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EVALUATION OF INTESTINAL PARASITE INFECTIONS IN HIV/AIDS PATIENTS ON ANTIRETROVIRAL THERAPY (ART) IN SELECTED HIV CLINICS IN CAPE COAST METROPOLIS.

By

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Intestinal parasites are a major concern in most developing countries. About 60% of the world's population are infected with intestinal parasites which are commonly associated with HIV/AIDS disease. This study was designed to assess the prevalence of intestinal parasite infections among adult HIV/AIDS patients on ART and those not on ART. This hospital-based prospective study was conducted in three selected HIV Clinics in the Cape Coast Metropolis. A total of 206 HIV positive patients aged from 18 to 65 years, of both sexes, were involved in the study from January 2011 to April 2012. Stool samples were collected from the participants and examined using direct wet mount, formol-ether concentration method, Kato-Katz technique and modified Ziehl-Neelsen (ZN) staining method. Blood samples were taken to analyze their CD4+ T-lymphocyte count at each encounter. The stool examination and blood analysis were repeated on their subsequent visits to the clinic for a period of six months. Questionnaire on sociodemographic and medical history of participants was administered. Data were analysed using SPSS version 19 software and p-value was set at $p<0.05$.The overall prevalence of intestinal parasites in this study was 47.6 % with a significant difference ($p=0.012$) between parasite-positive participants on ART (19.9%) and those not on ART (27.7%). Most of the parasite-positive participants (23.3%) had CD4+ counts below 200 cells/µL. Intestinal parasites detected in this study included: *Giardia lamblia* (18.0%), *Entamoeba histolytica* (12.1%), *Cryptosporidium* spp. (5.8%), *Isospora belli* (4.4%), *Strongyloides stercolaris* (3.9%), *Ascaris lumbricoides* (1.9%) and *Trichuris trichiura* (1.5%). *G. lamblia* and *E. histolytica* were the most prevalent. *Cryptosporidium* spp. ($p=0.021$) and *I. belli* ($p=0.020$) infections were significantly higher in participants who were not on ART, which participants were also significantly associated ($p=0.020$) with CD4+ counts below 200 cells/µL. *G. lamblia* ($p=0.002$), *E. histolytica* ($p=0.028$), *Cryptosporidium* spp. ($p=0.021$) and *I. belli* infections ($p=0.020$) were significantly associated with patients with diarrhoea stools. The overall prevalence of intestinal parasites of the study differed by ART status. Patients on ART had lower prevalence of intestinal parasites and most of the intestinal parasite infections were associated with lower CD4+ counts. This study has established that HIV/AIDS disease coexists with intestinal parasite infections, therefore, stool examination should be reinforced as part of the routine laboratory investigations for the management of HIV/AIDS patients.

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LIST OF ABBREVIATIONS

HIV - Human Immunodeficiency Virus

AIDS - Acquired Immunodeficiency syndrome

ART - Antiretroviral Therapy

CD4 - Cluster of Differentiation

UNAIDS - United Nations Programme on HIV/AIDS

NACP - National AIDS Control Programme

WHO - World Health Organization

GAC - Ghana AIDS Commission

MOH - Ministry Of Health

ELISA - Enzyme - Linked Immunosorbent Assay

PCR - Polymerase Chain Reaction

PFLP - Restriction Fragment Length Polymorphism

NCCLS - National Committee for Clinical Laboratory Standards

NRTIs - Nucleoside Reverse Transcriptase Inhibitors

NNRTIs - Non-Nucleoside Reverse Transcriptase Inhibitors

PIs - Protease Inhibitors

LMIC - Low and Middle Income Countries

GSS - Ghana Statistical Service

GHS - Ghana Health Service

CHRPE - Committee on Human Research Publications and Ethics

KNUST - Kwame Nkrumah University of Science and Technology

BD - Becton Dickinson

UCCH - University of Cape Coast Hospital

CRH - Central Regional Hospital

CCMH - Cape Coast Metropolitan Hospital

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Human Immunodeficiency Virus (HIV) is a retrovirus which causes Acquired Immunodeficiency Syndrome (AIDS) (Gurunathan *et al.*, 2009). AIDS is a clinical syndrome characterised by the progressive depletion of CD4+ T-lymphocyte population of the blood, leading to a progressive deterioration of the immune system and leaving the infected person vulnerable to a variety of infections (Gurunathan *et al.*, 2009). The clinical syndrome of AIDS was first recognised over 30 years ago and the discovery of HIV-1, the causative virus, followed soon after (Gelmann *et al.*, 1983). Since the beginning of the epidemic, about 75 million people have become infected with HIV and an estimated 36 million people have died of AIDS-related illnesses (UNAIDS, 2013).

The global statistics released by UNAIDS in 2013 indicated that in 2012, 35.3 million people were living with HIV/AIDS worldwide, with 2.3 million new infections and 1.6 million AIDS-related deaths (UNAIDS, 2013). In 2012, about 260,000 children were born with HIV, adding up to a total number of 3.3 million children less than fifteen (15) years living with HIV worldwide (UNAIDS, 2013). It was also estimated that 210,000 child deaths have occurred globally from HIV/AIDS in 2012, 90% of which occurred in sub-Saharan Africa (UNAIDS, 2013). Young people (15 years and above) accounted for around 40% of all new adult HIV infections worldwide (UNAIDS, 2013). The report also revealed that, adult HIV prevalence stood at 0.8% worldwide at the end of 2012 (UNAIDS, 2013).

Sub-Saharan Africa remains the region most greatly affected by HIV worldwide (UNAIDS, 2013). HIV/AIDS is one of the single most important health issues that disturbs the survival of millions in sub-Saharan Africa (UNAIDS, 2013). In 2012, sub-Saharan Africa accounted for 71% of HIV infections worldwide, 70% of new HIV infections among adults and 89% of new HIV infections among children (UNAIDS, 2013). It was reported again by UNAIDS that, in 2012, an estimated 1.6 million people were newly infected with HIV in sub-Saharan Africa, adding up to a total number of 25.0 million people living with HIV in the sub-region (UNAIDS, 2013). The sub-region also recorded an estimated 1.2 million adults and children deaths due to AIDS, accounting for 75% of the world's AIDS deaths in 2012 (UNAIDS, 2013).

The HIV/AIDS pandemic continues to confront the development and economy of many countries and Ghana is not an exemption (NACP, 2011). From 1986 to 2006, 121,050 cases of AIDS were reported by the National AIDS Control Programme (NACP), and in 2007, about 264,481 Ghanaians were estimated to be living with HIV (NACP, 2011). The estimated number of persons living with HIV and AIDS in Ghana in 2009 was 267,069, made up of 154,612 females and 112,457 males giving a female: male ratio of 1.4:1 (NACP, 2011). In the same year, there were 25,666 children living with HIV and estimated 3,354 children were newly infected. The annual AIDS deaths were estimated at 20,313 (NACP, 2011).

Presently the HIV prevalence in Ghana is 1.37% (GAC, 2013). It is firmly established within the whole society, that sub-populations with higher prevalence and risk of transmission constitute a reservoir for sustaining the epidemic (GAC, 2013). The estimated number of persons living with HIV/AIDS in Ghana in 2012 was 235,982

with 7,991 new HIV infections. This number was made up of 135,850 females and 100,132 males giving a female to male ratio of 1.4:1(GAC, 2013). In the same year, there were 27,734 children living with HIV and estimated 852 children were newly infected. The annual AIDS deaths were 11,655 which included 1,620 deaths of children (GAC, 2013).

HIV/AIDS patients can be managed by using antiretroviral therapy (ART) (Siddiqui *et al.*, 2007). Antiretroviral therapy is the use of anti-viral drugs to manage HIV/AIDS patients to help improve their immune system (Siddiqui *et al.*, 2007). The primary goal of ART for HIV infection is the suppression of viral replication (Siddiqui *et al.*, 2007). When the drugs are given in combination, HIV replication and immune deterioration can be delayed and the survival and quality of life improved (Siddiqui *et al.*, 2007). The use of ART by HIV/AIDS patients helps to improve their immune system, thus reducing their rate of having opportunistic infections including intestinal parasites (Vernazza *et al.*, 2000). ART can therefore contribute to control or reduce intestinal parasites in HIV/AIDS patients (Vernazza *et al.*, 2000).

Intestinal parasitic infections are among the most common infections world-wide (WHO, 2008). It is estimated that as much as 60% of the world's population are infected with intestinal parasites, which may play a significant role in morbidity (WHO, 2008). Opportunistic parasitic infections area common feature in HIV/AIDS infections where almost 80% of AIDS patients die of AIDS-related infections including intestinal parasites rather than of the HIV infection itself which usually occur late in the course of HIV infection when CD4+ T-cell count has been severely depleted mostlybelow200cells/µl (Shah *et al.*, 2003).

The rate of intestinal parasitic infection is remarkably high in Sub-Saharan Africa, where the majority of HIV and AIDS cases are concentrated and where factors including poverty and malnutrition could promote transmission of both infections in the region (UNAIDS, 2013). The incidence of intestinal parasitic infections is 50% in developed countries, whereas it reaches up to 95% in developing countries (UNAIDS, 2013). These infections are caused both by protozoa and helminths and the main clinical manifestation of the diseases caused by most of them is diarrhoea (Awole *et al.*, 2003). De Silva *et al*. (2003) gave an estimate that more than a quarter of the world's population were frequently infected with intestinal parasites and most of the people who are infected live in developing countries (De Silva *et al*., 2003).

Furthermore, in developing countries, acute gastroenteritis due to intestinal parasites is complicated and it is a major cause of illness, killing millions of AIDS patients annually (Adesiji *et al.*, 2007). Reports indicate that diarrhoea occurs in 30 – 60% of AIDS patients in developed countries, whereas it reaches up to 90% in developing countries (Siddiqui *et al.*, 2007). Opportunistic parasites have become the commonest pathogens affecting HIV infected patients, contributing to a major secondary aggravating factor of the disease (Feitosa *et al.*, 2001). These enteric infections frequently cause severe diarrhoea, which is often responsible for the seriousness of the disease and may sometimes lead to death (Feitosa *et al.*, 2001).

In general, diarrhoeal diseases are significant causes of morbidity and mortality in all age groups, but immunocompromised and paediatric patients experience more frequent and severe illnesses (Lekha *et al.*, 2008). Moreover, nowadays diarrhoea illnesses are becoming one of the most common clinically observable gastrointestinal

manifestations in AIDS patients, occurring at late stages of HIV infection; usually due to opportunistic infections (Evering and Weiss, 2006). Diarrhoea, both acute and chronic, affects 90% of people living with HIV/AIDS, causing significant morbidity and mortality (Lekha *et al.*, 2008). Intestinal parasitic infections, both protozoa and helminths are known to cause diarrhoea in patients (Gomez *et al.*, 1995). Moreover, parasites that are opportunistic in nature have been documented as a significant cause of diarrhoea in HIV/AIDS patients (Mohandas *et al.*, 2002).

Studies conducted in most African countries and elsewhere have demonstrated the presence of intestinal parasites as the cause of severe diarrhoea in HIV/AIDS patients (Gupta *et al*., 2008; Assefa *et al*., 2009; Kelly *et al*., 2009). Seventy percent (70%) of deaths from HIV occur in sub-Saharan Africa and one of the commonest complaints among these patients, which cause significant morbidity and mortality is diarrheoa (Nwachukwu and Okebe, 2008). Chronic diarrhoea can compromise the quality of life in both patients who are on antiretroviral therapy (ART) and those not on ART, since it can reduce the action of antiretroviral medication and nutrient absorption (Nwachukwu and Okebe, 2008).

In developing countries HIV infected patients are usually burdened with parasitic infection, which is a major health problem in sub-Saharan Africa (Smith *et al.*, 1998; Nworkediuko *et al.*, 2002). Among the parasitic infections are enteric parasites of helminths and protozoa (Nworkediuko *et al.*, 2002). It is known that helminths cause T-cell dysfunction, worsening the already devastated immune system of HIV patients (Borkow and Bentwich, 2004). Poverty and malnutrition are some of the predisposing factors that contribute to concomitant infections of both HIV and parasitic infections

in the sub region (Assefa *et al.*, 2009). Unfortunately, available data on the prevalence of intestinal parasitic infections are mostly centered on school-aged children (WHO, 2008).

HIV/AIDS infection manifests in individuals as serious gastrointestinal symptoms at various stages of the disease (Awadh and Anazi, 2009) and it is known that most opportunistic infections take place when the CD4+ T-cell count falls below 200 cells/ul (Assefa *et al*.,2009). Moreover, it has been established that 80% of T-cell population is found in the gastrointestinal tract making it an important site for HIV-induced immunodeficiency (Brenchley and Douek, 2008).

The devastating effect of HIV on the CD4+ T- cell, coupled with intestinal parasites, especially diarrhoea-causing parasites such as *Cryptosporidium*, will probably burden patients since conditions of diarrhoea will reduce ART and nutrient absorption (Smith *et al*., 1998). The infectious etiological agents include opportunistic and non-opportunistic agents that cause diarrhoea, which is a common presenting complaint in HIV infected individuals (Smith *et al*., 1998). Chronic diarrhoea, defined as persistence of diarrhoea beyond four weeks is a common symptom in HIV-infected patients in the tropics (Sarfati *et al.*, 2006). This may result in weight loss and wasting syndrome leading to profound morbidity and mortality (Modjarrad *et al.*, 2005; Sarfati *et al.*, 2006)

The presence of non-opportunistic parasites such as *Entamoeba histolytica*, *Giardia lamblia*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Ancyclostoma duodenale* in developing countries infect HIV/AIDS patients (Lucas,

1990). Opportunistic parasites play a major role in causing chronic diarrhoea accompanied by weight loss (Hammouda *et al.*, 1996). Among the species of opportunistic protozoa associated with diarrhoea in HIV/AIDS patients are; *Cryptosporidium parvum*, *Isospora belli*, *Microsporidium* species, and *Cyclospora* species (Assefa *et al*., 2009; Adamu and Petros, 2009). *Strongyloides stercoralis*, a nematode can cause diarrhoea and overwhelming infestation in patients with immunosuppressive disorders (Gupta *et al.*, 2008). Many studies of diarrhoeal disease in AIDS patients living in Africa were done in the pre-antiretroviral therapy (ART) era. That notwithstanding, there is little community-based information on the changes in susceptibility to intestinal parasite infections and diarrhoea in relation to the stage of HIV disease (Kalinkovich *et al.*, 1998).

HIV/AIDS patients in developing countries, unfortunately, continue to suffer the consequences of opportunistic parasites in the absence of ART (Maggi *et al.*, 2000). Patients who are enrolled into ART programmes with very low CD4+ T-cell counts have high risk of morbidity and mortality before ART (Lawn *et al.*, 2005). There is evidence that the control of these opportunistic parasitic infections in HIV-positive persons under highly active antiretroviral therapy (HAART) is also induced by the inhibition of the aspartylprotease of the parasites and by the reconstitution of the immune system of the patient (Alfonso and Monzote, 2011; Willemot and Klein, 2004). However, patients in resource-limited settings typically start ART programmes with advanced symptomatic disease and very low blood CD4+ T-cell counts which predisposes them to high rates of both clinical and subclinical opportunistic infections (Lawn *et al*., 2005).

An overlapping distribution of HIV and intestinal parasites becomes important because related infection of HIV and helminths may potentiate the virulence of each within a co- infected host (Modjarrad *et al*., 2005). It should be noted that these co-infection can have an influence on the intensity of HIV infection and the level of CD4+ T-cell count (Kaushal *et al.*, 2007). However, data on intestinal parasite infections and their relationship with diarrhoea and CD4+ T-cell count levels in HIV/AIDS patients in Ghana are limited and not elucidating (GAC, 2011).

1.2 STATEMENT OF PROBLEM

Intestinal parasites are prevalent in Ghana due to frequent shortage of clean drinking water, lack of proper sewage system and other unhygienic factors that increase the probability of infection (GHS, 2008). The unhygienic factors that contribute to high rate of intestinal parasite infections are common in the Cape Coast Metropolis (GHS, 2008). In recent times, several cases of HIV/AIDS infection have been recorded in hospitals and health centers in Ghana, with most of the cases in communities where there is migration of majority of people from neighbouring countries (GAC, 2011). The fishing activities of most of the fishermen and women in the Metropolis make them travel to and from neighbouring Ivory Coast which might contribute to the increase in the number of HIV cases in the Cape Coast Metropolis (GAC, 2011).

Routine stool examination has not been considered very important part of the routine tests done for HIV/AIDS patients who attend HIV Clinics, especially in Cape Coast Metropolis (Author, 2014). Therefore, there was the need to study the prevalence of intestinal parasites in the HIV/AIDS patients. The study was carried out to provide

data that will inform the Ghana AIDs Commission, National Aids Control Program and the ART service providers whether or not to add antihelminthic and antiprotozoan drugs to the ART regimen and ensure that routine stool examination is done regularly for all HIV/AIDS patients who attend the HIV Clinic.

1.3 JUSTIFICATION FOR THE STUDY

In hospital settings, prompt and accurate diagnosis of infection is critical for guiding clinical management of patients (Isenberg, 1998). The increase in the number of HIV/AIDS cases in Cape Coast Metropolis, with complications of gastroenteritis and diarrhoea call for proper investigation and diagnosis of the enteric infections in these patients. It has been reported that ART can reduce intestinal parasites in HIV/AIDS patients who are on the drug (Vernazza *et al.*, 2000). Currently in Ghana, there are very few or no reports detailing the interaction between HIV disease and intestinal parasites as well as the effect of ART on these parasites in HIV/AIDS patients who attend the HIV Clinic (GAC, 2011).

In addition, due to lack of adequate and appropriate techniques to diagnose some of the opportunistic intestinal parasites, there has not been much in-depth study on the relationship between HIV/AIDS disease and the prevalence of opportunistic intestinal parasites in Ghana (GAC, 2011). Thus, the purpose of this study was to assess the effect of ART on intestinal parasite prevalence and density in HIV/AIDS patients who attend HIV Clinic for treatment in the Cape Coast Metropolis, through follow up of the patients.

1.4 AIMS AND OBJECTIVES OF THE STUDY

The aim of the study was to determine intestinal parasite prevalence and density in HIV/AIDS patients who are on Antiretroviral Therapy (ART).

The specific objectives were:

1. To identify and quantify intestinal parasites in HIV/AIDS patients on ART in three selected HIV Clinics in Cape Coast Metropolis.
2. To determine the CD4+ T-lymphocyte counts of the patients and compare with the parasite density.
3. To compare the CD4+ counts of the patients with their ART status and parasite status.
4. To determine the sensitivity and specificity of the Wet Mount Method, Kato-Katz Technique, Modified Ziehl-Neelsen (ZN) Staining Method and the Formol-Ether Concentration Method for the diagnosis of intestinal parasites.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HIV/AIDS EPIDEMIC

2.1.1 HIV/AIDS Infection Worldwide

According to UNAIDS 2013 Report on the Global AIDS Epidemic, the estimated number of people living with HIV/AIDS worldwide in 2012 was 35.3 million, which included 3.3 million children under fifteen years and 32 million adults and adolescents (fifteen years and above) (UNAIDS, 2013). There were 2.3 million new infections in 2012 (UNAIDS, 2013). However, the new HIV infections among adults and adolescents decreased by 50% or more in 26 countries between 2001 and 2012 (UNAIDS, 2013). Moreover, new infections in children have declined by 52% since 2001, therefore 260,000 children became newly infected with HIV in 2012, a reduction from 550,000 in 2001 (UNAIDS, 2013).

The report also indicated that AIDS-related deaths have fallen by 30% since the peak in 2005. In 2012, 1.6 million people died from AIDS-related causes worldwide compared to 2.3 million deaths which occurred in 2005 (UNAIDS, 2013). This may be attributed to the fact that, in 2012, around 9.7 million people living with HIV/AIDS had access to antiretroviral therapy in low- and middle-income countries (UNAIDS, 2013). This indicates an increase from the 300,000 people who were receiving HIV treatment in 2002. Furthermore, in ten (10) low- and middle-income countries, more than 80% of those eligible were receiving antiretroviral therapy (UNAIDS, 2013).

According to the 2013 UNAIDS report, Sub-Saharan Africa remains the most affected region in the global AIDS epidemic (UNAIDS, 2013). The current estimates show that

sub-Saharan Africa accounted for 71% (25 million) of the people living with HIV worldwide and this includes 91% of the world's HIV-positive children (UNAIDS, 2013). Unlike other regions, the majority (61%) of people living with HIV in sub-Saharan Africa are women (UNAIDS, 2013). The report further estimated that, 1.6 million people in the region became newly infected and an estimated 1.2 million adults and children died of AIDS, accounting for 75% of the world's AIDS deaths in 2012 (UNAIDS, 2013).

2.1.2 HIV/AIDS infection in Ghana

The first reported cases of forty two HIV/AIDS patients in Ghana were recorded in 1986, mainly among women who had travelled outside the country (NACP, 2011). By the end of December 1999, a cumulative total of 37,298 cases had been recorded (NACP, 2011). Nearly 90% of the cumulative AIDS cases from 1986-1999 were between 15-49 years of age, with 63% of all reported HIV/AIDS cases being females (NACP, 2011). The National AIDS Control Programme (NACP) projected the average national prevalence rate to increase to 6.4% by 2004, 8.2% by 2009 and 9.5% by the year 2014 if the trend at that time continued (NACP, 2011).

According to the 2012 HIV Sentinel Survey and National HIV Prevalence, AIDS Estimates Reports released, the prevalence rate in Ghana dropped from 3.6 % in 2003 to 1.37% % in 2012 (GAC, 2013). An estimated 235,982 people made up of 27,734 children were living with HIV/AIDS in 2012 (GAC, 2013). Out of this number, 100,132 were males and 135, 850 were females. Two major age groups were most affected. These were 25-29 and 35-39 year olds. Each had prevalence of 3.5% (GAC,

2013). The survey also reported that, both HIV-1 and 2 were found among the Ghanaian population. HIV-1 is the predominant type accounting for about 96.8% (GAC, 2013). The prevalence of HIV-2 on the other hand is 1.4% while HIV-1 and 2 dual infection is estimated as 1.8%. Heterosexual transmission of HIV accounts for 75-80% of all HIV/AIDS infection (GAC, 2013). Vertical transmission (from mother to child) accounts for 15% while transmission through blood and blood products accounts for 5% (GAC, 2013).

2.2 OVERVIEW OF INTESTINAL PARASITIC INFECTIONS

2.2.1 Intestinal Parasitic Infections Worldwide

Intestinal parasitic infections are among the most common infections world-wide. It is estimated that some 3.5 billion people are infected, and 450 million are ill as a result of these infections worldwide (WHO, 2012). The rate of intestinal infection is extremely high in Sub-Saharan Africa, where the majority of HIV/AIDS cases are concentrated (WHO, 2012). These infections are caused both by protozoa and helminths and the main clinical manifestation of the disease caused by them is diarrhoea (Bundy *et al.*, 1992). In AIDS patients, opportunistic intestinal parasite infections cause severe diarrhoea, which compromise the absorptive function of the small intestine, that could lead to mortality of the patients (Bundy *et al.*, 1992).

Current estimates showed that at least more than one-quarter of the world's population is chronically infected with intestinal parasites and that most of these infected people live in developing countries (Franzer and Muller, 2011). However, intestinal parasites once considered to be controllable in developed countries remain a major cause of

morbidity and mortality worldwide. Remarkable expansion of the HIV/AIDS pandemic has brought about a significant change in the fauna of intestinal parasites all over the world (Gomez *et al*., 1995). Several other factors also contribute to the expansion and reinvasion of newly emerging intestinal parasites. Among these are, increasing migration of people due to political instability or wars, economical problems and travelling across developing countries are some of the main factors (Fincham *et al.*, 2003).

Furthermore, an increasing number of populations mainly in Africa and many parts of the developing world are severely immunocompromised because of HIV infection. As a result, some intestinal parasites are among the main health problems in HIIV/AIDS patients as concomitant infections due to depleted immunity. The opportunistic intestinal parasites are the major problems in such group of patients (Kaplan *et al.*, 1996).

2.2.2 Intestinal Parasitic Infections in Ghana

Like in many other developing countries, intestinal parasites are widely distributed in Ghana largely due to the low level of environmental and personal hygiene, contamination of food and drinking water that results from improper disposal of human excreta (GHS, 2008). In addition, lack of awareness of simple health promotion practices is also a contributing factor (GHS, 2008). Most health institutions lack appropriate and sensitive diagnostic methods to detect low levels of parasite burden (NCCLS, 1997). Furthermore, some of the diagnostic methods for specific

intestinal parasites, especially for the newly emerging opportunistic intestinal parasites, are not available to health facilities (NCCLS, 1997).

Intestinal helminths and protozoa which are commonly found in Ghana include: *Ascaris lumbricoides, Trichuris trichuria, Necator americanus /Acylostoma duodenale, Strongyloides stercoralis*, *Giardia lamblia* and *Entamoeba histolytica* (GHS, 2008). Common coccidian parasites that have emerged as a result of immunosuppression due to HIV include: *Isospora belli*, *Cryptosporidium spp.* and *Cyclospora cayetanensis*. (Assefa *et al*., 2009).

2.2.3 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 (WHO, 2012). With HIV/AIDS pandemic, many intestinal parasites have become opportunistic parasites causing uncontrollable life-threatening diarrhoea (Lindo *et al.*, 1998). Compared to developed countries, the prevalence of opportunistic intestinal parasites is expected to be higher in developing countries among HIV infected population (Lindo *et al.*, 1998). This is also reflected by the prevalence of opportunistic intestinal parasites in a given geographical locality among the general population (Lindo *et al*., 1998).

Furthermore, HIV infection has been shown to predispose the patient to intracellular opportunistic intestinal parasites such as *Cryptosporidium parvum, Isospora belli* (Wittner *et al.*, 1993). This does not seem to be the case with extracellular intestinal parasites such as *A. lumbricoides, T. trichiura*, *Hookworm*, *G. lamblia,* and others

(Wittner *et al.*, 1993). 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 *et al*., 1998). Clinical presentations of AIDS and the pathogens responsible in different geographical areas reflect the differing prevalence of opportunistic intestinal parasitic infections in a given community (Colebunders *et al.*, 1988).

2.2.4 Clinical indications of intestinal parasite infections

Intestinal parasite infections are mostly chronic and mild, and they are also usually asymptomatic or subclinical (Udonsi, 1984). However, several clinical signs and symptoms can occur in patients with moderate and heavy infections (Neva and Brown, 1994). During the first 1-2 weeks after a cutaneous infection with helminths such as *Strongyloides stercoralis, Necator americanus* and *Acylostoma duodenale,*, an intensely pruritic dermatitis will occur at the site of infection called ground itch and larval invasion of the lungs may produce respiratory symptoms called Löeffler or Löeffler-like syndrome (Neva and Brown, 1994). This syndrome is characterized by pneumonitis which can be accompanied by paroxysmal attacks of cough, coughing with blood-tinged sputum, wheezing, dyspnea, pleurisy, low grade fever, substernal pain, urticaria, asthma and eosinophilia (Coombs and Crompton, 1991). Adult worms in the intestine commonly cause abdominal pain (Spurchler, 1987). Other enteric symptoms reported include abdominal cramps/colic, intestinal blockage, nausea and/or vomiting (rarely), tenesmus, diarrhoea, constipation (occasionally) and dysentery (Vadlamudi *et al.*, 2006).

2.2.5 Immune Activation by Intestinal Parasites and its possible relation to HIV/AIDS Co-infection.

Different investigators have shown that infection by intestinal parasites enhances immune activation and has been suggested to contribute to progression of HIV infection (Bentwich *et al.*, 1995). In a study conducted in Ethiopia, there was a significant correlation between the number of excreted worm eggs and plasma viral load (Wolday *et al.*, 2002). Furthermore, there was 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 or were not present at all. These findings indicate that helminth infections may enhance HIV multiplication and increase plasma viral load, thereby contributing to HIV disease progression (Wolday *et al*., 2002).

Other studies have revealed that intestinal parasitic infections have interaction with immunological effectors such as T-cell subsets (CD4+ and CD8+) (Kalinkovich *et al.*, 1998). It has been indicated that even healthy Ethiopians (HIV Negatives) have lower CD4+ and higher CD8+ counts as compared to Europeans and other Africans and it is hypothesized that environmental pathogens such as intestinal parasites could play a role (Messele *et al.*, 1999).

2.2.6 Diarrhoea and HIV/AIDS

Diarrhoea is a common clinical manifestation of HIV infections both in the developing (90%) and the developed (30-50%) countries (Colebunders *et al.*, 1988). Its cause could be quite variable: bacterial, viral or parasitic (commonly opportunistic

intestinal parasites) (Germani *et al.*, 1998). Chronic diarrhoea lasting for more than one month is one of the major complaints of AIDS patients occurring in about 40% of cases and it is one of the WHO-staging criteria for AIDS (WHO, 2012).

Furthermore, it has been shown that at least 40-80% of AIDS patients report diarrhoeal episodes during their illness (Kelly, 1998). About 50% of chronic diarrhoea in AIDS patients may be explained by enteric infections with one or more species of pathogenic organisms, commonly opportunistic ones (Bartlett *et al.*, 1992). Gut architectural alteration secondary to local HIV infection, (usually referred to as HIV entropathy) a condition characterised with chronic diarrhoea in AIDS patients in whom no identifiable aetiological agent has been found for the diarrhoea (Bartlett *et al.*, 1992).

Patients with this syndrome have malabsorption, and small bowel histology revealing villous blunting and chronic inflammation (Kotler *et al.*, 1984). There is another view that enteropathy syndrome may not represent a direct effect of HIV on gut mucosa, but rather could be due to opportunistic enteric pathogens that are difficult to detect and therefore as yet undiagnosed (Dallabetta and Miotti, 1992). It is also held that HIV related entropathy is not only the cause of unexplained diarrhoea, but may also create favourable environment for the invasion of intracellular opportunistic intestinal parasites (Germani *et al.*, 1998).

2.3 COMMON INTESTINAL PARASITES IN HIV/AIDS PATIENTS

HIV/AIDS pandemic has brought about a great change in intestinal parasite fauna (Kelly, 1998). As the spectrum of immunodeficiency progresses, HIV infected

individuals become susceptible to a variety of opportunistic parasite infections that occur with greater frequency and severity (Kelly, 1998). Almost 80% of AIDS patients die from AIDS-related infections including intestinal parasites rather than HIV infection itself (Kelly, 1998). Several intestinal parasites previously considered non- pathogenic or with transient pathogenic potential in immunocompetent individuals are opportunistically becoming aggressive and causing debilitating illness in HIV/AIDS patients (Kaplan *et al.*, 1996). Most of these infections are caused by organisms that do not normally affect immunocompetent individuals (Kaplan *et al.*, 1996).

The principal pathogenic intestinal parasites commonly reported as opportunistic that cause chronic diarrhoea in HIV/AIDS patients are *Cryptosporidium parvum and Isospora belli*, *Cyclospora cayetanensis* and intestinal microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*) (Kaplan *et al.*, 1996). These infections usually occur late in the course of HIV infection when CD4+ T-cell count has been severely depleted (mostly below 200 cells/µLand in case of intestinal microsporidia below 100 cells/µL) (Kaplan *et al.*, 1996).

(a) Cryptosporidium parvum

Cryptosporidium parvum has been reported to be increasingly recognized agent of intestinal infection as a common cause of severe diarrhoea in immunocompetent and immunocompromised humans and domestic animals (Hunter and Nicholis, 2002). The major clinical symptoms are watery diarrhoea, malabsorption and wasting syndromes (Hunter and Nicholis, 2002). The severity of the disease depends on the immune status of the individuals (Martins and Guerrant, 1995). The nature of diarrhoea is usually

secretory or malabsorpative, voluminous, intractable, watery and often cholera-like (Clark and Sears, 1996). Severe weight loss (as much as 25kg) has been reported due to debilitating diarrhoea (Clark and Sears, 1996). Abdominal pain with cramps, low-grade fever, vomiting, nausea and anorexia accompanies the diarrhoea (Bartlett *et al.*, 1992). The diarrhoea can be bright yellowish-green in colour, offensive and may contain mucus (Bartlett *et al.*, 1992).

The development of chronic cryptosporidiosis in AIDS patients has been correlated with reduced CD4+cell count (Flanigan *et al.*, 1992). That is, the severity of the disease is manifested in AIDS patients usually when the CD4+ cell count is below 200cells/µL (Lopez-Velez *et al*.,1995; McDonald, 2000). The number of CD4+ cell count is higher in AIDS patients with diarrhoea when intestinal cryptosporidiosis is not involved. Flanigan *et al*. (1992) also reported that patients with CD4+ cell count of less than 180 cells/µL have persistent infection, while patients with CD4+ cell count greater than 200cells/µL have a transient or self- limited infection (Flanigan *et al*., 1992).

Some studies have indicated that self-limited cryptosporidiosis might be associated with a more intact immune system as reflected by a higher absolute CD4+ cell count (Flanigan, 1994). Flanigan *et al*. (1992) had also demonstrated that individuals with CD4+ cell count above 500 cells/µL do spontaneously clear *Cryptosporidium* infection, while it is only patients with CD4+ cell count 140cells/µL or less that develop chronic life threatening diarrhoea (Flanigan *et al*., 1992). Thus, the CD4+ cell count is used as a marker of the ability of an individual's immune system to respond appropriately to *Cryptosporidium* infection at the mucosal surface (McDonald, 2000).

(b) Cyclospora cayetanensis

Another newly defined coccidian opportunistic intestinal parasite in humans is *Cyclospora cayetanensis* (Curry and Smith, 1998). Cyclosporiosis is characterised by mild to severe watery diarrhoea, nausea, anorexia and abdominal cramps. It has been described from HIV/AIDS patients with protracted diarrhoea (Sifuenes-Osonio *et al.*, 1995). In immunocompetent individuals diarrhoea appears to be prolonged but self-limited, lasting from just over one week to a mean duration of about three weeks (Sifuenes-Osonio *et al.*, 1995). Although infections appear eventually to resolve spontaneously, in some cases, both in immunosuppressed and immunocompetent individuals, patients have been successfully treated with oral Trimethoprim-Sulfamethoxazole that produces a rapid improvement of the symptoms, but in AIDS patient recrudescence of the symptoms after treatment is a major problem (Sifuenes-Osonio *et al*., 1995). Cyclospora resembles *Cryptosporidium*, but the size varies ranging from 8 -10µm with 2 sporocysts and having 2 sporozoites in each sporocyst, whereas *Cryptosporidium* measures 4-6µm in size and has 4 naked sporozoites (Curry and Smith, 1998).

(c) Isospora belli

Isospora belli is another well-defined coccidian opportunistic intestinal parasite in HIV/AIDS patients and in some areas it is the cause of gastroenteritis (Lumb and Hardiman, 1991). It mostly causes watery diarrhoea and weight loss (Lindsay *et al.*, 1997). Diarrhoea produced by *I. belli* infection in AIDS patients is often secretory-like, without blood and leads to dehydration; low grade fever, eosinophlia, abdominal pain, vomiting and malaise are some of the symptoms reported (Lindsay *et al.*, 1997).

The transmission of *I. belli* in humans occurs via faecal-oral route, mainly by ingestion of infectious oocysts from contaminated food and/or water (Lindsay *et al.*, 1997). Diagnosis depends on microscopic identification of oocysts in the stool. Like *Cryptosporidium*, the oocysts of *I. belli* are acid fast during staining, but differ from oocyst of *Cryptosporidium* by their size and shape (Cheesbrough, 2009). The treatment of *I. belli* is by use of Trimethoprim-Sulfamethoxazole (Lindsay *et al.*, 1997). Prophylaxis for *Pneumocystis carinii* pneumonia in HIV/AIDS patients may effectively prevent the acquisition of primary *I. belli* infection or the recrudescence of existing infection (Lindsay *et al.*, 1997).

(d) Intestinal Microsporidia (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*)

Other important intestinal parasites in HIV/AIDS patient are the microsporidia (Curry and Smith, 1998). They are ubiquitous, obligate intracellular spore forming protozoan parasites increasingly detected as opportunistic pathogens in HIV/AIDS patients (Weber *et al.*, 1994). Since the onset of HIV/AIDS pandemic, a number of parasitic microsporidian pathogens of humans have been recognized (Weber *et al.*, 1994). The frequency with which they are encountered and reported in clinical practice and the intensity of infections with opportunistic microsporidian parasites in AIDS patients have tremendously increased (Weber *et al.*, 1994). Infection by *Enterocytozoon bieneusi* and / or *Encephalitozoon intestinalis* have been identified as main causes for watery diarrhoea and wasting syndrome in AIDS patients, when the CD4+ cell count is below 100 cells/μL (Asmuth *et al.*, 1994).

Infection in humans can occur in different tissues including the small intestine, kidney, cornea and liver with various clinical manifestations (Bryan, 1995). Diarrhoea and malabsorption are the most common clinical syndromes associated with intestinal microsporidial infections in AIDS patients (Bryan, 1995).

(e*) Blastocystis hominis*

Diarrhoeagenic intestinal parasites that were not recognized as such up to the recent past are emerging and increasing these days (Jelinek *et al.*, 1997). The problem has become more serious with onset of HIV/AIDS pandemic (Jelinek *et al.*, 1997) . Among these the status of *Blastocystis hominis* as a cause of diarrhoea is a controversial and not well-documented one (Jelinek *et al.*, 1997). Although *Blastocystis hominis* is often the most frequently reported from stool samples, its epidemiology is not clearly understood (Jelinek *et al.*, 1997). The reason behind this could be lack of appropriate information about the epidemiology of the parasite, conflicting and paradoxical ideas on its classification and pathogenicity (Jelinek *et al.*, 1997). Based on ultrastuctural and structural evidences, *Blastocystis hominis* has now been classified under protozoa (Stenzel and Boreham, 1996).

The association of *Blastocystis hominis* with diarrhoea in immunosuppressed patients has been suggested in one study among Tanzanian children with chronic diarrhoea (Cegielski *et al.*, 1993). Furthermore, molecular and immunological evidences have revealed that strain variation might be associated with pathogenic potentials (Kaneda *et al.*, 2002). It is generally accepted that *Blastocystis hominis* is transmitted by faecal-oral contamination, in a manner similar to other gastrointestinal protozoa (Leelayoova *et al.*, 2004).

Other intestinal parasite infections that remain prevalent in sub-Saharan Africa as well as Ghana include: *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercolaris, Taenia* spp, intestinal schistosomiasis infection caused by *Schistosoma mansoni* and hookworm infections (Brooker *et al.*, 2000). A record of spurious and genuine *Dicrocoelium* infections in man have been reported in Ghana (Odei, 1966) and elsewhere in Sierra Leone (King, 1971).

2.4 LABORATORY PROCEDURES FOR THE DIAGNOSIS OF INTESTINAL PARASITES

Definitive diagnosis of parasitic infections depends on demonstration of a stage of the parasite's life cycle in the human host (Garcia *et al.*, 2000). The adult worms, that inhabit the intestine, discharge the eggs or larvae they produce in faeces (Neva and Brown, 1994). Therefore, laboratory diagnosis of intestinal parasites is based on detection and identification of characteristic eggs or larvae in stool samples (Parija and Srinivasa, 1999). A wide variety of laboratory methods, including parasitologic, molecular, serologic and cultural approaches, have been developed over the years for diagnosis of intestinal parasites (Markell *et al.*, 1999).

2.4.1 Visual observation of nature of stool sample (Macroscopic examination)

Helminthic infections can induce digestive abnormalities and influence the nature or consistency of stool produced (Garcia, 2001). The macroscopic appearance of stool specimen can give a clue to the type of organisms present (Goodman *et al.*, 2007). Adult worms of *Ascaris, Enterobius* and tapeworm proglottids may be seen when

fresh specimens are visually examined. Fecal specimens are described as formed, semiformed, soft, loose, or watery (Goodman *et al.*, 2007).

2.4.2 Parasitological methods (Stool microscopy)

Microscopic or parasitologic diagnosis is generally sensitive, simple, and economical (Parija and Srinivasa, 1999). If performed correctly, stool microscopy offers many advantages over other diagnostic methods for detecting intestinal parasites (Bogoch *et al.*, 2006). Diagnostic tests involving microscopy include direct wet preparations, concentration methods and the Kato-Katz technique (Watson *et al.*, 1988).

2.4.3 Direct Wet Mount Method

Direct wet mount involves microscopic examination of fresh faecal specimens by wet preparations with physiological saline (saline wet mount) or iodine solution (iodine wet mount) or 1% aqueous solution of eosin (eosin wet mount) (Isenberg, 1998). The procedure provides rapid diagnosis for intestinal parasites when they are present in sufficient density in the faecal sample (Ukaga *et al.*, 2002).

The method is useful for detecting organism motility, including motile larval forms of *Strongyloides stercoralis* and trophozoites of intestinal protozoa (Watson *et al.*, 1988). The technique is also useful for diagnosis of parasites that may be lost in concentration techniques (Melvin and Brooke, 1985). It is particularly useful for the observation of motile protozoan trophozoites and the examination of certain diagnostically important objects such as Charcot-Leyden crystals and cellular exudates (Parija and Srinivasa, 1999).

2.4.4 Concentration Methods

Concentration techniques increase sensitivity of stool microscopy to allow the detection of small numbers of organisms that may be missed by using only a direct wet smear (Allen and Ridley, 1970). Basically, concentration techniques operate in two ways, either by sedimentation (Ritchie, 1948) in which the parasite sink to the bottom of the liquid suspension, or by flotation (Truant *et al.*, 1981) in which the parasite forms are suspended in a liquid of high specific density to make them buoyant and float to the surface where they are collected for examination (Truant *et al.*, 1981). The formalin-ether concentration procedure as described by Allen and Ridley (1970) provide the best diagnostic outcome in epidemiological studies (Akujobi *et al.*, 2005). The technique requires the use of formalin as a fixative and ether or ethyl acetate or gasoline as a lipid removing agent (Wirkom *et al.*, 2007). It uses formalin to fix and preserve the faecal specimen and ether or ethyl acetate to extract debris and fat from the faeces, leaving the parasites at the bottom of the suspension (Akujobi *et al.*, 2005). Authors consider the formol-ether concentration as the most effective technique that recovers the broadest range of organisms, and hence, the "gold standard" method of all parasitological techniques (Cheesbrough, 2009).

The advantages of this method are that it will recover most ova, cysts and larvae and retain their morphology, thereby facilitating identification (Neimeister *et al.*, 1987). There is less risk of infection from bacteria and viruses because they may not be able to survive the concentration process involved (Akujobi *et al.*, 2005). The concentration technique has additional advantage by allowing for transportation and storage after faeces are preserved in formalin (Oguama and Ekwunife, 2007).

2.4.5 Kato-Katz technique

The Kato-Katz technique is useful for the quantitative estimation of worm burdens (Markell *et al.*, 1999). It is especially useful for field surveys for helminth infections since it provides estimates of the intensity of infection (Markell *et al.*, 1999). According to Martin and Beaver (1968), the technique entails the examination of a standard sample (determined by the size of the template) of fresh faeces pressed between a microscope slide and a strip of cellophane that has been soaked in glycerine (Martin and Beaver, 1968).

Limitations of this method include difficulty in processing diarrhoeal stools and lack of sensitivity if only a single stool sample is examined (Hines and Nachamkin, 1996; Knopp *et at*., 2008). Counting of eggs in Kato-Katz smears can be a tedious and time consuming process, and can lead to technical errors (Kato and Miura, 1954). Other drawbacks of the method include high risk of infection for the technicians handling fresh stools (Ebrahim *et al.*, 1997). Hookworm eggs clear rapidly, and if slides are not examined within 30-60 minutes, the eggs may no longer be visible (Garcia, 2001). The technique is also known to be unsuitable for detection of cysts, larvae, small fluke eggs or thin-shelled eggs such as *Hymenolepis* species because eggs disappear during the clearing process in a short time of 30-120 minutes (Knopp *et al.*, 2008).

2.4.6 Serological (Immuno-diagnostic) methods

There are increasing availabilities of non-microscopic methods, such as DNA probes, direct fluorescent antibody methods and enzyme-linked immunosorbent assay (ELISA) (Farthing, 1994). An enzyme-linked immunosorbent assay (ELISA) using

larval antigen is employed for the diagnosis helminthic infections when larvae cannot be found through microscopy (Garcia, 2001).

Serological tests are of value in the diagnosis of acute trichinosis and strongyloidiasis (Smith and Bartlett, 1991). They have proved to be most useful for distinction between acute and chronic *Schistosoma mansoni* infection (Valli *et al.*, 1997). Serological methods are sensitive but are expensive for use in the developing world and may show cross reactivity with other helminthic infections. Another disadvantage of serodiagnositic approach is that tests might remain positive even after cure by chemotherapy (Knopp *et al.*, 2006).

2.4.7 Molecular diagnosis

Molecular techniques such as polymerase chain reaction (PCR) using primers derived from different genetic markers are useful diagnostic tools (Michaud *et al*., 2003). The technique is desirable in differentiating two morphologically identical species such as *A. duodenale* and *N. americanus* (De Gruijter *et al.*, 2005). PCR amplified fragments can be analyzed by using restriction fragment length polymorphisms (PCR-RFLP). PCR analysis has been used to distinguish the two human hookworms *Ancylostoma duodenale* and *Necator americanus* and from infection with *Oesophagostomum biurcum*, whose eggs are morphologically indistinguishable from hookworm (Verweij *et al.*, 2001). These "high-technology" methods are sensitive and specific, and allow distinction between morphologically identical parasite species. However, they are often too expensive for use in the developing world (Hotez *et al.*, 2006).

2.4.8 Cultural techniques (Coprocultures)

Faecal culture or coproculture involves in-vitro breeding of hookworm and other intestinal nematode larvae (Jozefzoon and Oostburg, 1994; Arcari *et al*., 2000). It is useful for detecting latent infections and for the diagnosis of *Ancylostoma duodenale, Necator americanus and Strongyloides stercoralis* infections (Hsieh, 1961; De Kaminsky, 1993). Coproculture is essentially a concentration method as the procedure is used for recovery of larvae when they are too scanty to be detected by other parasitological methods (Markell and Voge, 1976). Standard methods of culturing hookworm and *Strongyloides* larvae include the Baermann (Garcia, 2001), Harada-Mori test tube (Harada and Mori, 1955), agar-plate (Koga *et al*., 1990) and charcoal cultures (Smith and Bartlett, 1991), each of which has been modified in various ways to enhance yield or ease of maintenance (Hsieh, 1961; Koga *et al*., 1990). The specimen to be cultured must be fresh stool that has not been refrigerated, because some parasites (especially *Necator americanus*) are susceptible to cold and may fail to develop after refrigeration (Smith and Schad, 1990).

In general, cultural methods are not suitable for routine diagnostic practices or for screening asymptomatic patients because they require too much time to be clinically useful (Smith and Bartlett, 1991; Isenberg, 1998). Therefore faecal culture largely remains a research technique, where rapid results are not that important (Jozefzoon and Oostburg, 1994; WHO, 2006).

2.4.9 Choice of diagnostic method in routine parasitology laboratory

In hospital settings, prompt and accurate diagnosis of infection is critical for guiding clinical management of patients (Isenberg, 1998). The choice of a particular technique for routine use is influenced by its affordability (low cost), simplicity (ease of performance), sensitivity (effectiveness in detecting parasites in scanty numbers) and the level of technical skill involved (NCCLS, 1997; Melvin and Brooke, 1985; WHO, 2006).

In developing countries, routine methods are chosen largely on account of their affordability and rapidity (i.e. cheap, easy to carry out, and non-time-consuming procedure), and often disregarding the sensitivity and consequences of misdiagnosis that may ensue from employing a method of low sensitivity (Wirkom *et al*., 2007; Oguama and Ekwunife, 2007). For instance, many authors have indicated that the direct wet mount technique lacks sensitivity (Smith and Bartlett, 1991; Ukaga *et al*., 2002; Markell and Voge, 1976), yet because the method is inexpensive, simple and rapid to perform, hospital laboratories in developing countries rely on it as the main diagnostic tool for routine stool examinations (Cheesbrough, 2009).

2.5 THE ROLE OF ANTIRETROVIRAL THERAPY IN HIV/AIDS MANAGEMENT

There is currently no vaccine for HIV infection. One of the ways that an HIV/AIDS patient is managed is the use of Anti-Retroviral Therapy (ART) (Furtado *et al.*, 1999). The primary goal of ART for HIV infection is the suppression of the viral replication (Furtado *et al.*, 1999). When the drugs are given in combination, HIV replication and

immune deterioration can be delayed and the survival and quality of life improved (Furtado *et al.*, 1999).

However, there were changes in the strategies for the treatment of HIV infection when it was recognized that viral replication occurs during the years preceding the development of clinical disease (Furtado *et al.*, 1999). HIV infects cells with CD4+ receptors which are able to fuse with *gp 120* in the viral coat causing entry into the cells (Furtado *et al.*, 1999). Cells with this receptor in the body are predominantly T-lymphocytes which play a major role in cellular immunity in response to invasion of the body by foreign substances (Furtado *et al.*, 1999). Between the time of initial infection and the development of the clinical disease, the T-lymphocytes CD4+count progressively declines as a result of their destruction by the HIV after they have been used as site for viral replication (Furtado *et al.*, 1999). This results in the lesser number of T-lymphocytes production with a corresponding progressive suppression of the immune system (Furtado *et al.*, 1999).

Potent antiretroviral therapy against HIV infection results in a marked suppression of HIV-RNA concentration in the blood of infected individuals (Vernazza *et al.*, 2000). The effect of the therapy has also been associated with quite significant increases in absolute CD4+ count, CD4+ cell function and a reduction in mortality (Egger *et al.*, 1997). Currently, the standard care for the treatment of HIV-1 infection involves combination of antiretroviral drugs which includes the use of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) with either Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) or Protease Inhibitors (PIs) (Carpenter *et al*., 1998). The drugs which are combined for the treatment of HIV infection are: Zidovudine (ZDV),

Lamivudine (3TC), Stavudine (D4T), Efavirenz (EFV), Nevirapine (NVF), Combivir (CBV), and Abacavir (ABC) (WHO, 2012). Antiretroviral therapy has dramatically improved the survival of HIV infected individuals and is critically needed to save millions of lives (WHO, 2012). In a substantial fraction of patients receiving triple drug combination, HIV-RNA can no longer be detected in blood, even with the use of highly sensitive Polymerase Chain Reaction (PCR) technology (Savasi *et al.*, 2007).

In 2008, World Health Organization, (WHO), estimated that 2,015,000 people living with HIV/AIDS were receiving treatment in Low and Middle Income Countries (LMIC), representing 28% (24%-34%) of the estimated 7.1 million people in need (WHO, 2012). Antiretroviral therapy (ART), if prescribed and taken appropriately, is associated with dramatic reductions in blood HIV RNA load, often to undetectable levels (Kaul *et al.*, 2008). However, it is not established whether potent antiretroviral therapy is associated with a reduction of the infectiousness of treated individuals (Porco *et al.*, 2004).

2.6 CLASSES OF ANTIRETROVIRAL DRUGS

There are different classes of antiretroviral drugs that act at different stages of the HIV life-cycle.

(a) Nucleotide and Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

Drugs in this class inhibit reverse transcription by being incorporated into the newly synthesized viral DNA and preventing its further elongation (Weller and Williams, 2001). Examples of drugs in this class are zidovudine (AZT), stavudine (d4T),

lamivudine (3TC), emtricitabine (FTC), didanosine (ddl), abacavir (ABC) and tenofovir (TDF) (Weller and Williams, 2001).

(b) Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

Drugs in this class inhibit reverse transcriptase directly by binding to the enzyme and interfering with its function. Examples include nevirapine (NVP) and efavirenz (EFV). (Weller and Williams, 2001).

(c) Protease Inhibitors (PIs)

Protease inhibitors (PIs) target viral assembly by inhibiting the activity of protease which is an enzyme used by HIV to cleave nascent proteins for final assembly of new virons. Examples are saquinavir (SQV), indinavir (IDV) and nelfinavir (NFV) (Weller and Williams, 2001).

(d) Integrase Inhibitors

Drugs in this class inhibit the enzyme integrase, which is responsible for integration of viral DNA into the DNA of the infected cell. An example is raltegravir (De Soultrait *et al.*, 2002).

(e) Entry Inhibitors of Fusion Inhibitors

These drugs interfere with binding, fusion and entry of HIV-1 to the host cell by blocking one of several targets. Maraviroc and enfuvirtide are the two currently available agents in this class (Kilby *et al.*, 1998).

(f) Maturation Inhibitors

These drugs inhibit the last step in gag processing in which the viral capsid polyprotein is cleaved, thereby blocking the conversion of the polyprotein into the mature capsid protein (p24) (Weller and Williams, 2001). Because these viral particles have a defective core, the virion released consists mainly of non-infectious particles (Weller and Williams, 2001). There are no drugs in this class currently available, though two are under investigation, bevirimat and Vivecon (Weller and Williams, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was a hospital-base prospective study conducted in selected HIV Clinics in the Cape Coast Metropolis, Central Region. HIV patients aged 18 years and above attending the HIV Clinics for Antiretroviral Therapy from January 2011 to April 2011 were recruited after fulfilling the inclusion criteria. In this study, the first time of sample collection from the participants was called First Contact. Follow-ups were done for the participants from February 2011 to April 2012. These were described as Second Encounter, Third Encounter, Fourth Encounter and Fifth Encounter which represent the number of times samples were collected from the participants for the study. Stool samples were taken from the participants and examined for intestinal parasites at the beginning of the study. This was repeated on their subsequent visits to the Clinic for a period of six (6) months. Blood samples of the participants were taken to analyze their CD4+ T-lymphocyte count level at each time of the faecal specimen collection.

3.2 STUDY AREA

The study was carried out in three (3) selected HIV Clinics in the Cape Coast Metropolis which included Central Regional Hospital, Cape Coast Metropolitan Hospital and University of Cape Coast Hospital. The Cape Coast Metropolis is bounded on the south by the Gulf of Guinea, west by the Komenda / Edina / Eguafo / Abrem Municipal, east by the Abura / Asebu / Kwamankese District and north by the Twifu / Hemang / Lower Denkyira District. Cape Coast is the capital of the Central

Region(Ghana Districts, 2011). The Metropolis covers an area of 122 square kilometers and it is the smallest metropolis in the country with an estimated population of 169,894 (GSS, 2012).

The Cape Coast Metropolis experiences high temperatures year round. The hottest months are February and March, just before the main rainy season, while the coolest months are between June and August. The invariability of the climate in the metropolis is influenced more by rainfall than temperature. Cape Coast experiences relatively high temperatures throughout the year and is humid. Natural vegetation consists of shrubs, grasses and a few scattered trees. The present vegetation of the municipality consists of shrubs of about 1.5 meters high, grass and a few scattered trees. The original vegetation of dense shrubs, which the rainfall supported, has been replaced by secondary vegetation as a result of clearing for farming, charcoal burning, bushfires and other human activities. The major occupation of the people is fishing (Ghana Districts, 2011).

3.3 STUDY POPULATION

The study included all HIV patients who visited the HIV Clinics for antiretroviral therapy. A total of 206 participants of both sexes, aged 18 years and above, who were diagnosed and confirmed as HIV positive, and then referred to the HIV Clinics were involved in this study. The control group consisted of 60 HIV positive participants who were parasite-negative at the first contact of which 30 were on ART and 30 were not on ART. The sample size for the study was calculated using the formula below by Merrill (2009*)*.

Sample size: $n \geq \frac{Np(1-p)}{(N-1)D + p(1-p)}$

Where: $D = \frac{(Margin\ of\ Error)2}{Z\alpha/2}$

n: represents the sample size

D: represents the acceptable margin of error for the sample size being estimated.

Zα/2: represents the number of standard deviations relative to the mean of the standard normal curve corresponding to the level of confidence. In other words if the level of confidence is 95%, then α = 5%, and α/2 = 2.5%. Therefore the Z α/2 value is 1.96.

The Margin of Error: is a value added to and subtracted from the estimate which establishes an interval, which interval contains the true population parameter given a certain level of confidence. Using a level of confidence of 95% and an error margin of 5% or 0.05 the acceptable margin of error can be estimated.

P: is the prior assumption of the population parameter. If no information is available, **p** should be assumed to be 0.5.

N: represents the population size, which was recorded to be 4,192 (the total number of persons living with HIV/AIDS in the Cape Coast Metropolis as at December 2010) by statistical data of Cape Coast Metropolitan Directorate of Health Services.

Substituting the values into the above equation gives:

$$D = \frac{(0.05)2}{1.96}$$

Or D = 0.00128

$$n \geq \frac{(4192)(0.5)(0.5)}{(4191)0.00128 + 0.5(0.5)}$$

Or $n \geq 186.66 \geq 187$

The correct sample size should be 187. Merrill (2009) observed that, the sample size should be rounded up to the nearest integer.

From the statistical records of HIV Clinics in the Cape Coast Metropolis the dropout rate of patients was ten (10) percent (10%). The dropout rate of the study was calculated as a percentage of the sample size obtained and then added to it as follows:

Dropout rate = 10% of 187 = 10 / 100 x 187 = 18.7

Therefore the final sample size = 187 + 18.7 = 205.7, which was approximately 206.

3.4 INCLUSION AND EXCLUSION CRITERIA

The study involved one hundred and one (101) HIV patients who were referred to the HIV Clinic to start ART and one hundred and five (105) HIV patients who were on ART for less than six months. The exclusion criteria were HIV patients who were already on ART for more than six (6) months before the start of the study.

3.5 ETHICAL ISSUES

This study was approved by the Committee on Human Research, Publications and Ethics (CHRPE), of School of Medical Sciences, Kwame Nkrumah University of Science & Technology (KNUST), Kumasi. Permission to undertake the study at the HIV Clinics of the three hospitals was sought and granted by the hospital management. The participants enrolled in the study gave written informed consent after full explanation of the purpose and the techniques of the study was given to them. The study participants who had parasites in their stool samples were treated free of charge based on the Ghana Health Service treatment guidelines. The drugs

were administered by the clinicians working at the study sites. The full cost of treatment of each participant was absorbed by the principal investigator.

3.6 SAMPLING METHODS

The HIV/AIDS patients who reported at the HIV clinics were first examined by the nurses and clinicians in charge. They were then referred to the laboratory for investigations such as CD4+ count, full blood count, liver function test, renal function test, hepatitis B surface antigen test and sputum for Acid Fast Bacillus (AFB). The health status of all participants prior to enrolment into the study was assessed by the nurses and clinicians based on the results of the above investigations. A questionnaire was administered to gather information from all consenting participants on their age, sex, community of residence, living conditions, gastrointestinal clinical manifestation, and history of antiparasitic and antiretroviral drug use. The living condition of the participants was grouped into "poor" and "good" The "poor" living condition consisted of high degree of crowding, low quality of water supply and bad toilet facility whiles "good" living condition was made up of low degree of crowding, quality water supply and good toilet facility.

3.7 LABORATORY INVESTIGATIONS

3.7.1 Stool sample collection and processing

Patients who had consented to be participants of the study were provided with clean, dry, leak-proof, and wide-mouthed plastic specimen containers. They were given instructions on how to avoid contamination of stool sample with urine, and further instructed to collect about 10 grams of stool in the containers provided and to deliver them to the laboratory within two (2) hours after collection. Participants who were unable to produce specimens the same day in the hospital were allowed to send the specimen containers home and bring freshly passed stool sample the following day. Each specimen was labelled with a study number, date and time specimen was received. Participants who delivered inadequate stool specimens (about 10 grams was considered adequate for all the tests adopted for the study with enough remaining for preservation) and / or delivered them later than two (2) hours after collection were not included in the study (Morris *et al*., 1992).

3.7.2 Parasitological examinations

Four (4) parasitological methods were used for this study, namely the direct wet mount, Kato-Katz technique, formol-ether concentration method and modified Ziehl Neelsen (ZN) staining method. Each specimen was first examined macroscopically and its consistency or nature was recorded as either formed (F), semi-formed (SF), semi-formed with blood (SB), bloody-mucoid (BM), loose (L) or watery (W), in accordance with the description by Smith and Bartlett (1991). Samples were analyzed fresh, in batches, as soon as they were received; none was preserved in the refrigerator or any preservative added prior to processing, as this would kill laevae or motile

parasites which were present (Smith and Schad, 1990). The test procedures were carried out in accordance with standard protocols as described by Garcia (2001) and WHO (2006).

3.7.2.1 Direct wet mount and microscopy

A single stool sample was obtained in labelled specimen containers from all consenting patients selected for the study. A direct saline mount of each sample was prepared and microscopically checked for motile intestinal parasites. Lugol's iodine staining was done, after the stained concentrate was well mixed using a sterile Pasteur pipette. A drop was placed on a labelled clean dry 76mm x 26mm slide and covered with a 20mm x 20mm cover glass. The preparation was examined with Olympus light microscope using the low power (x10) and the high power (x40) objectives for helminth larvae and ova identification. The x10 and x40 objectives were used for searching and identification of the cysts and trophozoites of protozoa respectively. The number of cyst, ova, larvae or parasite density level was estimated as follows: Scanty = 1-3 parasites per preparation, Few = 4-10 parasites per preparation, Moderate = 11-20 parasites per preparation, Many = 21-40, Very many = >40 parasites per preparation (Cheesbrough, 2009).

3.7.2.2 Kato-Katz technique

A commercial disposable Kato-Katz kit (manufactured by AK Industriae Commercio Ltd., Belo Horizonte, Brazil) containing a cardboard template- with a hole of 6 mm diameter and 1.0mm thick, nylon screen of mesh size 200 µm, and hydrophilic cellophane strips of size 25x35 mm and 50 µm thick was used for this

study. Using an electronic weighing balance of 0.0001g sensitivity, the amount of sieved faecal sample delivered by the template was measured. The average amount was found to be 42 mg which was later used to calculate the eggs per gram (epg) of faeces (WHO, 2006).

The test was performed as follows; about 1g (1000 mg) of fresh stool was placed on a piece of paper or plastic sheet and a metal sieve was placed on top of it. The content was scrapped with an applicator stick (or with spatula) onto a 42 mg template with a hole on a centre of a microscope slide. The template was filled with the sieved stool using an applicator stick, and the excess faeces was removed from the edge of the hole. The scrapped faecal material on the slide was covered with the pre- soaked strip of cellophane tape (Soaking solution:1ml 3% aqueous malachite green, 100ml glycerol, 100ml distilled water). The stool was then pressed with another slide, in order to prepare an evenly distributed smear of approximately 1.5cm^2 to 2cm^2. After removing the slide, the prepared slide was allowed to stand for 15 minutes for the glycerol to clear the faeces. The slides were observed within 30 minutes of preparation. The whole slide was observed and eggs counted to determine the number of eggs per gram (epg) of stool (WHO, 2006).

The "epg" was calculated as follows: If "n" number of eggs of parasite species are found in 42 mg of stool specimen, then 1000 mg (1g) of the faecal specimen contains "n" X (1000/42) or ("n" X 24) epg. Because the same batch of Kato kit was used for this study, the multiplication factor of 24 (i.e. 1000 mg / 42 mg) was used for all parasite ova detected by the Kato-Katz method. The "epg" values obtained were used

to estimate the parasite infection density based on the classification developed by the (WHO, 2006).

3.7.2.3 Formol-ether concentration method

With the aid of an applicator stick, about1g of each fresh stool sample was emulsified in 3-4ml of 10% formalin and the content transferred into 10ml centrifuge tube. The content was mixed by shaking for 20 seconds and then sieved with double layer cotton gauze, collecting the sieved suspension in a beaker. The sieved suspension was poured back into the centrifuge tube and the debris discarded. Equal volume of ether (3-4ml) was added, mixed well and the content centrifuged at 3000 rpm for 1 minute. The supernatant was decanted and the tube placed in a rack. The sediment was transferred onto a slide, stained with iodine, covered with a coverslip and examined for the presence of ova or parasites under the light microscope at a magnification of X10 and X40 (Cheesbrough, 2009).

3.7.2.4 Modified Ziehl-Neelsen (ZN) staining method

A small portion of the fresh stool sample was processed for *Cryptosporidium parvum* and *Isospora belli* oocysts using the Ziehl-Neelsen (ZN) method with some modification. Thin smear was prepared directly from fresh stool as well as from sediment of concentrated stool and allowed to air dry. The slides were then fixed with methanol for 5minutes and stained with carbol fuchsin for 30minutes. After washing the slides in tap- water, they were decolourised with acid alcohol (99ml of 96% ethanol and 1ml HCl) for 1-3 minutes and counterstained in methylene blue for one minute. The slides were then washed in tap water and observed under light

microscope with a magnification of X10. Oocysts for *Cryptosporidium* appeared bright orange with clear halo against a blue background, measuring 4-6µm in size. For *Isospora* the oocysts were oval pink-red in colour, measuring 10-19µm by 20-33µm in size (Cheesbrough, 2009).

3.7.3 Blood sample collection and analysis

Blood samples were taken from all the study participants by aseptic technique into EDTA vacutainer tubes after they submitted their faecal specimen at the laboratory. Blood samples were collected from the ante cubital vein. Rubber tourniquet was applied for less than one minute and the site to be punctured cleaned with 70% methylated spirit. Two (2) ml of blood was taken into EDTA vacutainer tubes for the absolute cell counts. The samples were then mixed by hand to prevent clotting by turning the EDTA tubes up and down and then transferred onto mechanical mixer to dissolve the anticoagulant.

The absolute cell counts of CD4+ T-lymphocytes were analysed in the unlysed whole blood. The cell counts were determined using the Becton Dickinson (BD) FACSCount system (Becton, Dickinson and Company, Califonia, USA). The CD4+ count results obtained were grouped into three (3) immunity categories as follows: Low immunity= <200 cells /µL, Moderate immunity= 200–500 cells /µL, High immunity = >500cells/µL (WHO, 2012).

3.7.4 BD FACS Count System for CD4 Estimation

The BD FACSCount system is a compact, self-contained system, incorporating instrument, reagents, and controls, for automatic counting of CD4, CD8, and CD3 T lymphocytes. The BD FACS Count system uses whole blood, eliminating lyse and wash steps. A unique software algorithm automatically identifies the lymphocyte populations of interest. The BD FACS Count system precisely measures absolute numbers of CD4+ T-lymphocytes, the cellular parameter most closely associated with HIV/AIDS disease progression and therapy decisions. It gives consistent, accurate results even with low CD4+ counts. In addition, absolute counts of CD3+ and CD8+ T-lymphocytes, and the helper/suppressor ratio (CD4+: CD8+ T-lymphocytes) are measured. The BD FACS Count system offers a complete T-lymphocyte panel to monitor immune status. Standard Operating Procedures (SOPs) were followed to obtain valid results and quality control mechanisms including running of controls and standards were adhered to, in order to get accurate and reliable results according to the manufacture's protocol (BD Bioscience, 2005).

3.8 ADMINISTRATION OF DRUGS

The regimen of ART did not include administration of anthelmintic or antiprotozoan drugs. However, for the purpose of the study, all participants who were positive for intestinal parasites were treated free with antihelmintic or antiprotozoan drug. The ART, antihelmintic and antiprotozoan drugs were administered by clinicians at the HIV Clinics.

3.8.1 Antiretroviral Therapy (ART)

The ART was administered by Clinicians at the HIV Clinics. The eligibility criterion was a patient with CD4 count less than 350 cells/μL. The regimen of ART is a triple therapy; that is three (3) drugs were used. The following triple therapy regimens were recommended for use:

(a) First Line Antiretroviral (ARV) drugs.

1. **First choice drugs**: **First option** – Zidovudine – Adult, 300 mg twice daily, plus Lamivudine – Adult, 150 mg twice daily, plus Nevirapine – Adult, 200 mg once daily for first 14 days then (if no rash present) 200 mg twice daily.

   Second option - Zidovudine – Adult, 300 mg twice daily, plus Lamivudine – Adult,150mg twice daily, plus Efavirenz.– Adult, 600 mg once daily.
2. **Second choice drugs: First option** - Stavudine – Adult, body weight under 60 kg, 30 mg twice daily, 60 kg and over, 40 mg twice daily, plus Lamivudine – Adult, 150 mg twice daily, plus Nevirapine – Adult, 200 mg once daily for first 14 days then (if no rash present) 200 mg twice daily.

   Second option - Stavudine – Adult, body weight under 60 kg, 30 mg twice daily, 60 kg and over, 40 mg twice daily, plus Lamivudine – Adult, 150 mg twice daily, plus Efavirenz – Adult, 600 mg once daily.

(b) Second Line Antiretroviral (ARV) drugs.

1. **First Alternative: First option** - Abacavir – Adult, 300 mg twice daily, plus Tenofovir – 300 mg once daily, plus Nelfinavir – Adult, 750 mg three times daily or 1250 mg twice daily.

Second option - Abacavir – Adult, 300 mg twice daily, plus Tenofovir – 300 mg once daily, plus Lopinavir – Adult, 400 mg twice daily.

2. **Second Alternative: First option** – Didanosine – Adult, body weight under 60 kg, 125 mg twice daily, 60 kg and over 200 mg twice daily, plus Abacavir – Adult, 300 mg twice daily, plus Nelfinavir – Adult, 750 mg three times daily or 1250 mg twice daily.

   Second option - Didanosine – Adult, body weight under 60 kg, 125 mg twice daily, 60kg and over 200 mg twice daily, plus Abacavir – Adult, 300 mg twice daily, plus Lopinavir – Adult, 400 mg twice daily.

3.8.2 Antihelminthic drug

The adult dose of albendazole (manufactured by SmithKline Beecham Laboratoires Pharmaceutiques, Nanterre Cedex, France), which is 400 mg or two 200 mg tablets or 20 ml suspension, was given as a single dose in cases of *A. lumbricoides* and *T. trichiura*. In cases of *S. stercoralis*, albendazole 400 mg tablet was given as a single dose for three (3) consecutive days. If the patient was not cured on follow-up after three weeks, a second course of treatment was added.

3.8.3 Antiprotozoan drug

The adult dose of Secnidazole (manufactured by Unichem Laboratories Ltd., Mumbai, India) which is 2g tablet was given as a single dose for the treatment of *G. lamblia* and *E. histolytica.* A combination therapy of oral Trimethoprim (160mg) and Sulfamethoxazole (800mg) was given at a dosage of four (4) times a day for ten (10) days for the treatment of *Cryptosporidium* spp. and *I. belli.*

3.9 STATISTICAL ANALYSIS

The data obtained from 206 samples were analyzed with SPSS version 19 software, (Statistical Package for Social Sciences). Descriptive Statistics was performed and reported using frequency tables, pie charts, bar charts and cross tabulation. Pearson Chi-square model, (χ^2), was conducted to measure the association between two categorical variables. Also chi-square goodness of fit-test was conducted to measure the significant difference within a dichotomous variable (parasite infection status, diarrhoea status and ART status). Data on age was analysed as a continuous variable while data on intestinal parasites density was analyzed as both continuous and categorical variables; where groups consisted of scanty, few, moderate and many. Mann-Whitney U test was also performed to measure the significant difference of parasite density and CD4 counts levels between participants on ART and those not on ART.

Cross tabulation with bonferroni post hoc analysis was performed to determine the significant differences of intestinal parasite prevalence among the CD4 categories. Significant differences were identified with p-value < 0.05 at 95% confidence interval. P-values provide a sense of the strength of the evidence against the null hypothesis. The null hypothesis was that, there is no significant difference between the prevalence of intestinal parasites in study participants on ART and those not on ART. The smaller the P value, the more strongly the test rejects the null hypothesis, that is, the hypothesis being tested. The most commonly used level of significance is 0.05. The significance level was set at 0.05, and a *P value* of less than 0.05 was considered

significant. Therefore, the null hypothesis will be rejected in favour of the alternative hypothesis.

The statistical tools below were used to determine the degree of accuracy of three parasitological diagnostic methods used in the study, which were direct wet mount method, kato-katz technique and formol formol-ether concentration method, which was used as the "gold standard" method. These tools include sensitivity, specificity and predictive values.

3.9.1 Sensitivity

Sensitivity is the ability of a test to correctly identify all positive samples. It is calculated as the number of true positive samples detected by that test being evaluated, divided by the number of samples identified by the reference method as positive, expressed as a percentage (Hoehler, 2000). High sensitivity allows accurate detection of disease, and therefore reduced incidence of misdiagnosis.

3.9.2 Specificity

Specificity is the ability of a test to detect correctly all negative samples. It is calculated as the number of true negative samples recognized by the test being evaluated, divided by the number of samples identified by the reference test as negative, expressed as a percentage. High specificity suggests that the test is good for "ruling out" disease (Hoehler, 2000).

3.9.3 Predictive values

Positive predictive value (PPV) expresses the proportion of persons with positive test who have the disease condition, that is, the probability that a positive test is a true positive. Negative predictive value (NPV) is the probability that a negative test is a true negative, or the proportion of persons with negative test who do not have the disease condition. Both PPV and NPV can be calculated using the table below (Hoehler, 2000):

Table 3.1 Determination of PPV and NPV.

Disease condition	**Test Outcome**	
	Positive	Negative
Present	**a**	**b**
Absent	**c**	**d**

They were calculated using the formula: PPV= a/(a+b) or true positive/all negative, and NPV=d/(d+c) or true negative/all negative.

CHAPTER FOUR

4.0 RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

Figures 4.1 to 4.3 below illustrate the demographic characteristics of the study participants which included; Gender, age and the community of residence respectively. The sex distribution of the studied participants in each hospital is shown in Figure 4.1 below. A total of 206 HIV positive individuals participated in this study. Among these, 41 (19.9%) were males and165 (80.1%) were females. Out of the total studied participants, University of Cape Coast Hospital (UCCH) recorded the highest attendance of 60.7%, followed by Central Regional Hospital (CRH) with 32.0% and Cape Coast Metropolitan Hospital (CCMH) with 7.3% (Figure 4.1).

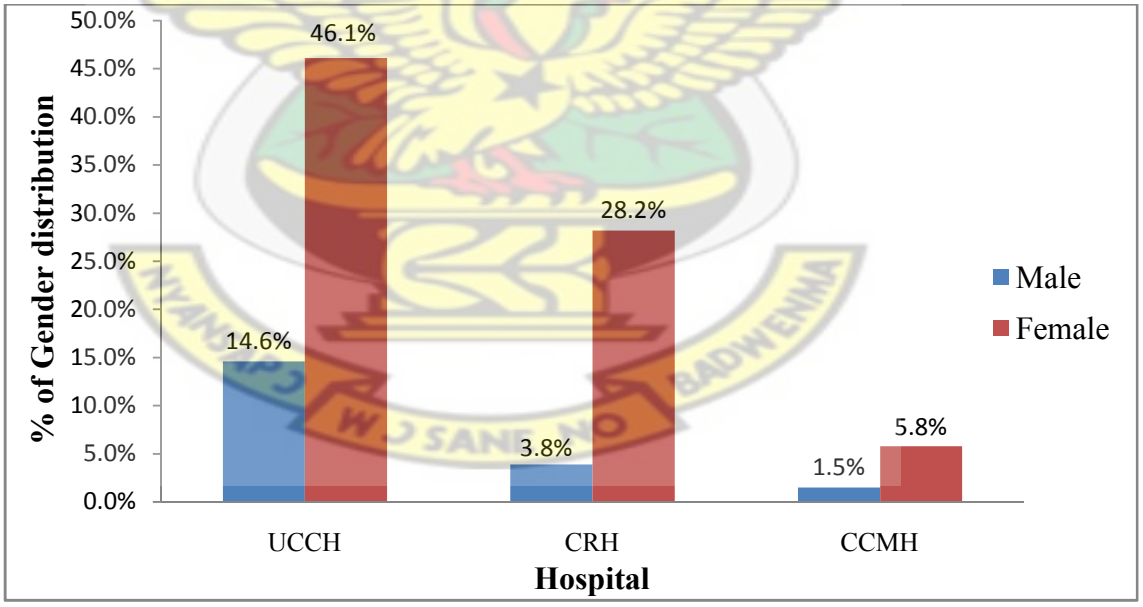

Figure 4.1: Gender distribution of participants according to hospitals studied

4.1.1 Age distribution of participants.

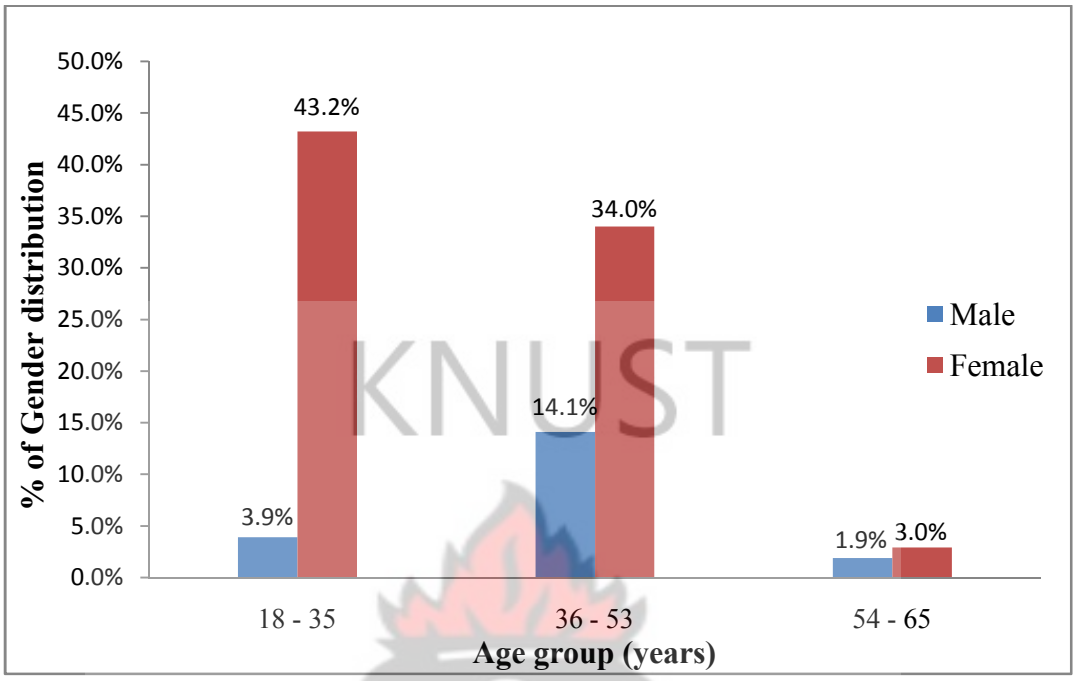

Figure 4.2: Age distribution stratified by gender of participants

Figure 4.2 above shows the age distribution stratified by the gender of the study participants**.** The mean age of the study participants was 42 years. A total of 97 (47.1%) participants were in the age group18 to 35 years, with 43.2% being females and 3.9% males. 99 (48.1%) participants were in the age group 36 to 53 years, with 34.0% being females and 14.1% males. Also, 10 (4.9%) participants were in the age group 54 to 65 years, with 3.0% being females and 1.9% males (Figure 4.2).

4.1.2 The community of residence of participants

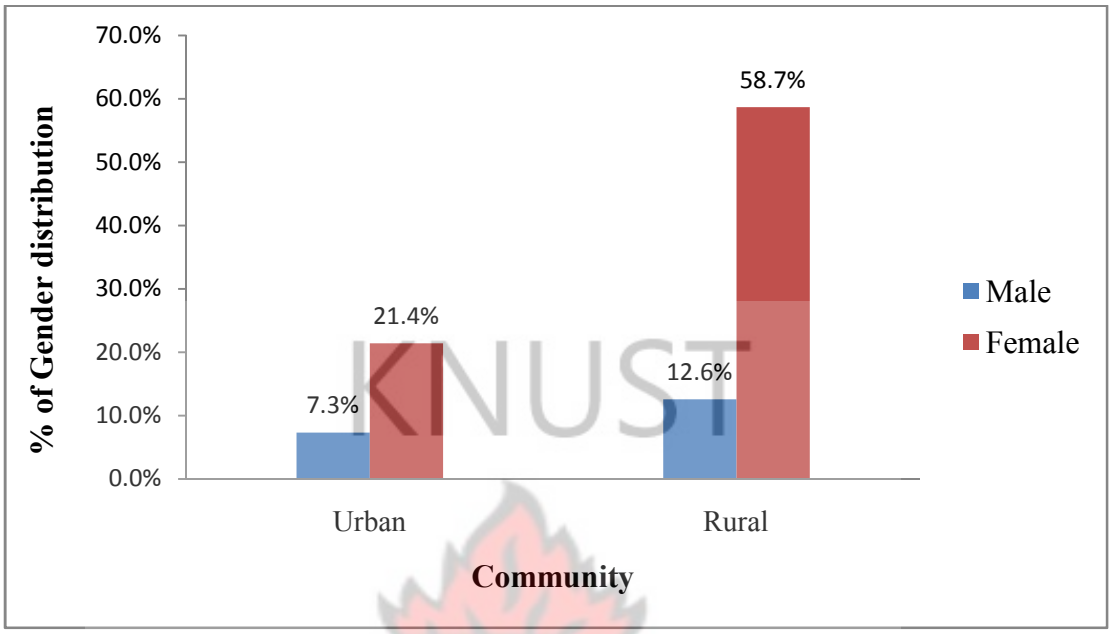

Figure 4.3: Community of residence of participants stratified by gender

The community of residence in relation to the gender of study participants is shown in Figure 4.3 above. Out of the total of 206 participants, 147 (71.4%) were from the rural community, which had 58.7% being females and 12.6% males, and 59 (28.6%) were from the urban community, which also had 21.4% being females and 7.3% males.

4.2 USE OF ANTIHELMINTHIC DRUG AMONG STUDY PARTICIPANTS

Among the 206 study participants, 52 (25.3%) were found to have taken some form of antihelminthic drug in the past 3 months preceding the study. Out of these, 9 (4.4%) were parasite-positive, whiles 43 (20.9%) were parasite-negative. Also, 154 (74.7%) had not used any antihelminthic drug prior to the study, of which 89 (43.2%) were parasite-positive and 65 (31.5%) were parasite-negative (Table 4.1).

Table 4.1: Antihelminthic drug usage and parasite status of study participants

	Antihelminthic Usage		Total
Parasite status	**Yes**	**No**	
	n (%)	**n (%)**	**n (%)**
Positive	9 (4.4)	89 (43.2)	98 (47.6)
Negative	43 (20.9)	65 (31.5)	108 (52.4)
Total	**52 (25.3)**	**154 (74.7)**	**206 (100.0)**

4.3 PREVALENCE OF INTESTINAL PARASITES AMONG STUDY PARTICIPANTS

The overall prevalence of intestinal parasites of the study was 47.6 % out of 206 HIV positive study participants. This prevalence was not statistically significant (p=0.486) when compared to 108 (52.4%) of participants with no parasite infection (Figure 4.4).

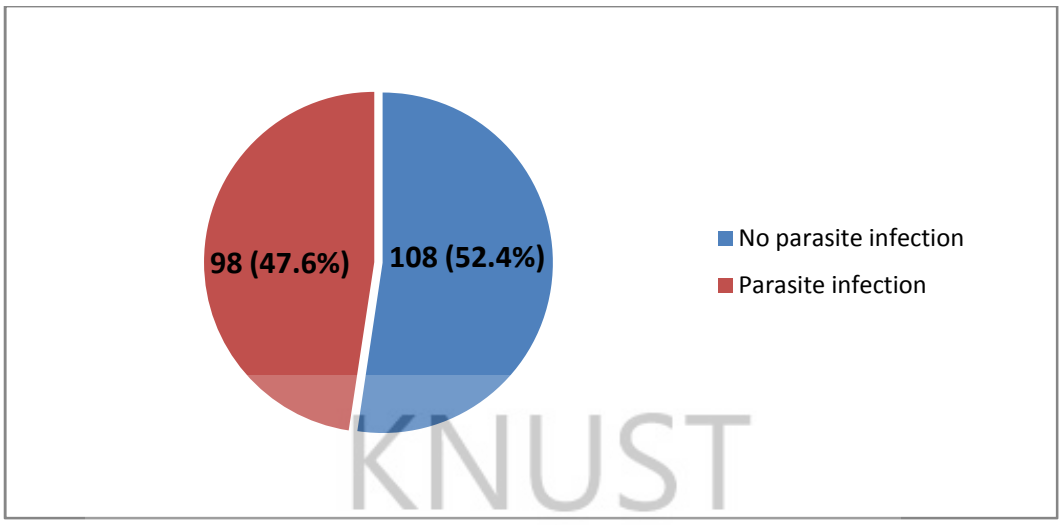

Figure 4.4: Overall Prevalence of intestinal parasite infection of participants in the study

Among the 98 (47.6%) parasite-positive study participants, 41 (19.9%) were on ART whiles 57 (27.7%) were not on ART (Figure 4.5). There was a significant difference (p=0.012) between the two groups.

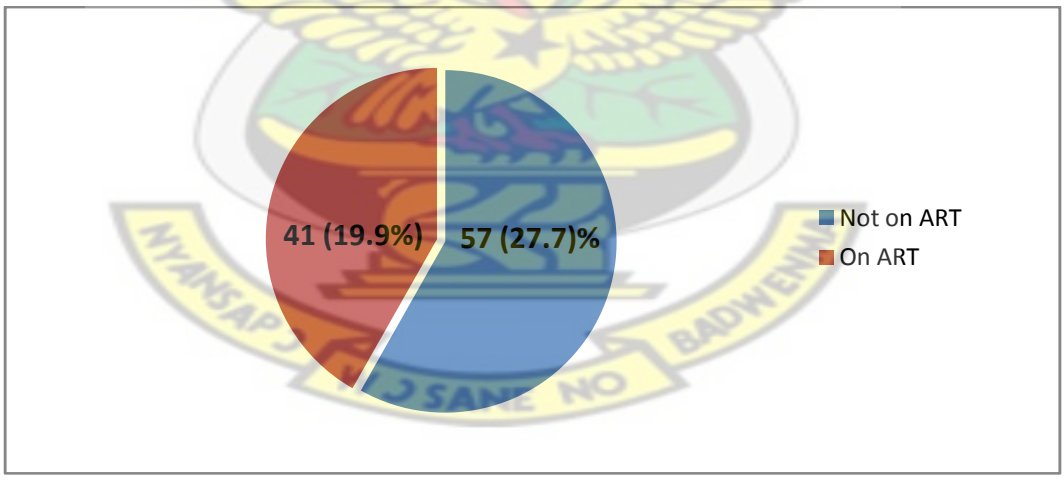

Figure 4.5 Distribution of intestinal parasite infection of study participants per ART status

Figure 4.6 below represents the seven (7) species of intestinal parasites which were detected in 98 (47.6%) out of the 206 study participants in this study. The parasites

included: *Giardia lamblia,* 37 *(*18.0%), *Entamoeba histolytica,* 25 (12.1%), *Cryptosporidium* spp., 12 (5.8%), *Isospora belli,* 9 *(*4.4%), *S. stercolaris,* 8 (3.9%), *Ascaris lumbricoides*, 4 (1.9%) and *Trichuris trichiura,* 3 (1.5%). The most prevalent intestinal parasites in this study were *G. lamblia* (18.0%) and *E. histolytica* (12.1%) (Figure 4.6).

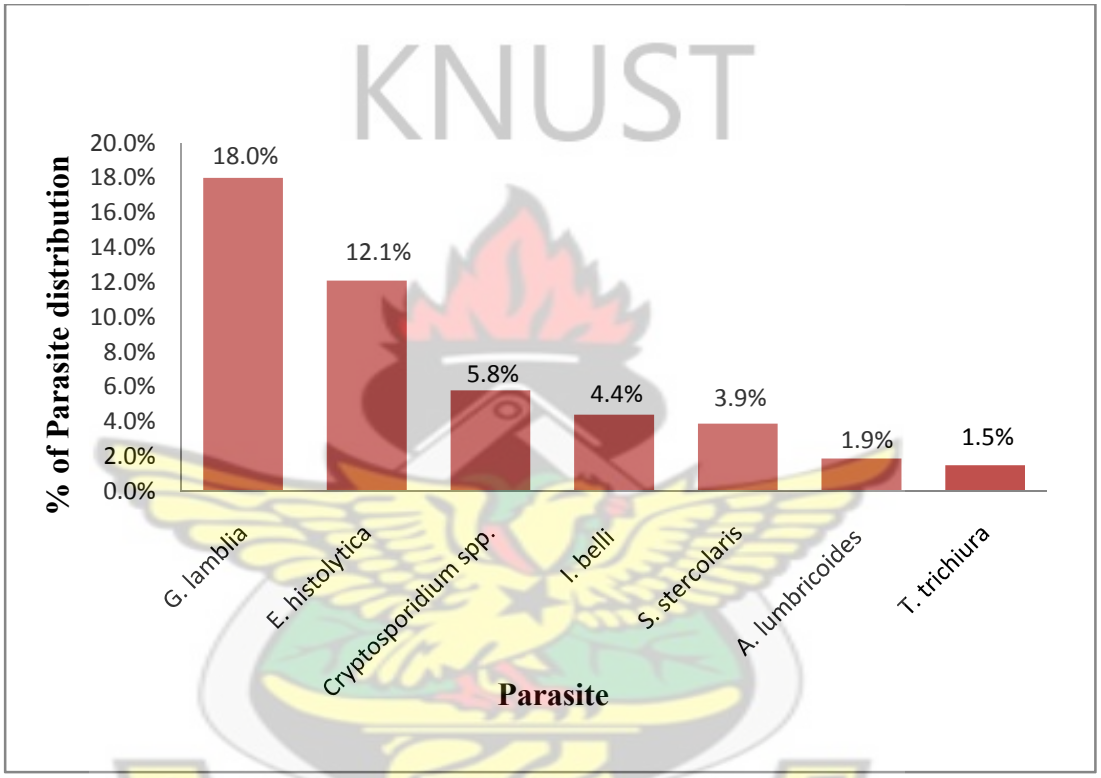

Figure 4.6: Prevalence of various intestinal parasites detected in the study

4.4 PREVALENCE RATES OF INTESTINAL PARASITES BY THE PARASITOLOGICAL METHODS USED

The overall prevalence of intestinal parasites detected by the formol-ether concentration (gold standard) method was 39.8%. The direct wet mount and Kato-Katz methods gave overall prevalence of 16.0% and 3.4% respectively. The

prevalence of the seven (7) intestinal parasites detected by the three methods is presented in Table 4.2.

Table 4.2: Prevalence of intestinal parasites stratified by the parasitological methods used

Parasite	Formol-ether conc. N=206 n (%)	Direct Wet Mount N=206 n (%)	Kato-Katz N=206 n (%)
G. lamblia	37 (13.6)	15 (7.3)	0 (0.0)
E. histolytica	25 (8.7)	11 (5.3)	0 (0.0)
Cryptosporidium	3 (1.5)	0 (0.0)	0 (0.0)
I. belli	2 (1.0)	0 (0.0)	0 (0.0)
S. stercolaris	8 (3.9)	4 (1.9)	0 (0.0)
A. lumbricoides	4 (1.9)	3 (1.5)	4 (1.9)
T. trichiura	3 (1.5)	0 (0.0)	3 (1.5)
Total	**82 (39.8)**	**33 (16.0)**	**7 (3.4)**

4.4.1 Test performance of direct wet mount

The performance of direct wet mount was evaluated in relation to formol-ether concentration which was the gold standard test and the evaluation results are shown in Table 4.3.

Table 4.3: Performance of direct wet mount against the gold standard method

Gold standard / Reference Method		Direct wet mount test		Total
		Positive	Negative	
Formol-ether concentration method	Positive	33	49	**82**
	Negative	0	124	**124**
Total		**33**	**173**	**206**

Positive Predictive Value, PPV=40.2% (33/82).

As shown in Table 4.3 above, the wet mount method detected a total of 33 intestinal parasites as against 82 by the formol-ether concentration method (gold standard). The evaluation results gave sensitivity and specificity of the wet mount method as 40.2% (33/82) and 100% (124/124), respectively.

4.4.2 Performance of Kato-Katz method for detection of intestinal helminth parasite infection

As shown in Table 4.4, both the Kato-Katz and formol-ether concentration methods showed good agreement for detection of intestinal helminth parasites such as *A. lumbricoides* (1.9%) *T. Trichiura* (1.5%).

Table 4.4: Kato-Katz method versus the gold standard for detection of intestinal helminth parasites

Parasite	Formol-ether conc. N=206 n (%)	Kato-Katz N=206 n (%)
S. stercolaris	8 (3.9)	0 (0.0)
A. lumbricoides	4 (1.9)	4 (1.9)
T. trichiura	3 (1.5)	3 (1.5)
Total	**15 (7.3)**	**7 (3.4)**

Positive Predictive Value, PPV= 46.7% (7/15)

A comparison of the performance of the Kato-Katz versus the formol-ether concentration method (gold standard) results gave sensitivity of 46.7% (7/15) (Table 4.4). The Kato-Katz method was good in detecting only *A. lumbricoides* and *T. trichiura* in this study.

4.4.3 Performance of modified Ziehl-Neelsen (ZN) staining method for detection of intestinal protozoan parasite infections

Table 4.5 shows that, the modified Ziehl-Neelsen staining method was good for the detection of opportunistic intestinal parasites such as *Cryptosporidium spp* (5.8%) and *I. belli* (4.4%).

Table 4.5: Modified Ziehl-Neelsen (ZN) staining method versus the gold standard for detection of intestinal protozoan parasites

Parasite	Formol-ether conc. N=206 n (%)	Modified Ziehl-Neelsen (ZN) N=206 n (%)
G. lamblia	37 (13.6)	0 (0.0)
E. histolytica	25 (8.7)	0 (0.0)
Cryptosporidium	3 (1.5)	12 (5.8)
I. belli	2 (1.0)	9 (4.4)
Total	**67 (32.5)**	**21 (10.2)**

Positive Predictive Value, PPV= 31.3% (21/67)

The performance of the modified Ziehl-Neelsen staining method was compared with the formol-ether concentration method (gold standard) results which gave a sensitivity of 31.3% (21/67) (Table 4.5). The modified Ziehl-Neelsen (ZN) staining method was good in detecting only *Cryptosporidium* and *I. belli* in this study.

4.5 PREVALENCE OF INTESTINAL PARASITES ACCORDING TO THE HOSPITALS STUDIED

The intestinal parasites detected were grouped according to the hospitals attended by the patients, which is presented in Table 4.6 below.

Table 4.6: Prevalence of intestinal parasites stratified by the hospitals

	Study Sites			
	UCCH	**CRH**	**CCMH**	**Total**
Parasite	**n (%)**	**n (%)**	**n (%)**	**n (%)**
G. lamblia	22 (10.7)	12 (5.8)	3 (1.5)	**37 (18.0)**
E. histolytica	14 (6.8)	8 (3.9)	3 (1.4)	**25 (12.1)**
Cryptosporidium	6 (2.9)	5 (2.4)	1 (0.5)	**12 (5.8)**
I. belli	5 (2.4)	3 (1.5)	1 (0.5)	**9 (4.4)**
S. stercolaris	3 (1.5)	4 (2.0)	1 (0.4)	**8 (3.9)**
A. lumbricoides	2 (0.95)	2 (0.95)	0 (0.0)	**4 (1.9)**
T. trichiura	1 (0.5)	2 (1.0)	0 (0.0)	**3 (1.5)**
Total	**53 (25.7)**	**36 (17.6)**	**9 (4.3)**	**98 (47.6)**

UCCH- University of Cape Coast Hospital, CRH- Central Regional Hospital, CCMH- Cape Coast Metropolitan Hospital.

The University of Cape Coast Hospital (UCCH) recorded the highest prevalence of 25.7%, followed by Central Regional Hospital (CRH) with a prevalence of 17.6% and Cape Coast Metropolitan Hospital (CCMH) recorded the lowest prevalence of 4.3% out of the total prevalence obtained in the study (Table 4.6). There were significant differences between the prevalence of intestinal parasites obtained from University of Cape Coast Hospital (UCCH) compared to Cape Coast Metropolitan Hospital (CCMH) (p=0.001) and that obtained from Central Regional Hospital (CRH) compared to Cape Coast Metropolitan Hospital (CCMH) (p=0.001). However, there was no significant difference (p=0.090) between the prevalence obtained from University of Cape Coast Hospital (UCCH) and the Central Regional Hospital (CRH) (Table 4.6).

4.6 INTESTINAL PARASITE INFECTION AND DIARRHOEAL STATUS OF PARTICIPANTS

The tables below (Table 4.7 and 4.8) show the relationships between intestinal parasite infection and diarrhoeal status of the study participants at the first encounter.

Table 4.7: Parasite infection and diarrhoeal status of participants

	Diarrhoeal status		Total
Parasite status	**Diarrhoea**	**Non-Diarrhoea**	
	n (%)	**n (%)**	**n (%)**
Positive	70 (34.0)	28 (13.6)	98 (47.6)
Negative	31 (15.0)	77 (37.4)	108 (52.4)
Total	**101 (49.0)**	**105 (51.0)**	**206 (100.0)**

$X^2(1) = 37.529, (P = 0.001)$

Out of 206 participants, 101 (49.0%) had diarrhoea. Of these, 70 (34.0%) were parasite-positive and 31 (15.0%) were negative. 105 (51.0%) did not have diarrhoea, and of these, 28 (13.6%) were parasite-positive and 77 (37.4%) were negative. The number of parasite-positive participants with diarrheoa stools, 70 (34.0%) was significantly higher (p=0.001) than those without diarrheoa stools, 28 (13.6%) (Table 4.7).

Table 4.8: Intestinal parasites detected and diarrhoeal status of participants

	Diarrhoeal status		
	Diarrheoa	Non-diarrheoa	
Parasite	**n (%)**	**n (%)**	**P value**
G. lamblia	28 (13.6)	9 (4.4)	0.002
E. histolytica	18 (8.7)	7 (3.4)	0.028
Cryptosporidium	10 (4.8)	2 (1.0)	0.021
I. belli	8 (3.9)	1 (0.5)	0.020
S. stercolaris	6 (2.9)	2 (1.0)	0.157
A. lumbricoides	0 (0.0)	4 (1.9)	0.219
T. trichiura	0 (0.0)	3 (1.5)	0.625
Total	**70 (34.0)**	**28 (13.6)**	**0.001**

Table 4.8 above shows the significant differences of intestinal parasites detected in parasite-positive participants with diarrhoea and those with no diarrhoea stools. There were significantly high levels of *G. lamblia* (p=0.002), *E. histolytica* (p=0.028), *Cryptosporidium* (p=0.021) and *I. belli* infections (p=0.020) in participants with diarrhoea stools than those without diarrhoea stools. However, there were no significant differences of the levels of *S. stercolaris* (p=0.157), *A. lumbricoides* (p=0.219) and *T. trichiura* (p=0.625) infections, between the participants with diarrhoea and non-diarrhoea stools (Table 4.8).

4.7 INTESTINAL PARASITE INFECTION AND ART STATUS OF PARTICIPANTS

The table below (Table 4.9) shows intestinal parasite infection and ART status of participants at the first encounter.

Table 4.9: Parasite status and ART status of participants

	ART status		Total
Parasite status	**On ART**	**Not on ART**	
	n (%)	**n (%)**	**n (%)**
Positive	41 (19.9)	57 (27.7)	98 (47.6)
Negative	64 (31.1)	44 (21.4)	108 (52.4)
Total	**105 (51.1)**	**101 (49.0)**	**206 (100.0)**

$X^2(1) = 6.241, p = 0.012$

Out of the 206 participants, 105 (51.1%) were on ART, and of these, 41 (19.9%) were parasite-positive and 64 (31.1%) were negative. 101 (49.0%) were not on ART, and of these, 57 (27.7%) were parasite-positive and 44 (21.4%) were negative. The number of parasite-positive participants who were not on ART, 57 (27.7%) was significantly higher (p=0.012) than those on ART, 41 (19.9%) (Table 4.9).

Table 4.10 below shows the relationship between intestinal parasites detected in parasite-positive participants on ART and those not on ART.

Table 4.10: Intestinal parasites detected and ART status of participants

	ART status		
	On-ART	Not on-ART	P value
Parasite	n (%)	n (%)	
G. lamblia	21 (10.2)	16 (7.8)	0.250
E. histolytica	14 (6.8)	11 (5.3)	0.549
Cryptosporidium	2 (0.9)	10 (4.8)	0.021
I. belli	1 (0.5)	8 (3.8)	0.020
S. stercolaris	3 (1.5)	5 (2.4)	0.480
A. lumbricoides	0 (0.0)	4 (1.9)	0.219
T. trichiura	0 (0.0)	3 (1.5)	0.625
Total	**41 (19.9)**	**57 (27.7)**	**0.012**

On-ART=On Antiretroviral therapy, Not on-ART=Not on Antiretroviral therapy, Significant difference exist at p <0.05.

Out of the 206 participants, 57 (27.7%) who were not on ART was significantly higher (0.012) than 41 (19.9%) who were on ART. With respect to the parasites detected, there were significantly low levels of *Cryptosporidium* (p=0.021) and *I. belli* (p=0.020) infections in participants who were on ART compared to those who were not on ART. However, there were no significant differences of levels of *G. lamblia* (p=0.250), *E. histolytica* (p=0.549), *S. stercolaris* (p=0.480), *A. lumbricoides* (p=0.219) and *T. trichiura* (p=0.625) infections, between parasite-positive participants who were on ART and those not on ART (Table 4.10).

4.8 INTESTINAL PARASITE INFECTION AND CD4+ COUNT LEVELS OF PARTICIPANTS

The table below (Table 4.11) shows intestinal parasite infection and CD4+ count levels of participants at the first encounter.

Table 4.11: Relationship between parasite status and CD4+ count levels of participants at first encounter

	CD4+ count level			Total
Parasite status	**<200 /µL**	**200–500 /µL**	**>500 /µL**	
	n (%)	**n (%)**	**n (%)**	**n (%)**
Positive	48 (23.3)	46 (22.3)	4 (1.9)	98 (47.6)
Negative	3 (1.5)	36 (17.5)	69 (33.5)	108 (52.4)
Total	**51 (24.8)**	**82 (39.8)**	**73 (35.4)**	**206 (100.0)**

CD4+ count: >500 cells/µL- High Immunity, 200–500 cells/µL- Moderate Immunity, <200 cells/µL –Low Immunity.

Out of the 206 participants, 51 (24.8%) had CD4+ count below 200 /µL, and of these, 48 (23.3%) were parasite-positive and 3 (1.5%) were negative. Also, 82 (39.8%) participants had CD4+ count level of 200-500/µL, and of these, 46 (22.3%) were parasite-positive and 36 (17.5%) were negative. Lastly, 73 (35.4%) participants had their CD4+ count above 500 /µL, and of these, 4 (1.9%) were parasite-positive and 69 (33.5%) were negative. The number of parasite-positive participants who had their CD4+ counts below 200 /µL were significantly higher (p=0.001) than parasite-negative participants in the same CD4+ count category. Conversely, the number of parasite-negative participants with CD4+ counts above 500 /µL were significantly

higher (p=0.001) than parasite-positive participants in the same CD4+ count category. However, there was no significant difference (p=0.125) between parasite-positive and negative participants who had CD4+ count level of 200-500/µL (Table 4.11).

4.9 ART STATUS AND CD4+COUNT LEVELS OF PARTICIPANTS

Table 4.12 describes the association between ART status and CD4+ counts of participants at the first encounter.

Table 4.12: Relationship between ART status and CD4+ count levels of participants at first encounter

	CD4+ count level			Total
ART status	**<200 /µL**	**200–500 /µL**	**>500 /µL**	
	n (%)	**n (%)**	**n (%)**	**n (%)**
On ART	6 (2.9)	64 (31.1)	35 (17.0)	105 (51.0)
Not on ART	45 (21.8)	18 (8.7)	38 (18.4)	101 (49.9)
Total	**51 (24.8)**	**82 (39.8)**	**73 (35.4)**	**206 (100.0)**

CD4+ count : >500 cells/µL- High Immunity, 200–500 cells /µL- Moderate Immunity, <200 cells/µL – Low Immunity.

Out of the 206 participants, 51 (24.8%) had CD4+ counts below 200 /µL, and of these, 6 (2.9%) were on ART which was significantly lower (p= 0.001) than 45 (21.8%) who were not on ART. Also, 82 (39.8%) participants had CD4+ counts of 200-500/µL, and of these, 64 (31.1 %) were on ART, which was significantly higher (p=0.002) than 18 (8.7%) who were not on ART. Lastly, 73 (35.4%) participants had CD4+ counts above

500 /μL, and of these, 35 (17.0%) were on ART, which was not significantly different (p=0.569) from 38 (18.4%) who were not on ART (Table 4.12).

4.10 INTESTINAL PARASITE INFECTION AND LIVING CONDITIONS OF PARASITE-POSITIVE PARTICIPANTS

Table 4.13 below shows the relationship between the levels of intestinal parasites in participants living in "good" and "poor" conditions as defined in the methods.

Table 4.13: Intestinal parasites detected and living conditions of participants

	Living condition		
	Poor	**Good**	**P value**
Parasite	**n (%)**	**n (%)**	
G. lamblia	36 (17.5)	1 (0.5)	0.001
E. histolytica	19 (9.2)	6 (2.9)	0.009
Cryptosporidium	8 (3.9)	4 (1.9)	0.248
I. belli	7 (3.4)	2 (1.0)	0.096
S. stercolaris	7 (3.4)	1 (0.5)	0.034
A. lumbricoides	4 (1.9)	0 (0.0)	0.219
T. trichiura	3 (1.5)	0 (0.0)	0.625
Total	**84 (40.8)**	**14 (6.8)**	**0.001**

From table 4.13 above, out of the 206 participants, 84 (40.8%) parasite-positive participants were living in "poor" conditions compared to 14 (6.8%) who were living in "good" conditions. The participants who were living in "poor" conditions had significantly higher (p=0.001) number of intestinal parasite than those living in "good" conditions. With respect to intestinal parasites detected, there were

significantly high levels of *G. Lamblia* (p=0.001), *E. histolytica* (p=0.009) and *S. stercolaris* (p=0.034) infections in participants living in "poor" conditions than those living in "good" conditions. However, there were no significant differences of the levels of *Cryptosporidium* (p=0.248), *I. belli* (p=0.096), *A. lumbricoides* (p=0.219) and *T. trichiura* (p=0.625) infections between the participants living in "poor" and "good" conditions (Table 4.13).

4.11 EVALUATION OF PARASITE DENSITY, CD4+ COUNT LEVELS AND ART STATUS AMONG PARASITE-POSITIVE PARTICIPANTS

Table 4.14 below shows the intestinal parasite density and ART status of the parasite-positive participants at the first encounter.

Table 4.14: Parasite density level and ART status at first encounter

	Parasite density level				Total
ART Status	**Scanty**	**Few**	**Moderate**	**Many**	
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
On ART	9 (4.4)	23 (11.2)	9 (4.4)	0 (0.0)	41 (19.9)
Not on ART	4 (1.9)	8 (3.9)	36 (17.5)	9 (4.4)	57 (27.7)
Total	**13 (6.3)**	**31 (15.1)**	**45 (21.8)**	**9 (4.4)**	**98 (47.6)**

X^2 (3) = 32.639, p = 0.01.

Out of the 98 parasite-positive participants, 13 (6.3%) had scanty parasite density, and of these, 9 (4.4%) were on ART and 4 (1.9%) were not on ART. 31 (15.1%) had few parasite density, and of these, 23 (11.2%) were on ART and 8 (3.9%) were not on ART. 45 (21.8%) had moderate parasite density, and of these, 9 (4.4%) were on ART

and 36 (17.5%) were not on ART. Lastly, 9 (4.4%) parasite-positive participants who were not on ART had many parasite density. Interestingly, the parasite-positive participants who were not on ART, 57 (27.7%) were significantly higher (p=0.01) than those on ART, 41 (19.9%) at the first encounter (Table 4.14).

4.11.1 CD4+ count levels and ART status of parasite-positive participants

Table 4.15 below shows the CD4+ count levels and ART status of the parasite-positive participants at the first encounter. It must be noted that, participants who had CD4+ counts less than 350 cells/µL at the first encounter were put on ART before the second encounter.

Table 4.15: CD4+count level and ART status at first encounter

	CD4+ count level			Total
ART Status	**<200/µL**	**200-500/µL**	**>500/µL**	
	n (%)	**n (%)**	**n (%)**	**n (%)**
On ART	2 (1.0)	33 (16.0)	6 (2.9)	41 (19.9)
Not on ART	46 (22.3)	11 (5.4)	0 (0.0)	57 (27.7)
Total	**48 (23.3)**	**44 (21.4)**	**6 (2.9)**	**98 (47.6)**

Out of the 98 parasite-positive participants, 48 (23.3%) participants had CD4+ counts below 200 cells/µL, and of these, 2 (1.0%) were on ART and 46 (22.3%) were not on ART. 44 (21.4%) participants had CD4+ counts of 200-500 cells/µL, and of these 33 (16.0%) were on ART and 11 (5.4%) were not on ART. 6 (2.9%) had CD4+ count above 500 cells/µL, who were all on ART. There were significant differences between

participants on ART and those not on ART with CD4+ counts below 200 cells/μL (p=0.001), those with CD4+ counts of 200-500 cells/μL (p=0.011) and those with CD4+ counts above 500 cells/μL (p=0.034) (Table 4.15).

4.12 FOLLOW UP ON PARASITE INFECTION AND CD4+ COUNT OF PARASITE-POSITIVE PARTICIPANTS

The results obtained on the follow up of parasite-positive participants before and after they were given treatment for intestinal parasites is shown in Table 4.16 below.

Table 4.16: CD4+ count and parasite status of parasite-positive participants

CD4+ count / Parasite status of participants	1st Encounter	Follow up		
		1st	2nd	3rd
	N (Mean)	N (Mean)	N (Mean)	N (Mean)
CD4+ count (On-ART)	41 (323.4)	41 (485.9)	41 (715.2)	41 (802.7)
Parasite status (On-ART)	41 (6.2)	41 (4.5)	0 (0)	0 (0)
CD4+ count (Not on-ART)	57 (151.0)	57(333.9)	57(619.5)	57(785.9)
Parasite status (Not on-ART)	57 (13.7)	57 (9.3)	0 (0)	0 (0)

The average CD4+ count of parasite-positive participants on ART increased significantly from the first encounter to the third follow up time points (p=0.001, 0.001 and 0.002 respectively) (Table 4.16). Also, with respect to participants who were not on ART, the average CD4+ counts increased significantly from the first encounter to the third follow up time points (p=0.001, 0.002 and 0.025 respectively) (Table 4.16). The increase in the average CD4+ counts of the participants who were

not on ART occurred, when 26 (12.6%) of them became eligible and were put on ART after the first encounter and the remaining 31 (15.1%) started ART after the first follow up. They continued with the ART throughout the study period.

It can be seen from the table 4.16 above that, the average parasite density of participants on ART and those not on ART decreased from the first encounter to the first follow up with no significant difference between them (p=0.186). However, when the two groups of participants were compared, there was a significant difference between the average parasite density within the first encounter (p=0.001) and within the first follow up (p=0.012) (Table 4.16). The parasite density became negative at the second and third follow up time point after the parasite-positive participants were given antihelminthic or antiprotozoan drugs after first follow up.

4.13 FOLLOW-UP ON PARASITE DENSITY AND CD4+ COUNT LEVELS OF PARASITE-NEGATIVE PARTICIPANTS USED AS CONTROLS

The results below (Table 4.17) show the follow up of parasite-negative participants who were on ART and those not on ART at first encounter but became parasite-positive in the course of the study.

Table 4.17: CD4+ count and parasite status of parasite-negative participants

CD4+ count / Parasite status of participants	1st Encounter	Follow up			
		1st	2nd	3rd	4th
	N (Mean)	N (Mean)	N (Mean)	N (Mean)	N (Mean)
CD4+ count (On-ART)	30 (346.4)	30 (416.4)	30 (458.9)	30 (544.3)	30 (609.4)
Parasite status (On-ART)	0 (0)	2 (1.45)	2 (1.30)	0 (0)	0 (0)
CD4+ count (Not on-ART)	30 (557.4)	30 (477.4)	30 (443.5)	30 (481.1)	30 (523.4)
Parasite status (Not on-ART)	0 (0)	16 (5.37)	18 (5.60)	0 (0)	0 (0)

The average CD4+ counts of the parasite-negative participants on ART increased significantly from the first encounter to the fourth follow up time points (p=0.001, 0.002, 0.003 and 0.016 respectively). With respect to participants not on ART, the average CD4+ counts decreased progressively from the first encounter (557.4) to the first follow up (477.4) and from the first follow up (477.4) to the second follow up (443.5) encounter, with significant differences between the follow up time points (p=0.001 and 0.002 respectively). However, the average CD4+ counts increased from the second (443.5) to the third follow up (481.1) and from the third (481.1) to the fourth follow up (523.4), but there were no significant differences between the follow up time points (p=0.409 and 0.283 respectively) (Table 4.17). The parasite-negative participants who were not on ART at the first encounter because they had CD4+ counts above 350 cells/μL, became eligible and were put on ART after the second follow up and they continued throughout the study period.

It can be seen from the table 4.17 above that, 30 participants each from each group were parasite-negative at the first encounter and there was no significant difference

between them. However, 2 (1.0%) of the participants on ART became parasite-positive at the first and second follow up time points with low parasite density. Conversely, at the same time point, 16 (7.8%) participants who were not on ART became parasite-positive at the first follow up and 18 (8.7%) became positive at the second follow with moderate parasite density. There was a significant difference between the parasite-positive participants on ART and those not on ART within the first follow up ($p=0.002$) and within second follow up ($p=0.001$) (Table 4.17). The parasite density became negative at the third and fourth follow up time points after the parasite-positive participants were given antihelminthic or antiprotozoan drugs after the second follow up.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Introduction

This study reports the prevalence of intestinal parasites among HIV/AIDS patients with special emphasis on the ART status and CD4+ count levels of the patients who visited three (3) selected HIV clinics in the Cape Coast Metropolis in the Central region of Ghana.

The use ART to managed HIV/AIDS patients helps to improve their immune system and reduce their rate of having opportunistic infections including intestinal parasites (Vernazza *et al.*, 2000). In the absence of ART, HIV/AIDS patients in developing countries unfortunately continue to suffer from the consequences of opportunistic parasites. In countries where antiretroviral drugs are widely available, the prevalence of these parasites has greatly decreased (Gurunathan *et al.*, 2009). In this study, the effect of ART on intestinal parasite prevalence and density in HIV/AIDS patients was investigated.

5.2 HIV INFECTION AND THE DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

In this study it was found that, HIV infection was significantly ($p=0.01$) higher in females than in males, giving a female: male ratio of 4:1 (Figure 4.1). The 2009 HIV Sentinel Survey and National HIV/AIDS Prevalence Estimates reported that, the number of persons living with HIV/AIDS in Ghana was 267,069, made up of 154,612 females and 112,457 males, giving a female: male ratio of 1.4:1 (NACP, 2011). This

national ratio though lower than the one obtained in this study still emphasizes the fact that females are more infected by HIV than males. The difference between the female and male HIV infection prevalence in this study could be due to a social factor, such as one man who is infected with HIV having unprotected sex with more than one female partner in the Cape Coast Metropolis (NACP, 2011). The common behavior of most men in this region is having multiple female sexual partners and this might have contributed to the high HIV prevalence rate in females in this study (NACP, 2011).

Women face greater biological susceptibility to HIV infection through heterosexual intercourse than do men (Lagarde *et al.*, 2003). Women's greater biological susceptibility has been attributed to the following factors; the greater exposed surface area in the female genital tract compared to the male genital tract, higher concentrations of HIV in seminal fluids than in vaginal fluids, larger amount of semen than vaginal fluids exchanged during intercourse, and the greater potential for injury to the cell wall during intercourse for women compared to men (Pettifor *et al*., 2005).

The gender difference of HIV prevalence obtained in this study is similar to a study by KNBS and Macro (2010) in Kenya, in which HIV prevalence rate for adult women was almost double that for men, which represented a female-to-male ratio of 2:1. It was reported by UNAIDS (2013) that, in some countries including Lesotho, Namibia, and Zimbabwe with high HIV prevalence, there were an estimated three women infected with HIV for every two men and among young adults, HIV-infected women outnumbered HIV-infected men by a ratio of 3:1(UNAIDS, 2013).

In this study, the age groups with high HIV prevalence were 18-35 and 36-53 years (Figure 4.2). It was reported in the 2011 HIV Sentinel Survey and National HIV Prevalence AIDS Estimates in Ghana that, two major age groups were most affected by HIV/AIDS, which were 25-29 and 35-39 year olds (NACP, 2011). However, the peak ages for HIV infection were 25-29 years for females and 30-34 years for males (NACP, 2011). The age grouping in this current study though wider than the nationwide report still contain the two major age groups which were most affected by HIV/AIDS. The high prevalence of HIV infection found in the age groups in this study could be due to the fact that it is the most sexually active age group. People within this age group may have unprotected sex with more than one sexual partner which might contribute to the increase in HIV infection (NACP, 2011).

This study showed that a significantly high (p=0.02) number of participants were from rural communities (Figure 4.3). This is similar to a study done in Ethiopia by Adamu and Petros (2009), in which 71.5% of the participants were from rural communities and 28.5% were from urban communities. This is because, most of the study participants who resided in the rural communities were fishermen and women who had a history of travelling to and from neighbouring Ivory Coast through their fishing activities and they might have contracted the disease from there (Ghana Districts, 2011). Another reason is the high illiteracy rate and lack of entertainment activities in the rural communities and as a result they use sexual activities as a means of entertainment (Liu *et al*., 1998). In most cases they indulge in unprotected sex with more than one sexual partner which could lead to the spread of the virus in these communities (Liu *et al*., 1998).

5.3 PREVALENCE OF INTESTINAL PARASITES AMONG THE STUDY PARTICIPANTS

The overall prevalence of intestinal parasites of the study was 47.6 % out of 206 participants (Figure 4.4). This was not statistically significantly (p=0.49) different compared with 52.4% of participants who had no parasite infection. This is very worrying. It was observed that, 43.2% of the parasite-positive participants had not used any antihelminthic drug at the first encounter (Table 4.1). This was because stool routine examinations were not being done for the patients (Author, 2014). With respect to ART treatment, out of the 206 participants, 64 (31.1%) of the parasite-negative participants were already on ART at the first encounter whiles 57 (27.7%) of the parasite-positive participants were not on ART at the first encounter (Table 4.9). The high number of parasite-negative participants recorded in this study could be explained by the fact that, most of the participants who were parasite-negative were on ART which helped to enhance their immunity to enable them to deal with intestinal parasite infection (Rodrigues *et al*., 2008).

The study also showed that, participant's parasite infection was significantly (p=0.01) associated with their ART status (Table 4.9). This finding was in line with previous studies done in Brazil and in different parts of Ethiopia where those studies indicated a significant decrease of intestinal parasite in the patients on ART (Rodrigues *et al*., 2008, Assefa *et al*., 2009). This was attributed to the fact that, the use of ART provides an improvement in immunological conditions of the patients and better response to infections including intestinal parasites (Rodrigues *et al.*, 2008). Better clinical handling of patients with constant updating of protocols for treatment and prophylaxis besides

their better follow up through laboratory tests can help reduce the rate of intestinal parasite infections (Assefa *et al.*, 2009).

The prevalent rate of intestinal parasites in this study was similar to studies done in Ethiopia (44.8%) by Awole *et al.* (2003) and in Cameroon (47.5%) by Sarfati *et al.* (2006). However, this was lower than studies done in Brazil where they had a prevalence of 63.9% (Rodrigues *et al.,* 2008), and also in selected ART centers of Adama, Afar and Dire-Dawa in Ethiopia (52%) by Adamu and Petros (2009) and in different parts of Ethiopia (57.2%) by Assefa *et al.* (2009). The difference in this study compared to other studies elsewhere might be due to geographical differences, sample sizes and the time lapse for recruiting participants; for instance, while the recruitment time for those studies were averagely a year, only four months were used for recruiting participants in this study.

The most prevalent intestinal parasites identified in this study were *G. lamblia* (18.0%) and *E. histolytica* (12.1%) (Figure 4.6). The opportunistic parasites identified were *Cryptosporidium* (5.8%) and *I. belli* (4.4%) (Figure 4.6). A significant number (p=0.01) of parasite-positive participants (40.8%) were living under unhygienic or "poor" sanitary conditions (Table 4.13); where there was improper disposal of rubbish or human excreta which served as good habitats for intestinal parasites (Adamu and Petros, 2009). It was observed in this study that most of the participants with diarrheoa who were significantly associated with *G. lamblia* (p=0.01), *E. histolytica* (p=0.03), *Cryptosporidium* (p=0.02) and *I. belli* (p=0.02) infections were also living under unhygienic or "poor" sanitary conditions (Table 4.13) (Adamu and Petros, 2009). Conversely, participants living in "good" hygienic

conditions had less diarrhoea and less intestinal parasite infections (Table 4.7 & 4.13) (Adamu and Petros, 2009).

Gupta *et al*. (2008) reported that, several species of protozoa have been associated with acute and chronic diarrhoea in HIV patients who lived under unhygienic or bad sanitary conditions. These include; *Cryptosporidium, I. belli, G. lamblia*, *E. histolytica, Microsporidia* spp*, Cyclospora* spp., *B. hominis*, and *Dientamoeba* spp. The finding in this study was also in line with a study in Ethiopia which revealed that, 80% of HIV/AIDS patients with diarrhoea who were living under unhygienic or poor sanitary conditions were significantly (p=0.02) associated with *G. lamblia, Cryptosporidium* and *I. belli* infections (Adamu and Petros, 2009). The association between poor sanitary conditions and intestinal parasite infection has also been indicated by other studies in selected ART centers in Ethiopia (Awole *et al.*, 2003). In Adama, Afar and Dire-Dawa ART centers in Ethiopia, a joint percentage prevalence of 87.5% has been reported whiles in other parts of Ethiopia, 87.6% of parasite-positive patients were living in poor sanitary conditions (Adamu and Petros, 2009; Assefa *et al*., 2009).

5.4 RELATIONSHIP BETWEEN INTESTINAL PARASITE INFECTION, CD4+ COUNT LEVEL AND ART STATUS

A significant number of parasite-positive participants in this study had CD4+ count levels of 200-500 cells/µL and below 200 cells/µL (p=0.021 and 0.001 respectively) (Table 4.11). It was found that, participants with *Cryptosporidium* and *I. belli* were significantly (p=0.01) associated with CD4+ counts below 200 cells/µL (Table 4.11).

In addition, *Cryptosporidium* and *I. belli* infection among participants not on ART were found to be significantly associated (p=0.02) with CD4+ counts below 200 cells/µL (Table 4.11). The association between opportunistic intestinal parasites, ART status and CD4+ count has been explained by various reporters.

Wittner *et al* (1993) reported that, HIV infection has been shown to predispose patients to intracellular opportunistic intestinal parasites such as *Cryptosporidium* and *I. belli*. However, it was indicated by Alfonso and Monzote (2011) and Willemot *et al.* (2004) that, opportunistic parasites are known to resolve spontaneously with immune restoration among HIV/AIDS patients on ART. Flanigan (1994) again demonstrated that, patients with CD4+ counts above 500cells/µL do spontaneously clear *Cryptosporidium* and *I. belli* infection, while it was only patients with CD4+ counts below 200 cells/µL who developed chronic life threatening diarrhoea caused by these opportunistic parasites. The above finding confirmed why *Cryptosporidium* and *I. belli* infection in patients not on ART were found to be significantly associated with low CD4+ counts in this study (Lekha *et al*., 2008).

The association of opportunistic parasites with CD4+ counts below 200 cells/µL in this study was also in line with studies done elsewhere which include, that of India (83%) by Dufera *et al* (2008), in selected ART centers of Adama, Afar and Dire-Dawa in Ethiopia (62.5%) by Adamu and Petros (2009) and also indifferent parts of Ethiopia (76.9%) by Assefa *et al.* (2009). Another study done in Benin City, Nigeria by Akinbo *et al.* (2010) also reported that *Cryptosporidium* and *I. belli* infections were significantly associated (p=0.01) with CD4+ counts below 200 cells/µL.

This study has also shown significant difference of *Cryptosporidium* (p=0.02) and *I. belli* (p=0.02) prevalence rate between the participants on ART and those not on ART (Table 4.10). This is an indication that ART can contribute to the reduction of some opportunistic intestinal parasites infections (Willemot *et al.*, 2004) However, for other intestinal parasites detected in this study (*G. lamblia, E. Histolytica* and *S. stercolaris*) there were no significant (p=0.250, 0.549 and 0.480 respectively) differences between those on ART and those not on ART (Table 4.10). This finding was in agreement with a study done in Ethiopia by Telele *et al.* (2010), which also reported that, there were no significant (p>0.05) differences of species specific intestinal parasites between the two groups of patients.

Furthermore, it was found in this study that, the participant's CD4+ count levels were significantly dependent (p=0.01) on whether they were on ART or not. Out of 105 participants who were on ART, 61.0% recorded CD4+ counts of 200-500 cells/µL whiles only 5.7% had CD4+ countsbelow200cells/µL (Table 4.12). Conversely, out of 101 participants who were not on ART, only 17.8% recorded CD4+ counts of 200-500cells/µL whiles 44.6% had CD4+ counts below 200cells/µL (Table 4.12). In a similar study done in Ethiopia by Assefa *et al.* (2009), it was also reported that, 52.9% of patients who were on ART recorded CD4+ counts of 200–500 cells/µL and 12.5% of them had CD4+ counts below 200cells/µL. Again, 21.3% of patients who were not on ART recorded CD4+ counts of 200–500 cells/µL and 41.2% of them had CD4+ counts below 200 cells/µL. This indicates that, ART can improve the immunity of the patients for them to have a progressive increase in their CD4+ count levels and its absence can lead to a decrease in the CD4+ count level in the patients (Sucilathangam *et al*., 2011).

5.5 EVALUATION OF PARASITE DENSITY, ART STATUS AND CD4+ COUNT LEVELS IN PARASITE-POSITIVE PARTICIPANTS

In this study, it was observed that, out of the 98 (47.6%) parasite-positive participants, 9.2% and 23.5%of those on ART had moderate and few parasite density levels respectively whiles 36.7% and 8.1% of those not on ART had moderate and few parasite density levels respectively at the first encounter (Table 4.14). This shows that, most of the parasite-positive participants who were on ART recorded few parasite density level whiles in contrast most of parasite-positive participants who were not on ART recorded moderate parasite density level. This indicates that the ART helped to reduce the parasite density level in the parasite-positive participants in the ART group. This could be explained by the account given by Rodrigues *et al.* (2008) that, the use of ART provides an improvement in immunological conditions of the patients and reduce the establishment of intestinal parasite infections.

The study has also shown that, in relation to CD4+ count level at the first encounter, 33.7% and 2.0% of the parasite-positive participants who were on ART recorded CD4+ counts of 200-500 cells/µL and below 200 cells/µL respectively whiles in contrast 11.2% and 47.0% of those who were not on ART recorded CD4+ counts of 200-500 cells/µL and below 200 cells/µL respectively (Table 4.15). The results showed that, most of the parasite-positive participants who were on ART had good immunity, whiles most of those who were not on ART had poor immunity as indicated by their CD4+ count levels. This finding was in line with the assertion made by Egger *et al.* (1997) that, the effect of the ART is associated with quite significant increases in

absolute CD4+ count, CD4+ cell function and an improvement in the immunity of the patients.

5.6 THE IMPACT OF ART ON PARASITE DENSITY AND CD4+ COUNT LEVEL

This assessment was done on the follow ups of parasite-positive participants from the first encounter to the third follow up. The average CD4+ count of parasite-positive participants on ART was significantly (p=0.001) higher than those not on ART throughout the follow up time points (Table 4.16).The results showed that, ART helped the participants in that group to have a progressive increase in their CD4+ count level throughout the study period. Conversely, the average parasite density level of parasite-positive participants on ART was significantly lower than those who were not on ART at the first encounter (p=0.001) and first follow up (p=0.012) (Table 4.16). This can be explained by the fact that, the patients on ART had high CD4+ count levels (above 350cells/μL per WHO protocol, WHO, 2012) which gave them good immunity and this helped to reduce the establishment of intestinal parasites infection in those patients as reported also by Rodrigues *et al.* (2008).

The results of the follow up done for parasite-negative participants showed that, the average CD4+ counts of the parasite-negative participants on ART increased significantly from the first encounter to the fourth follow up time point (p=0.001, 0.002, 0.003 and 0.016 respectively) whiles that of participants not on ART decreased significantly from the first encounter to the first follow up (p=0.001) and from the first follow up to the second follow up (p=0.002) (Table 4.17). This eventually increased

from the second to the fourth follow up time points after they became eligible (CD4+ count below 350cells/μL per WHO protocol, WHO, 2012) and were put on ART after the second follow up. The results showed that, the participants in the ART group were supported by the ART which enable them to have a progressive increase in their CD4+ count throughout the study period.

During the follow up of the parasite-negative participants, it was found that, out of the 30 participants who were on ART, 2(6.7%)became parasite-positive at the first and second follow up time points with low parasite density (Table 4.17). Conversely, at the same time point, out of the 30 participants who were not on ART, 16(53.3%) became parasite-positive at the first follow up and 18(60%) became positive at the second follow up with moderate parasite density (Table 4.17). There was a significant difference between the parasite-positive participants of the two groups at the first follow up (p=0.002) and the second follow up (p=0.001) (Table 4.17).

The deduction obtained from the results was that, ART helped to improve the immunity of patients and therefore reduced the establishment of intestinal parasite infection in those patients, but those not on ART were susceptible to intestinal parasite infections. This made it necessary for them to start ART after the second follow up which helped to improve their immunity and enhanced their capacity to deal with subsequent infections. This is an indication that, though the ART helped to reduce the intestinal parasite density level it could not cure them completely, therefore the parasite-positive participants were given antihelminthic and antiprotozoan drugs after the first follow up (Table 4.16) and the second follow up (Table 4.17) respectively.

After successful treatment of the intestinal parasites, their parasite status became negative at the next follow up time points till the end of the study.

5.7 COMPARISM OF THE DIAGNOSTIC METHODS USED

The overall prevalence of intestinal parasite infection as detected by the formol-ether concentration (which was used as the gold standard method for the study), direct wet mount and Kato-Katz methods were 39.8%, 16.0%and 3.4%, respectively (Table 4.2). The prevalence of opportunistic intestinal parasites detected by the modified Ziehl Neelsen (ZN) staining technique was 10.2%. Analysis of the diagnostic performance of direct wet mount, Kato-Katz method and modified Ziehl Neelsen (ZN) staining technique gave sensitivitiesof40.2%, 46.7% and 31.3% respectively. It was observed that, direct wet mount, Kato-Katz method and modified Ziehl Neelsen (ZN) staining technique were better in the detection of intestinal protozoan parasites, intestinal helminth parasites and opportunistic intestinal parasites respectively.

The study has revealed that, the direct wet mount was as good as the gold standard for the detection of intestinal protozoan parasites such *G. lamblia* and *E. histolytica* and motile larval forms of *S. stercoralis*. Similar observation was reported by Watson *et al.* (1988) that the direct wet mount method is useful for detecting organism motility, including motile larval forms of *S. stercoralis* and particularly useful for the observation of motile trophozoites of intestinal protozoan parasites. Melvin and Brooke (1985) also reported that, the method is also useful for diagnosis of parasites that may be lost in concentration techniques. The overall prevalence rate of 16.0% by the direct wet mount method for intestinal parasites observed in this study could be

due to the fact that majority of infected patients have low parasite burden.

The Kato-Katz technique and formol-ether concentration techniques showed good agreement for detection of intestinal helminth parasites such as *A. lumbricoides* and *T. trichiura*. Compared with the formol-ether concentration method, which was used as the gold standard test, the Kato-Katz method gave a good sensitivity of46.7% for detection of *A. lumbricoides* and *T. trichiura* infections (Table 4.4). This observation demonstrates a good agreement between the Kato-Katz and formol-ether concentration methods for the diagnosis of major soil-transmitted helminth infections, but lack of sensitivity of the Kato-Katz method for the detection of other helminth parasites such as *S. stercoralis* as observed in this study.

According to Markell *et al.* (1999), the Kato-Katz technique is useful for field surveys for helminth parasite infections since it provides estimates of the intensity of infection. Siegel *et al.* (1990) and Booth *et al.* (2003) also reported the difficulty in processing diarrhoeal stools and the lack of sensitivity if only a single stool sample is examined by the Kato-Katz technique.

The modified Ziehl Neelsen (ZN) staining method was good for the detection of opportunistic intestinal parasites such as *Cryptosporidium* and *I. belli*. A comparison of the performance of the modified Ziehl Neelsen (ZN) staining method and the gold standard results gave sensitivity of31.3% in this study (Table 4.5). Adamu and Petros (2009) and Assefa *et al.* (2009) also reported the use of the modified Ziehl Neelsen (ZN) staining method to detect *Cryptosporidium* and *I. belli* infections in studies conducted in different parts of Ethiopia. It is therefore imperative that this method be

used in all parasitological laboratories, especially for the detection and confirmation of opportunistic intestinal parasites in HIV/AIDS patients.

The shortfall of all the four (4) methods is that, they could not differentiate *E. histolytica* which is very pathogenic from *E. dispar* which is not pathogenic. Real-time PCR and enzyme-linked immunosorbent assay-based kits can be used to differentiate between *E. histolytica* and *E. dispar* (Leo *et al*., 2006).

CHAPTER SIX

6.1 CONCLUSIONS

Conclusively, the overall prevalence of intestinal parasites in this study was 47.6% with a significant difference (p=0.012) between the parasite-positive participants on ART and participants not on ART. The most predominant intestinal parasites were *G. lamblia* and *E. histolytica,* which also occurred in both groups (ART and non-ART) without any significant difference (p=0.250 and p=0.549 respectively) between the two groups. Infection with opportunistic parasites, *Cryptosporidium spp* and *I. belli* were significantly associated (p=0.02) with participants who were not on ART in this study.

The study has revealed that, most of the intestinal parasite infections were associated with lower CD4+ counts in patients not on ART. This indicates that, when the immune status of HIV/AIDS patients is supported with ART it can help reduce the level of intestinal parasite infection. It has also been confirmed that, living under unhygienic or poor sanitary conditions are likely to predispose patients to intestinal parasite infections. The study also affirms that ART can help reduce the intestinal parasite density in infected HIV/AIDS patients. However, since the ART alone could not eliminate the parasites, there was the need to treat the specific parasites with anthelminthic or antiprotozoan drugs.

Additionally, the study showed that stool examination is very important in determining the possible causes of diarrhoea in HIV/AIDS patients. This study has revealed that, three less expensive parasitological methods namely; formol-ether

concentration, direct wet mount and modified Ziehl Neelsen (ZN) staining technique were used for the detection of opportunistic and other intestinal parasites which coexist with HIV disease.

6.2 RECOMMENDATIONS

The importance of testing for intestinal parasites in HIV/AIDS patients must be strongly emphasized and the necessity of increasing awareness among clinicians regarding the occurrence of these intestinal parasites in this population cannot be over emphasized. All diagnostic techniques for parasite detection especially opportunistic parasites should be made widely available at HIV clinics. It is recommended that clinicians caring for HIV/AIDS patients request routine stool examination for the specific diagnosis of opportunistic intestinal parasites, especially in patients at the symptomatic stage (CD4+T-cell count: 50-200 cells/µl) of HIV disease (WHO, 2012).

The high prevalence of *G. lamblia* suggests a potential contamination of drinking water in the catchment areas of our study. The public health division of the Ghana Health Service in the Metropolis should continue to emphasize the importance of environmental and personal hygiene and collaborate with other Government agencies to provide quality drinking water, good waste management systems, good garbage / faecal disposal systems amongst others to help alleviate the burden of intestinal parasite infections.

Routine stool examination should be enforced and performed regularly during the follow-up of HIV/AIDS patients who attend HIV clinic for early diagnosis and treatment of the specific parasites in these patients. Since HIV/AIDS disease coexist

with intestinal parasite infections as indicated in this study, it is imperative that the National AIDS control Programme provides the necessary logistics required to diagnose specific opportunistic intestinal parasites. This should include; PCR, Isoenzyme Analysis and Antigen detection which has proven to be a very effective means of diagnosing intestinal parasites. Training programs should be held for laboratory professionals to improve their knowledge on these special diagnostic procedures aimed at diagnosing specific opportunistic intestinal parasites.

There is the need for the Ghana AIDs Commission, National Aids Control Programme and the ART service providers to consider adding anthelminthic and antiprotozoan drugs as part of the drug regimen to treat specific intestinal parasites which are known to coexist in HIV/AIDS conditions.

6.3 LIMITATIONS

In this study, direct wet mount, Kato-katz technique, formol-ether concentration method and modified Ziehl-Neelsen (ZN) staining method were used for the detection of common and opportunistic intestinal parasites. I did not use water-ether sedimentation method for *Microsporidia,* adhesive tape or anal swab for *Enterobius vermicularis* and other methods like molecular techniques and immunoflouscent techniques sensitive for parasite detection. Therefore, the prevalence of intestinal parasites among the study participants may have been underestimated. A well designed study employing all the other diagnostic techniques would be needed to determine the true prevalence of intestinal parasites in HIV/AIDS patients in Ghana.

This study did not exclude other causative agents of diarrhoea and therefore the association of parasites identified with diarrhoea should be made cautiously. A well designed study to consider other causes of diarrhoea would be needed to determine the association of specific intestinal parasites with diarrhoea.

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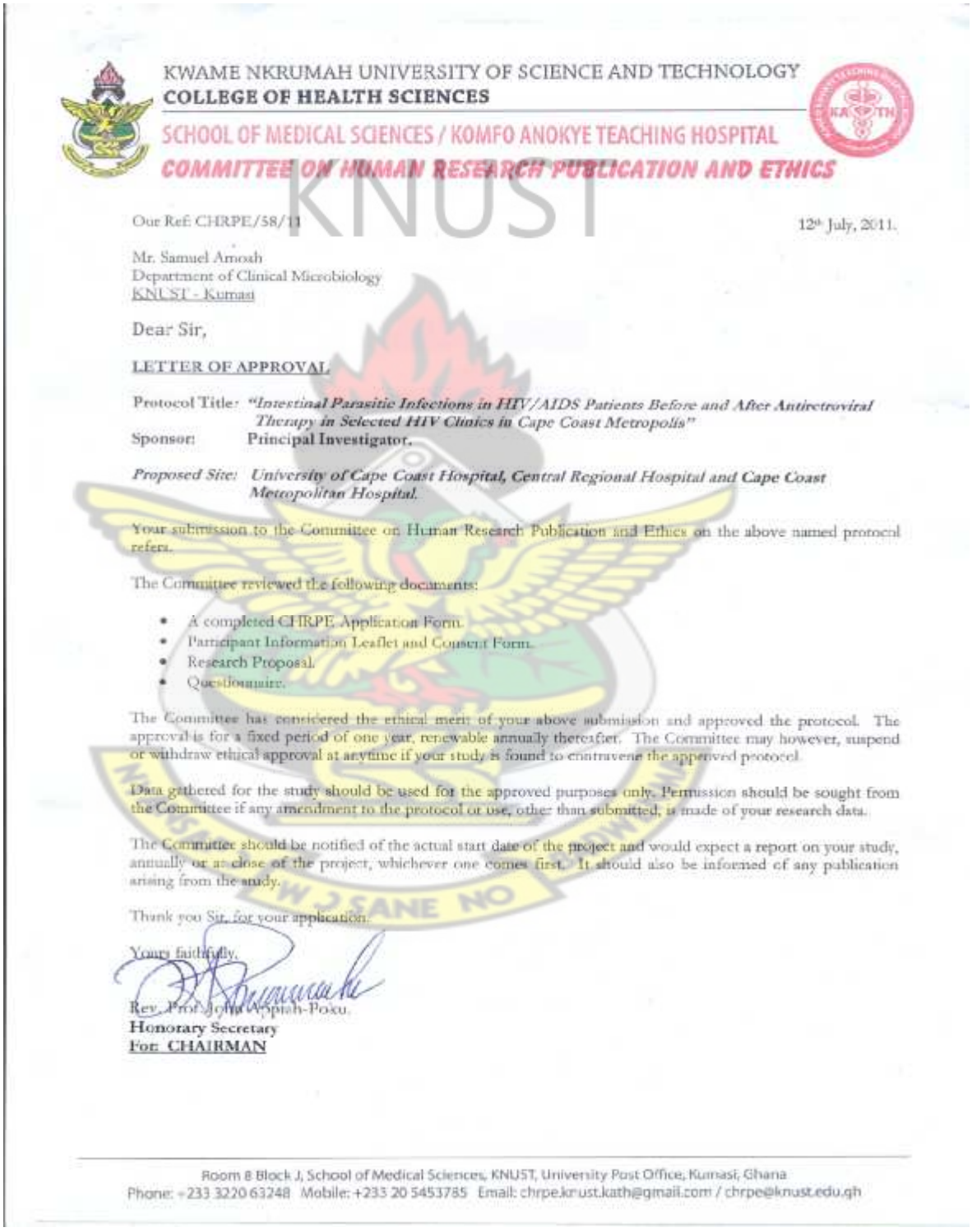
APPENDICES

Appendix 1: Ethics Approval letter for the study



KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS

Our Ref: CHRPE/58/11 — 12th July, 2011.

Mr. Samuel Amoah
Department of Clinical Microbiology
KNUST - Kumasi

Dear Sir,

LETTER OF APPROVAL

Protocol Title: *"Intestinal Parasitic Infections in HIV/AIDS Patients Before and After Antiretroviral Therapy in Selected HIV Clinics in Cape Coast Metropolis"*
Sponsor: **Principal Investigator.**

Proposed Site: ***University of Cape Coast Hospital, Central Regional Hospital and Cape Coast Metropolitan Hospital.***

Your submission to the Committee on Human Research Publication and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Proposal.
- Questionnaire.

The Committee has considered the ethical merit of your above submission and approved the protocol. The approval is for a fixed period of one year, renewable annually thereafter. The Committee may however, suspend or withdraw ethical approval at anytime if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Thank you Sir, for your application.

Yours faithfully,

Rev. Prof. John A. Ofosu-Poku.
Honorary Secretary
For: CHAIRMAN

Room 8 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

Appendix 2: Participant Information Leaflet

<u>This leaflet must be given to all prospective participants to enable them know enough about the research before deciding to or not to participate</u>

Title of Research:

Evaluation of intestinal Parasite Infections in HIV/AIDS patients on antiretroviral therapy (ART) in selected HIV Clinics in Cape Coast Metropolis.

Name(s) and affiliation(s) of researcher(s):

This study is being conducted by Mr. Samuel Amoah of University of Cape Coast Hospital and Dr. Alexander Debrah of KNUST, Kumasi.

Background (Please explain simply and briefly what the study is about):

Human Immunodeficiency Virus (HIV) is the virus that causes Acquired Immune Deficiency Syndrome (AIDS). HIV destroys the biological ability of the human body to fight off opportunistic infections such as enteric parasitic infections. A person can be infected with HIV for a long time without showing any symptoms of the disease. An individual is said to have developed AIDS when he or she presents with a combination of signs and symptoms and has a positive HIV antibody test. Parasitic infections are caused by both protozoa and helminths and the main clinical manifestation is diarrhoea. This infection usually occurs in the intestinal tract, and is one of the sources of disease in HIV patients with diarrhoea as a common complaint with variable severity. Specific intestinal parasites can be identified in HIV/AIDS patients with persistent diarrhoea. These include: *Cryptosporidium, parvum lsospora belli, Microsporidia species*, *Giardia intestinalis, Entamoeba histolytica, Cyclospora species, Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichuria, Hymnolepis nana, Schistosoma mansoni* and *Hook worm species.* Antiretroviral therapy (ART) is the use of anti-viral drugs to manage HIV/AIDS patients to help improve their immune system, thus reducing their rate of having opportunistic infections including diarrheogenic intestinal parasites. ART can therefore contribute to

control or reduce intestinal parasites in HIV/AIDS patients. Since ART does not have direct effect on intestinal parasitic infections, this study aims at finding out the extent to which ART can reduce intestinal parasites in HIV/AIDS patients.

Purpose(s) of research:

The purpose of this study is to assess intestinal parasite prevalence and density in HIV/AIDS patients who come for treatment at HIV Clinics in the Cape Coast metropolis.

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

We will involve 206 participants (HIV/AIDS patients) who will be selected after answering a questionnaire. All the study participants will be expected to be on ART, but those who will be found positive for any intestinal parasite will be given standard antihelmithic drug. Stool samples will be taken from the participants and examined for intestinal parasites at the beginning of the study, which will be repeated again on their subsequent visits to the Clinic at two (2) monthsinterval for a period of one (1) year. Blood samples of the participants willalso be taken to analyse their CD4+ T-lymphocyte count level at each time of the faecal specimen collection.

Risk(s):

The process of venous sample taking will be painful. In some individuals, there may be mild swellings at the site of puncture which will disappear in a short while. The process of faecal sample collection will not be painful but a little inconvenient. The specimen will be sent to the laboratory as soon as they are brought to the Clinic.

Benefit(s):

The goal of this study is to investigate whether or not ART can reduce intestinal parasitic infections in HIV/AIDS patients.The participants who will be found to have intestinal parasites will be given antihelmithic drugs free by the Principal Investigator through the Clinician in-charge at HIV Clinic.

Confidentiality:

Names of participants will not be included in this study. All information collected in this study will be given code numbers. Data collected cannot be linked to you in anyway. No name or identifier will be used in any publication or reports from this study. Records will be kept for two years in the sole custody of the principal investigator and the co-investigator. There will be strict confidentiality and limited access to information. Information will however be available to the investigators only.

Voluntariness:

Taking part in this study should be out of your own free will. You are not under obligation to participate. Research is entirely voluntary.

Alternatives to participation:

If you choose not to participate, this will not affect your treatment in this hospital/institution in any way.

Withdrawal from the research:

You may choose to withdraw from the research at anytime without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal:

There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you without identifiers (name etc), before you chose to withdraw, may have been modified or used in analysis reports and publications. These cannot be removed anymore. We do promise to make good faith effort to comply with your wishes as much as practicable.

Costs/Compensation:

Since this is a self sponsored research and participants will be expected to come for their routine clinics, no monetary compensation will be given to them, however, those who will be found to have intestinal parasites will be treated free of charge by the Principal Investigator, in collaboration with the Clinician in-charge at the HIV Clinic.

Contacts:

If you have any question concerning this study, please do not hesitate to contact Mr Samuel Amoah of University of Cape Coast Hospital on this telephone number: 0244862715.

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel: 3220 63248 or 020 5453785

Appendix 3: Consent Form

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including that about risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

To all PIs, please select and use as appropriate: (delete whichever provision below that does not apply to your study)

WITNESS' SIGNATURE (maintain, if participants could be non-literate): _____

WITNESS'NAME:_____

Appendix 4: Questionnaire

This project is being conducted at the Department of Clinical Microbiology, KNUST to study the effect of antiretroviral therapy on intestinal parasite infections among HIV/AIDS patients. The information that you provide will contribute to our knowledge in how susceptible an HIV/AIDS patient is to intestinal parasite infection, its relationship with ART and CD4+T-lymphocyte count.

Participation in this study would involve completing this questionnaire

Participant's No:_____

Date of Interview: _____

Name of Facility:_____

Name of community where you live: _____

A. Patient Information

1. Sex: 1. Male ☐ 2. Female ☐

2. Age: 1.18-35yrs ☐ 2.36-53yrs ☐ 3.54-65yrs ☐ 4.66 and above ☐

3. Marital status: 1.Single ☐ 2.Married ☐ 3.Divorced ☐ 4.Widowed ☐ 5.Concubine ☐

4. Are you pregnant?: 1.Yes ☐ 2.No ☐ 3.Non-Applicable(N/A) ☐

5. Residential Address: _____Town /Village:_____ District_____

6. Current Occupation: 1.Student ☐ 2.Trader ☐ 3.Public servant ☐ 4.Other ☐ (Specify) ___

7. Educational level: 1.Primary ☐ 2. Secondary ☐ 3.Tertiary ☐ Other ☐ (Specify)___

8. Lifestyle: 1.Poor ☐ 2.Less Affluent ☐ 3.Affluent ☐ 4.More Affluent ☐

9. History of trans-boarder migration: 1.Yes ☐ 2.No ☐

B. General hygiene

10. Type of water used for drinking and other domestic purposes?:1.Pipe borne ☐
2.Borehole ☐ 3.Well ☐ 4.River ☐ 5.Other ☐ (Specify) _____
11. Type of toilet facility: 1.Private WC ☐ 2.Privatepit latrine ☐ 3.Public WC ☐
4. Public pit latrine ☐ 5.PublicKVIP ☐ 6.Others ☐(Specify)_____

C. Personal hygiene

12. Do you wash hands with soap before eating?: 1.Yes ☐ 2.No ☐
13.Do you wash hands with soap after visiting the toilet?: 1.Yes ☐ 2. No ☐
14.Do you have a pet in your home?: 1.Yes ☐ 2.No ☐ If yes go to Q. 15.If no go to Q. 16.
15.If yes, what animal?: 1.Cat ☐ 2.Dog ☐ 3.Others ☐ (Specify) _____
16.Do you rear animals in the compound where you live?: 1.Yes ☐ 2.No ☐
If yes go to Q. 17.Ifnogo to Q. 18.
17. What type of animal?:1.Goat ☐ 2.Sheep ☐ 3.Cattle ☐ 4.Fowl ☐ 5.Others ☐
(Specify) __

D. Gastrointestinal tract manifestation

18. How many times do you defecate in a day?: 1.Once ☐ 2.Two times ☐
3. Three time ☐ 4.Four times or more ☐
If three times or more go to Q19.Iftwo times or less go to Q 20.
19. What is the period of diarrhoea?: 1.Less than three weeks ☐
2. Three weeks or more ☐
20. What is the consistency or nature of your stool?: 1.Loose ☐ 2.Watery ☐
3. Semi-formed ☐ 4.Formed ☐ 5.Mucoid ☐ 6.Others ☐
(Specify)_____
21. Any GIT disease symptoms?: 1.Yes ☐ 2.No ☐

If yes go to Q 22. If no go to Q 23.

22. Specify symptom or symptoms: 1.Abdominal pain ☐ 2.Nausea or vomiting ☐

3. Gas or bloating ☐ 4. Stomach pain or tenderness ☐

E. Medication History

23 Antiretroviral Therapy?: 1.Yes ☐ 2.No ☐

If yes go to Q 24.Ifnogo to Q 25.

24. If yes how long have you been on the drug?: 1. 1-3 months ☐ 2. 3-6 months ☐

3. 6-12 months ☐ 4. more than 1 year ☐

25. Are you on prophylaxis?1.Yes ☐ 2.No ☐

If yes go to Q 26.Ifnogo to Q 27.

26. Which type of drug(s)? Specify:_____

27. Have you taken antihelminth or antiprotozoa drug in the past 3 months?: 1.Yes ☐

2. No ☐

28. Any other medical complains? Specify?_____

F. Laboratory investigations

29. Can you provide stool specimen for routine examination? Yes ☐ No ☐

If no, what are the reasons? _____

30. Can we take your blood sample for CD4 Count? Yes ☐ No ☐

If no, what are the reasons? _____

G. ForLaboratory useonly

31. CD4+T-cell count:1. >500cells/ul ☐ 2. 200-500cells/ul ☐ 3.<200cells/ul ☐

32. Stool R/E (maro): 1.L ☐ 2.W ☐ 3.SF ☐ 4.F ☐ 5.M ☐ 6.BS ☐

33. Stool R/E (micro): 1.Giardia intestinalis ☐ 2.Other flagellates ☐

3. Entamoeba ☐ 4. Ascaris ☐ 5.Strogyloides ☐ 6. Hookworm ☐

7. Other helminths ☐ 8. Isospora ☐ 9.Cryptosporidium ☐ 10.Cyclospora ☐

11. Microsporadia ☐ 12. Other protozoa ☐

34. Infection intensity (parasite density): 1. 1-3 parasites per preparation ☐

2. 4-10 parasites per preparation ☐ 3. 11-20 parasites per preparation ☐

4. 21-40 parasites per preparation ☐ 5. Over 40 parasites per preparation ☐

Appendix 4: Materials used for the study

4.1 Equipment:

i. Binocular Light Microscope (with 10X,40X, and 100X objectives)
ii. Centrifuge, with head and cups to hold 15ml conical tubes
iii. FacsCount Machine (BD FacsCount)
iv. Electronic pipette
v. Vortex mixer
vi. Coring station
vii. Stop watch

4.2 Reagents:

viii. Diethyl ether
ix. Formalin (10%)
x. Lugol's iodine solution (1%)
xi. Physiological saline solution, isotonic (0.85% NaCl)
xii. Formol Saline (10%)
xiii. Carbol Fuchsin stain
xiv. Methylene blue (0.3%)
xv. Acid alcohol (3%) (99ml of 96% ethanol and 1ml HCl)
xvi. Glycerol-malachite green solution (1%)
xvii. Methanol
xviii. Methylated spirit
xix. CD4 reagent
xx. CD4 control beads
xxi. FacsFlow

4.3. Other laboratory supplies:

i. Kato-Katz set (Helm R test kits: from Brazil AK Industriae Commercio Ltd., Belo Horizonte, Brazil; containing a 41.7mg cardboard template-with a holeof7.5mm diameter and 1.0mmthick, number 105-sized nylon mesh screen, hydrophilic cellophane strips of size 25x35 mm and 50 µm thick).

ii. Applicator sticks, wooden.
iii. Beakers (50ml, 100ml and 500ml).
iv. Centrifuge tubes 15ml (ether resistant).
v. Cover slips (22 X 22mm)
vi. Slides
vii. Slide File
viii. Pipette tips
ix. BD vacutainer needles
x. BD EDTA vacutainer tubes
xi. Examination Gloves
xii. Disinfectant (Izal)
xiii. Distilled water
xiv. Stool specimen containers
xv. Disposable plastic Pasteur (transfer)pipettes
xvi. Dropping/'Squeeze' bottles, 250 ml.
xvii. Face shield/Mask
xviii. Filter paper (WhatmannNo.3).
xix. Forceps.
xx. Gauze.
xxi. Glazed tile.
xxii. Markers for indelible labelling.
xxiii. Measuring Cylinders (200ml and 1000ml)
xxiv. Microscope slides (75mmX 25mm)
xxv. Pergamon absorbent sheets.
xxvi. Slide boxes.
xxvii. Staining jar with lid.
xxviii. Test tube rack

Appendix 5: Preparation of Reagents

5.1. Formol Saline, 10%v/v

Preparation of Physiological saline, 8.5g/l (0.85%w/v)

Sodium chloride……8.5g
Distilled water…….1000ml
To make500ml of10%v/v Formol Saline:
Physiological saline……450ml

Formaldehyde solution, concentrated……..50ml

1. Measure the physiological saline and transfer it to a leak-proof bottle.

2. Measure the formaldehyde solution and add to the saline. Mix well.

3. Label the bottle and store at room temperature in a safe place.
   The reagent is stable indefinitely.

5.2. Carbol Fuchsin stain

To make1115ml:
Basic Fuchsin……10g
Ethanol or methanol, absolute……...10g
Phenol…….....50g
Distilled water …….1 liter

1. Weigh the basic fuchsin on a piece of clean paper (preweighed), and transfer to a bottle of at least 1.5 liter capacity
2. Measure the ethanol (ethyl alcohol) or methanol (methyl alcohol), and add to the bottle. Mix at intervals until the basic fuchsin is dissolved.
3. With great care, weigh the phenol in a beaker. Measure the water, and add some of it to the beaker to dissolve the phenol. Transfer to the bottle of

stain, and mix well.

4. Add the remainder of the water, and mix well.
5. Label, and store at room temperature. The stain is stable indefinitely.

5.3. Methylene blue, 3g/l (0.3% w/v)

To make1 liter

Methylene blue…….3g

Distilled water……..1litre

1. Weigh the methylene blue on a piece of clean (preweighed paper), and transfer to a bottle of 1litre capacity.
2. Measure the water, and add about a quarter of it to the bottle. Mix until the dye is fully dissolved.
3. Add the remainder of the water, and mix well.
4. Label the bottle and store at room temperature

5.4. Glycerol-malachite green solution

1. A stock solution of malachite green, 1% solution is prepared as follows:

Malachite green crystals…….1g

Distilled water ……100ml

2. A working solution of glycerol-malachite green solution is prepared as follows:

Glycerol ……..100ml

Malachite green, 1% stock solution…….....1ml

Distilled water ……100ml

The glycerol, malachite green stock solution and distilled water are mixed and poured into a 250ml glass stoppered bottle, and then labeled as "GLYCEROL-MALACHITE GREEN SOLUTION". It is mixed gently before use.