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THE PREVALENCE OF INTESTINAL PARASITIC INFECTION AND THEIR ASSOCIATION WITH THE T-CELL $CD4^+$ COUNTS OF HIV/AIDS INFECTED PATIENTS IN KUMASI

BY

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Declaration

I hereby declare that, this thesis is my original work towards the MPhil (Clinical Microbiology) degree and that, no aspect of this work has been previously published or presented by any other person in any university to the best of my knowledge, except in places where references have been duly cited in the text.

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Abstract

Intestinal parasitic co-infections with HIV, if left untreated appear to speed up the progression of the HIV infection. However, to date, there have been very few studies conducted in quite resource limited settings to determine the interaction of intestinal parasitic co-infection with HIV/AIDS, especially in places where HIV/AIDS management largely depends on $CD4^+$ T-lymphocyte cell counts and WHO clinical staging. The study evaluated the prevalence of intestinal parasite infection in HIV/AIDS infected individuals and its effect on the immune status, using their T-cell $CD4^+$ count as a parameter. Stool and blood samples were collected from 855 HIV infected patients and 100 HIV negative individuals, between January, 2012 and February, 2013. Each stool sample was preserved in Sodium acetate, Acetic acid and Formalin solution (SAF) within 30 minutes of collection. This was later analysed at the Kumasi Centre for Collaborative Research into tropical medicine (KCCR) laboratory, using the Formol ethyl acetate concentration technique and Polymerase Chain Reaction (PCR) in accordance with the standard protocol.

There was a 20.3% prevalence of intestinal parasites among the study individuals. *Endolimax nana* recorded the highest prevalence of 6.6%, followed by *Entamoeba coli* with 4.9% and *Balantidium coli* recorded the lowest prevalence of 0.1% for the formol-ethyl aecetate concentration technique. The PCR technique focused on *Entamoeba dispar, Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium parvum.* Of the four parasites *Entamoeba dispar* recorded the highest prevalence of 10.4% and *E. histolytica,* recorded the lowest prevalence of 1.6%. Interestingly, for the intestinal helminths, only hookworm was found with a prevalence of 0.2%. There was no significant relation between the CD4 T-cell counts of the study individuals and the intestinal parasites detected, except for *C. parvum*, which was recorded in individuals with T-cell $CD4^+$ counts, below 200 cells/mm^3.

CHAPTER ONE

1.0 Introduction

1.1 Background to the study

Intestinal parasitic infections are among the most prevalent and widespread of chronic human infections worldwide, causing considerable medical and public health problems (Brooker *et al*., 2004). Estimates show that at least more than quarter of the world's population is chronically infected with intestinal parasites, and the majority of these people live in the developing countries (Fincham *et al.*, 2003). It is known that lack of access to potable water, illiteracy, poverty, poor personal and environmental hygiene and a hot and humid tropical climate are some of the factors that enhance intestinal parasite infections (Bethony *et al.*, 2006). Intestinal parasites still remain a major cause of morbidity and mortality in developing countries, reaching up to a rate of 95% in some countries (Stephenson *et al.*, 2000).

A wide variety of intestinal parasites are prevalent in different parts of the world. These include *Ascaris lumbricodes, Entamoeba histolytica/dispar, Cyclospora cayatanensis, Giardia lamblia, Cryptosporidium parvum, Hymenolepis nana,* and hookworm (Stepek *et al*., 2006). *Enterobius vermicularis* is more prevalent in temperate areas (Vermud and Wilson, 2000) and *Ascaris lumbricoides* is more common in tropical regions (Stepek *et al.*, 2006). The highest rates of Ascaris infection have been reported in China, Southeast Asia and coastal regions of West and Central Africa (Lindo *et al*., 1998). Hookworm infections are most common in sub-Saharan Africa, South China, and Southeast Asia (Kaplan *et al*., 1996). In industrialized countries the prevalence of intestinal parasites such as *Giardia* ranges from 2%-5% (Wiwanitkit, 2001). Global infections reported for some of the common intestinal parasites are *Ascaris* (20%), hookworm (18%), *Trichuris trichura* (10%) and *Entamoeba histolytica* (10%) (Absar *et al.*, 2010). In Ghana, estimates show that majority of the

population are infected with either pathogenic or non-pathogenic strains of intestinal parasites (Ayeh-kumi *et al.,* 2009).

Human Immunodeficiency virus (HIV) infection is still a global health issue with over 33.3 million people infected and over 2 million people dying from it each year (UNAIDS and WHO, 2007). Sub-Saharan Africa remains by far the worst affected region, harboring more than two thirds of the worldwide HIV/AIDS infection (UNAIDS and WHO, 2007). Dramatic expansion of HIV/AIDS pandemic has brought about a significant change in the fauna of intestinal parasites all over the world (National AIDS/STD Control Programme, 2001). An increasing number of people in developing countries especially in Africa are immune compromised due to HIV/AIDS infection (Lucas, 1990; Suryawanshi, 2012). As a result, intestinal parasitic infections are among the major public health issues in HIIV/AIDS patients due to their compromised immunity (Goodgame, 1996; Kaplan *et al.*, 1996).

The rate of intestinal parasitic infection is remarkably high in sub-Saharan Africa, where the majority of HIV/AIDS cases are concentrated (WHO, 2002). Individuals with HIV/AIDS are mostly threatened by a number of diseases including others caused by different kinds of biological agents (Nwachukwu and Okebe, 2008). The progressive decline and ultimate collapse of immune system functions, which are characteristic for HIV/AIDS infection, usually result in morbidity and death due to opportunistic bacterial, viral, and parasitic infections (Ramakrishnan *et al.,* 2007). Gastrointestinal parasites and HIV/AIDS infection have major effects on the host's immune response and co-infection is widespread (Bundy *et al.,* 2000). With the progressive development of HIV/AIDS, once the patient's T-cell $CD4^+$ counts have fallen below 200 cells/mm^3, patients are likely to be co-infected with bacteria, parasites, or viruses. Such co-infections generally are the main cause of death in HIV/AIDS patients (Morris *et al.,* 2004; Nielsen *et al.,* 2007). Several parasites that are frequently

encountered in persons living with HIV/AIDS include hookworm, *Isospora belli, G. lamblia, Microsporidia spp., T. trichiura, E. histolytica, A. lumbricoides, Strongyloides stercoralis and Cryptosporidium spp.* (Okodua *et al.,* 2003).

Another group of parasites that is known to cause morbidity in HIV/AIDS patients worldwide is the opportunistic intestinal parasites (Wolday *et al*., 2002). These include the Coccidians, *Cryptosporidium parvum, Cyclospora cayetanensis, Enterocytozoon bieneusi, Encephalitozoon intestinalis and Isospora belli* (Weiss and Keohane, 1997). These infections usually occur late in the course of HIV/AIDS infection when T-cell $CD4^+$ counts have been severely depleted mostly below 200 cells/mm^3 and in case of intestinal Microsporidia below 100 cells/mm^3 (Ramakrishnan *et al*., 2007).

Some studies have indicated that compared to the general population, there is relatively lower prevalence of non-opportunistic extracellular intestinal parasites in HIV/AIDS patients (Lindo and Lee 2001). Although differences in exposure may not be ruled out, it is suggested that HIV/AIDS induced entropathy may not create conducive environment for the establishment of extracellular intestinal parasites (Wiwanitkit, 2001).

Clinical manifestations of HIV/AIDS mostly result from the reactivation of pre-existing latent pathogens as the individuals become immune suppressed (Wiwanitkit, 2001). They may also be caused by exposure to locally predominant pathogens. Hence, clinical presentations of HIV/AIDS and the pathogens responsible in different geographical areas may reflect the difference in prevalence of intestinal parasitic infections in a given community (Lindo *et al.,* 1998).

The advent of newly improved diagnostic techniques such as ELISA and PCR to identify intestinal parasites has enhanced their detection and recognition both in immune suppressed and immune competent individuals (Grossman *et al*., 2002).

1.2 Rationale of the study

Intestinal parasites, either backed by HIV/AIDS or independent, have continued to be a major cause of morbidity and mortality in HIV/AIDS infected individuals worldwide (Habtamu and Kloos, 2006). Intestinal parasitic infections are the most serious among all the superimposed infections in HIV/AIDS patients and they claim a number of lives every year (Okodua *et al.*, 2003). Therefore effective detection and treatment are important components that reduce disease complications and prolong the life span of these individuals (Harinda, 2008). Parasitic infections particularly helminths sometimes cause chronic immune activation (Borkow and Bentwich, 2004; Grossman *et al.*, 2002). Though evidence for such effect is somehow inadequate, such immune modulation is shown to increase host susceptibility; thereby promoting HIV/AIDS infection and disease progression (Kalinkovich *et al.*, 2001).

The HIV/AIDS pandemic has in a way also altered the fauna of intestinal parasite infection. This is because the eggs and cysts of most parasites are passed out in the stools of most affected individuals (Kalinkovich *et al.*, 1998). These can easily contaminate food and drinking water sources, thereby being transmitted from one individual to another in a community (Djauzi, 2006).

Ghana is among the sub-Saharan African countries with overlapping rate of HIV/AIDS and parasitic infections (Disease control unit, Ministry of health, 2001). However, very little have been done in this area to ascertain whether HIV/AIDS populations in the country are independent of growing intestinal parasites infection (Gyasi, *et al.*, 2000).

In a study conducted in Ethiopia it was established that there was a significant relationship between the number of excreted worm eggs and plasma HIV viral load (Wolday *et al.*, 2002). It also showed a significant reduction of plasma HIV viral load in individuals from whom helminth infections were eradicated, as compared to those in whom helminth infections

persisted. (Wolday *et al.,* 2002). These findings indicated that helminth infections may enhance HIV/AIDS multiplication and increase plasma viral load, thereby contributing to HIV/AIDS disease progression (Wolday *et al*., 2002). Other studies have also shown that intestinal parasitic infections have interactions with immunological effectors such as T-cell subsets ($CD4^+$ and $CD8^+$) (Kalinkovich *et al.,* 1998). In another study conducted in the same country it was established that immunodeficiency in HIV/AIDS patients increased the risk of intestinal parasite infection (Shimelis *et al*., 2009). Patients having T-cell $CD4^+$ counts less than 200 cells/mm^3 had the highest rate of infection of about six fold higher compared with those with T-cell $CD4^+$ counts more than 500 cells/mm^3 (Shimelis *et al*., 2009). Between 30%-60% of HIV/AIDS infected individuals suffer from chronic infectious diarrhea; out of this about 80% are as a result of intestinal parasite infection, and this can result in significant morbidity and mortality (Cotte *et al.,* 1993; Djauzi, 2006). Opportunistic parasites such as *Cryptosporidium parvum.* and Microsporidia can spread to various organs, including the bronchia, bile and liver ducts, producing symptoms specific to the affected organs (McGowan *et al.,* 1993). This study therefore was intended to provide a reflection of the situation in Kumasi, Ghana.

1.3 Aims and Objectives

1.3.1 Aim

The main aim of the study was to determine the prevalence of intestinal parasite infection and their association with the T-cell $CD4^+$ counts of HIV/AIDS infected patients attending the Komfo Anokye Teaching Hospital in Kumasi.

1.3.2 Objectives

The specific objectives of the study were to:

- Identify the various kinds of intestinal parasites present in HIV/AIDS patients.
- Assess the relation between intestinal parasite infections and the T-cell $CD4^+$ count of HIV/AIDS positive individuals.
- Determine the association between opportunistic intestinal parasites and the level of progression of HIV in an individual.
- Compare the sensitivity and specificity of standard microscopy and polymerase chain reaction (PCR).

CHAPTER TWO

2.0 Literature Review

2.1 Intestinal Parasite Infection

A parasite is an organism that lives on or in another organism, usually larger known as the host and derives nutrients and causes harm to that organism (Nishimura and Hung, 1997). The term infection is used mostly in association with endoparasites (Capello *et al*., 1995). Endoparasites are parasites that live in the body of an organism (Capello *et al*., 1995).

Intestinal Parasite Infections are infections found in the intestinal tract of the host organism (Nwachukwu and Okebe, 2008). Intestinal parasitic infections have a worldwide distribution with high prevalence in people with poor living conditions, poor environmental sanitation, low socio-economic status, improper garbage disposal and unsafe water supply (Yanoviak, *et al.*, 2008). There are two classes of intestinal parasites; intestinal protozoans and intestinal helminths (Getz, 2011).

2.2 Intestinal Protozoan Infection

Protozoans are free-living, unicellular eukaryotic organisms (Ryan and Ray, 2004). They vary in sizes from 5 µm to 2 ml and have an inner layer of cytoplasm known as endoplasm and an outer layer of ectoplasm (Valerio *et al*., 2002). Most protozoans reproduce sexually by meiosis and asexually by binary fission (Winstead, 2001). There are two diagnostic life forms of protozoans, the motile active feeding trophozoites and the non-motile infective form known as the cyst (Valerio *et al*., 2002). Protozoans mostly form cysts when exposed to harsh conditions like drought (Valerio *et al*., 2002). There are various classes of intestinal protozoans; intestinal flagellates, intestinal amoeba and the coccidians (Nappi and Vass, 2002).

2.3 Intestinal Flagellates (*Giardia lamblia*)

2.3.1 Epidemiology

Giardia spp. is one of the most common intestinal protozoans with worldwide coverage, found mostly in warm climates and the common intestinal protozoan found in the United States of America (USA) (Juranek, 1995). An estimated 200 million people are infected with *Giardia lamblia* each year (Swarbrick, *et al*., 1997). It is more prevalent in developing countries with poor sanitation and places lacking clean drinking water (Hill *et al*., 2006). In all regions the highest prevalence is found among children, with as high as 10%-30% prevalence (Hill *et al*., 2006). A prevalence of 43.7% is reported for *Giardia lamblia* infection in Senegal, Zimbabwe recorded a prevalence of 19.4% and 19.7% in Egypt (Magambo *et al*., 1998). The cysts of *Giardia lamblia* are found in water surfaces worldwide even those of high quality and have been found in surface waters from the Artic to the tropics (U.S. EPA, 1998).

2.3.2 Morphology

Giardia lamblia is a flagellated tear-shaped protozoan which causes giardiasis in the small intestine (Dib *et al*., 2008). The parasite has two life forms, a motile active feeding trophozoite and the non-motile infective cyst (Dib *et al*., 2008). It belongs to the class Zoomastigophorea, the order Diplomonadida and the family Hexamitidae (Hill *et al*., 2006).

The trophozoites of *Giardia lamblia* are flattened and pear shaped measuring 15 μm long, 9 μm wide and 3 μm thick and bilaterally symmetrical (Hill *et al*., 2006). At the anterior side, are four pairs of flagella which aid in movement and a ventral disc which aid in attachment to the intestinal epithelial cells of the host (Dib *et al*., 2008). It has two nuclei and two slender median

rods (Robertson *et al*., 2010). Using their four pairs of flagella for locomotion, they attach themselves to the mucosa of the duodenum by their disc-like suckers (Dib *et al*., 2008).

The cysts of *Giardia lamblia* is egg-shaped measuring 8-14 µm by 7-10 µm with very thick walls which aid in survival outside of the host organism (Swarbrick *et al*., 1997). The cyst can survive outside of the body for several weeks under favourable conditions and is the infective life form of the organism (Swarbrick *et al*., 1997). It has four nuclei which are not so visible and also remains of the axonemes, the cyst may appear to shrink from the cell wall (Mitchell *et al.*, 2008). The cysts are mostly found in stool samples (Robertson *et al*., 2010).

2.3.3 Life Cycle

The cysts are responsible for the transmission of Giardiasis (Mitchell *et al.*, 2008). When the cysts are ingested either by faecal-oral route, through contaminated water or food, it passes through the duodenum and gets into the small intestines (Swarbrick *et al*., 1997). In the small intestines, it encysts releasing the trophozoites, which begins to move and feed (Mitchell *et al*., 2008). After feeding, they multiply by binary fission in the lumen (Mitchell *et al*., 2008). Cyst formation is triggered by the dehydration of the content of the gut as it moves through the large intestine (Robertson *et al*., 2010). The resulting trophozoites and cysts are egested through the faeces (Mitchell *et al*., 2008).

2.3.4 Pathology

The parasite causes the disease, giardiasis (Godfray and Charles, 2004). Infection with *Giardia lamblia* is mostly asymptomatic, however in severe infection with the parasite, the trophozoites are mostly found in the stool of the host (Curtale *et al*., 1998). The trophozoite is the major cause

of diarrhoea; these trophozoites do not survive in the environment (Curtale *et al*., 1998). Diarrhoea is as a result of malabsorption and hypersecretion (Musher and Musher, 2004). The small intestine is the site for major abnormalities associated with giardiasis (Curtale *et al*., 1998). Asymptomatic infection is as a result of continued transmission of the parasite cyst (Musher and Musher, 2004).

2.3.5 Clinical Manifestations of the disease

Infection with *Giardia lamblia* is mostly asymptomatic (Curtale *et al*., 1998). However, in acute infection with the parasite, one may experience; diarrhoea, greasy stools that tend to float, abdominal disorders, flatulence and dehydration (Robertson *et al*., 2010). Giardiasis can also cause weight loss and malabsorption of fats and lactose (Robertson *et al*., 2010). In children, severe infection might retard the physical and mental growth (Gardener and Hill, 2001).

2.3.6 Laboratory Diagnosis

Trophozoites can be found in fresh diarrhoea stool by the examination of wet saline preparations (Gardener and Hill, 2001). Giardiasis stool ranges from profuse and watery to greasy and foul smelling (Hill *et al*., 2006). Trophozoites can also be found in jejunal asprate recovered by the string test and examined microscopically for motile trophozoites (Hill *et al*., 2006). Cyst can be found in formol-ether stool concentrate (Gardener and Hill, 2001). Conventional methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) tests are also available (Nwachukwu and Okebe, 2008). These tests have very high sensitivity (Hill *et al*., 2006).

2.3.7 Treatment

Giardiasis is mostly treated with nitromidazole medication such as metronidazole, tinidazole and albendazole (Hill *et al*., 2006). The most widely used medication is metronidazole however a single dose of tinidazole is also considered a first-line agent (Hill *et al*. 2006).

2.3.8 Prevention

Avoid contact with contaminated and untreated water or food, wash hands with soap under running water after defecation and before and after eating (Gardener and Hill, 2001).

2.4 Intestinal Amoebae

Amoebae are single celled organisms of the genus protozoa and phylum Sarcodina (Ryan and Ray, 2004). The most prominent feature of amoeba is their pseudopodia, which is used for movement and feeding (Robertson *et al*., 2010). Common amoebae found in the intestinal tract include *Entamoeba histolytica/dispar, Endolimax nana, Entamoeba coli* and *Entamoeba hartmani* (Robertson *et al*., 2010).

2.5 *Entamoeba histolytica*

2.5.1 Epidemiology

Entamoeba histolytica has been separated from *Entamoeba dispar* on the basis of genetic differences (Ryan and Ray, 2004). *E. dispar* and *E. histolytica* are morphologically the same but *E. dispar* is non-pathogenic whilst *E. histolytica* is the pathogenic form of the parasite (Ryan and Ray, 2004).

Amoebiasis caused by *E. histolytica* is the third parasitic disease responsible for mortality worldwide after malaria and schistosomiasis (Hill *et al*., 2006). It affects about 180 million people worldwide of which 110,000 die from it each year (Voigt *et al*., 1999). It has a worldwide distribution but highly endemic in Africa especially the tropical areas and in places with poor sanitation and low socioeconomic status (Voigt *et al*., 1999).

2.5.2 Morphology

The trophozoites of *E. histolytica* can be up to 60 µm in diameter and moves in a unidirectional manner (Voigt *et al*., 1999). It is a non-flagellated protozoan parasite with a single nucleus (Gaucher and Chadee, 2003). When observed under the microscope, the trophozoites are seen to have a clear visible centrally placed karyosome (Ryan and Ray, 2004). It has a clear pseudopodia and the host's red blood cells can be seen in the in the cytoplasm (Ryan and Ray, 2004). The trophozoites move by extending a pseudopodium and pulling the rest of the body forward this movement is called ameboid movement (Ryan and Ray, 2004).

The cyst of *E. dispar* is 10-15 µm in diameter and contains 1-4 nuclei (Ryan and Ray, 2004). Chromatid bodies are usually present in immature cyst as elongated bars and glycogen is usually diffused (Voigt *et al*., 1999).

2.5.3 Life Cycle

Entamoeba histolytica has no sexual means of reproduction (Caler and Lorenzi, 2010). It reproduces asexually by binary fission (Caler and Lorenzi, 2010). Like most protozoans, the infective form is the cyst, which is responsible for transmission (Godfray and Charles, 2004).

When the cyst is ingested in contaminated food and water it excyst in the small intestines releasing four trophozoites which immediately starts feeding and movement (Ryan and Ray,

2004). The trophozoites migrate to the large intestine especially the colon, where it multiplies by binary fission (Ryan and Ray, 2004). The trophozoites ingest red blood cells and absorb their nutrients (Ryan and Ray, 2004). Under unfavourable conditions some trophozoites form cysts and these pass out into the environment through the faeces of the host (Ryan and Ray, 2004).

2.5.4 Pathology

The disease caused by *E. histolytica* is known as Amoebiasis (Ryan and Ray, 2004). The trophozoites migrate to the colon where they perforate the lamina propria creating "flask shaped" ulcers (Hill *et al*., 2006). Acute amoebiasis is characterised by severe dysentery with bloody stool (Hill *et al.*, 2006). Chronic amoebiasis is characterised with gastrointestinal diaturbances (Goodgame, 1996). Occasionally trophozoites migrate to the liver where they cause liver abscess (Hill *et al.*, 2006). This is characterised by enlarged tender liver with pain in the upper hypochondrium (Godfray and Charles, 2004).

2.5.5 Clinical Manifestations

Clinical symptoms of amoebiasis are generally seen after about a month of contact with the parasite (Devinder *et al*., 1996). Symptoms of mild amoebiasis may include abdominal discomfort, loose stool and excess gas (Hill *et al*., 2006). In acute amoebiasis, symptoms like dysentery with blood in stool, vomiting, fever and nausea may occur (Hill *et al*., 2006). Amoebic ulcers may also occur in the colon and rectal area (Devinder *et al*., 1996). In hepatic amoebiasis symptoms such as weight loss and a very tender liver may be seen (Shahram and Petri, 2008).

2.5.6 Laboratory Diagnosis

Trophozoites can be found in fresh stool by the examination of wet saline preparations (Ryan and Ray, 2004). In the case of amoebic dysentery, stool samples should be examined within 20 minutes of being passed to identify motile trophozoites (Hill *et al.*, 2006). *Entamoeba histolytica* trophozoites can also be identified in liver or lung aspirates microscopically (Hill *et al.*, 2006). The non-pathogenic *Entamoeba dispar* is morphologically similar to the pathogenic *Entamoeba histolytica*, hence can be differentiated by immunologic or molecular analysis such as; polymerase chain reaction (PCR), indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) (Devinder *et al*., 1996).

2.5.7 Treatment

In intestinal infection with *E. histolytica*, drugs like metronidazole, ornidazole and tinidazole are used, which work effectively against the trophozoites (Shahram and Petri, 2008). A combination of the nitromidazole derivatives with paromomycin is effective against the cyst as well (Shahram and Petri, 2008). In liver abscess, 800 mg of metronidazole is the drug of choice (Shahram and Petri, 2008).

2.5.8 Prevention

Avoid contact with contaminated water (Godfray and Charles, 2004). Improved socioeconomic standards and proper personal and communal hygiene should be practiced (Godfray and Charles, 2004).

2.6 Intestinal Coccidia

Coccidias are single celled, obligate intracellular organisms of the phylum apicomplexa (Juranek, 1995). Some of the organisms of this group include *Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis* and *Sarcocystis hominis* (Juranek, 1995). It reproduces by both sexual and asexual reproduction (Jurenek, 1995).

2.7 *Cryptosporidium parvum*

2.7.1 Epidemiology

Cryptosporidium parvum has a cosmopolitan distribution and is responsible for most water borne outbreaks in the United Kingdom (Juranek, 1995). It is the common cause of diarrhoea in both adults and children (Huang and White, 2006). It is more prevalent in immunosuppressed individuals across the globe (Goodgame, 1996). It is found mostly in places with poor sanitation (Juranek, 1995). The parasite is transmitted through the faecal oral route and can survive in food, water, soil and vertebrate host (Huang and White, 2006). In developing countries, a seroprevalence of 60%-70% of the population have circulating antibodies for the parasite with 30%-35% of the population in developed countries also having circulating antibodies of the parasite (Tzipori and Griffiths, 1998). A high prevalence of HIV/AIDS in developing countries is associated with a high prevalence of *Cryptosporidium parvum* infection in these areas (Hunter and Nichols, 2002).

2.7.2 Morphology

Cryptosporidium parvum is a microscopic obligate intracellular parasite that carries out its entire life cycle in a single host (Juranek, 1995). The infective stage of the parasite is the oocyst

(Tzipori and Griffiths, 1998). The oocyst has a very thick outer wall that enables it to survive long periods of time outside of its host (Hunter and Nichols, 2002). The oocyst is also resistant to many disinfectants, especially chlorine (Hunter and Nichols, 2002). It is 4-6 µm in diameter and round or oval in shape and has resemblance to yeast or fungal spores (Goodgame, 1996). The oocyst is mostly refractile at wet smear (Theodos, 1998). Small vacuoles or black dots could be seen in oocyst after modified acid fast staining (Goodgame, 1996). The sporozoites and merozoites have an apical complex (microneme, conoid and preconoidal ring) at the anterior part (Tzipori and Widmer, 2000).

2.7.3 Life Cycle

The life cycle of *Cryptosporidium* parvum begins with the ingestion of oocysts by a suitable host (Juranek, 1995). The oocysts, excyst in the gut, releasing four sporozoites which infect the epithelial cells (Theodos, 1998). In these cells, the parasites multiply by asexual means known as schizogony or merogony producing merozoites and by sexual means known as gametogony, producing microgamonts (male) and macrogamonts (female) (Juranek, 1995). These microgamonts produces microgametes (Juranek, 1995). The microgametes then fertilises the macrogametes producing a zygote (Juranek, 1995). The zygote goes through two asexual divisions producing two kinds of oocyst (Goodgame, 1996). The thick walled oocyst which is normally excreted from the host through faecal matter and the thin walled oocyst, which normally causes autoinfection in the host (Goodgame, 1996).

2.7.4 Pathology

The disease caused by the parasite, *Cryptosporidium parvum* is known as cryptosporidiosis (Theodore, 1998). The pathogenesis of the disease is not fully understood (Tzipori and Widmer, 2000). It is regarded as a minimal invasive pathogen since it invades the luminal surface of epithelial cells of the intestinal tract (White *et al*., 1994). The oocyst can induce infection with as few as 10 oocysts (Tzipori and Ward, 2002). Infection of the intestinal cells by the parasite can result in blunting of the intestinal villi, hyperplasia and inflammation of the cells (Chen *et al*., 2001). Epithelial cells apoptosis due to the parasite can also occur (Chen *et al*., 2001).

2.7.5 Clinical Manifestations

Cryptosporidium parvum causes acute gastro enteritis (Theodore, 1998). It is characterised by watery offensive diarrhoea, abdominal pain, anorexia, vomiting and nausea (Tzipori and Griffiths, 1998). In immunocompetent individuals, symptoms are usually mild and persist for about two weeks whilst in immunosuppressed individuals, symptoms are severe and can persist for over a month and some never clear the infection (Tzipori and Griffiths, 1998). It is the most common cause of diarrhoea in immunosuppressed individuals worldwide (Goodgame, 1996).

2.7.6 Laboratory Diagnosis

Oocysts of *Cryptosporidium parvum* can be identified microscopically in stool samples by the modified acid fast staining test and also by phase-contrast microscopy (Goodgame, 1996). Conventional methods such as enzyme linked immunosorbent assays (ELISA), direct immunofluoresence assays (DFA), indirect fluorescent antibody test (IFA) and polymerase chain

reaction (PCR) can also be used in the detection of *Cryptosporidium parvum* parasite (Goodgame, 1996).

2.7.7 Treatment

There is no specific treatment for cryptosporidial infection because the disease is self-limiting (Goodgame, 1996). Some exceptions require the maintenance of fluid balance and such treatments are available (Hunter and Nichols, 2002). However that is not the case in immunosuppressed patients like AIDS patients (Goodgame, 1996). A high dose of albendazole (800 mg, twice daily) has been shown to improve symptoms in four Zambian AIDS patients (Kelly *et al*., 1998). Paromomycin in a dose of 35 mg per day has limited but beneficial effect on oocyst shedding and stool frequency in AIDS patients (White *et al*., 1994).

2.7.8 Prevention

The most common preventive measure for cryptosporidiosis is the practice of good personal hygiene (Juranek, 1995). People should avoid contact with public pools and also the in-take of water treated with only chlorine, since the oocyst of *Cryptosporidium parvum* is chlorine resistant (White *et al*, 1995). The oocyst can be killed by 5% ammonia in the environment and also by desiccation (White *et al*, 1995).

2.8 Helminthic Infection

Helminths are eukaryotic worm like organisms that live in and feed on the host (Valerio *et al*., 2002). According to their shape, helminths are divided into two main groups, these are the round worms known as nematodes and the flatworms also known as platyhelminths (Cheesbrough,

1992). According to their medical importance, they are divided into three main groups; the nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes) (Cheesborough, 1992). Most of the parasites of medical importance to this work are found in the group of nematodes (Capello *et al*., 1995). Hence our focus will be on the nematodes.

2.9 Nematodes (Roundworms)

Intestinal nematodes mature into adults in the intestinal tract of man (Capello *et al*., 1995). The larval forms of the parasites are widely distributed throughout the body (Hotez *et al*., 2005). The adult larval forms are large enough to be seen with the naked eye, whilst the eggs of these parasites, though bigger than protozoan cyst, is also microscopic and cannot be seen with the unaided eye (Capello *et al*., 1995). Some examples of parasites in this group include; *Ascaris lumbricoides, Trichuris trichuria, Strongyloides stercoralis* and Hookworm (Hill *et al*., 2006).

2.10 *Strongyloides stercoralis*

2.10.1 Epidemiology

Strongyloides stercoralis parasite has a worldwide distribution, but has high prevalence in tropical and subtropical regions (Daubenton *et al*., 1998). An estimated 30-100 million people are infected with the parasite worldwide (Stepek *et al*., 2006). The parasite is mostly under diagnosed because of its many asymptomatic cases (Keiser and Nutman, 2004). High prevalence of the infection has been associated with low socioeconomic status, alcoholism and male gender (Keiser and Nutman, 2004). Like most soil-transmitted helminths, transmission is associated with hygiene, making children most vulnerable to the parasite (Stepek *et al*., 2006). Persons

involved with occupations like; farming and coal mining, which have increased contact with soil contaminated with human faeces, are more prevalent to the parasite (Keiser and Nutman, 2004). It is responsible for 60-85% of mortality in immunocompromised patients (Daubenton *et al*., 1998).

2.10.2 Morphology

The parasite is one of the smallest pathogenic nematode, known to infect humans (Keiser and Nutman, 2004). The body cavity of the parasite is known as a pseudocoel (Roberts *et al*., 2005). The parasite is generally long and cylindrical with four layers of cuticle (Barnes, 1980). On the surface of the parasite are amphids which act as chemoreceptors and open to the outside through pores at the anterior end (Roberts *et al*., 2005). The mouth of *S. stercoralis* opens into a buccal capsule, which is long and lacks a bulb at its posterior end, from this food moves into the oesophagus, which is about one third of the body length (Borkow and Bentwich, 2004). The oesophagus connects to the intestine by an oesophago-intestinal valve (Fincham *et al*., 2003). The anterior most region of the intestine is the ventricular which has a secretory function (Fincham *et al*., 2003). The intestine ends in the rectum and the alimentary canal opens through the anus (Roberts *et al*., 2005). The worm has lateral "alae", which can be seen as ridges across the body in some cases (Robert *et al*., 2005). The adult female worm ranges from 2-2.5 mm in length (Barnes, 1980). The infective form of the parasite is the female filariform larvae, the males are known to be non-parasitic (Fincham *et al*., 2003). The female filariform larvae are slender and fast moving, they are 50 µm in diameter and between 350-600 µm in length and slightly larger than the males (Fincham *et al*., 2003). The adult female worm may live up to five years (Fincham *et al*., 2003). The rhabditiform larvae are smaller and slower and ranges from

250-300 µm in length and 60 µm in diameter (Roberts *et al*., 2005). The eggs of *S. stercoralis* are oval in shape, thin shelled and transparent (Roberts *et al*., 2005).

2.10.3 Life Cycle

The life cycle of *S. stercoralis* has both parasitic and free living stages (Nandy *et al*., 1995). **Free-living cycle:** Adult female worms in the small intestine lay eggs in the intestinal mucosa, these eggs hatch into rhabditiform larvae, which are excreted in stools (Nandy *et al*., 1995). Under moist warm environmental conditions, the rhabditiform larvae either moult twice into infective filariform, this is known as direct development or moult four times into free living adult worms (Fincham *et al*., 2003). Harsh environmental conditions are known to be a stimulus that encourages the development of parasitic stages (Fincham *et al*., 2003). Sexual reproduction takes place in the free living form (Fincham *et al*., 2003).

Parasitic cycle: Filariform larvae in contaminated soil penetrate the human skin and are transported to the lungs (Roberts *et al*., 2005). In the lugs they penetrate the alveolar spaces and are carried through the bronchial tree to the pharynx (Roberts *et al*., 2005). The larvae are then swallowed and reach the small intestine (Roberts *et al*., 2005). In the small intestine, they moult twice to become the adult female worm (Nandy *et al*., 1995). The female adult worm lives threaded in the epithelium of the small intestine (Roberts *et al*., 2005). The female adult worm produces eggs by parthenogenesis, which hatches into rhabditiform larvae (Fincham *et al*., 2003). The rhabditiform larvae are either passed out in stool or remain in the intestinal tract and cause autoinfection (Fincham *et al*., 2003). In autoinfection, the rhabditiform larvae develops into infective filariform larvae, which can either penetrate the intestinal mucosa, in this case

known as internal autoinfection or the perianal skin, in this case is known as external autoinfection (Nandy *et al*., 1995).

2.10.4 Pathology

The disease caused by *S. stercoralis* is known as strongyloidiosis (Fincham *et al*., 2003). The mode of transmission of the parasite is by the filariform larvae (Roberts *et al*., 2005). Acute strongyloidiasis can cause wheezing, epigastric tenderness and low-grade fever whilst chronic strongyloidiasis can cause chronic urticaria and larva currens (Roberts *et al*., 2005). Sloughing of the intestinal mucosa may occur along with ulceration in the small intestine (Roberts *et al*., 2005). S. *stercoralis* is able to remain in its host for years without being detected because of its asymptomatic nature in some cases and this may cause lethal hyperinfection syndrome in immunosuppressed patients (Nandy *et al*., 1995).

2.10.5 Clinical Manifestation

Many individuals may initially be asymptomatic to the infection (Nandy *et al*., 1995). There may be itching, swelling and mild haemorrhage on the skin, at the site of penetration of the parasite (Roberts *et al*., 2005). In the case of intestinal infection, symptoms may include; diarrhoea, vomiting, weight loss and red hive-like appearances near the area of the anus (Nandy *et al*., 1995). Symptoms of lung infection may include; cough, wheezing and chronic bronchitis (Nandy *et al*., 1995).

2.10.6 Laboratory Diagnosis

S. stercoralis larvae can be identified microscopically in concentrated stool samples or by direct wet mount preparation (Nandy *et al*., 1995). Duodenal aspirates can also be examined microscopically for *S. stercoralis* larvae (Nandy *et al*., 1995). Conventional methods such as PCR and ELISA can also be used to detect the parasite (Keiser and Nutman, 2004).

2.10.7 Treatment

The drug of choice for the treatment of strongyloidiasis is ivermectin for one to two days; this drug kills the worms in the small intestine (Nandy *et al*., 1995). Thiabendazole can also be administered twice daily for two to three days (Nandy *et al*., 1995). Albendazole is also an effective drug against *S. stercoralis* infection (Nandy *et al*., 1995).

2.10.8 Prevention

Practice of good personal hygiene is a means of control against infection with *S. stercoralis* (Fincham *et al*, 2003). Good sanitary and public health facilities are also a means of prevention (Fincham *et al*., 2003).

2.11 Hookworm

2.11.1 Epidemiology

There are two main species of hookworm that infect man; these are *Ancylostoma duodenale* and *Necator americanus* (Chan *et al*., 1994). Hookworm is the second most common helminthic parasite known to man and affects almost 600 million people worldwide including children (Chan *et al*., 1994). It has a global distribution but mostly in areas of moist warm climate and in

the poorest regions of the world (Bethony *et al*., 2006). Hookworm prevalence in sub-Saharan Africa is nearly 30% and as high as 50% in the north eastern part of Ghana (Humphries *et al*., 2011). *A. duodenale* is more prevalent in India, North Africa, and the Middle East whilst *N. americanus* is more prevalent in sub Saharan Africa, Southeast Asia, Indonesia, China and the Americans (Hotez *et al* 2005). Transmission is by contact with soil contaminated with infective larvae (Macdonald *et al*., 2002).

2.11.2 Morphology

The hookworm is a parasitic nematode that lives in the small intestine (Hotez *et al*., 2004). The adult worm is long cylindrical and transparent in appearance (Hotez *et al*., 2004). The adult female worm is mostly longer and stouter than the male worm and measures about 1 cm (Bethony *et al*., 2006). The adult male worm measures about 0.5 cm and is rarely seen because it dies right after mating (Bethony *et al*., 2006). The tail of the male is curved and on each side of the anterior end of the body are cuticular extensions known as cephalic alae (Borgoine *et al*., 2011). The egg of the hookworm is 50 µm by 25 µm in size, colourless, thick and has the appearance of a persimmon seed (Brooker *et al*., 2004). The egg is mostly passed out in faeces and they later hatch into larvae (Bundy *et al*., 2000).

2.11.3 Life Cycle

The eggs of hookworm are mostly passed in stool, under favourable conditions of moisture and optimal temperature the eggs hatch into the first stage rhabditiform larvae (Brooker *et al*., 2004). After 5-10 days, the larvae moult twice to become the third stage infective filariform larvae (Brooker *et al.,* 2004). The larvae can survive for about three to four weeks in the environment,

under favourable conditions (Bethony *et al*., 2006). The larvae penetrate the skin of the host and are carried through the blood vessels to the heart through to the lungs (Bethony *et al*., 2006). In the lungs, they penetrate into the pulmonary alveoli and ascend to the pharynx and are swallowed (Hotez *et al*., 2004). The larvae get into the small intestine where they mature into adults and attach to the intestinal walls with resultant blood loss by the host (Brooker *et al.*, 2004). In the small intestine, the male locates the female, they mate and produce eggs which are passed out in faeces (Brooker *et al*., 2004).

2.11.4 Pathology

Several pathological changes occur in the host during the migration of hookworm (Hotez *et al*., 2004). These changes may include small haemorrhages and leukocytic or eosinophilic infiltrations where larvae pass through the alveolar walls of the lungs (Hotez *et al*., 2005). Larval migration through the respiratory tract may also result in irritation of the bronchial and tracheal mucous membranes (Brooker *et al*., 2004). In the duodenum and jejunum, hookworms attach themselves to the intestine by engulfing a part of the intestinal mucosa in their buccal cavities (Hotez *et al*., 2005). At the point of attachment, there is usually some bleeding and inflammatory reactions (Brooker *et al*., 2004).

2.11.5 Clinical Manifestations

When the filariform larvae penetrate the skin, they may cause a stinging sensation, irritation, erythema, oedema and papulovessicular eruption also known as ground itch (Pawlowski *et al*., 1991). These symptoms mostly occur in visitors from endemic areas but rarely in people who live in endemic areas (Pawlowski *et al*., 1991). Migration of the larvae through the respiratory

tract may cause coughing and during the intestinal phase, there may be duodenal-type pain, ingestion, loss of appetite or diarrhoea (Hotez *et al*., 2004). The most serious consequences of hookworm infection are chronic blood loss from the duodenum and jejunum (Pawlowski *et al*., 1991). Untreated blood loss can lead to development of iron deficiency anaemia (Hotez *et al*., 2004). There is also loss of serum proteins which may result in severe hypoalbuminaemia (Pawlowski *et al*., 1991).

2.11.6 Laboratory Diagnosis

The standard method of laboratory diagnosis of hookworm is by identifying the eggs in stool samples, using the microscope (Hotez *et al*., 2005). In light infections, the concentration technique is usually recommended as eggs may be difficult to find in stool (Hotez *et al*., 2005).

2.11.7 Treatment

Hookworm infection is mostly treated with mebendazole, which works by blocking the uptake of nutrients by the worm thereby killing the worms. Albendazole also works in eliminating the worms in the host (Hotez *et al*., 2005). Iron supplements may also be prescribed for anaemia caused by hookworm infection (Hotez *et al*., 2005).

2.11.8 Prevention

Practice of good personal hygiene is the sure means of preventing infection with hookworm (Bundy *et al*., 2000). Avoid walking barefooted in damp soil (Bundy *et al*., 2000). Proper

sanitation is also very important in keeping your environment free of hookworm (Bundy *et al*., 2000).

2.12 Intestinal parasite infection in Ghana

Few data exist on the general prevalence of intestinal parasite infection in Ghana (Ayeh-Kumi *et al*., 2009). Annan *et al* (1986) reported a 63% prevalence of intestinal helminthic infection among school children, Nelly *et al* (2009) reported a 25.7% prevalence of intestinal helminthic infection among pregnant women in Ghana and Ayeh-Kumi *et al* (2009) reported 21.6% prevalence of intestinal parasite infection among food vendors in Accra, Ghana.

2.13 Human Immunodeficiency Virus (HIV) infection in Ghana

Human Immunodeficiency Virus (HIV) is the virus that causes Acquired Immune Deficiency Syndrome (AIDS) (Burton *et al*., 2002). The virus destroys the biological ability of the human body to fight against infections (Abass and Litchman, 2003). In Ghana a person is said to have developed AIDS when he or she presents with a combination of signs and symptoms and has a positive HIV antibody tests (Disease Control Unit, Ministry of Health, 2010).

These are grouped into major and minor signs and symptoms.

The major signs and symptoms include:

- Prolonged fever (more than one month)
- Prolonged and chronic diarrhoea (usually over a month)
- Significant weight loss more than 10% body weight (over a period of time)

The minor signs and symptoms include:

- Persistent cough over a month

- Persistent skin infection
- Aggressive skin cancer (Kaposi Sarcoma)
- Oral thrush (Candidiasis)
- Recurrent shingles (‘Ananse”)
- Enlargement of the lymph glands

An individual with two of these major signs and symptoms and two of the minor signs and symptoms plus a positive HIV antibody test is said to have the disease (Health Research Unit, Ministry of Health, 1996).

The HIV pandemic began in Ghana in the mid 1980’s and the first AIDS case was diagnosed in the country in 1986 (Nabila *et al*., 2001). So far about 2% of the entire Ghanaian population is infected with HIV/AIDS and most of these people do not even know they carry the virus (Disease Control unit, Ministry of Health, 2010). The common type of HIV present in Ghana is HIV I forming about 96% of the total positive cases in the country and dual infection of HIV I and II being 2.6% (Nabila *et al*., 2001). The estimated number of persons living with HIV and AIDS in Ghana in 2009 was 267,069 out of which 25,666 were children (Disease Control Unit, Ministry of Health, 2010). The majority of the infections (80%) are transmitted through heterosexual contact, with mother-to-child transmission being about 15% and through other means being about 5% (Disease Control Unit, Ministry of Health, 2010).

The true number of cumulative AIDS cases in the country is not known but according to information provided by the Ghana AIDS Commission; about 185,000 people had developed AIDS as at the year 2009 and many more have contracted the virus since then (Disease Control unit, Ministry of Health, 2010). However these are crude estimates; since majority of these

people do not know their HIV status and the scare is that these people are likely to spread the infection, hence increase the number of infected individuals in the country (Gyasi *et al*., 2001).

2.14 Intestinal parasitic infections in HIV/AIDS Patients

The public health importance of intestinal parasites as a major concern in most developing countries has been pronounced with the co-occurrence of malnutrition and HIV/AIDS (Nwachukwu and Okebe, 2008). As compared to developed countries, the prevalence of intestinal parasite infection is higher in developing countries, especially among HIV/AIDS infected population (Nwachukwu and Okebe, 2008). This is reflected by the prevalence of intestinal parasites in a given geographical locality among the general population (Lindo and Lee, 2001). HIV/AIDS infection has been shown to predispose the patient to some opportunistic intestinal parasites such *as Cryptosporidium parvum, Isospora belli* and *Cyclospora cayetanensis* (Goodgame, 1996). This does not seem to be the case with exracellular intestinal parasites such as *Ascaris lumbricoides, Trichuris trichiura*, Hookworm Spp. and *Giardia lamblia* (Harinda, 2008).

Some studies have indicated that compared to the general population, there is relatively lower prevalence of non-opportunistic extracellular intestinal parasites in HIV/AIDS patients (Akinbo *et al.,* 2010). Most clinical manifestations of HIV/AIDS patients result either from the reactivation of pre-existing latent pathogens, as the individuals become immunosuppressed or is caused by exposure to locally predominant pathogens (Lindo *et al*., 1998). Consequently, clinical presentations of HIV/AIDS and the pathogens responsible in different geographical areas reflect the differing prevalence of opportunistic intestinal parasitic infections in a given community (Lindo *et al.*, 1998).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Site

The study was carried out at the HIV clinic of the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. Kumasi is the second largest commercial city in Ghana and the capital of Ashanti region and has a population of 2,035,064 (Population and Housing Census, 2010). The hospital is the second largest teaching hospital in Ghana after the Korle Bu Teaching Hospital in Accra (KATH official website, 2014). The geographical location, of the 1000 bed capacity hospital makes it accessible and a preferred referral centre for most areas in the Ashanti region and beyond (KATH official website, 2014). The HIV clinic of KATH serves about 9000 HIV positive patients both in Kumasi and beyond. The clinic is organized 3 days in a week, that is, Monday, Wednesday and Friday (KATH official website, 2014).

3.2 Study Population

Based on the inclusion and exclusion criteria of the study, a total of 855 HIV patients and 100 non-HIV individuals were recruited for the study, over a period of 13 months, that is from January 2012 to February 2013 after informed written consents were obtained from each participant. They then responded to a standard questionnaire on medical history and basic socio-demographic characteristics (age, sex, education, socioeconomic status, residence, etc.) and any other information relevant to the study. The inclusion and exclusion criteria for the recruitment of patients included:

Inclusion criteria:

I. Able and willing to give informed written consent

II. HIV infection present

III. Age ≥ 18 yrs

IV. **Exclusion criteria:**

I. Not willing or able to comply with study procedures

II. Active opportunistic infection or other acute systemic infection (e.g. Pneumonia) or malignancy (e.g. lymphoma).

III. Age < 18 yrs

The inclusion and exclusion criteria for the recruitment of the 100 HIV negative individuals included;

Inclusion criteria:

I. Age ≥ 18 yrs

II. Able and willing to give informed written consent

III. HIV infection absent

Exclusion criteria:

I. Age < 18 yrs

II. Not willing or able to comply with study procedures

III. HIV infection present

3.3 Study Design

This was a long term cross-sectional study.

3.4 Ethical Approval

The Committee on Human Research, Publications and Ethics of the School of Medical Sciences (SMS) of the Kwame Nkrumah University of Science and Technology in Kumasi, after reviewing the study protocol approved the study. Permission to undertake the study at the KATH was granted by the hospital management and the organizers of the HIV clinic in KATH.

3.5 Sample Collection and Handling

Clean, sterile and dry stool containers were given to study participants after receipt of a fully-filled informed consent. Instructions were given on how to take the sample to avoid contamination of the stool sample with urine or any unwanted material and on the amount of stool sample to collect. Study participants who were unable to produce stool specimens on the same day were given the containers to send home and bring freshly passed stool sample the next day not less than two hours after it had been passed. Each specimen container was labelled with the participant's study identity number and date sample was received. Samples that were contaminated with urine as well as specimen containers with inadequate stool samples were rejected.

Aliquots were made from the stool samples collected. Two aliquots each per participant into 2 ml eppendorf tubes; an aliquot of 0.2 g and another aliquot of 1.0 g were made. These were stored at -80° C and Sodium acetate, Acetic acid and Formalin (SAF) solution was added to the rest of the stool specimen and observed later. About 5 ml blood samples were also taken from the patients for T-cell $CD4^{+}$ counts.

3.6 Laboratory Procedures

3.6.1 Concentration Technique (Formol ethyl acetate concentration)

A portion of each stool sample fixed with Sodium acetate, Acetic acid and Formalin (SAF) solution was processed as described by the Ridley-Allen method, (1970). Using applicator sticks a portion of the stool sample was transferred into a centrifuge tube and emulsified with 7 ml SAF solution. This kills and fixes all organisms in the stool samples. This was then filtered through metal gauze to get rid of all lumpy residues centrifuged, discarding the supernatant.

About 7 ml saline solution was added to the pellet to wash the sample of all unwanted material and 2 ml ethyl acetate added as a lipid removing agent. This mixture was shaken thoroughly and centrifuged. After centrifugation 4 layers were observed, the top 3 layers were discarded leaving the bottom layer.

Few drops of the deposit were transferred onto a slide and Lugol's iodine added for contrast and covered with a cover slip. This was then examined microscopically for helminthic eggs and protozoan cysts using ×10 for observation or detection and ×40 objective lens for confirmation of helminthic eggs and ×100 for confirmation of protozoan cysts.

3.6.2 Molecular Analysis of Stool Samples

QIAamp DNA stool mini kit (QIAGEN®, Hilden, Germany) was used for the DNA extraction.

There were two major steps in the isolation process: Extraction and Purification.

3.6.2.1 DNA Extraction

In the primary steps of the protocol, stool samples were lysed in 1.4 ml of buffer ASL. This lysed any human, bacterial or any pathogenic cell present. This was incubated at an optimal temperature of 70 °C for 5 minutes. It was then spun down and the supernatant pipetted into a new tube.

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions (Lipp *et al.*, 2001). These substances present in the stool sample were adsorbed to an InhibitEX matrix provided in a tablet form in the QIAGEN® kit. After adsorption, the InhibitEX was pelleted by centrifugation and the supernatant transferred into a new tube. About 15 µl Proteinase K and 200 µl buffer AL were added to the sample. This denatured and digested the proteins at 70 °C incubation for 10 minutes, and at this stage also the cells ruptured, exposing the DNA. About 200 µl ethanol (96-100%) was added to the lysate to precipitate the digested proteins and concentrate the nucleic acids (Poms *et al.*, 2001).

3.6.2.2 Purification

The complete lysate was applied to the QIAamp spin column. In the spin column was a silica membrane, which is positively charged (Prado *et al.*, 2002). The principle of this method is reversible binding of DNA to the silica membrane at high concentration of chaotropic salts, present in buffer AL, rendering the DNA negatively charged (Fujiwara *et al.*, 2005). Negatively charged DNA molecules then bind to the positively charged silica membrane during a brief centrifugation step.

DNA bound to the membrane was washed in two centrifugation steps. Optimized wash conditions were done using 500 µl each of the two wash buffers to ensure complete removal of any residual impurities without affecting DNA binding (Lipp *et al.*, 2001).

The final step involved the release of pure DNA from the silica. Purified, concentrated DNA was eluted from the spin column in 200 µl buffer AE elution buffer (Tris 10mM Cl; 0.5mM EDTA; pH: 9.0). This was eluted into a 1.5 ml eppendorf tube and used for the Real-Time Multiplex PCR (Prado *et al*., 2002).

3.6.2.3 Polymerase Chain Reaction (PCR)

DNA amplification was performed using Real Time multiplex PCR which is a quantitative form of PCR for the detection of four different protozoan parasites at the same time. These protozoans were *Entamoeba histolytica, Entamoeba dispar, Giardia lamblia* and *Cryptosporidium parvum*. The procedure follows the general principle of polymerase chain reaction; its key feature is that the amplified DNA is detected as the reaction progresses in real time.

Amplification of PCR was performed in a volume of 25 µl reaction mixture in 0.2 ml eppendorf tubes each containing 0.75 µl of each of the four primer mix (forward, reverse and probe for each primer), 12.50 µl of HotStarTaq Mastermix 2× which contain 10× PCR buffer, dNTPs and the HotstarTaq, 3.50 µl of $MgCl_2$ 25mM, 3.50 µl of nuclease free water and 2.50 µl of the DNA sample. This was run using Rotor-Gene 6000 Series Software 1.7.

The PCR cycling parameters were 40 cycles with an initial hold temperature of 95 $^{\circ}$C for 15 minutes, with denaturation at 95 $^{\circ}$C for 15 seconds and primer annealing at 64 $^{\circ}$C for 30 seconds,

elongation at 72 °C for 30 seconds and a 0.5 °C decrease in temperature for each cycle for 9 cycles.

After the ninth cycle there was a new hold temperature of 40 °C for 30 seconds and the cycle repeated itself till the fortieth cycle. The whole amplification process took 2 hours.

After the amplification process, the run was analysed on the Corbett®. The results were shown in a tabular form on the Corbett®, with the positive and negative controls passing out correctly, and the positives for each parasite also showing with their corresponding cycle threshold (Ct).

3.6.3 $CD4^+$ (Cluster of Differentiation) T-cell $CD4^+$ Count

The absolute T-cell $CD4^+$ of the study participants were measured using the FACScan® flow cytometry (Becton Dickinson Immunocytometry system, San Jose, CA., USA). The patients' T-cell $CD4^+$ counts were measured following the manufacturer's protocol.

Blood samples were taken into EDTA tubes. About 50 µl of the blood was pipetted into the reagent tubes and these were incubated in the dark at room temperature for 60 minutes. After incubation 50 µl of fixative solution was added to the reagent tubes by reverse pipetting, this was then mixed thoroughly by vortexing. The samples were then run on the FACSCount and the results printed out for each patient.

3.7 Data Analysis

The data for 955 samples were entered into an excel spread sheet and grouped. The data were analysed using SPSS statistical software version 17.0 (Stata Corporation, Texas, USA). Student's

t-test was used to compare continuous variables. Proportions were compared using chi-square tests. A univariate Mantel-Haenzel analysis was performed to examine the association between intestinal parasite infection and T-cell $CD4^+$ counts. P-values of <0.05 were considered to be statistically significant.

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the formol-ethyl acetate technique were calculated as follows with the concentration technique being the "gold standard test".

Concentration technique

Polymerase Chain Reaction (PCR)	Positive (+)	Negative (-)
Disease (+)	a	b
No Disease (-)	c	d

Sensitivity = True Positive/Condition Positive, $a/(a+c)x100$

Specificity = True Negative/Condition Negative, $d/(b+d)x100$

PPV = True Positive/Test Outcome Positive, $a/(a+b)x100$

NPV = True Negative/Test Outcome Negative, $d/(c+d)x100$

CHAPTER FOUR

4.0 RESULTS

4.1 Socio demographic characteristics of study participants

A total of 955 individuals were recruited for the study, out of which 855 were HIV/AIDS Positive and 100 were HIV/AIDS Negative. There were 26% (253) males and 74% (702) females. Their ages ranged from 19 to 85 years, with a mean age of 39 years and the modal age group of 30-39 (Table 1). The proportion of males to females was 1:3. The number of females was higher in all age groups compared to the males, and the age group that had the highest frequency of males was 40- 49 years (Table 1). The study volunteers were generally young, with 83.1% of them being below 50 years, and the rest being 50 years and above. Only one was 85 years (Table 1).

In all, the age group with the highest population among the HIV Positive individuals was 30-39 year group with a percentage of 37.3% (Table 1). The HIV Negative individuals on the other hand showed a high frequency in the 19-29 year group with 53% and a low frequency in two year groups, both with a percentage of 6%. The age group 19-29 years also had the highest frequency of both male and female populations of 54.5% and 52.2%, respectively, among the negative cases.

Table 1: Demographic characteristics of the study participants.

	HIV POSITIVE			HIV NEGATIVE		
Age Groups	Males	Females	Total	Males	Females	Total
19-29	7(3.2%)	98(15.4%)	105(12.3%)	18(54.5%)	35(52.2%)	53(53%)
30-39	62(28.2%)	257(40.5%)	319(37.3%)	8(24.2%)	19(28.4%)	27(27%)
40-49	98(44.5%)	184(29%)	282(33%)	2(6%)	6(9%)	8(8%)
50-59	46(20.9%)	82(12.9%)	128(15%)	2(6%)	4(6%)	6(6%)
60 and above	7(3.2%)	14(2.2%)	21(2.4%)	3(9.1%)	3(4.4%)	6(6%)
Total	220 (100%)	635 (100%)	855(100%)	33(100%)	67(100%)	100(100%)

4.2 Clinical Stage of the Patients

Of the 955 individuals, 855 were HIV positive patients. According to the World Health Organisation (WHO), there are four main clinical stages of the disease. Based on this, the patients were grouped under the various stages. In all 61.6% of the individuals had stage 1 of the disease, 10.7% had stage 2 and 13.6% had stage 3 and 0.7% in stage 4 of the disease (Table 2). Of the 855 HIV positives, the staging of 114 patients could not be retrieved from their hospital folders.

Table 2: WHO, HIV staging of the patients

WHO Staging	Frequency (%)
1	527 (61.6)
2	92 (10.7)
3	116 (13.6)
4	6 (0.7)
Unclassified	114 (13.4%)
Total	855 (100%)

4.3 HIV disease Type

Of the 855 patients only 634 representing, 74.2% had their HIV type recorded in their hospital folder. HIV type 1 disease was more prevalent in the study participants, recording a highest frequency of 68.8%. The HIV type of 25.8% could not be retrieved from their hospital folders (Table 3).

Table 3: HIV Disease type of the Patients

HIV TYPE	FREQUENCY (%)
1	589 (68.8)
2	17 (2.0)
1+2	28 (3.3)
Unclassified	221 (25.8)
Total	855 (100)

4.4 T-cell $CD4^+$ count

The T-cell CD4 counts were also categorized under four main groups (Table 4). T-cell $CD4^+$ count category, 500 cells/mm^3 and above had the highest frequency of 33.4% and category 200-349 cells/mm^3 had the lowest frequency of 20% (Table 4).

Table 4: T-cell $CD4^+$ count of the patients

CD4 Count (cells/mm^3)	Frequency (%)
Below 200	220 (25.7)
200 – 349	171 (20.0)
350 – 499	179 (20.9)
500 and above	285 (33.4)
Total	855 (100)

4.5 Intestinal Parasites Detected from the Formol-ethyl acetate Concentration Technique

There was a general prevalence of 20.3%. The HIV/AIDS Positive group had a prevalence of 20.1% and the negative control group had a prevalence of 22%. *E. nana*, which is a non-pathogenic intestinal parasite, had the highest frequency in both groups as can be seen from Table 5, with a percentage of 6.6%. The organism with the second highest percentage of prevalence was *E. coli*, with a percentage of 4.9%. *B. coli* which is also a non-pathogenic intestinal parasite had the lowest frequency and was detected only in the HIV/AIDS positive individuals with a percentage of 0.1% (Table 5). Hookworm was the only helminthic parasite detected with a percentage of 0.2% in the HIV positive individuals. Two pathogenic intestinal protozoans were reported, *E. histolytica/dispar* and *G. lamblia* both with percentages of 1.9% in

HIV/AIDS positive group and *G. lamblia* only appearing in the HIV/AIDS negative group, with a percentage of 1%. The *E. histolytica/dispar, B. coli* and Hookworm were also reported in the HIV/AIDS negative group (Table 5).

Table 5: Intestinal parasites among the study individuals using the formol ethyl-acetate concentration technique.

Intestinal parasite	HIV positive (%) (n=855)	HIV negative (%) (n=100)	Total (%) n=955	P-value
E. coli	41 (4.8)	6 (6)	47 (4.9)	0.598
E. nana	54 (6.3)	9 (9)	63 (6.6)	0.306
C. mesnili	31(3.6)	3 (3)	34 (3.5)	0.749
E. histolytica/dispar	16 (1.9)	0	16 (1.7)	0.168
B. coli	1 (0.1)	0	1 (0.1)	0.732
G. lamblia	16 (1.9)	1 (1)	17 (1.8)	0.533
Hookworm	2 (0.2)	0	2 (0.2)	0.628
I. butschlii	11 (1.3)	3 (3)	14 (1.5)	0.171
Total	172 (20.1)	22 (22)	194 (20.3)	

4.6 Relationship between T-cell $CD4^+$ count and Intestinal parasites

Chi square test of association between T-cell $CD4^+$ counts and the intestinal parasites recorded, showed no significant association, with a p-value of 0.8 (Confidence Interval of 95.5%) (Table 6). The T-cell $CD4^+$ category which had the highest frequency of intestinal parasitic load was

500 cells/mm^3 and above, with a percentage of 32.6%, with those in category of below 200cells/mm^3 having the lowest frequency of 19.8% as shown in Table 6 below.

Table 6: Correlation between T-cell CD4$^+$ Categories and the Intestinal Parasites

	CD4 Categories				
Organisms	Below 200	200-349	350-499	500 and above	Total
E. coli	7 (4.1%)	12 (7.0%)	8 (4.7%)	14 (8.1%)	41 (23.8%)
E. nana	12 (7.0%)	15 (8.7%)	7 (4.0%)	20 (11.6%)	54 (31.4%)
C. mesnili	6 (3.5%)	8 (4.7%)	8 (4.7%)	9 (5.2%)	31 (18.0%)
E. histolytica/dispar	4 (2.3%)	3 (1.7%)	4 (2.3%)	5 (2.9%)	16 (9.3%)
B. coli	0	1 (0.6%)	0	0	1 (0.6%)
G. lamblia	4 (2.3%)	4 (2.3%)	3 (1.7%)	5 (2.9%)	16 (9.3%)
Hookworm	0	0	2 (1.2%)	0	2 (1.2%)
I. butschlii	1 (0.6%)	3 (1.7%)	4 (2.3%)	3 (1.7%)	11 (6.4%)
Total	34 (19.8%)	46 (26.7%)	36 (20.9%)	56 (32.6%)	172 (100%)

*n=172

4.7 Correlation between Age and the T-cell CD4$^+$ categories

On the other hand the correlation of the ages and the T-cell CD4$^+$ count of the study participants showed a significant relation between the ages of the patients and their T-cell CD4$^+$ counts, having a p value of 0.004 (Confidence Interval of 95%) (Table 7).

Table 7: Correlation between Age and the T-cell $CD4^+$ categories

Age Categories	Below 200	200-349	350-499	500 and above	Total
18-29	27 (3.2%)	20 (2.3%)	21 (2.5%)	37 (4.3%)	105 (12.2%)
30-39	85 (9.9%)	65 (7.6%)	67 (7.8%)	102 (11.9%)	319 (37.3%)
40-49	73 (8.5%)	52 (6.1%)	61 (7.1%)	96 (11.2%)	282 (33.0%)
50-59	33 (3.9%)	32 (3.7%)	23 (2.7%)	40 (4.7%)	128 (15.0%)
60 and above	2 (0.2%)	2 (0.2%)	7 (0.8%)	10 (1.2%)	21 (2.5%)
Total	220 (25.7%)	171 (20%)	179 (20.9%)	285 (33.3%)	855 (100%)

*n= 855

4.8 Real Time Multiplex Polymerase Chain Reaction (RT-PCR)

The other test that was used was the Real Time multiplex PCR, which focused on four different intestinal parasites. This test comparatively yielded more positives among the four organisms than the formol-ethyl acetate concentration technique.

The PCR technique gave a general prevalence of 19.4%. As can be seen from Table 8 below, there were 166 positive cases of intestinal parasites among the study individuals. *E. dispar* which is a non-pathogenic parasite, gave a prevalence of 10.4% in the general population. *E. histolytica* had a prevalence of 1.6% in the HIV/AIDS positive group with no prevalence in the HIV negative group. *Cryptosporidium parvum* had a prevalence of 1.8% with no positives in the control group (Table 8).

Chi-square test of association between T-cell $CD4^+$ count and *C. parvum* proved significant with a p-value of 0.004. All of the 15 positives of *C. parvum* occurred in patients with T-cell $CD4^+$ counts below 100 cells/mm^3.

A chi-square test of association between the T-cell $CD4^+$ categories and the intestinal parasites that were reported by the RT-PCR, showed no statistical relation, with a p-value of 0.8.

Table 8: Frequency of the organisms detected by the RT-PCR technique

Organism	HIV positive (%) (n=855)	HIV negative (%) (n=100)	Total (%)	P-value
C. parvum	15 (10.1)	0	15 (9.0)	0.68
E. dispar	78 (52.7)	11(61.1)	89 (53.6)	0.35
E. histolytica	14 (9.5)	0	14 (8.4)	0.56
G. lamblia	41 (27.7)	7 (38.9)	48 (29.0)	0.57
Total	148 (100)	18 (100)	166 (100)	

4.9 Comparison of PCR and Concentration Technique

The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of the concentration technique against the PCR test were 27.4%, 85.9%, 21.5% and 89.3% respectively (Table 9).

The sensitivity of the concentration technique was low (27.4%) with a high specificity of 85.9%.

Table 9: Comparison of Formol-ethyl acetate concentration technique against the PCR

		Polymerase chain reaction (PCR)		
		Negative (-)	Positive (+)	Total
Formol-ethyl acetate concentration technique	Negative (-)	511	61	572
	Positive (+)	84	23	107
	Total	595	84	679

CHAPTER FIVE

5.0 Discussion

Intestinal parasites are common causes of public health problems in HIV/AIDS patients (Brooker *et al*., 2004). Detecting these parasites and understanding the status and significance of the infections they cause will greatly help in proper management and treatment of HIV/AIDS patients. The general trend in prevalence and species of intestinal parasites fauna has dramatically changed with the HIV/AIDS epidemic globally (WHO Expert Committee, 2002).

This study sought to look at the prevalence of intestinal parasites and if there is any relationship between the parasitic load and the T-cell $CD4^+$ counts of HIV/AIDS patients presenting for treatment at the Komfo Anokye Teaching Hospital (KATH). Different studies have indicated that intestinal parasites are common causes of public health problems in HIV/AIDS individuals (Akinbo *et al.,* 2010).

5.1 Socio demographic characteristics

The high female population than males, could be that more women are affected with the disease that men. According to the Global Coalition on Women and AIDS (GCWA) an estimated 57% of adults living with HIV/AIDS in Africa are women (UNICEF, 2008). Another contributing factor to the larger proportion of the study participants being women could also be the existence of a special occupation known as commercial sex workers, which has over 85% of them being women who sell sex to men for a fee, thereby increasing the risk of infection among women (Whitworth *et al*., 2000).

The population of the studied participants was generally a young population with 83.1% of them being below 50 years. This is to be expected because although HIV/AIDS is prevalent in all age groups, it is more pronounced among those who are within the reproductive and productive age

group and this is below 50 years (Mohandas *et al*., 2002). Data from the United States of America showed that 64% of reported HIV infections occurred among youths aged 20 to 24 years, with 57% among ages 30 to 35 years (CDC, 2002). In Nigeria, as it is in many sub-Saharan African countries, prevalence of HIV/AIDS is predominant in the age group 15-34 years (National Youth Service Corps/UNICEF, 2007).

The highest population of females that were infected was in the 30-39 years age group. This is because most women are in their prime at that age group and sexually active (UNICEF, 2008).

5.2 Clinical Diagnosis of the Patients

Majority of the patients were in stage 1 of the disease. This stage is mostly classified as asymptomatic, that is showing no or very little signs of the infection. This is because most of the patients were on Antiretroviral Therapy (ART). These ARTs tend to strengthen the immune system of the individual by reducing the HIV viral load in the blood and enabling the immune system to recover by the increase of the T-cell CD4. This then enables the individual to fight most infections that can be threatening. It also stabilizes and prolongs the latent state of the infection, thereby moving others from lower stages to a higher stage. This could also account for most of the study individuals having T-cell CD4 counts of 500 cells/mm^3 and above.

The next highest CD4 category was those with CD4 counts of below 200cells/mm^3. HIV stage is not linear to CD4 increase or decrease. An individual can have a low CD4 count but be classified under stage 1, based on how much of the symptoms of the infection, the individual exhibits. This could be a factor for more individuals (61.6%) classified under stage 1 and a comparatively lower individuals (33.3%) having T-cell CD4 counts of above 500 cells/mm^3.

The HIV type that was common among the study individuals was HIV type 1. Globally the most widespread HIV infection is the type 1, having a global prevalence of 33 million and over 2.7 million people being infected with this type every year (UNAIDS/WHO, 2009).

Both HIV type 1 and 2 are common in Africa (Okodua *et al*., 2003). HIV type 2 is found more specifically in West Africa than other parts of the world (Niama *et al.*, 2006), hence accounting for the presence of both HIV type 1 and 2 among the study individuals. About a quarter of the study individuals had no records of their HIV type. This is because this information was retrieved from the patients' hospital folder. Hence the study had no direct control over that information or how that data was recorded.

5.3 Parasitic load among Study Participants

Two main tests were used in the identification of intestinal parasites: these were the formol-ethyl acetate concentration technique and the RT-PCR.

The concentration technique is considered the "gold standard" method for the identification of intestinal parasites (Wiebe *et al.*, 1999). The RT-PCR test was used to complement areas where the formol-ethyl acetate concentration test could not be used to differentiate between *Entamoeba dispar* and *Entamoeba histolytica*, because these two parasites are morphologically the same (Lindo *et al*., 1998). The PCR test was also used to identify *Cryptosporidium parvum* which is an opportunistic infection in HIV patients.

5.3.1 Intestinal parasites detection from the formol-ethyl acetate concentration technique

The prevalence of intestinal parasites was generally low among the study individuals, especially intestinal helminths, which recorded only hookworm with a frequency of 1.2%. The generally low prevalence of intestinal parasites could be due to the fact that all of the study participants were adults above 18 years. Also most of these patients reside in the urban areas, precisely Kumasi and Obuasi, and have some level of formal education, so have some basic knowledge about personal hygiene, hence reducing the risk of infection among the study participants (Rosen *et al.*, 2008).

Secondly these are individuals who are very much aware of their clinical status, attended regular HIV clinic, organized by the hospital, where they are taught on how to care for themselves properly in order to live long and healthy and hence are able to avoid infection with these parasites (Hedden *et al.*, 2009).

Another factor that could have also contributed to the general low prevalence of intestinal parasite was the interval between the time of passage of the stool sample and the time it got to the hospital. Some of the study participants were unable to produce stool samples on the day of recruitment. These individuals were given sterile containers to take home and bring fresh samples the next morning. Since these patients could not be monitored in their homes to ascertain the exact time of passage of the stool samples. Some of the samples could have gotten to the laboratory after 2 hours of its passage, hence most of the organisms being likely lost already before the tests.

The general low prevalence of intestinal helminths could be due to the fact that, anti-helminthic drugs are easily accessible in the country, unlike protozoans whose drugs are not readily

available. Intestinal helminths are also known to be highly prevalent in children than in adults (Okyay *et al.*, 2004).

In all, the non-pathogenic intestinal protozoans were more than the pathogenic ones, *E. nana* was the most prevalent parasite followed by *E. coli.* This could be due to their non-pathogenic nature, hence can be present with no clinical manifestations unlike the pathogenic ones that present with clinical manifestations so are easily detected and treated. These parasites can be present in the individual for a long time without really causing any complications for the host so remain unnoticed.

The results showed no significant relation between the T-cell CD4 counts of the individuals and the kind of intestinal parasites that were recorded. The T-cell CD4 count category that had the highest frequency of intestinal parasites was the category 500 cells/mm^3 and above. It was highest because it had the highest number of individuals in that category.

Cryptosporidium parvum was not found in the controlled group. This was probably because none of the study participants in this group had T-cell CD4 counts of below 200 cells/mm^3. It is known that this parasite is common in individuals whose immune systems have been compromised (Akinbo *et al*., 2010). *Entamoeba histolytica* which is a pathogenic intestinal parasite was also not found in the control group.

5.3.2 Intestinal parasites detected from the Real Time Multiplex PCR

The PCR focused on four intestinal parasites, *G. lamblia, E. dispar, E. histolytica* and *C. parvum.* As was to be expected the PCR gave a higher yield of intestinal parasites compared to the concentration technique.

The PCR was able to distinguish between *E. dispar* which is non-pathogenic parasite from the pathogenic *E. histolytica* because of the different primer sequences that was used for the two intestinal parasites. The PCR comparatively yielded more positives than the concentration technique. Whilst the concentration technique works on the principle of the presence of the whole organism, PCR is able to detect even the smallest fragment of the organism's DNA present in the stool isolate, hence making it highly sensitive and able to detect more parasites than the concentration technique.

Another organism that PCR was able to detect was *Cryptosporidium parvum*. This organism yielded 15 positive cases with a very interesting trend. All the positive cases were found in patients with T-cell CD4 counts of below a 100 cells/mm^3 and in stage 4 of the disease. This confirms reports from various articles that *Cryptosporidium parvum* is an opportunistic intestinal parasitic infection found in individuals whose immune system have been compromised like HIV/AIDS patients (Kulkarni *et al*., 2007; Anand *et al*., 2002).

5.4 Comparison of PCR and Concentration technique

The concentration technique which was the "gold standard" was compared to the RT-PCR and gave a sensitivity and specificity of 27.4% and 85.9% respectively. The Positive Predictive Value and Negative Predictive Value were 21.5% and 89.3% respectively. The percentage sensitivity of the concentration technique clearly shows that is less sensitive as compared to the PCR technique.

Although there are some characteristics that make the concentration technique a preferable choice, the PCR proven to be more sensitive for the diagnosis of intestinal parasite.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusion

In conclusion, both pathogenic and non-pathogenic intestinal parasites including *E. nana* which had the highest prevalence and *B. coli* which had the lowest prevalence were detected in HIV/AIDS positive patients at a prevalence of 20.3%. There was no significant relationship between intestinal parasite infections and T-cell $CD4^+$ counts of HIV/AIDS positive individuals. There was a significant relationship between disease progression in HIV/AIDS patients and opportunistic intestinal parasites as determined by the prevalence of *C. parvum* in patients with T-cell $CD4^+$ counts below 200 cells/mm^3. PCR was a superior method of parasite detection to microscopy as adjudged by the specificity and sensitivity of the two tests. This study showed that non-pathogenic intestinal protozoans like *Endolimax nana* and *Entamoeba dispar* are more prevalent in HIV/AIDS patients in Kumasi than pathogenic intestinal protozoans.

6.2 Reccommendations

1. Routine stool diagnosis should be included in the treatment of patients in stages III and IV of HIV/AIDS infection.
2. Patients in stages I and II of HIV/AIDS infection who show any signs of intestinal parasite infection should be given the appropriate intervention.
3. HIV/AIDS patients on ARTs should be encouraged to take their medications seriously as they were found to be working effectively in boosting the immune system of the individuals in the study.

4. Stool samples should be brought to the laboratory in a timely manner to prevent degradation of parasites which affect diagnosis.

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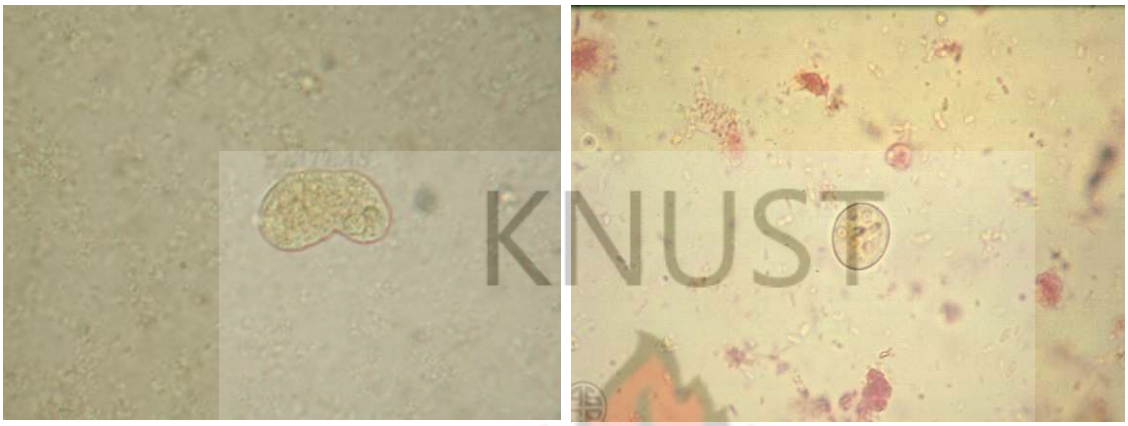
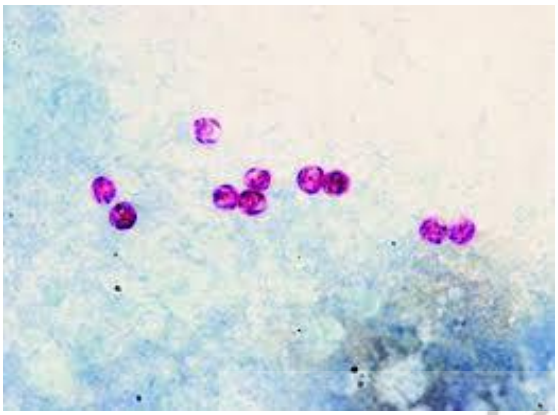
Appendix

Diagnostic characteristics of some intestinal parasites found in stool samples (Courtesy, CDC, 2006).

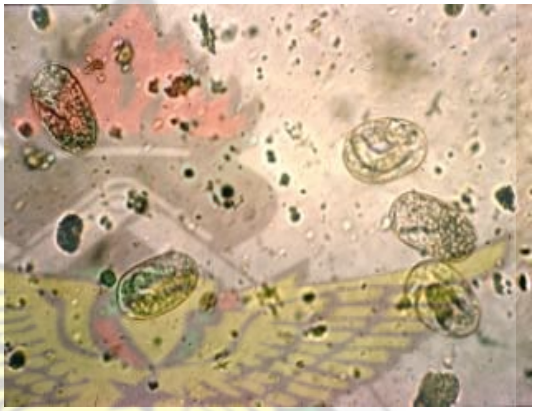


Trophozoite and cyst of *Entamoeba histolytica/dispar*

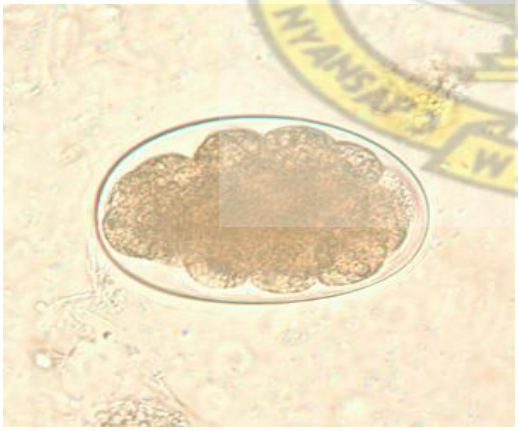
Trophozoite and cyst of *Giardia lamblia*



Oocyst of *Cryptosporidium parvum* stained with Ziehl-Neelsen staining

Adult worm and ova of *Strongyloides stercoralis*




Egg and L_1 rhabditiform larva of Hookworm