
Wusasa	2 (1.09)	1	50.0		
Samaru	3 (1.64)	3	100.0		
Total	**183**	**78**	**42.62**		