KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

PREVALENCE OF GASTROINTESTINAL PARASITES AND URINARY TRACT INFECTIONS AMONG HIV SEROPOSITIVE PATIENTS IN RELATION TO THEIR IMMUNE LEVELS AT THE BOMSO SPECIALIST HOSPITAL, KUMASI GHANA

BY

GARIBA GEORGE APAAKALI LAAREY

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN THE DEPARTMENT OF CLINICAL MICROBIOLOGY, SCHOOL OF MEDICAL SCIENCES

BY

GARIBA GEORGE APAAKALI LAAREY

MAY, 2014

DECLARATION

I declare that the experimental work described in this thesis was carried out by me, under the supervision of Prof. S. C. K. Tay, Associate Professor at the Department of Clinical Microbiology, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology.

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Head of Department

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DEDICATION

I dedicate this work to my wife Christiana Yaa Ayoma, my younger brother, Augustine Apolinya Gariba and my sisters, Achaalie and Baby Gariba.

ACKNOWLEDGEMENT

May all glory be to God, the Father Almighty for whom I have gotten this far.

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ABSTRACT

Gastrointestinal parasitic infections (GPI) and urinary tract infections (UTI) have contributed to the progression of HIV causing significant morbidity and mortality among HIV positive patients. This study aimed at detecting the prevalence of GPI and UTI among HIV seropositive patients.

A prospective cross-sectional study was conducted among 256 HIV positive patients attending the Outpatient Department of the Bomso Specialist Hospital, Kumasi, Ghana from November 2010 to April 2011. Venous blood, stool and midstream urine samples were collected from each patient. Stool samples were processed using the formol-ether concentration technique and stained by the Modified Ziehl-Neelson staining procedure. CD4 T-cell counts were measured by the FACS Count System using the FACS Flow Cytometer. Urine specimens were examined microscopically and cultured for pathogens.

The overall prevalence of GPI was 18.8%. The most common intestinal parasites were Giardia lamblia 19 (39.6%) and Entamoeba histolytica 11 (22.9%). Other parasites included Ascaris lumbricoides (6.3%), Strongyloides stercoralis (4.2%), Taenia spp (4.2%), Cryptosporidium parvum (2.1%), Trichuris trichiura (2.1%), and Isospora belli (2.1%). Age and gender did not have any significant association with GPI ($p>0.05$) although higher prevalence was observed among females (12.9%) than male (5.9%) and age group 36-45 (6.6%). Most diarrhoea causing parasites were Giardia lamblia (100%), followed by Entamoeba histolytica (36.4%), A. lumbricoides (33.3%) and S. stercoralis (31.6%). Participants with CD4 counts <200 cells/μL had higher and significant ($p<0.0001$) prevalence rates of parasitic infection (8.6%) than those without infection (4.3%). Mean CD4 count was lower (275.8 ± 18.0) and statistically significant ($p<0.0001$) among participants with intestinal parasite infections than those without the infections (485.3 ± 21.5). Urban settlement, pipe borne water usage, preparing food in open space, use of KVIP, diarrhoea and the knowledge of dewormers had a significant association with GPI ($P<0.05$). Most of the UTI was due to Escherichia coli (29.4%) followed by Staphylococcus aureus (20.6%) and Candida albicans (20.6%). A prevalence of 8.8% was recorded for Klebsiella pneumonia, Proteus vulgaris and Salmonella typhi isolates while Shigella spp were the least isolated pathogen (2.9%). A significant ($p<0.0001$) and higher proportion of females (59.8%) than males (0.8%) had bacterial infections. The age group with

highest frequency was 36-45 (5.5%), the most infected group (54.5%) of UTI was observed amongst participants with CD4 counts <200 cells/μL. UTI infected participants recorded a lower level of CD4 count (273.2± 20.1) than those without the infections (472.5 ± 14.4). The overall prevalence of UTI was 13.3%.

The co-existence of GPI and UTI among HIV infected patients in the study area is significant. This calls for intensive public health education on improvement of environmental sanitation and good personal hygiene practices. HIV patients undergoing HAART must also be examined for GPI and UTI.

Keywords: Gastrointestinal parasite, urinary tract infection, Human immunodeficiency virus, CD4 count.

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ABBREVIATIONS

AIDs	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
CCR5	C-C Chemokine Receptor Type 5
CD4	Cluster of Differentiation 4
CFU	Colony Forming Units
CLED	Cysteine Lactose Electrolyte Deficient
FACS	Fluorescence Activated Cell Sorting
GIT	Gastrointestinal Tract
GPI	Gastrointestinal Parasite Infection
HIV	Human Immunodeficiency Virus
IDU	Infectious Disease Unit
INR	Immunological Non-Responders
KNUST	Kwame Nkrumah University of Science and Technology
MPhil	Master of Philosophy
NACP	National AIDS Control Programme
SMS	School of Medical Sciences
TSI	Triple Sugar Iron
UNAIDS	United Nations Programme on HIV/AIDS
USA	United States of America
UTI	Urinary Tract Infection
WHO	World Health Organization

Chapter 1

INTRODUCTION

GENERAL INTRODUCTION

Gastrointestinal parasitic infections are widely distributed public health burden (Savioli *et al.*, 1992). In an article published by World Health Organization in 1987, it was suggested that about 25% of the world's population is chronically infected with enteric parasites. These infections are appreciably higher in developing countries probably due to poverty, overcrowding coupled with poor environmental sanitation and the high prevalence in the general populace (Mohandas *et al.*, 2002). However, intestinal opportunistic parasitic infections in human immunodeficiency virus (HIV) infected subjects present commonly as diarrhoea (Mohandas *et al.*, 2002).

Reports indicate that diarrhoea occurs in 30-60% of AIDS patients in developed countries and up to 90% in developing countries (Framm and Soave, 1997).

Since the first described cases of Acquired Immune Deficiency Syndrome (AIDS), a high prevalence of gastrointestinal infections has been reported in such patients in the form of diarrhoea associated parasitosis (Al-Megrin, 2010). Thus infections in the gastrointestinal tract play a fundamental role in the morbidity and mortality of AIDS patients (Smith *et al.*, 1988; Quinn, 1996), the incidence of which was 50% in developed countries, whereas it reaches up to 95% in developing countries such as in the sub-Saharan Africa (Smith *et al.*, 1988; Quinn, 1996). Many factors including poverty and malnutrition have been noted to have promoted the spread of infections in this region (Grossman *et al.*, 2002; Borkow and Bentwich, 2004). It was suggested, therefore, that attempts to improve the underlying conditions may revert the situation (Al-Megrin, 2010).

In a study conducted by Mannheimer and Soave, (1994) it was noted that the incidence and prevalence of an infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community.

Several opportunistic species of protozoa have been documented to be associated with acute and chronic diarrhoea in HIV diseases (Goodgame, 1996). These include; *Cryptosporidium parvum, Isospora belli, Microsporidia species, Entamoeba coli, Cyclospora species, Blastocystis hominis* and *Dientamoeba fragilis* (Goodgame, 1996).

Non opportunistic parasites such as *Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis* and *Ancylostoma* species are frequently encountered in developing countries but are not currently considered opportunistic in immunocompromised patients (Kulkarni *et al.*, 2009). In such patients, the opportunistic intestinal parasites probably play a major role in causing chronic diarrhoea accompanied by weight loss (Kulkarni *et al.*, 2009). Besides protozoa, the nematode *Strongyloides stercoralis*, a ubiquitous parasite in Tropical and Sub-Tropical areas, can cause diarrhoea and overwhelming infestation (hyper infection syndrome) in patients with a variety of immunosuppressive disorders, including HIV/AIDS (Essex, 1994).

The Acquired Immunodeficiency Syndrome (AIDS) is caused by the Human Immunodeficiency Virus I and II (HIV I and II). The virus was first described in 1981 and the Type I isolated in 1983 (Sharp and Hahn, 2011). It infects certain types of white blood cells, principally the T-Helper lymphocytes, monocytes and macrophages (CD4 cells), which all have important functions in the immune system, the disruption of which lies at the heart of the immunodeficiency that characterises AIDS (Pinsky and Douglas, 2009).

AIDS is regarded as one of the leading public health concerns in the world particularly in sub-Saharan Africa where it is reported to be endemic (66.27%) (UNAIDS/WHO, 2002). Once infected, the subject remains so throughout life, and if the infected persons are left untreated within a decade, majority of them will develop opportunistic infections due to the compromised immune systems caused by the virus (Jawetz *et al.*, 2004). Most infected subjects experience loss of weight, persistent catarrh followed by coughing and diarrhoea during some stage of the infection (Jawetz, 2004).

Worldwide, the toll of HIV disease is enormous (UNAIDS/WHO, 2002). As of 2007, there was an estimated 33.2 million people worldwide living with HIV/AIDS and at least

21.8 million died of the disease (UNAIDS/WHO, 2002). The AIDS epidemic is most severe in sub-Saharan Africa, where 22 million of the world's 33.2 million people with HIV/AIDS live (Kilmarx, 2009)

Other major factors which include poverty, lack of good personal hygiene practices and malnutrition could promote the spread of infections in the region and attempts to improve the underlying conditions may revert the situation (Grossman *et al.*, 2002; Borkow *et al.*, 2004). Studies which investigated the existence of interaction between HIV and parasitic infections in co-infected individuals suggest that these infections cause chronic immune activation (Shapira-Nahor *et al.*, 1998; Kalinkovich *et al.*, 2001; Secor *et al.*, 2003). Though proving existence is insufficient, such immune modulation was shown to increase host susceptibility, thereby, promoting HIV infection and disease progression (Shapira-Nahor *et al.*, 1998; Kalinkovich *et al.*, 2001; Secor *et al.*, 2003).

HIV Sero-negative individuals can be infected with entero-parasites such as; *Giardia lamblia, Trichomonas hominis, Entamoeba histolytica (E. histolytica), Balantidium coli, Taenia species, Strongyloides stercoralis, Ancylostoma species, Ascaris lumbricoides,* and *Trichuris trichiura* which may or may not cause diarrhoea (Cheesbrough, 2004). Urinary pathogens that infect man include parasites (both helmiths and protozoa), Candida species and bacteria (Cheesbrough, 2005). Parasites that are associated with urinary tract infection also include *Schistosoma haematobium, Trichomonas vaginalis* and the fungus *Candida albicans* (Cheesbrough, 2005). Bacteria commonly associated with urinary tract infections (U.T.I) includes *Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Proteus vulgaris and Staphylococcus aureus* whiles *Neisseria gonorrhoea* is less common (Cheesbrough, 2005). Co infections with intestinal parasites can have an influence on the intensity of the HIV sero positive subjects, especially on the CD4 T – cell count and viral load (Faye *et al.*, 2010).

A study conducted by Gazzard *et al.*, (2008) noted that the gastrointestinal tract is a common site for clinical expression of HIV infections. It was also observed that about 80% of CD4 T-cells are found in the gastrointestinal tract (GIT), and the depletion of the these involve the entire GIT (Brenchley and Douek, 2008) It is a known fact that

opportunistic infections virtually take place when the CD4 T-cell count is less than 200cells/ul (Assefa *et al.*, 2009).

AIM AND OBJECTIVES

1.1.1 Main Aim

This study was conducted to determine the prevalence of gastrointestinal parasites and urinary tract pathogens in HIV patients in relation to their ages, sex, and immune levels in the study area.

1.1.2 Specific objectives

1. To evaluate the prevalence of gastrointestinal parasitic and urinary tract infections in HIV Sero-positive patients enrolled in the study area.
2. To determine the immune levels (CD4-T cell counts) of the patients enrolled in the study group.
3. To relate the prevalence of infections to the immune levels of the patients according to
   a. Sex
   b. Age Groups

JUSTIFICATION OF THE STUDY

The Human Immunodeficiency Virus (HIV) which is a Lentivirus belongs to the group of Retroviruses (Abass and Lichtman, 2003). Lentiviruses include Visna and Bovine, Feline and Simian Immunodeficiency Virus (SIV) are all capable of long-term cytopathic effects and produce slowly progressive fatal diseases that include wasting syndrome and central nervous system degeneration (Abbas and Lichtman, 2003). It is well known that most HIV/AIDS patients die from opportunistic infections (Harms *et al.*, 1991). It is also known that HIV infections lead to T-cell dysfunction which in turn leads to increased risk of diarrhoea (Kelly and LaMont, 2008). Stringer *et al.*, (2009) noted that some parts of Africa currently has seen a reduction in the incidence of diarrhoeal diseases associated

with HIV infection, however, people with HIV/AIDS related diarrhoea still remain significant.

Sub-Saharan Africa, a region of intestinal helminth endemicity among both rural and urban settings, especially urban poor (Chan, 1998) are disproportionally burdened by infection with human immunodeficiency virus (HIV), with an estimated 70% of the world's cases (UN Joint Report on HIV/AIDS; 2002) and in 11% of the world's population (U.S. Census Bureau; 2002).

An overlapping distribution of the two pathogens becomes important because concomitant infection with HIV and helminths may potentiate the virulence of each within a coinfected host (Bentwich *et al.*, 2000). However, the few studies published to date indicate that helminth infections may occur with equal frequency and intensity in HIV-infected and HIV-uninfected persons (Feitosa *et al.*, 2001).

Despite increasing interest in the pathologic interactions between the two infections, no epidemiological studies have assessed the prevalence of helminth infections in relation to the immune status in HIV-infected patients in the study area.

An MPhil study conducted at the Komfo Anokye Teaching Hospital (KATH) in an unpublished paper found no parasites in urine samples of HIV/AIDS patients. The study observed pus cells and yeast – like cells in the concentrated urine deposits under the microscope. It is therefore prudent to include studies on urinary pathogens in order to establish the cause of the pus cells being present in urine samples of HIV patients.

Chapter 2

LITERATURE REVIEW

EPIDEMIOLOGY AND HISTORY OF HIV

It has been documented by (UNAIDS/WHO, 2002) that HIV infection is a worldwide problem in the present day. About 33.2 million people are infected globally of which sub Saharan Africa accounts for about 22 million (66.2%) of this number (UNAIDS, 2007). In South and East Asia about 4.9 million people are currently living with HIV (UNAIDS, 2007). Four hundred thirty-two thousand people were estimated to have been newly infected in 2007 (UNAIDS, 2007). About 302,000 people are believed to have died of AIDS in 2007 (UNAIDS, 2007). In India, where the infection rate is less than 1%, an estimated 2.5 million people were living with HIV in 2006, a number second only to South Africa (UNAIDS, 2007). The official estimate is that 700,000 people living in China are currently HIV-infected (UNAIDS, 2007). Given poverty, lack of prevention measures, and limited medical care, this sets the stage in Southeast Asia and perhaps China for a repetition of the disaster occurring in Africa (UNAIDS, 2007). The HIV epidemic is severe in several Caribbean Island nations, where it is estimated that 230,000 people are living with HIV/AIDS (UNAIDS, 2007). In Haiti, 3.8 of every 100 adults are infected; in the Bahamas, 3.3 in 100; in Trinidad and Tobago, 2.6 in 100; and in the Dominican Republic, 1.1 in 100 (UNAIDS, 2007). By the mid-2000s, the HIV epidemic had grown fastest in Eastern Europe and Central Asia, though the rate of new infections has decreased (UNAIDS 2007). There were 150,000 new cases of HIV in this area in 2007, bringing the total number of HIV-infected people to 1.5 million, a 150% increase since 2001(UNAIDS, 2007). The use of intravenous drugs by drug addicts has been driving the HIV epidemic in this part of the world (UNAIDS, 2007). In the Middle East and North Africa, it is estimated that 380,000 people are living with HIV(UCSF, 2008), about 25,000 people died of AIDS in 2007 and 35,000 people became newly HIV-infected (UCSF, 2008).

PATHOGENESIS

2.2.1 HIV AND THE BRAIN

Studies conducted by Nelson *et al.*, (1988), Pomerantz *et al.*, (1987) and Elder and Sever, (1988), noted that the virus also infects other cells such as the lining of the intestinal wall and the nerve cells.

It was, therefore, suggested by Bacellar *et al.*, (1994) that the damage caused by the virus in the intestinal wall contributes to diarrhoea and severe weight loss. HIV can directly damage the central nervous system (CNS), including the brain resulting in some opportunistic infections, particularly cryptococcus, toxoplasmosis, and progressive multifocal leucoencephalopathy (PML) (Bacellar *et al.*, 1994). Problems range from very mild memory disruption to very severe memory difficulties and confusion (Elder and Sever, 1988; Grant and Heaton, 1990).

Studies show that serious cognitive symptoms generally develop late in the course of HIV disease and after the appearance of other physical symptoms due to the HIV infection (Miller *et al.*, 1990; Sacktor *et al.*, 2002).

2.2.2 OPPORTUNISTIC INFECTIONS AND HIV

An immunocompromised system may result in the appearance of certain cancers and disease conditions caused by opportunistic infections (Glatt *et al.*, 1988) which are currently the cause of most deaths of AIDS patients (Glatt *et al.*, 1988). Pathogens that cause opportunistic infections in HIV/AIDS subjects include parasites such as *Toxoplasma gondii*, *Microsporidia* and *Cryptosporidia* (Mofenson *et al.*, 2005). The former results in brain and central nervous system complications while the latter two cause diarrhoea and severe weight loss (Mofenson *et al.*, 2005). Bacteria such as *Mycobacterium avium* complex (MAC), the cause of liver and bone marrow disease, *Campylobacter* and *Clostridium difficile* cause gastrointestinal disease such as diarrhoea and weight loss (Mofenson *et al.*, 2005).

Fungi such as *Histoplasma* species and *Pneumocystis jirovecii* are associated with lung and multi-organ infections (Ramos-e-Silva *et al.*, 2010). Viruses include Adenovirus, the

cause of gastrointestinal disease, J.C. virus which results in Progressive Multifocal Leucoencephalopathy (PML), Cytomegalovirus which is associated with infections of the retina of the eye, colitis and encephalitis (Sweet, 1999) . However, treatment of most opportunistic infections has improved radically since the early days of the epidemic, as rates of these infections have dropped due to effective antiretroviral treatment which prevents the deterioration of the immune system (CDC, 2002).

It was originally thought that HIV involves a period of latency, however, it is now well established that the virus attaches to the CD4 T-cells, the monocytes and macrophage lineage cells and chemokine receptors, during acute infections (Sedaghat and Siliciano, 2004; Downs, 2010) which leads to the destruction of these cells and consequent weakening of the immune system (Sedaghat and Siliciano, 2004; Downs, 2010). Chronic HIV infection was attributed to the infection of the monocytes and macrophage lineage cells, and were thought to be the possible major reservoir of viral replication and therefore contributing to immune deficiency (Lackner and Veazey, 2007). It has recently been observed that the CD4 T-cells which bear the CCR5 HIV co-receptors are primary targets of HIV (Douek *et al.*, 2003). It was also estimated that nearly 80% of the T-cell population is found in the gastrointestinal tract (GIT) (Downs, 2010).

As many as 30% of people living with HIV/AIDS (PLWHA) on antiretroviral therapy (ART), fail to reconstitute CD4 T-cells, despite HIV viraemia control, and are described as immunologically non-responders (INRs) (Rusconi, 2012). The INRs have an increased risk of HIV/AIDS progression and therefore, there is a high probability of opportunistic infections in such subjects (Rusconi, 2012). In order to access the level of immunity in HIV/AIDS subjects, a T-cell count or a CD4 T-cell count is conducted (Cao and Lu, 2005).

2.2.3. CD4 LYMPHOCYTE T-CELL AND HIV/AIDS

CD4 T-helper cells are critical in helping the body mount an effective immune response as they signal the immune system to 'turn on' in order to fight infections. Paradoxically, CD4 cells are also the major targets of HIV (Pinsky and Douglas, 2009). A CD4 cell count measures the number of CD4 cells per cubic millimeter or micron-litre of blood (Alimonti *et al.*, 2003).

As the disease progresses, it destroys the CD4 cells and eventually interferes with the functioning of the immune system (Alimonti *et al.*, 2003).

The CD4 cell counts are therefore used to measure the extent of damage, if any, that has been caused to the immune system (Alimonti *et al.*, 2003).

Normal CD4 counts are between 500 and 1,500 cells per cubic millimeter (mm^3) or micron-litre (ul) (Castro *et al.*, 1993). When a CD4 cell count falls below 200 cells/ul, the infected subject is rendered susceptible to a variety of opportunistic infections, and is considered to have developed AIDS (Castro *et al.*, 1993). The immune status can also be measured by determining the percentage of CD4 cells present in the total number of lymphocytes. A normal percentage is between 32 and 68% (Welker and Rubsamen-Waigmann, 2003).

2.2.4. VIRAL LOAD AND HIV/AIDS

Another means of measuring the immune level is by determining the viral load in the blood of the infected subject, a method which counts the copies of the virus in a cubic millimeter of blood (Pinsky and Douglas, 2009). It is a direct measurement of how active infection is and a very good predictor of how fast HIV disease is progressing or likely to progress (Pinsky and Douglas, 2009). A high viral load such as above 10,000 copies means that there is a high level of virus in the blood and other tissues of the body, many T-helper cells are therefore being infected and destroyed, and the immune system is in danger of significant damage (Pinsky and Douglas, 2009). A low viral load less than 10,000 copies means there is a smaller amount of virus in the blood, thus fewer T-helper cells are being destroyed and much less damage to the immune system is occurring. Studies show that less than 5% of all AIDS-defining complications occur in people with a viral load less than 5, 000 copies (Dornadula *et al.*, 2001). Data about viral load and CD4 cell counts from a large cohort of patients have been used to predict the likelihood of the development of an AIDS-defining opportunistic infection in the absence of treatment (Mellors *et al.*, 1997).

Viral load determination, in combination to other laboratory tests and clinical markers, may be a consideration in the decision to start anti-retrovirals. It is particularly critical for the evaluation of response to therapy (Pinsky and Douglas, 2009).

HIV AND GASTROINTESTINAL PARASITE INFECTIONS

Gastrointestinal parasitic infections in HIV/AIDS patients are almost universal and significant disease occurs in 50-90% patients (Awole *et al.*, 2003).

Diarrhoeas are manifestations or life threatening complication of infections with HIV sometimes during the course of the disease (Oguntibeju, 2006).

Pathogens causing diarrhoea have been found in 30-80% of patients depending on the extent of the study and patients characters, these pathogens include opportunistic agent that consistently cause severe, chronic or frequent gastrointestinal disease and non-opportunistic agents that cause acute treatable diarrhea illness (Awole *et al.*, 2003). The etiology of such diarrhea could either be parasitic, bacterial, fungal and enteric or the HIV itself may contribute to it (Awole *et al.*, 2003).

Numerous studies, since the discovery of HIV/AIDS, have documented intestinal parasites as being frequently associated with the features of HIV infected patients with severe diarrhoea (Wuhib *et al.*, 1994).

In an article published by the WHO, (2002) it was documented that HIV infection was recognised in Sub-Saharan Africa more than two decades ago and today around 42 million people are estimated to be infected with the virus in this area alone (WHO, 2002).

It was also noted by Eamsophana *et al.*, (1987) that developing countries have been the areas mostly infected by HIV/AIDS and worsened by the numerous endemic parasitic infections.

In addition to the endemicity of enteroagents in these countries, the serious economic hardships contribute to increase in infections and co-infections with the virus (Ntigwa *et al.*, 2000).

With this relative high prevalence of parasitaemia in tropical countries, HIV/AIDS infections and parasitic infections frequently overlap (Awole *et al.*, 2003).

In a study conducted by Benwich *et al.,* (1995) it was assumed that based on the immunological characteristics of the HIV/AIDS infections, the history of parasitic infections will be altered in an unfavourable way because the majority of such infections requires a competent immune response to contain the pathogen. The study also noted that just as the consequences of HIV on parasitic infections have attracted much interest, the impact parasites may have on the replication of the virus and the eventual progression to AIDS has also gained attention.

Other studies conducted by Escobedo *et al.,* (1999) entero agents such as *Cryptosporidium parvum, Isospora belli* and *Strongyloides stercoralis* among others are related to the gastrointestinal changes among patients infected with the virus. Some of which occurred with elevated prevalence in these patients in the years preceding the introduction of the Highly Active Antiretroviral Therapy (HAART) (Escobedo *et al.,* 1999).

In Ethiopia, a study conducted by Endeshaw *et al.,* (2006) identified *microsporidium* to be the common cause of chronic diarrhoea and severe weight loss in advanced HIV/AIDS patients.

A study conducted in Nigeria by Okodua *et al.,* (2004) reported intestinal parasitic infections to have been a major source of tropical disease especially among HIV patients. In their study, it was found that, from a total of 215 subjects, comprising of 35 seronegative and 180 seropositive patients, the parasitic infection rate among HIV seropositive subjects (49.9%) was statistically higher than that among HIV seronegative subjects (25.6%), ($P< 0.05$).

A similar study conducted by Adjei *et al.,* (2003) at the University of Ghana Medical School, demonstrated the prevalence of *Cryptosporidium* species.

It has been documented in studies by Mohammed Awole *et al.,* (1994) that no etiological agent was found in 15 – 50% of patients with chronic diarrhoea.

The most commonly (up to 60%) diagnosed pathogens were *Cryptosporidium parvum* and *Isospora belli. Giardia lamblia* and *Entamoeba histolytica* often associated with diarrhoea in tropical countries were common in HIV/AIDS patients except

Cryptosporidium parvum in Buenos Aires and *E. histolytica* in Maryland and Cote D'ivoire (Mohammed Awole *et al.,* 1994).

Strongyloides stercoralis is also found in a higher proportion (17%) in Cote D'ivoire (Awole *et al.,* 1994). *Cyclospora cayetanensis,* also known to cause prolonged watery diarrhoea in travelers and HIV/AIDS subjects has limited epidemiological relevance especially in association with HIV infection (Mohammed Awole *et al.,* 1994).

A study conducted in Thailand shows prevalence of *Cyclospora cayetanensis* 2.21% in HIV infected patients (Tarimo *et al.,* 1996 and Ortega *et al.,* 1993).

The causes of chronic diarrhoea are perhaps the most satisfying to diagnose because with the exception of Cryptosporidiosis and HIV related enteropathy, good response to treatment can be expected (Ortega *et al.,* 1993). Unfortunately, all etiological agents cannot be diagnosed in Africa on routine basis due to limited diagnostic facilities and trained personnel (Ortega *et al.,* 1993).

The geographical distribution of intestinal parasites have been shown to coincide with that of HIV/AIDS especially with CD4 T-cell counts under conditions of poverty stricken countries in Sub Saharan Africa which has therefore led to increase interest in the pathological interaction between parasitic infections and HIV/AIDS particularly in adults

(Kipyegen *et al.,* 2013; Teklemariam *et al.,* 2013).

(Ntigwa *et al.,* 2000) in a survey aimed at studying parasitic infections and their correlation with the immune status in a cross-sectional study using urine, sputum, stool and blood samples from HIV infected patients found that, out of a total of 56 HIV positive patients with a mean age of 37 years, no parasites were found in sputa. *Schistosoma haematobium* was found in one urine sample, from the stool analysis, 17 patients were infected by at least one of the following parasites; *Cryptosporidium* (19.6%), *Microsporidia* (10.7%), *Entamoeba coli* (15.4%), *Entamoeba histolytica* (7.7%) (Ntigwa *et al.,* 2000). On analysis the probability of finding Cryptosporidium significantly increased with decrease in CD4 T cell count (Ntigwa et al., 2000).

In a similar study conducted by Ramakrishnan *et al.*, (2007), it was observed that parasitic infections are more common in HIV infected patients than in HIV negative patients in South India probably due to difference in immunological profile.

A study of stool samples of sixty HIV infected patients at different immunity levels was conducted by Wiwanitkit, (2001) at King Chulalonkon Memorial Hospital Thailand. Each person was examined for CD4 T Cell count and screened for diarrhea. It was found that the prevalence of intestinal parasites among HIV patients in this study area was about 50%.

Furthermore, non-opportunistic intestinal parasitic infections such as hookworm, *Opisthorchis viverrini* and *Ascaris lumbricoides* were common in HIV infected persons in all the immunity levels with or without diarrhoea symptoms (Wiwanitkit, 2001)

Opportunistic intestinal parasitic infections such as *Cryptosporidium parvum, Isospora belli,* and *Microsporidium* significantly predominate in the low immunity group of patients with diarrhoea (Wiwanitkit, 2001). It was therefore suggested that opportunistic protozoa infections should be suspected in any low immuned group of HIV infected patients, especially those presenting with diarrhoea, whiles the importance of tropical epidemic non opportunistic intestinal parasitic infections among HIV infected patients should not be neglected (Wiwanitkit, 2001).

HISTORY AND EPIDEMIOLOGY OF HIV

The viruses are of zoonotic origin, principally affecting cats and monkeys, especially the chimpanzee family (Harinda, 2008) which is the reservoir of the Simian Immunodeficiency Virus (SIV). Two closely related types, designated HIV I and II have been identified (Gilbert *et al.*, 2003). HIV I is by far the most common cause of AIDS, but HIV II which differs in generic structure and antigenecity causes a similar clinical syndrome (Gilbert *et al.*, 2003).

Theory has it that the first infections were passed from the blood of chimpanzees to humans when hunters ate the flesh of the infected animals (Harindra, 2008). The virus probably penetrated the human body through wounds of the hunters (Harindra, 2008).

Wolfe *et al.,* (2004) in their study established that each time the virus passes from the chimpanzee into a human host, it mutates into a different strain.

In Africa, human infections occurred in rural areas in the early 1930 but passed unnoticed (Chitnis *et al.,* 2000).

Infected immigrants from rural areas (Hunter, 2006) introduced the virus into densely populated areas where the cultural acceptance of prostitution promoted its spread worldwide.

An unusual number of young homosexual male heroin addicts and haemophiliac in the United States of America were noted to be dying of normal benign opportunistic infections in the late 1970s and early 1980s. Their symptoms define a disease, acquired immunodefiency syndrome (AIDS) (Hunter, 2006).

The first recognised cases of AIDS therefore occurred in the U.S.A in 1981 (Sharp and Hahn, 2010), and the HIV Type I isolated in 1983 (Jawetz *et al.*, 2004).

The viral particles that initiate infection are usually found in the blood, semen, or other body fluids of the infected individual and are introduced into another individual by sexual contact, sharp object prick or by transplacental route (vertical transmission) (Donegan *et al.*, 1990; Kuplan and Heimer, 1995; Varghese *et al.,* 2002; Coovadia, 2004).

The infection begins when the glycoprotein on the viral envelope binds to both CD4 and a co-receptor that is a member of the chemokine receptor family (Chan and Kim, 1998; Wyatt and Sodroski, 1998).

After the virus completes its life cycle in the infected cell, free virions are released from infected cells and bind to uninfected ones thus propagating the infection (Abass and Lichtman, 2003), which when left untreated within a decade develops into AIDS (Jawetz *et al.,* 2004)

Most infected individuals will progress from asymptomatic to become symptomatic, and the majority of them will ultimately succumb to the disease (Lawn, 2004). Diseases directly related to AIDS mainly consist of opportunistic infections, cancers and the direct

effect of the virus itself on the central nervous system (CNS) (Abass and Lichtman, 2003).

However, there are cases of long term survivors (Graber *et al.,* 2009), some of which results from infections with HIV strains that lack a functional protein designated "nef protein" (Papkalla *et al.,* 2002). This resistance to the virus correlates with a lack of expression of the chemokine co-receptor for the virus (Papkalla *et al.,* 2002).

Between two to four weeks of HIV infection, the infected individual may develop initial symptoms which resemble those of influenza or infectious mononucleosis with 'aseptic' meningitis or a rash which could occur up to three months after infection (Abass and Lichtman, 2003).

These symptoms stem from immune responses initiated by a wide spread infection of the lymphoid tissue (Abass and Lichtman, 2003) and subside spontaneously after two to three weeks. This is followed by a period of asymptomatic infection or a persisted generalised lymphadenopathy that may last for several years (Abass and Lichtman, 2003; Burton *et al.*, 2002) during which period the virus replicates in the lymphoid tissue (Burton et al., 2002).

The onset of symptoms correlates with a reduction of CD4-T cell counts to less than 450 cells/µL and increased viral load count in the blood (Pentaleo *et al.,* 1993) which signifies a deterioration of the immune system resulting in increased susceptibility to opportunistic pathogens such as Candida species, herpes virus or intracellular bacteria (Abass and Lichtman, 2003).

The term "full blown AIDS", as used by Buchbinder *et al., (*1994) to describe the progress of HIV disease from an asymptomatic infection to profound immunosuppression, occurs when the CD4 T-cell counts are less than 200cells/µL. This involves the onset of more significant diseases, such as HIV wasting syndrome (weight loss and diarrhoea for more than a month) with the occurrence of indicator diseases such as Kaposi sarcoma or specific opportunistic diseases especially Pneumonia due to *Pneumocystic carinii*, *Mycobacterium avium-intracellare* complex infection and severe cytomegalovirus disease (Awadh and Anazi, 2009).

A number of HIV specific markers have been used for staging and monitoring progression of HIV infection and assessing response to therapy but the most common cellular marker adopted in Ghana is the CD4 T-cell count (Dar and Singh, 1999). CD4 T-cell is one of the several glycoproteins termed "Cluster of Differentiation (CD) antigens", expressed on the surface of lymphocytes (Abass and Lichtman, 2003). CD4 T-cell serves as a receptor for HIV infection (Gupta and Gupta, 2004).

The median interval between HIV I infection and the development into AIDS in adults is 10-11 years (Munoz *et al.,* 1989). Some rapidly progress to AIDS in less than 5 years (Phair *et al.,* 1992).

The biological etiology for this variability is unknown (Sheppard *et al.,*1998). However, exposure to microbial agents has been suggested as one of the probable factors leading to AIDS progression (Webster *et.al.,* 1989).

Estimation of CD4 T-cell is one of the measures of ascertaining the immune competence of HIV infected individuals throughout the broad spectrum of HIV disease (WHO, 1990). In early HIV infection, the number of leucocytes and lymphocytes, including T-cells and their subsets are normal (Abass and Lichtman, 2003). However, the number and percentage of CD8 T-cell subsets begins to increase dramatically soon after seroconversion in the initial few months (Abass and Lichtman, 2003).

These cells operate by killing infected CD4 T-cells thereby partially controlling the infection while simultaneously contributing to the destruction of the immune system (Abass and Lichtman, 2003).

GASTROINTESTINAL TRACT INFECTIONS (GI)

Most gastrointestinal tract infections are caused by parasites that are cosmopolitan in distribution (Cheesbrough, 2005).

Human intestinal parasites are either single-cell organisms (Protozoa) or multicellular organisms (helminths) that inhabit the small and large intestines of their hosts where they use the stool or blood from the intestinal wall as its food (Ryan and Ray, 2004).

Protozoa are single-cell organisms and multiply spontaneously inside its host thereby causing serious infections to develop rapidly which can be directly infectious to humans when passed out in faeces into the environment (Cheesbrough, 2005). Helminths however, require a period of maturation while in the soil in the form of ova or larvae where they become infectious (Cheesbrough, 2005). Transmission occurs when the host comes into contact with the infected faeces through contaminated soil, food or water (Cheesbrough, 2005).

Other parasites such as Taenia species require an intermediate host to complete its life cycle (Robert *et al.*, 2005)

The most common mode of gastrointestinal parasitic infections is by the faeco-oral route whereby water, food and hands are contaminated with faecal material and then transferred to the mouth and subsequently ingested (Cheesbrough, 2005).

A number of gastrointestinal infections can reach epidemic proportions with rare protozoa infections only now being understood as they are appearing as a concomitant infection in people with depressed immune responsiveness such as HIV/AIDS (Nwachukwu and Okeke, 2008).

By classification, gastrointestinal parasites are either helminths or protozoa (Cheesbrough, 1992).

Helminthiasis is reported to be endemic in Africa (Cheesbrough, 1992). The helmiths of medical importance are classified into three groups; Cestodes (tape worms), eg. *Taenia solium*, Trematodes (flukes), eg. *Fashiola hepatica* and the nematodes (round worms), eg. *Necator americanus* commonly known as hookworm (Cheesbrough, 1992).

Ryan and Ray, (2004) classified helminths into two groups according to their shape, i.e. flat or roundworms. The flukes and tape worms are included in the group of flat worms or platyhelminths, owing to their usual flattened, bilateral symmetrical shapes without through body cavities and are hermaphrodite except the Schistosomes (Cheesbrough, 1992).

The round worms are distinct, more tubular with distinct sexes (Ryan and Ray, 2004). Matured tape worms have no digestive system, but possess a small head (scolex) with two or four suckers and a circle of hooks which it uses to attach to the host's intestinal wall (Cheesbrough, 1992).

They absolve food nutrients directly from the host's gut contents through their surfaces (Ryan and Ray, 2004). The mature tape worm is characterized by its generally long and tape-like body (strobila) consisting of numerous units (proglottids) (Cheesbrough, 1992).It owes its name to the tape-like structure of its body (Ryan and Ray, 2004).

Roundworms on the other hand have a developed digestive system with a mouth and anus and are all soil-transmitted helminths (Cheesbrough, 1992). The final host of all the helminths of medical importance is man, except the dog tape worm (*Echinococcus granulosus*) which man is an accidental intermediate host (Cheesbrough, 1992).

The ova of all intestinal helminths are finally excreted in stools (Cheesbrough, 1992). It was suggested by Cheesbrough, 2005 that through proper sanitary disposal of human excreta, control of most of the helminths can be achieved with the exception of *Enterobius vermicularis* and the Schistosomes which is a water borne infection.

Enterobius vermicularis

The matured female is a small yellowish-white worm of length between 8-13mm with a thin and pointed tail. The matured male is relatively shorter, measuring between 2-5mm with a rather coiled tail equipped with a single copulatory spicule. The adult worms are identified by the double bulb esophagus and a pair of cervical alae in the anterior end (Cheesbrough, 2005).

Enterobius vermicularis, often known as the pin worm, causes Enterobiasis. This worm is world-wide in distribution (Cheesbrough, 2005). It is probably the oldest demonstrated helminth in man with its eggs found in a 10,000-year-old coprolite (Ryan and Ray, 2004).

Infections are more common in young children who live under poor unhygienic conditions, probably due to auto infections (Cheesbrough, 2005) which are caused by the presence of its eggs around the anal area which causes intense itching or irritation and

scratching of the infected area leading to contamination of the fingers (Cheesbrough, 2005). Air-borne contamination is also possible whereby the eggs may be taken into the air, inhaled and swallowed (Cheesbrough, 2005) while preparing beds contaminated with the eggs of the parasite.

Life Cycle

After ingestion, the infective eggs hatch into larvae in the small intestine and develop into fully matured worms in the large intestine and mostly inhabiting the caecum (Cheesbrough, 2005).

After copulation, the female worms migrate further down to the rectum, pass onto the anal area and lay their eggs in the perianal skin, and within about 6 hours, eggs develop infective larvae (Cheesbrough, 2005).

Occasionally, infective eggs may hatch around the perianal skin and migrate back to the intestine where they develop into adult worms, a situation referred to as autoinfection (Cheesbrough, 2005). The infective eggs can remain viable on clothing and in dust for several weeks (Cheesbrough, 2005). They thrive best under warm and humid conditions (Cheesbrough, 2005).

Once an infection is introduced into a household, other members of the family may spontaneously be infected (Ryan and Ray, 2004). The entire life cycle of the worm is completed in six weeks when eggs are produced after infection (Cheesbrough, 2005).

Infections with this worm does not normally result in serious disease but manifests in hypersensitivity (perianal itching) mostly at night due to migration of the gravid female from the large intestine down to the anal region (Ryan and Ray, 2004). This may lead to irritability and other minor complains, but in severe infections, the intense itching may lead to scratching and secondary bacterial infections (Ryan and Ray, 2004).

In infected females, the worm may enter into the genitalia and cause vaginitis, granulomatous endometritis or salpingitis (Ryan and Ray, 2004). It is also suggested that these migrating gravid females might carry with them enteric bacteria into the urinary bladder in young women which might induce acute bacterial infections of the urinary tract

(Ryan and Ray, 2004). Worms in the appendix may be associated with appendicitis (Cheesbrough, 2005).

Since infections can be spontaneous due to the eggs being infective very soon after being laid, control and preventive measures are required to combat the spread of infections (Ryan and Ray, 2004).

These include measures such as deworming all members of a family in which infections have been identified and the practice of good personal hygiene, such as washing of anal skin each morning as well as clothes worn at night (Cheesbrough, 2005).

Enterobiasis is diagnosed by microscopically demonstrating ova of the worm in samples collected from the infected perianal skin or clothing worn at night and the adult worm recovered in faecal material or during clinical examination (Cheesbrough, 2005).

Treatment

Infections with this parasite seldom produces serious disease (Ryan and Ray, 2004), but in cases where it does, satisfactory drug such as pyrantel pamoate and mebendazole are used for treatment (Ryan and Ray, 2004).

Many authorities believe that all members of a family or other cohabiting group should be treated simultaneously (Ryan and Ray, 2004). In severe infections, treatment after 2 weeks is recommended (Ryan and Ray, 2004).

Strongyloides stercoralis

Strongyloidiasis is the infection caused by the nematode *Strongyloides stercoralis* or otherwise the dwarf thread worm, with a worldwide distribution and particularly endemic in many tropical and sub-tropical countries (Cheesbrough, 2005).

The worm is also noted to be endemic in developing countries where it is widely associated with conditions of HIV/AIDS (Gomez et al, 1995).

Known to be a soil-borne infection, *Strongyloides stercoralis* infects its host by the filariform larvae penetrating the unbroken skin from infested surroundings (Cheesbrough, 2005).

Auto-infection may also occur by either the first stage larvae (rhabditiform) developing into the filariform stage in faecal material present on perianal skin and or the development of the rhabditiform larvae into the filariform larvae in the intestine and subsequently penetrating the skin or the gut wall respectively (Cheesbrough, 2005).

Transmission through breast feeding is also known to occur, usually called Trans mammary infections (Cheesbrough, 2005).

The development of rhabditiform larvae into the infective (filariform) larvae in the intestine in the case of autoinfections may also favour interpersonal transmission of the parasites (Cheesbrough, 2005). The larvae present in the anal region of the infected male or female could penetrate the penis of the partner during anal sexual intercourse, meaning that males could have greater chances of being reinfected by having sodomy relations with both sexes (Dias *et al*., 1992).

Worldwide, *Strongyloides stercoralis* has infected millions of people most of whom are found in Tropical and Sub-tropical regions (Keisser and Nutman, 2004). Infections have however been reported in Temperate zones as well (Walzer *et al*., 1982).

Occupations in which there is increased contact with soil contaminated especially with human waste such as use of human excreta as organic manure in farming, and promiscuous defecation activities increase the risk of being infected (*Walzer et al.*, 1982).

Factors such as behavioural or socioeconomic may vary the prevalence of infections with this worm among ethnic groups, whiles other researchers suggest that differences in skin types may result in more or less resistance to larvae penetration (Keiser and Nutman, 2004). Hyperinfection syndrome of this worm has mortality rates ranging from 15% to as high as 87% (Marcos *et al.*, 2008).

Life Cycle

The filariform larvae, on gaining access to its host enters the superficial blood vessels and follow a heart-lung migration, during which period they develop up to the trachea where they are coughed up and swallowed (Cheesbrough, 2005).

The larva matures in the intestinal tract and the female gets embedded in the intestinal mucosa where they lay their eggs (Cheesbrough, 2005). The rhabditiform larvae hatch out of the eggs as soon as the eggs are laid. They develop in the intestine into the filariform stage and subsequently cause autoinfection or are passed out in the faeces (Cheesbrough, 2005).

Rhabditiform larvae which may be excreted in faeces are capable of developing into filariform larva within 3-4 days under suitable conditions (Cheesbrough, 2005). These larvae can remain infective in the soil for several months before the life cycle resumes (Cheesbrough, 2005). In favourable climatic conditions, the worm can follow a free-living existence for several generations and instead of developing into the filariform stage, the rhabditiform larvae develops directly into mature egg-producing worms in the soil (Cheesbrough, 2005).

The auto infectious life cycle aspect of this infection causes the infection to persist for longer periods after the host has left an endemic area (Ryan and Ray, 2004). Infections with Strongyloides are usually asymptomatic, but in some cases it may result in mild cutaneous and/or abdominal symptoms (Cheesbrough, 2005). Recurrent urticaria, often involving the buttocks and wrists is the most common cutaneous manifestation (Ryan and Ray, 2004).

Pulmonary symptoms are rare in light infections where as relatively heavy adult infections involving the intestinal mucosa can cause abdominal cramps which are worsened with food ingestion (Ryan and Ray, 2004). Nausea, diarrhoea, gastrointestinal bleeding accompanied with weight loss readily occur (Cheesbrough, 2005).

Autoinfections with *Strongyloides stercoralis* can be overwhelming and sometimes fatal in conditions of immunosuppression including HIV/AIDS during which period the larvae

can be found in most tissues and serous cavities such as the liver, Central Nervous System (CNS), peritoneum and kidney (Cheesbrough, 2005).

Bacteraemia may as well develop due to the entry of enteric flora through the disrupted mucosal barriers (Ryan and Ray, 2004).

The infection is diagnosed by microscopically demonstrating the larvae in freshly voided infected stool samples and duodenal aspirates (Cheesbrough, 2005). The ova are not detectable due to their spontaneous hatching ability mentioned earlier in the text.

An ELISA system for detecting antibodies to excretory-secretory antigens of Strongyloides is a sensitive method of diagnosing uncomplicated infections (Cheesbrough, 2005).

Control of this infection is by deworming even the asymptomatic state due to the potential for fatal hyperinfections (Steinmann *et al.*, 2008).

Treatment

Treatment should be given to all patients in both symptomatic and asymptomatic states because of the potential for fatal hyper infection (Steinmann *et al.*, 2008).

The drugs of choice are mebendazole given 100mg twice daily for 3 consecutive days or thiabendazole at 25mg/kg body weight twice daily for 2 days (Steinmann *et al.*, 2008). However, in disseminated disease, treatment should be extended for 5-9 days (Steinmann *et al.*, 2008). Albendazole given 400mg daily for 3 consecutive days is also effective. Levamisole is only effective in about 50% of the cases and is therefore not a drug of choice (Steinmann *et al.*, 2008).

Trichuris trichiura

Trichuris trichiura which is of world-wide distribution is the cause of Trichuriasis (Cheesbrough, 2005). Also known as the whip worm, it is reported to be the most

prevalent helminth in the Caribbean and also wide spread in the Tropical Africa and South East Asia where warm humid condition prevail (Cheesbrough, 2005). It is rarely found in arid areas and high altitudes.

Infections with this worm are concentrated in areas where indiscriminate defecation occurs with prevalence rates rising as high as 80% (Ryan and Ray, 2004).

The intensity of infections is generally low but adult worms may live between 4-8 years (Ryan and Ray, 2004). The mature female worm measures up to 50mm in length whiles the mature male measures up to 40mm and possess a characteristic whip like shape (Cheesbrough, 2005).

The tail of the male is coiled with a single spicule whiles that of the female is straight (Ryan and Ray, 2004).

Life Cycle

After ingestion of the infective eggs, the larvae hatch out in the small intestine and penetrate the intestinal villi where further development takes place.

The larvae then migrate from the small intestine to the large intestine or the caecum and develop into adult worms and attach to the mucosa of the colon by their thin anterior end (Ryan and Ray, 2004).

The female remains in the caecum and releases its eggs into the lumen of the gut and are subsequently passed out of the body with faecal material (Ryan and Ray, 2004). Unlike the eggs of the *Enterobius vermicularis*, the eggs of *Trichuris trichiura* when laid are immature and takes up to ten days to develop fully into the embryonated and infective stage (Ryan and Ray, 2004). The matured infective eggs may be picked up by hands especially by children who play in the sand and agricultural workers and passed to the mouth and the infection repeats itself (Ryan and Ray, 2004).

Infections may occur in areas where human excreta is used as organic fertilizer, raw fruits and vegetables may also be contaminated and later be ingested (Ryan and Ray, 2004).

Clinical Manifestation

Light infections with this worm produce few symptoms, but modest to heavy infections can cause abdominal cramps and diarrhoea often with blood being voided (Ryan and Ray, 2004). In rare occasions, some children may harbour up to 800 worms (Ryan and Ray, 2004). In such situation, the entire colon is parasitized with significant damage to the mucosa resulting in chronic diarrhoea, blood loss and anaemia (Ryan and Ryan, 2004).

The force of the faecal stream on the worms may produce prolapse of the rectum, particularly when the host strains during defecation or child birth (Ryan and Ray, 2004).

Laboratory Diagnosis

Diagnosis of infections of *Trichuris trichiura* involves the microscopical demonstration of the eggs in faecal materials (Cheesbrough, 2005). The female worm produces numerous eggs, therefore concentrated techniques are not required to detect significant infections (Cheesbrough, 2005).

Infections can be controlled by deworming the infected individual or those at high risk while prevention can be achieved by good personal hygiene practices and discouraging the use of untreated human excreta as organic fertilizer (Cheesbrough, 2005).

Treatment

Infections should not be treated unless they are symptomatic (Ryan and Ray, 2004). Mebendazole is the drug of choice; albendazole is thought to be equally effective (Ryan and Ray, 2004).

Although the cure rate is only 60-70%, more than 90% of the adult worm are usually expelled, rendering the patient asymptomatic (Ryan and Ray, 2004).

Prevention of infections can be achieved through public health education on personal hygiene practice and improved sanitation facilities.

Ascariasis

Ascariasis is one of the leading helminth diseases found in developing countries (Ryan and Ray, 2004). This disease condition is caused by *Ascaris lumbricoides* also known to be the largest intestinal round worm (Cheesbrough, 2005). It measures up to 40mm in length with the male being shorter than the female (Cheesbrough, 2005).

Ascaris lumbricoides is known to be one of the commonest and most wide spread of all human helminthic parasites, particularly prevalent in regions of poor environmental sanitation (Cheesbrough, 1992).

Infections occur when a person ingests the infective eggs in contaminated food or hands contaminated with faecal material containing the infective eggs (Cheesbrough, 1992).

The adult female worm is prolific and produces numerous eggs which are surrounded by a strong protective coat, an adaptation which enables them to remain viable in the environment for several years (Cheesbrough, 1992).

It is this adaptation that has contributed to the wide spread and often than not the heavy infections found in rural areas of developing countries especially in young children who practice geophagy (Cheesbrough, 1992).

After the infective eggs are ingested, the larvae hatch in the small intestine and penetrate the intestinal blood vessels where they develop whiles undertaking the heart-lung migration characteristic of intestinal nematodes (Cheesbrough, 1992).

On reaching the trachea, the larvae are coughed up and swallowed down the small intestine where they remain and mature up into adult worms. Eggs are produced after about eight weeks of infection when the female produces numerous quantities (about 200,000 eggs per day) which are passed out in faecal material (Cheesbrough, 1992).

Under warm and humid conditions, the eggs develop and within 30-40 days of being passed out each egg contains an infective larvae (Cheesbrough, 1992).

The larva does not hatch until the egg is swallowed (Cheesbrough, 1992). Even though it thrives best in warm humid conditions, it seems not to depend so much on the climate, a

phenomenon that explains why infections with this worm are common all over Africa (Cheesbrough, 1992).

The life span of the adult worm is about 1-2years (Ryan and Ray, 2004).

Clinical Manifestation

Light infections of Ascariasis are usually asymptomatic or if any at all they are not characteristic even though temporal symptoms such as coughing occur during the heart-lung larval migration (Ryan and Ray, 2004). Adult worms are occasionally vomitted or passed out in stools (Cheesbrough, 1992).

In very heavy infections, complications may occur due to the migration of the adult worms or the larvae to the lungs through the hepatic blood vessels causing tissue destruction (Ryan and Ray, 2004).

Diagnosis is not usually established until after a few weeks later when the worms are matured and produce eggs that can be demonstrated in faecal material (Cheesbrough, 1992).

Intestinal obstruction may occur at the ileocecal junction by a large ball of worms, just as wondering worms can block the bile duct which results in destructive jaundice (Cheesbrough, 2005). The migration of the worms into the liver results in liver abscess (Cheesbrough, 2005).

The worms feed on the food nutrients consumed by the host resulting in malnutrition such as in "Kwashiorkor" and Vitamin A deficiency (Cheesbrough, 2005).

Laboratory Diagnosis

The laboratory diagnosis of Ascariasis is based on microscopic demonstration of the eggs in faecal material and or identifying the worms expelled through the mouth or the anus (Cheesbrough, 2005).

Larvae can be found in sputa and or gastric aspirates of infected individuals before eggs appear in faeces (Ryan and Ray, 2004).

Abdominal radiographs may also reveal masses of worms in gas filled loops of bowel in patients with intestinal obstruction (Ryan and Ray, 2004).

Control is usually by deworming the infected person while good personal hygienic practices are advised (Cheesbrough, 1992).

Treatment

Ascariasis should always be treated to prevent potentially serious complications (Ryan and Ray, 2004). Both mebendazole and albendazole are effective except during pregnancy where they are contraindicated and in heavy infections in which ectopic migration may be provoked (Ryan and Ray, 2004). Piperazine is safe during pregnancy (Ryan and Ray, 2004).

Mebendazole is the drug most commonly used because it is a broad spectrum antihelminthic (Steinmann *et al.*, 2008). It is given in a dose of 100mg twice daily for 3 days.

Levamisole at a dose of 5mg/kg body weight can alternatively be given in a single dose or albendazole 400mg given also in a single dose (Steinmann *et al.*, 2008). Peperazine 150mg/kg body weight can be administered as a single dose and not exceeding 4g. Pyrantel pamoate is also highly effective (Steinmann *et al.*, 2008).

Hookworm

Hookworm, a soil born infection is caused by *Necator americanus* and *Ancylostoma duodenale* (Cheesbrough, 2005). The former is more common in the Far East, South Asia, The Pacific Islands, Tropical Africa, Central and South America whiles the latter is found in the Middle East around the Mediterranean and North China but can also be found with *Necator americanus* in Africa, South East Asia, the Pacific Islands, and in South America (Cheesbrough, 1992).

An estimated 1.3 billion people are affected by this disease condition (Ryan and Ray, 2004). As of 1990, an estimated 7% (41 million) of the world's preschool children, 26%

(239 million) of school age children and 44.3 million of the developing world are infected with Hookworm (Ryan and Ray, 2004).

124.3 million pregnant women harboured the infection (Holland and Kennedy, 2002).

Life Cycle

The eggs are already embryonated when passed out with faecal material (Kayser *et al.*, 2001). They hatch into the rhabditiform larvae and bury themselves in the moist dump soil where they develop into the infective sheathed filariform stage (Kayser *et al.*, 2001).

It takes up to seven days for the development from the rhabditiform larvae into the infective filariform stage (Cheesbrough, 1992) which may attach themselves to grass or remain in the soil (Ryan and Ray, 2004). An infective filariform larva penetrates the unbroken skin, reaches the blood vessels and follows a heart-lung migration up to the trachea whiles developing (Cheesbrough, 1992).

From the trachea, the larvae are coughed up and swallowed back and reaches the small intestine within 3-5days after penetration of the skin (Kayser *et al*., 2001).

In the intestine, the larvae develop into the mature worm and attach itself to the intestinal mucosa by means of hooks in its buccal cavity ((Cheesbrough, 1992). The period of development from skin penetration to the appearance of eggs in the faeces is about 6-8weeks, but may be longer with infections of *Ancylostoma duodenale* (Ryan and Ray 2004).

The larvae of *Ancylostoma duodenale* can survive and develop directly in the intestinal mucosa into mature worms if swallowed (Cheesbrough, 1992). The mature worms can survive over 10years, but usually the life span for *Necator americanus* is 2-5 years whiles it is up to 6-8 years for *Ancylostoma duodenale* (Ryan and Ray, 2004).

Clinical Manifestation

In the vast majority of cases, infections of hookworm are asymptomatic (Ryan and Ray, 2004) but the infective larvae may cause a skin reaction at the point of larval penetration, usually referred to as the "ground itch" (Cheesbrough, 1992). Itchy erythematous papules

appear at the site as well as tracts of subcutaneous migration which is similar to cutaneous larva migration (Ryan and Ray, 2004), commonly found between the toes and on the sole of the feet (Holland and Kennedy, 2002).

During the heart-lung migration of the larvae, there may be coughing, wheezing, eosinophilia and occasionally mild transient pneumonia, a condition which develops less frequently in hookworm infections than in Ascariasis (Cheesbrough, 1992).

Mucoid stool with blood is passed during heavy infections (Holland and Kennedy, 2002) which may be mistaken for a symptom of duodenal or gastric ulcers (Ryan and Ray, 2004). The mature worms suck blood from the intestines resulting in the loss of blood, iron, and protein especially albumin from the host, a cause of iron deficiency anaemia in the host (Holland and Kennedy, 2002).

The evolution of the anaemia is slow and because of the physiological adjustment it evokes, the patient can continue to walk about with a surprisingly low haemoglobin level usually referred to as "walking anaemia of hookworm" (Ryan and Ray 2004).

Laboratory Diagnosis

Diagnosis in the laboratory usually involves the demonstration of eggs in infected faecal material (Cheesbrough, 1992). Species differentiation of eggs is not possible but the larvae when freshly hatched can be differentiated (Cheesbrough, 1992).

Control measures include deworming of the infected individuals whiles prevention is achieved by advising communities on the use of untreated human excreta as fertilizer and the wearing of Wellington boots and wearing shoes by both adults and children whiles working on farms.

Treatment

Steinmann *et al.*, (2008) recommended that infections with hookworm can be eradicated by the use of the following antihelminthic drugs which are safe and highly effective.

Mebendazole 100mg twice daily for 3 days; Pyrantel pamoate 10mg/kg body weight daily for 3 days; Levamisole 3 tablet adult single dose which can be used in case of mixed infections and Bephenium.

Prevention requires public health education on the proper disposal of human excreta in order to improve sanitation. Re-infection is very likely if a community does not improve its methods of faecal disposal (Ryan and Ray, 2004). In situations where anaemia is evident, treatment should aim at eliminating the worms and correcting the anaemia (Ryan and Ray, 2004).

Iron deficiency is corrected by the administration of oral iron for at least 3 months (Holland and Kennedy, 2002). Even when anaemia is severe, patients respond rapidly to oral iron therapy (Holland and Kennedy, 2002).

Folate deficiency may occur as a result of increased bone marrow activity when the iron deficiency is being corrected (Holland and Kennedy, 2002).

Intestinal Protozoa

The disease condition Amoebiasis is caused by the protozoa *Entamoeba histolytica* of which only a few species are pathogenic (Cheesbrough, 1992).

The first case in North America was reported in 1890 by Sir William Oster when he observed amoeba in the stool and fluid from an abscess from a physician who lived in Panama (*Bhanu et al.*, 2011). The protozoan was later given the name *Entamoeba histolytica* by Fritz Schaudin in 1903 (Saklavalva, 1993).

The cyst was documented as the infective form in the Philippines by Walker and Sellards in 1913 whiles the life cycle was later established in 1925 by Dobell (Bhanu *et al.*, 2011).

Entamoeba histolytica infections are endemic in many parts of tropical and subtropical Africa, Asia, Mexico, South America, and China (Cheesbrough, 1992). It is estimated that about 10% of the world's population is infected with this protozoan (Cheesbrough, 1992).

Its prevalence is as high as 50% in areas of Central and South America, Africa and Asia (Stanley, 2003).

In Egypt, 38% of individuals presenting with acute diarrhoea in an outpatient clinic were found to have amoebic colitis (Stanley, 2003).

The highest prevalence of amoebiasis is in developing countries where barriers between human faeces and food and water supplies are inadequate (Ryan and Ray, 2004). In most cases, Amoebiasis are asymptomatic, but dysentery and invasive extra intestinal disease can occur (Cheesbrough, 1992).

Amoebic liver abscess is the most common manifestation of invasive amoebiasis, but other organs may also be involved, including the pleuropulmonary cavity, cardiac, cerebral, renal urogenital and cutaneous sites (Ryan and Ray, 2004).

Asymptomatic *Entamoeba histolytica* infections seem to be region-dependent, as high as 11% in Brazil (Fotedar *et al.*, 2007). In western countries, it is estimate that 20%-30% of men who have sex with men are colonized with *Entamoeba despair* (Fotedar *et al.*, 2007). Amoebiasis is second to malaria in terms of protozoa-associated mortality (Bhanu *et al.*, 2011).

The combined prevalence of amoebic colitis and amoebic liver abscess is estimated to be as high as 40-50 million cases annually worldwide resulting in about 40,000 – 100,000 deaths (Li and Stanley, 1996; Stanley, 2003).

Asymptomatic intestinal Amoebiasis occurs in about 90% of infected individuals (Bhanu *et al.*, 2011). However, only 4% - 10% of individuals with asymptomatic Amoebiasis who were monitored for one year eventually developed colitis or extra intestinal disease (Fotedar, 2007).

Mortality rates due to amoebic liver abscess has fallen to 1-3% in the last century following the introduction of effective treatment (Stanley, 2003).

Nevertheless, amoebic liver abscess is complicated by sudden intraperitoneal rupture in 2-7% of patients, leading to a higher mortality rate (Stanley, 2003). In Japan and Taiwan, immunosuppression due to HIV infection is a risk factor for invasive extra intestinal

Amoebiasis (Hung *et al.*, 2005), whiles in India *Entamoeba histolytica* recorded a prevalence rate of 7.1% in HIV infected patients with a higher prevalence of 23% in HIV sero-negative patients (Gupta *et al.*, 2008).

Entamoeba histolytica is a frequently identified parasite in HIV infected individuals with diarrhoea in India and other parts of the world (Prasad, 2000).

Stool surveys in the United States indicate that 1-5% of the population have *Entamoeba histolytica*, most of which are non-pathogenic *Entamoeba dispar* (Ryan and Ray, 2004).

Life Cycle

The faeco-oral route is the mode of transmission of infections where the infective cyst is ingested in food, water or from hands contaminated with infected faeces (Cheesbrough, 2005).

Following ingestion, each cyst excysts in the large intestine to produce daughter cells which repeatedly multiply and form single-nucleated cyst that subsequently develop into the infective cyst which has four nuclei (Cheesbrough, 1992).

Once cysts are formed, they do not develop into amoeba again in the same host (Cheesbrough, 1992), but are excreted in the faeces ready for ingestion by another host (Ryan and Ray, 2004).

Cysts can survive and remain infective for several weeks in sewage and water (Cheesbrough, 2005). The trophozoites that are passed out in faecal material are not infective to other hosts but they rather die rapidly (Cheesbrough, 1992).

Formerly, a pathogenic invasive and a non-pathogenic strain was thought to exist and by the use of iso enzyme-electrophoretic techniques, these two strains have now been recognized as separate species (Cheesbrough, 2005).

Entamoeba histolytica is the invasive pathogenic species and *Entamoeba dispar* has been designated the non-invasive non-pathogenic species (Cheesbrough, 2005). The two species are morphologically identical so as their cysts which are indistinguishable

microscopically. However the trophozoite of *Entamoeba histolytica* can be differentiated by the presence of red blood cells within the parasite (Cheesbrough, 2005).

Clinical Manifestation

Severe intestinal Amoebiasis causes overwhelming inflammation of the colon which can be fatal, but occurs occasionally (Cheesbrough, 1992).

Rectal bleeding without diarrhoea can occur, especially in children (Cheesbrough, 1992). Only approximately 10-30% of patients with amoebic colitis develop fever (Adams and MacLeod, 1997).

Weight loss and anorexia may occur. Necrotizing colitis manifests as severe bloody diarrhoea and widespread abdominal pain with evidence of peritonitis and fever (Ryan and Ray, 2004).

Mondal *et al.*, (2006) suggested that predisposing factors for fulminant colitis included very young age, poor nutrition, pregnancy and the use of corticosteroids.

Invasive Amoebiasis may also result in amoebic liver abscess provided an intestinal ulcer reaches a blood vessel (Cheesbrough, 1992) from where the amoebae may enter the blood stream and be carried to the liver and other parts of the body where they can form abscesses. It is however, a relatively rare complication of invasive Amoebiasis (Cheesbrough, 1992).

The most typical presentations of amoebic liver abscess are fever, right upper quadrant pain, and tenderness of the abdomen of less than 10 days duration (Ryan and Ray, 2004).

Unlike with amoebic colitis, amoebic liver abscess is associated with fever in 80-90% of cases (Ryan and Ray, 2004). Cough can occur but jaundice is unusual (Gillespie and Pearson, 2001) except in severe cases where patients with large or multiple abscesses may become jaundiced and anaemic (Cheesbrough, 1992).

Gillespie and Pearson, (2001) suggested that about sixty to seventy (60-70%) of cases associated with amoebic liver abscess do not have concomitant colitis, although a history of dysentery within the previous year may be obtained.

Amoebic liver abscess may manifest years after travel to or residency in an endemic area (Ryan and Ray, 2004).

Laboratory Diagnosis

Amoebiasis is mostly diagnosed by demonstrating microscopically the cysts and/or trophozoites from fresh stool samples or rectal scraping (Cheesbrough, 1992).

The microscopic examination for trophozoites from a single fresh stool sample in amoebic colitis is only 33-50% sensitive (Fotedar *et al.*, 2007), whiles examination of three samples over a period of about 3 days improves the detection rate up to 85-95%.

Unlike in Shigellosis where leukocytosis is pronounced, there are few leukocytes found in amoebic colitis because pathogenic strains of *Entamoeba histolytica* are able to destroy polymorphonuclear cells and macrophages (Cheesbrough, 1992).

The demonstration of the parasite from aspirates of a liver abscess is only about 20% sensitive (Fotedar *et al.*, 2007) while the presence of intracytoplasmic leukocytes in trophozoites is typically diagnostic of *Entamoeba histolytica*, although recent studies have demonstrated same for *Entamoeba dispar* (Fotedar *et al.*, 2007).

The World Health Organization (W.H.O.) has however, recommended that intestinal amoebiasis be diagnosed with an *Entamoeba histolytica* – specific test such as PCR, thus rendering the classic stool examination for cysts and trophozoites obsolete (Fotedar *et al.*, 2007).

Antigenic Tests

Enzyme-linked Immunosorbent Assay (ELISA) is used to detect antigens from *Entamoeba histolytica* in fresh stool samples (Fotedar *et al.*, 2007).

Antigen-based ELISA kits employing monoclonal antibodies against the GAL/Gal NAC-Specific lectin of *Entamoeba histolytica* (E. histolytica II, TechLab, Blacksburg, VA) yields an overall sensitivity of 70-100% and specificity of 93-100% (Fotedar *et al.*, 2007).

A sensitivity of 96-100% was achieved using the same kit on aspirates of serum and liver from patients with amoebic liver abscesses respectively (Fotedar *et al.*, 2007).

Other stool kits meant for the detection of monoclonal antibodies against the serine-rich antigen of *Entamoeba histolytica* (Optimum S kit, Merlin Diagniostika, Bornheim-Hersel, Germany) or against other specific antigens (Entamoeba CELISA-PATH, Cellabs, Brookavale, Australia, ProSpecT EIA, RemleInc, Lenexa, KY).

No specific antigenic test is available for the detection of the non-pathogenic *Entamoeba dispar* and *Entamoeba moshkovskii* from clinical samples (Fotedar *et al.*, 2007).

Serological Diagnosis

Multiple serological assays are available for the diagnosis of Amoebiasis (Fotedar *et al.*, 2007). These include ELISA, the most widely used assay worldwide employed for the measurement of the present of serum antilectin antibodies (IgG) (Fotedar *et al.*, 2007).

The sensitivity for detection of antibodies to *Entamoeba histolytica* in patients with amoebic liver abscess is 97.9% whereas the specificity is 94.8% (Fotedar *et al.*, 2007). False negative results can occur within the first 7-10days after infection (Fotedar *et al.*, 2007). Immunofluorescence from the Immunofluorecescent Assay (IFA) technique is also rapid, reliable, and equally reproducible, achieving a sensitivity and specificity of 93.6% and 96.7% respectively in cases of amoebic liver abscess (Fotedar *et al.*, 2007).

The Indirect Haemaglutination Assay (IHA) is very specific (99.1%) but rather less sensitive than the ELISA (Fotedar *et al.*, 2007).

Immunoelectrophoresis, counter-immunoelectrophoresis (CIE), and gel diffusion techniques employ the precipitation property of antigen-antibody complex in agar-gel plates (Fotedar *et al.*, 2007). The CIE technique has been shown to have a sensitivity of 100% in invasive amoebiasis but found to be time-consuming (Fotedar *et al.*, 2007).

Seropositivity prevalence is very high in endemic areas, which has limited antibody-based testing for the diagnosis of active infection, since antibodies once produced can persist for several years after infection (Fotedar *et al.*, 2007).

Polymerases Chain Reaction (PCR)

A wide variety of polymerase chain reaction (PCR) methods targeting different genes, including a small-subunit rRNA gene (18s rDNA), 30-kDa antigen gene, serin-rich gene, chitinase gene, haemolysin gene, and extrachromosomal circular DNA, have been employed for the detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkorskii* (Fotedar *et al.*, 2007).

Sensitivities vary according to sampling and the specific target gene used (Fotedar *et al.*, 2007). *Entamoeba histolytica* is identified in various types of clinical specimen including faeces, tissues and aspirates from liver abscess using PCR (Fotedar *et al.*, 2007). When performed on faeces, it yields a sensitivity that is similar to that of stool antigen-based assay (Fotedar *et al.*, 2007).

Treatment

It was recommended by Stanley, (2003) that in order to eradicate asymptomatic amoebiasis, luminal agents such as iodoquinol, paromomycin, diloxanidefuroate should be used for treatment of infected patients. He based his recommendation on two counts; first of them is that invasive disease may develop and secondly that the shedding of *Entamoeba histolytica* cysts into the environment is a public health burden.

Asymptomatic *Entamoeba dispar* infections should not be treated (Stanley, 2003) but public health education should be pursued since it is a marker of faecal-oral contamination. For the treatment of *Amoebic colitis*, a nitromidazole derivative is used first, followed by a luminal agent in order to eradicate colonization (Bhanu *et al.*, 2011; Stanley, 2003).

Amoebic liver abscess can be treated without drainage, with just a single dose of metronidazole (Stanley, 2003) with clinical effervescence occurring during the first 3-4 days of treatment.

Metronidazole failure may be an indication for surgery (Stanley, 2003). Disseminated amoebiasis should also be treated with metronidazole, which can cross the brain-blood barrier (Stanley, 2003).

Stanley, (2003) also recommended that in cases where perforated viscus is implicated, empirical bacterial agents should be used concomitantly.

Giardiasis

Giardiasis, an infection caused by the intestinal flagellate *Giardia lamblia* or otherwise *Giardia intestinalis* is worldwide in distribution and is particularly common in tropical and sub-tropical countries where water supplies and the environment become contaminated with faecal material (Cheesbrough, 1992).

This parasite is most often recovered from individuals with asymptomatic colonization in the intestinal tract or in cases of acute or chronic diarrhoeal illness (Moore *et al.*, 1969). In endemic areas, young children are more frequently infected than adults (Cheesbrough, 2005).

Giardia lamblia occurs in humans and the infection may be passed from primates, dogs or cats to humans (Ryan and Ray, 2004). It was first described in 1681 by Van Leeuwenhoek from his own stool (Hill, 2005).

HIV patients who are immunosuppressive are at risk of infections if exposed to dogs and cats which are kept in homes as pets (Ryan and Ray, 2004). Infections with *Giardia lamblia* are endemic in areas of the world where there is poor environmental sanitation (Ryan and Ray, 2004).

The disease is a significant cause of morbidity and water-borne and food –borne outbreaks are common (Ryan and Ray, 2004; Craun, 1990, Cheesbrough, 1992). It is particularly a significant pathogen for people who are malnourished, immunodeficient, and people with cystic fibrosis (Flannagan, 1992).

Giardia lamblia is genetically heterogeneous, with two major genotypes, A and B, found in both humans and animals (Morgan, 1994; Caccio and Ryan, 2008). Five other genotypes exist (C-G) and are host-specific (Caccio and Ryan, 2008).F

This parasite can cause abdominal discomfort, severe diarrhoea, flatulence, nausea, vomiting, weight loss, and malabsorption with lactose intolerance in children less than 3 years of age (Cheesbrough, 2005).

In individuals who are immunosuppressed, with gastrointestinal disorders or intestinal bacterial infections tend to be more susceptible to infections with *Giardia lamblia* (Cheesbrough, 2005).

The diarrhoea causing mechanism and low intestinal absorption by the parasite is not clearly understood. However one possible explanation is a number of multiple factors such as age of the cyst, host immunity and its genetic variability (Caccio and Ryan, 2008).

Ryan and Ray, (2004) also suggested that the mechanism by which *Giardia lamblia* causes diarrhoea and malabsorption are probably multifactorial and are yet to be fully elucidated.

Although the parasite appears to alter epithelial structure and function which leads to malabsorption, diarrhoea can also occur in individuals in the absence of obvious light microscopic changes in the small intestinal structure (Buret, 2008).

Marked or moderate partial atrophy of the villi in the jejunum can be observed in histological sections from asymptomatic individuals who are infected (Buret, 2008).

Ryan and Ray (2004); Buret (2008) all suggested that effects in the lumen may contribute to malabsorption and the onset of diarrhoea in addition to the disruption of the mucosal epithelium.

Morphology

The vegetative form of *Giardia lamblia* flagellates measure about 9-20um in length (usual size is 10-12um) (Cheesbrough, 1992) and have a characteristic concave shape at the front end.

It possesses eight (8) flagella which enables it to have a rotating and twisting movement which some workers refer to liken the movement of a falling leaf (Cheesbrough, 1992).

The cysts of *Giardia lamblia* which are the infective stages of the parasite are small and oval measuring about 10x16 um and contain four (4) nuclei which are relatively difficult to see under the light microscope (Cheesbrough, 1992). The parasite also contains the remains of axonemes and parabasal bodies which stain well with iodine and the threadlike remains of the flagella also visible (Cheesbrough, 1992).

Epidemiology

Giardia lamblia has been found in mammals including domestic and farm animals such as cats, dogs, livestock, bears and guinea pigs respectively (Ryan and Ray, 2004). Studies by Dykes (1980) also found the flagellates in treated sewage water and wildlife.

The organism is implicated in 25% of cases of gastrointestinal disease and presents asymptomatically (Farthing, 1994). Nwachukwu and Okeke (2008) documented that about 70% of HIV-infected individuals contract at least one pathogen causing diarrhoea.

Chronic Giardiasis is one of the most common causes of diarrhoea in HIV infected subjects (Tosones, 1993). Tosones (1993) also documented that 94.4% of patients infected with *Giardia lamblia* were symptomatic and that absolute CD4 T-cell count was 84.2/cm3.

Tosomes (1993) in that study therefore concluded that *Giardia lamblia* infection is higher in HIV infected patients than in non-infected patients in age matched general population with a prevalence of 30% and 9.7% respectively.

Mode of Infection

The infective stage of *Giardia lamblia* is the cystic form and is transmitted by the faeco-oral route through food, water or hands contaminated with infected faeces (Cheesbrough, 2005). The parasite is shed in faeces as an environmentally robust cyst ready to infect a new host (Cheesbrough, 2005).

On reaching the duodenum of the infected host, it undergoes rapid mitosis (Cheesbrough, 2005). Each of the two daughter cells produced attaches themselves to the epithelium by means of adhesive discs and feed on the epithelia cells (Cheesbrough, 2005).

In about 72 hours turn over time, the trophozoites get detached from the intestinal epithelium, probably due to the rapid turnover and undergo further mitotic division in the intestinal lumen (Cheesbrough, 2005).

These trophozoites may be transported with the intestinal contents and excreted in faeces during periods of diarrhoea, but they do not survive long outside the host. Some of them encyst during the passage through the intestine and leave the host with the faeces as cysts (Cheesbrough, 2005).

The cysts of *Giardia lamblia* are most often recovered from formed stools than the trophozoites (Cheesbrough, 2005).

These cysts are elliptical and measure about 8-12um in length and 7-10um in width, with the cystic wall having a thickness of 0.3-0.5um with a fibrillous structure (Cheesbrough, 2005).

Two to four nuclei are usually found in each cyst, together with axonemes of the flagella of the trophozoite. These cysts, once excreted in faeces are directly infective (Cheesbrough, 2005).

Laboratory Diagnosis

Giardiasis is diagnosed by demonstrating the cysts of the parasite in formed infected stools or finding the motile trophozoits in diarrhoeic stools (Cheesbrough, 1992).

The motile flagellates can also be found from duodenal aspirates by means of the Entero-test technique for the recovery of *Giardia lamblia* in duodenal contents (Cheesbrough, 1992).

The most sensitive and specific technique employed in the diagnosis of the Giardiasis is the Enzyme Linked Immunosorbent Assay (ELISA) which is used in the detection of *Giardia lamblia* antigen in faeces (Green et al., 1985).

The assay has sensitivity and specificity of 98% and 100% respectively and directly detects active infection since it involves the detection of antigens (Cheesbrough, 1992).

Trichomoniasis

One other flagellate of medical interest and for that matter parasitological study is *Trichomonas vaginalis*. Unlike other flagellates which are G.I.T. borne, *Trichomonas vaginalis* is a flagellate that mostly inhabits the urogenital tract of both women and men (Cheesbrough, 1992).

This parasite is distributed worldwide and unlike *Giardia lamblia* it is not specifically common in tropical countries (Cheesbrough, 1992).

Trichomonas vaginalis whose infective form is the trophozoite form is transmitted sexually and lives and multiply in the urogenital tract of the infected host and has no cystic form (Cheesbrough, 1992).

Studies conducted in the Ivory Coast by Laga *et al*., (1993) found an association between HIV and *Trichomonas vaginalis* infections, which suggested a two to three fold increase in HIV infected subjects.

In Tanzania, a similar study by Laga *et al*., (1993) found the flagellate to be more common in women infected with HIV than non-infected subjects.

Trichomonas vaginalis causes acute inflammation of the vagina with a yellowish-green purulent discharge in about 40% of infected women but usually asymptomatic in men, occasionally causing urethritis without purulent discharges (Cheesbrough, 1992).

Laboratory Diagnosis

The presence of Trichomoniasis is confirmed by finding the vegetative forms of the parasite in vaginal and urethral discharges of infected women and men respectively (Cheesbrough, 1992), but in severe infections the parasites are observed in urine deposits under the light microscope (Cheesbrough, 1992).

COCCIDIA

Gupta *et al.*, (2008) in their study documented gastrointestinal (GI) opportunistic infections commonly encountered at various stages of HIV infection. In view of the suppressive nature of the virus and direct contact with the environment, the gastrointestinal tract is readily accessible and a common site for clinical expression of a HIV (Awadh and Anazi, 2009).

Cryptosporidium parvum, *Isospora belli* and *Cyclospora cayetanensis* are the common intestinal coccidian parasites that have emerged as a result of immunosuppression due to HIV (Assefa *et al.*, 2009).

Isosporiasis

This opportunistic disease condition is cause by *Isospora belli* which is an Apicomplexa belonging to the class Sporozoa, the sub-class Coccidia, and the family Eimeriidae and causes a self-limiting diarrhoea illness in immunocompetent individuals. It may cause chronic life-threatening diarrhoea and dehydration in immunosuppressed individuals (DeHovitz *et al.*, 1986).

Other species of Isospora infects reptiles, birds, and mammals (Kirkpatrick, 1988). Only *Isospora belli* is known to infect humans (Lindsay *et al.*, 1997).

Epidemiology

Isospora was first noted as a human pathogen among military personnel during World War I (Woodcock, 1915).

It is a rarely serious infection in immunocompetent hosts (Cheesbrough, 2005). Infections with *Isospora belli* are endemic in tropical and subtropical countries particularly Central and South America, Africa and South East Asia (Cheesbrough, 2005; Lindsay *et al.*, 1997) and have been associated with cholecystitis in patients with HIV/AIDS (Debra *et al.*, 1994).

Somachai *et al.*, (2007) found the prevalence of *Isospora belli* among HIV patients in Thailand to be 60% and 0.02% in non-HIV patients with 86.8% of HIV patients being immunocompromised.

A similar cross-sectional study conducted by Certad *et al.*, (2003) in Venezuela showed a higher prevalence of the parasite whiles (0.2-1.0)% of HIV/AIDS patients in the United States of America had *Isospora belli* infection (Robert *et al.*, 2002).

Between 1985 and 1992, 1.0% of HIV/AIDS patients were known to harbour oocysts of the parasite in Los Angeles (Sorvillo *et al.*, 1995) whiles in developing countries 8-40% of patients with HIV/AIDS have *Isospora belli* infection and occurs up to 15% in HIV patients in Haiti (DeHovitz *et al.*, 1986).

In Southern India, 11.7% out of 111 HIV infected patients harboured the oocysts of *Isospora belli* (Mukhopadhya *et al.*, 1999). *Isospora belli* is the most frequently recovered parasite in HIV infected individuals with diarrhoea in India and other parts of the world (Prasad, 2000).

Mode of Infection

The life cycle of *Isospora belli* begins with the ingestion of the sporulated oocyts, which is the infective stage of the parasite, in food, water, or hands which have been contaminated with infected faecal material (Cheesbrough, 2005).

On reaching the small intestine, the oocysts liberate sporozoites, probably in response to bile in the small intestine, which then colonizes the enterocytes of the proximal small intestine (Somachai *et al.*, 2007).

Within the enterocytes, the sporozoites develop into trophozoites and under asexual multiplication (Schizogony) producing numerous merozoites which invade previously uninfected cells (Rosiere *et al.*, 2003).

Shortly thereafter, sexual multiplication (sporogony) begins, a process which generates immature oocysts that may pass out into the environment through faeces (Cheesbrough, 2005). These immature oocytst in the environment then mature into infective oocysts

outside the body in about 2-3 days after being passed out (Cheesbrough, 2005; Morakote *et al.*, 1987).

This opportunistic infection usually causes gastrointestinal illness that is characterized by loose stools or watery diarrhoea and is often associated with abdominal pain, malabsorption, weight loss and peripheral eosinophilia (Stark *et al.*, 2009; Robert *et al.*, 2002).

The disease condition is self-limiting in immunocompetent adults (Certad *et al.*, 2003) whiles in patients with HIV/AIDS and other immunodeficient states, the illness is chronic and may be associated with severe dehydration and debilitating effects (DeHovitz *et al.*, 1986).

Clinical Manifestation

Patients with Isosporiasis usually present with a mild crampy abdominal pain and produces profuse watery stools which have a foul-smelling odour (Robert *et al.*, 2002). Clinical symptoms include foul smelling flatulence, anorexia and low grade fever (Stark *et al.*, 2009).

Dehydration may be evident in immunosuppressed individuals with severe or long-lasting disease, otherwise minimal abdominal tenderness may be present (Robert *et al.*, 2002).

Laboratory Diagnosis

Isosporiasis can be diagnosed by demonstrating the oocysts, usually the immature stages in stools, under the light microscope though matured forms may occasionally be seen (Cheesbrough, 1992). The matured oocysts measure 30 by 12um and have a thin translucent wall and two spherical sporocysts each of which contains 4 crescentric sporozoites (Somachai *et al.*, 2007).

Physiological saline preparations are usually employed in routine diagnosis (Cheesbrough, 1992), however staining techniques such as Auramine-rhodamine fluorescence, modified Kinyoun acid fast, haematoxylin/eosin, Giemsa and or Carbol

fuschin are employed to make permanent slides of the parasite (Somachai *et al.*, 2007; Robert *et al.*, 2002).

Charcot-Leyden crystals are often observed (Robert *et al.*, 2002) whiles mid eosinophilia may be revealed in about 50% of cases.

Serological tests for detection of antigens or antibodies are not yet available but the detection of oocyts using PCR has been lately developed (TenHove *et al.*, 2008; *Robert et al.*, 2002; Muller *et al.*, 2000).

From biopsies of the colon, the electron microscope can be helpful in finding the parasite, but that is rather labour intensive and non-specific (Robert *et al.*, 2002).

Treatment

Administrating a dosage of 160mg of Trimethoprim-sulfamethoxazole (TMP-SMZ) daily for 10 days generally improves infections with Isosporiasis even though the infection is self-limiting (Robert *et al.*, 2002). Patients who are not treated remain sick for considerably longer periods (Robert *et al.*, 2002).

Rehydration therapy is the most urgent intervention in infected children and severely immunocompromised patients (Ryan and Ray, 2004; Washington *et al.*, 2006).

Immunocompetent host generally respond very rapidly to therapy with symptomatic improvement within 7 days (Washington *et al.*, 2006), whiles the immunosuppressed host responds less rapidly, taking up to 4 weeks to get well (Robert *et al.*, 2002).

Trimethoprim-sulfamethoxazole is chosen as the first line of treatment because it is the best studied and most widely available drug (Ryan and Ray, 2004). It is being used as prophylaxis for Pneumocystis infections by many patients (Ryan and Ray, 2004).

An alternative for long-term prophylaxis is pyrimethamine with sulfadiazine or sulfadoxine (Washington *et al.*, 2006).

Infections with *Isospora belli* can be controlled by the practice of good personal hygiene and public health education in areas where these infections are endemic (Cheesbrough, 1998).

Cryptosporidiosis

Cryptosporidiosis is the disease condition caused by *Cryptosporidium parvum* which infects a wide range of animals with human infections thought to be a zoonosis, but person to person infection may also occur (Cheesbrough, 1992).

Ten recognized species of Cryptosporidium exist based on host specificity, morphology and molecular biological studies, which besides humans, the parasite can infect a wide variety of animals such as mammals, birds and reptiles alike (White, 2005). It is pathogenic in immunocompromised hosts (Robert *et al.*, 2002).

Out of the lot, only two species of cryptosporidium infects humans mainly, ie. *Cryptosporidium hominis* (previously genotype I), which infects only humans, and *Cryptosporidium parvum* (previously genotype II) which infects both humans and animals (Flynn, 1997). *Cryptosporidium canis* infects dogs as well as humans as a zoonosis (White, 2005). Additional subspecies of *Cryptosporidium parvum* have been identified in stools of HIV/AIDS patients (Robert *et al.*, 2002).

Epidemiology

The genus Cryptosporidium consists of a group of protozoan parasites within the protist subphylum Apicomplexa, class Sporozasida and subclass Coccidiasina (Robert *et al.*, 2002).

The parasite was first identified in the stomachs of mice in 1907 and the name Cryptosporidium later proposed in 1912 (Keush *et al.*, 1995).

The organism was associated with gastrointestinal disease in humans in 1976 (Flanigan and Soave, 1993). With the advent of HIV/AIDS epidemic, *Cryptosporidium parvum* infections became increasingly recognized as one of the major causes of diarrhoeal illness in immunocompromised patients and widely distributed in HIV/AIDS patients (Fisseha *et al.*, 1999; Gupta *et al.*, 2008;).

The prevalence of the parasites appears to be greater in less developed countries, probably due to the lack of clean water, poor sanitary facilities, overcrowding and the close proximity of animal reservoirs to residences (Ryan and Ray, 2010; Brett *et al.*, 2008).

Younger children of age two and below are more susceptible to infections probably because they mostly practice geophagy and the lack of protective immunity (Cheesbrough, 2005; Clark, 1999).

The excretion of oocysts is found in 4-11% of the population in less developed countries and sero prevalence of antibodies may be as high as 50% in China and nearly all children living in urban slums in Brazil (Ryan and Ray, 2004; Bett *et al.*, 2003). In the developed world, sporadic outbreaks and epidemics of cryptosporidiosis occurs in the United States of America (U.S.A) (Brett *et al.*, 2008) where the largest outbreak of the disease occurred in Milwaukee, U.S.A. in 1993 (MacKenzie *et al.*, 1995).

In Africa and Haiti, more than 50% of HIV/AIDS patients develop chronic Cryptosporidiosis with about 10% having a fulminant course (Ryan and Ray; 2004). The parasite is frequently recovered from HIV/AIDS patients in India and other parts of the world (Prasad *et al.*, 2000).

Mode of Infection

Cyrptosporidium parvum is transmitted through the faeco-oral route by the ingestion of the infective oocysts in food, water, or from hands contaminated with infected faecal material (Cheesbrough, 1992).

Its life cycle is completed in a single host which includes both stages of sexual (sporogony) and asexual (merogony) reproductive cycles (Ryan and Ray, 2004). The ingested oocysts are activated in the stomach and the upper intestines and releases four motile sporozoites per oocyst (Ryan and Ray, 2004) which then binds to the receptors on the surface of the intestinal epithelial cells (Cheesbrough, 1992).

The parasite is able to infect and reproduce in the epithelial cell lining of the gastrointestinal tract without causing cytopathic effects (Ryan and Ray, 2004).

Two morphologically significant forms are known to exist, the thin-walled asexual stage oocysts which excysts within the same host (causing auto-infection) and the thick walled sexual stage oocysts which are shed into the environment through faeces (Robert *et al.*, 2002).

Clinical Manifestation

In immunocompromised individuals, the incubation period is 2-14days but symptoms may develop within 2-10 days after infection with the parasite (Brett *et al.*, 2008) whiles it last from several days to five weeks in the immunocompetent host (Jokipii and Jokipii, 1986).

Shortness of breath may develop in immunocompromised patients infected with *Cryptosporidium parvum* (Hunter and Nicholas, 2002).

Diarrhoea with or without crampy abdominal pain, may be intermittent and scanty or continuous, watery, and copious and sometimes the diarrhoea is mucoid (Robert *et al.*, 2002). Diarhoea can persist longer in individuals who are immunosuppressed (Brett *et al.*, 2008).

The volume of fluid losses through diarrhoea may be as high as 25 litres per day in particularly HIV/AIDS patients whiles the shedding of oocysts can persist for as long as two weeks after clinical improvement (Ryan and Ray, 2004).

Fever may be of low grade or may not exist at all in sporadic cases but may occur in 30-60% of patients during outbreaks (Rachel *et al.*, 2011). Nausea and vomiting are present in 50% of cases (Forsythe, 2010). Malaise may be reported (Ryan and Ray, 2004).

Approximately 15% of patients with HIV/AIDS may present with fever, right upper-quadrant pain, jaundice, nausea and vomiting (Wolska-Kusnierz *et al.*, 2007). Most infections are self-limiting in patients with CD4 T-cell counts greater than 200/ul just similar to in immunocompetent hosts (Wolska-Kusnierz *et al.*, 2007).

Biliary involvement is associated with significantly low CD4 T-cell counts, and patients present with cholecystitis, sclerosis, cholangitis, or pancreatitis whiles other patients

develop chronic diarrhoeal illness with frequent foul-smelling and bulky stools accompanied by weight loss (Ryan and Ray, 2004).

Immunocompetent patients usually present with subclinical or milder illness that lasts for less than 5 days in waterborne outbreaks (Craun, 1990; Brett *et al.*, 2008).

Although healthy individuals can become ill from outbreaks of infections due to water contamination, immunodeficiency puts an individual at a higher risk for cryptosporidiosis, particularly for more severe and disseminated disease (Brett *et al.*, 2008).

Immunosuppression may be congenital or may be secondary to HIV, cancer therapy, diabetes mellitus or bone marrow or solid organ transplantation (Hunter and Nicholas, 2002).

Laboratory Diagnosis

Diagnosis of Cryptosporidiosis is established by demonstrating the oocysts in infected stools under the light microscope (Brett *et al.*, 2008; Cheesbrough 2005). Patients may be asked to submit several stool samples over several days as detection of Cryptosporidium can be difficult (Bronsdon, 1984).

In addition to the routine saline preparation of stools for detection of the parasite, samples are examined using different techniques such as the Carbol fuschin acid fast staining, direct fluorescent antibody, and enzyme immunoassays for the detection of Cryptosporidium antigens (Garcia *et al.*, 1983; Bronsdon, 1984; Werber *et al.*, 1991).

The availability of several preservatives such as 10% buffered formalin solution, Sodium Acetate Formalin (SAF) or Merthiolate-Iodine-Formalin (MIF) makes it possible for stool samples to be preserved if immediate examination is not possible. Unpreserved samples could also be refrigerated for short periods to delay deterioration of the parasites (Cheesbrough, 2005).

Even though oocysts can be detected in unconcentrated faecal specimen due to its numerous numbers in acute infections, other workers are of the view that concentration by the Formol-ether concentration technique is preferable (Garcia *et al.*, 1983; Casemore

et al., 1985; Cheesbrough, 1992) where optimal centrifugation time and speed for 10 minutes at 500g are critical for concentrating Cryptosporidium oocysts (Cheesbrough, 1992).

The modified Zeinhl-Neelson's staining technique is useful for the identification of oocysts of Cryptosporidium species, *Isospora belli* and cyclospora species which may be difficult to detect with routine stains such as trichrome (Garcia *et al.*, 1983; Bronsdon, 1984).

Other types of clinical specimen such as duodenal fluid, bile, pulmonary samples (induced sputum, bronchial washings and biopsies) may also be stained (Garcia *et al.*, 1983; Bronsdon, 1984). An alternative is the sucrose floatation method or the formalin ethyl acetate technique used in concentrating stool before staining with modified Kinyoun acid-fast stain (Garcia *et al.*, 1983).

This technique stains the oocysts red or pink against a background colour of blue or green counter stained faecal debris or yeast cells (Werber *et al.*, 1991; Garcia *et al.*, 1983). The Enzyme Immunoassay (EIA) technique used in detecting antigens of the parasite in stool is much more specific and reliable than the monoclonal antibody-based fluorescence conjugated stain for oocysts in stool. The former is widely available commercially (Weitraub, 2006).

Due to the intermittent shedding of oocysts, at least three stool specimen collected on separate days must be examined before declaring test results negative (Casemore *et al.*, 1985). Due to the non-invasiveness of the parasite under the epithelial layer of the intestinal mucosa, faecal leucocytes are usually not present in stool specimen (Cheesbrough, 1992).

The oocyst are small, measuring between 4 and 6 um in diameter and can be missed by inexperienced workers without a very careful examination of the preparation under the microscope (Cheesbrough, 2005).

Electron microscopy of stool or biopsy specimen can also be performed for direct visualization of oocysts (Casemore *et al.*, 1985).

Epidemiological studies have classified the parasite into heterogeneous species with most of them being associated with zoonotic transmission (Morgan *et al.*, 2000). PCR assay is used in research purposes for species identification (Morgan *et al.*, 2000).

Treatment

There is no reliable treatment for Cryptosporidiosis (Xiao and Ryan, 2004; Soave, 1990; Clark, 1999). It is known that within the host cell, a unique parasitophorous vacuole compartment is established thus sheltering the parasite from antimicrobial drugs (Griffiths *et al.*, 1998). It was therefore suggested by Clark, (1999) that supportive therapy is the key component in managing Cryptosporidiasis.

The administration of fluid and electrolyte is critical especially in AIDS patients in whom the infection causes protracted diarrhoea resulting in severe loss of water (Fayer, 1997; Clark, 1999).

Nonspecific antidiarrhoeal agents such as Ostreotide, a somatostatin analogue and a substance pantagonist, suppress diarrhoea in chronic Cryptosporidiosis (Clark, 1999). Immunocompetent subjects recover without treatment (Clark, 1999).

Matured epithelial cells located at the tips of the intestinal villi are preferentially lost, hence, enzymes expressed on these cells, which includes lactase are lost. These losses eventually lead to secondary lactose intolerance (Dourus, 2010).

Biliary involvement in Cryptosporidiosis which is found in some HIV infected patients requires specific intervention such as cholecystectomy and retrograde cholangiopancreatography (RECP) (Blumberg *et al.*, 1984).

Nitazoxanide has been approved for the treatment of children with diarrhoea due Cryptosporidium infections (Clark, 1999; Fox and Saravolatz, 2005).

Clinical trials with the agent showed a significant reduction in the duration of diarrhoea caused by the parasite (Fox and Saravolatz, 2005).

Smith *et al.*, (1988) and Mofenson *et al.*, (2005) noted that antiretroviral treatment of AIDS patients has seen improvement, probably due to the general improvement of the immune function.

A combination therapy of Paromomycin and Azithromycin (Hicks *et al.*, 1996) for 4 weeks followed by Paromomycinmonotheraphy for up to 8 weeks has been successfully used in adult patients with AIDS (Clark, 1999).

Since available data suggests that infections with *Cryptosporidium parvum* occurs mostly in immunocompromised subjects, the best approach to the prevention of the infection in HIV infected patients is to maintain the good immune system through HAART administration (Clark, 1999).

Clark, (1999) also noted that the administration of Clarithromycin which was aimed at preventing infections with mycobacterium avium cellulorare in severely immunocompromised individuals also has a protective effect against Cryptosporidium infections.

Cyclospora cayetanensis

Cyclospora cayetanensis which was originally considered to be a cyanobacterium or a coccidian-like body (Ashford *et al.*, 1993) has been classified as a coccidian parasite that infects mainly the gastrointestinal tract of immunocompetent and immunocompromised hosts alike (Cheesbrough, 2005).

The parasite was first recovered in human faeces in 1979 (Ashford *et al.*, 1993) and since the emergence of the HIV/AIDS epidemic, it has been increasingly recognized as an enteric pathogen (Mannheimer and Soave, 1994). The organism colonizes the small intestine of its host and causes flu-like symptoms accompanied by nausea, vomiting and explosive diarrhoea normally lasting for 3 weeks in immunocompletent hosts whiles in the immunocompromised, particularly HIV/AIDS individuals who are commonly infected, diarrhoea may be prolonged, lasting between 4 and 6 weeks sometimes causing biliary disease (Washington *et al.*, 2006).

Since its earlier description as a gastrointestinal illness in 1979, the parasite has gained international recognition especially among travellers (Blans *et al.*, 2005). Cyclosporiasis has been reported in travellers returning from Mexico, South East Asian countries and Puerto Rico (Blans *et al.*, 2005).

The parasite has been recognized as a waterborne pathogen with reports suggesting its association with waterborne outbreaks worldwide (Ortega *et al.*, 1993).

Cyclospora species are round to ovoid parasites that measure 8-10um in diameter (Cheesbrough, 2005) and are variably acid fast (Garcia *et al.*, 1983). The parasite is characterized by an anterior polar complex that allows penetration into host cells, but its life cycle and the mechanisms by which it interacts with human host target cells to cause disease are not clearly understood (Forsythe, 2010).

Epidemiology

Cyclosporiasis is endemic in Haiti, Nepal and Peru with a strong seasonal predominance during rainy, spring and summer months (Hoge *et al.*, 1993; Lopez *et al.*, 2003). The oocysts shed in faeces of the infected host undergo sporulation to maturity outside the host within 3-5 days in the environment and seasonal pattern suggests that oocysts may survive for extended periods in the environment (Sterling and Ortega, 2004).

People living in the tropics and subtropics are at risk of infection because of the endemicity of Cyclosporiasis in some developing countries (Soave *et al.*, 998).

The disease is also thought to be at its peak in certain seasons but it is not clearly understood (Sterling and Ortega, 2004). Humans are the only known hosts of *Cyclospora cayetanensis* (Eberhard *et al.*, 2000).

Mode of Infection

Infections with *Cyclospora cayetanensis* is by ingestion of the infective sporulated oocysts in food, water and from hands contaminated with infected faecal material (Cheesbrough, 2005).

The ingested oocysts on reaching the small intestine excysts and release sporozoites which colonize the small intestinal mucosa (Ortega *et al.*, 1997). The parasite exogenously sporulates and produces two sporocyts per oocyst (Levine, 1973).

The incubation period between acquisition of infection and the onset of symptoms averages approximately 7 days (Soave, 1996) and the oocysts shed in faeces for more than 3 weeks (Forsythe, 2010).

It has been established that the presence of distinctive intracellular asexual merozoites and sexual gametocyte stages are requisite forms for completion of the life cycle within a single host (Sterling and Ortega, 2004).

Direct person to person (faeco-oral) transmission of Cyclospora is most likely (Forsythe, 2010). However, indirect transmission can occur if an infected person contaminates the environment and oocysts are sufficient thereafter under favourable conditions become infective (Forsythe, 2010).

Clinical Manifestation

The disease manifests as protracted and relapsing enteritis in patients who are immunosuppressed (Cheesbrough, 2005). Symptoms may develop spontaneously or gradual and may be of a relatively short duration or last for an average of 7 weeks in immunocompetent hosts (Sterling and Ortega, 2004).

The parasite causes watery diarrhoea with frequent, sometimes explosive stools which can result in dehydration and weight loss (Forsythe, 2010).

The diarrhoea may be associated with one or more non-specific symptoms, including intermittent crampy abdominal pain, nausea, vomiting, low grade fever, malaise, anorexia, bloating, flatulence, and profound fatigue (Forsythe, 2010; Washington *et al.*, 2006).

Biliary disease with right upper quadrant pain, raised alkaline phosphatase and thickened gall bladder on ultrasound findings have been reported in an immunocompromised host infected with Cyclospora (Debra *et al.*, 1994).

Small-bowl biopsy reveals pathologic changes, including blunting and atrophy of the villi, acute and chronic inflammation, and hyperplasia of crypts. Severity of the histopathologic findings correlates with the severity of clinical symptoms, including malabsorption (St. Georgiev, 1993).

Immunosuppression is a risk to chronic Cyclosporiasis in endemic areas (Stark *et al.*, 2009). If untreated, the illness may last for a few days to a month or longer, and may follow a remitting-relapsing course (Forsythe, 2010).

Reports of asymptomatic carriers of oocysts are available particularly in communities where the disease is endemic (Eberhard *et al.*, 1999; Ortega *et al.*, 1993).

Laboratory Diagnosis

Diagnosis of Cyclosporiasis is based on the demonstration of the oocysts in faecal material under the light microscope (Cheesbrough, 2005) however using the modified Zeihl-Neelson or Kinyoun acid fast staining technique is more appropriate.

A Polymerase Chain Reaction (PCR) assay for detecting oocysts in food stuffs has recently been described (Relman *et al.*, 1996).

Treatment

In immunocompetent hosts, Cyclosporiasis is self-limiting (Sterling and Ortega, 2004) lasting several days to 2 weeks. Infected immunocompromised hosts and children require as a matter of urgency rehydration therapies (Sterling and Ortega, 2004) before treatment is sorted for.

Trimethoprim-sulfamethoxazole (TMP-SMZ) has proven to be effective in managing infections with this parasite in infected hosts (Bouree *et al.*, 2007).

The administration of TMP-SMZ reduces shedding of oocysts from 9 days to 2 days and inhibits the diarrhoea within 2 days (Bouree *et al.*, 2007).

Immunocompromised hosts require TMP 160mg and SMZ 180mg three times daily for up to 10days and may continue for longer periods, followed by prophylaxis to prevent recurrent infections (Sterling and Ortega, 2004). One study has indicated that for patients who are allergic to sulpha drugs or cannot tolerate them, especially AIDS patients, Ciprofloxacin is an alternative (Verdier *et al.*, 2000).

OVERVIEW OF ENTERIC PARASITES IN HIV/AIDS

Mohandas *et al.*, (2002) in a study conducted among HIV/AIDS patients in Northern India reported that out of 36 patients (30%) who tested positive for intestinal parasites, Cryptosporidium was the commonest parasite (10.8%), followed by *Giardia lamblia* (8.3%) with *Cyclospora cayetanensis* and *Blastocystis hominis* each recording 3.3% of patients, while *Isospora belli* and *Enterocytozoon beneusi* were each detected in 2.5% of patients.

Other parasites observed in their study were *Entamoeba histolytica* and *Entamoeba dispar* in two cases each and hook worm in one patient.

It was also noted that out of the 36 patients who harboured intestinal parasites, 27 (75%) had diarrhoea and the most common parasite associated with this diarrhoea was *Cryptosporidium parvum.*

Prasad *et al.*, (2002) in previous studies in Northern India conducted among HIV patients, reported *Isospora belli* as the most frequent parasite followed by *Cryptosporidium parvum.*

The most predominant parasite encountered in a cross sectional study in Thailand was *Cryptosporidium*, with over 30% prevalence (Saksirisampant *et al.*, 2009).

Microsporidia of the genus *Enterocytozoan beneusi* recorded a prevalence of 5.6% followed by *Blastocystis hominis*, *Cyclospora cayetanensis* and *Isospora belli* having a prevalence of 2.2%, 1.1% and 1.1% respectively (Saksirisampant *et al.*, 2009) with the only helminth infection detected being *Opistorchis sinensis*, recording a prevalence of 2.2%.

In an earlier study carried out by Saksirisampant *et al.*, (2009) among HIV patients who presented with diarrhoea, *Cryptosporidium* was the predominant parasite (19.2%), followed by *Isospora belli* (14.5%), *Giardia lamblia* (3.8%), *Entamoeba histoblytica* (0.9%) and *Iodamoeba butschili* (0.3%) (Saksirisampant *et al.*, 2009).

In Sub Saharan Africa, a survey of intestinal parasitic infections among a rural setting in Tanzania revealed the presence of 8 protozoa and 7 helminths out of 287 subjects (Gomez *et al.*, 1995). The prevalence of *Entamoeba histolytica* in HIV negative and positive patients was 25.1% and 12.5% respectively ($p< 0.04$) (Gomez *et al.*, 1995).

On the other hand, *Cryptosporidium parvum*, *Isospora belli* and *Strongyloides stercoralis* prevalence were higher in HIV-positive than HIV-negative patients ($p<0.1$) (Gomez *et al.*, 1995)

The prevalence of the two opportunistic protozoa was also higher in AIDS patients than in HIV- positive patients without AIDS.

Specific anti-*Cryptosporidium parvum* IgG antibodies were detected by ELISA in 18% and 56% of HIV-negative and HIV-positive patients respectively, thus confirming the high number of contacts between this parasite and humans (Gomez *et al.*, 1995).

Specific anti- *Encephalitozoon cuniculi* and anti-*Encephalitozoon* IgG antibodies were detected by Immunoflourescent assays (IFA) in 18% and 19.1% of subjects respectively, without any correlation with HIV and malaria infections (Gomez *et al.*, 1995).

In Zambia, Modjarrad *et al.*,(2005) documented a prevalence of 24.9% of intestinal helminth infections among HIV infected adults, from which 39 (52.7%) were *Ascarsis*

lumbricoides and 29 (39.2%) were infected with hookworm, whiles *Strongyloides stercoralis, Schistosoma mansoni, Hymenolepis nana* and *Taenia species* were much lower.

In similar studies conducted in Cameroon by Sarfati *et al.,(2006)* among 154 HIV-infected adults, a prevalence rate of 33% was reported. Opportunistic protozoa were found in 9.7% of the patients although as high as 53% presented with diarrhoea.

Of the protozoan species recorded in the study, *Enterocytozoon bineusis* was found in 8 (5.1%) patients, *Cryptosporidium parvum* in 6 (3.9%) patients and *Isospora belli* in 3 (1.9%) patients.

A higher prevalence (32%) of opportunistic protozoa among patients with CD4 T-cell counts less than 50/mm^3 was recorded while half of the patients that had *Cryptosporidium* infection presented with diarrhoea (Sarfati *et al., 2006).*

There has been a significant reduction of infection with *Strongyloides stercoralis, Ascaris lumbricoides,* hookworm, *Trichuris trichiura, Giardia lamblia, Entamoeba histolytica, Isospora belli* and *Cryptosporidium* following the onset of Anti-retroviral drugs therapy (Bachur *et al.,* 2008). Anti-retrovirals help in the control of HIV infection and the reconstitution of the immune system of the patients. Modifying the morbid-mortality profile among these patients has reflected in the reduced occurrence of opportunistic infections, including those caused by enteroparasites (Willemont and Klein, 2004). Bachur *et al.,* (2008) demonstrated a remarkable reduction in the number of parasitic infections among HIV patients in this Anti- Retroviral therapy era.

HIV AND URINARY TRACT INFECTIONS

Parasitic agents that cause Urinary Tract Infections (UTI) are *Schistosoma haematobium, Trichomonas vaginalis* and *Candida albicans* (Cheesbrough, 2004). Schistosomiasis is the second most prevalent tropical disease after malaria and affects approximately 200 million people in Africa, Asia, South America, and the Caribbean's (Harms and Feldmeier, 2002).

Morbidity depends on the Schistosoma species involved, the intensity of the infection, the topographic site affected by the eggs and the immune responsiveness of the host (Karanja *et al.*, 1998). So far there are only indirect hints that Schistosomiasis may have a negative impact on HIV infection, and as shown in a study in Kenya by egg excretion per worm pair was reduced with decreasing CD4 T-cell count. Egg excretion rates were significantly correlated with CD4 levels (Karanja *et al.*, 1998).

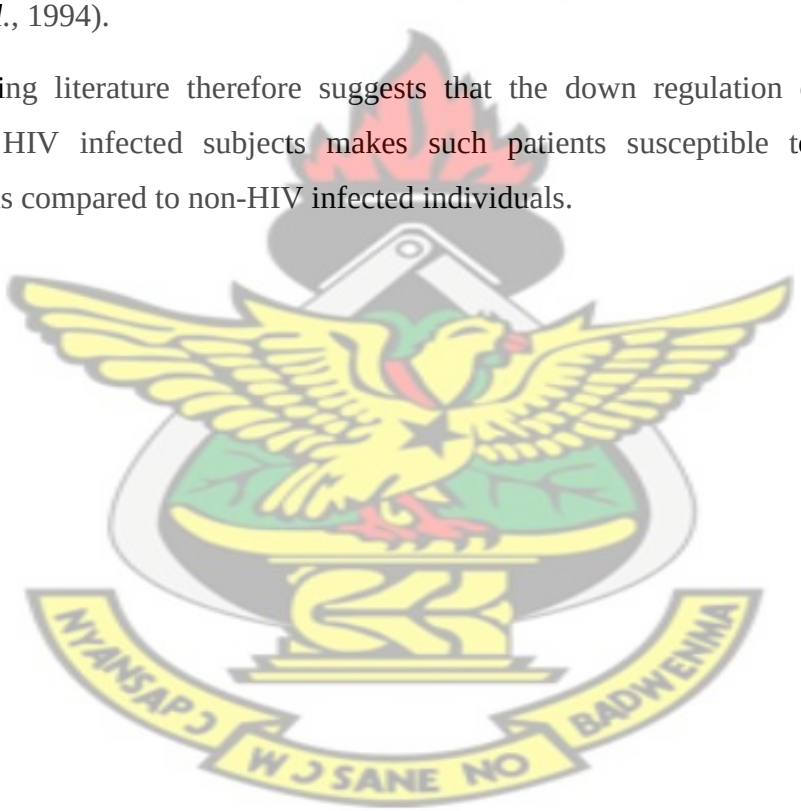
A study was conducted in two villages in Zambia by Mwanakasale *et al.*, (2013) to compare the efficacy of praziquantel in the treatment of Schistosomiasis in people with and without infection. Five hundred and seven individuals infected with *Schistosoma haematobium* were enrolled and followed up for as long as twelve months after treatment with a single dose of praziquantel. Seventy three were coinfected with HIV. The study demonstrated that praziquantel is still very effective in the treatment and control of Schistosomiasis even when there is coinfection with HIV (without symptoms and signs of HIV/AIDS disease). Resistance to reinfection with *Schistosoma haematobium* is not altered in subjects coinfected with HIV (without symptoms and signs of HIV/AIDS disease) (Mwanakasale *et al.*, 2013).

Trichomonas vaginalis (TV) is a protozoan parasite transmitted principally through vaginal intercourse (Sorvillo *et al.*, 2001). Contaminated towels, douche equipments and examination instruments may also be a source of infection (Jawetz, 2004). Infection with the organism, sometimes asymptomatic in men can cause vaginitis in women and urethritis in men (Sorvillo *et al.*, 2001). Despite a relative paucity of studies on the prevalence and incidence of Trichomoniasis, recent publications suggest that *Trichomonas vaginalis* is one of the most common sexually transmitted infections (STI) in the United States of America, with estimated five million new cases occurring annually (Sorvillo *et al.*, 2001). Although the organism appears to be highly prevalent and has a widespread geographical distribution, *Trichomonas* has not been the focus of intensive study nor of active control programs (WÃ¸lner-Hanssen *et al.*, 1989).

The neglect is likely a function of the relatively mild nature of the disease, the lack of effect on fertility and the historic absence of association with adverse birth outcome (although recent data suggest a possible casual role in low birth weight and prematurity) (Cotch *et al.*, 1997).

A cross-sectional study conducted by Laga *et al.*, (1993) on 1,209 female sex workers in the Ivory Coast found an association between HIV and *Trichomonas vaginalis* infections, suggesting a two to three fold increase in HIV infected subjects. In a similar cross-sectional study conducted in Tanzania by Laga *et al.*, (1993) among 359 women admitted for gynaecological conditions, *Trichomonas vaginalis* was found to be more common in women infected with HIV. Infections with this organism typically elicits an aggressive local cellular response with inflammation of the vaginal epithelium and exocervix in infected women and the urethra of infected men (Sardana *et al.*, 1993). The inflammatory response induces a large infiltration of leucocytes including HIV target cells such as CD4 T-cell bearing lymphocytes and macrophages to which HIV can bind and gain access (Kreiss *et al.*, 1994).

The foregoing literature therefore suggests that the down regulation of the immune system of HIV infected subjects makes such patients susceptible to opportunistic organisms as compared to non-HIV infected individuals.



Chapter 3

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

This cross-sectional study was conducted at the Bomso Specialist Hospital, Kumasi in the Ashanti Region from November 2010 to April 2011.

This is a medium sized health facility of sixty bed capacity and located behind the west end of the Kwame Nkrumah University of Science and Technology (K.N.U.S.T), Kumasi. Its size notwithstanding, the hospital offers mainly specialist services which include Surgery, Obstetrics and Gynaecology (O&G), Medical Unit, Paediatric, General Medicine, Maternal Health Care and Infectious Disease Unit (I.D.U). It also offers Laboratory and Radiological Services. Other units include Out Patients and In-Patients Departments and a Pharmacy with an Accounts Unit attached. The hospital has been running an Infectious Disease Unit for several years and has a large number of HIV Sero-positive clients most of whom are on Anti Retro Viral Therapy (ART).

STUDY POPULATION AND SUBJECT SELECTION

A total of 256 patients were enrolled in the study. This group comprised HIV Sero-positive patients who were attending the Infectious Disease Clinic during the period of the study. Only patients who gave written consent and also satisfied the inclusion criteria were enrolled in the study. The study patients were interviewed using a structured questionnaire which included age, sex, occupation, and other socioeconomic parameters. Information was obtained on demographic characteristics, present and past knowledge of deworming, antibiotic treatment, diarrhoea, marital status, personal hygienic practices, and type of toilets used. Patients on antibiotic treatment and those with history of being dewormed within the last three months were excluded from the study. Diarrhoea was defined as two or more liquid or three or more soft stools passed per day.

3.1.1 Inclusion Criteria

- Patients who gave a written consent to the study.
- Those who were not on antibiotic treatment during the period of the study.
- Patients who had no history of being dewormed within three months prior to the study.

- Patients who had just been tested positive prior to the study and referred to the clinic for treatment to start.

3.1.2 Exclusion Criteria

- Patients who did not consent to the study.
- Patients who were on antibiotic treatment.
- Patients who have been dewormed within three months prior to the study.

ETHICAL CONSIDERATION

The study protocol was approved by the Committee on Human Research, Publications and Ethics of the School of Medical Sciences (SMS), Kwame Nkrumah University of Science and Technology (KNUST) and the ethical review committee of the Bomso Specialist Hospital, Kumasi. After explaining the significance, purpose and benefits to be derived from the study a written informed consent was obtained from study participants. Confidentiality of the study was paramount and maintained.

SAMPLE COLLECTION AND PROCESSING

3.1.3 STOOL SAMPLE

Fresh stool samples were collected into clean dry leak proof plastic containers. Stool samples were divided into two parts, one of which was preserved with 10% Formol-saline. The fresh unpreserved portion was set aside for wet mount preparation and was immediately examined microscopically for parasites as per the following procedure.

TECHNIQUES USED IN THE LABORATORY INVESTIGATION S

A. MACROSCOPIC EXAMINATION OF STOOL SAMPLES

The freshly voided stool samples were first of all inspected macroscopically for the presence of blood, mucus, tape worm segments, adult *Ascaris lumbricoides* or the pin worm *Enterobius vermicularis.*

These features served as a guide to what may be expected to be seen microscopically.

B. MICROSCOPIC EXAMINATION OF STOOL SAMPLES

DIRECT SALINE MOUNTS

1. 1gm of the freshly collected stool samples was placed in clean leak proof 5 millilitre plastic tube whose cap contained a long spoon-like end used to emulsify the specimen in the tube.
2. About 2mls of saline from a wash bottle was added to the specimen and emulsified.
3. A dropper was used to fetch the emulsified specimen on to two different clean grease-free slides.
4. A drop of 1% neutral red stain was added to one slide and mixed with a cover slip and the cover slip applied on it. A cover slip was also applied onto the second slide with the content unstained.
5. The preparations were examined under the microscope for motile trophozoites, cysts of protozoa, and the ova or larvae of helminths.

The neutral red stained mount was used in detecting the cysts and trophozoites of protozoa which are difficult to find in ordinary saline mounts. Any parasites seen were recorded.

FORMOL ETHER CONCENTRATION

The preserved portions of the stool samples were examined using the formol-ether concentration technique as outlined in WHO Standard Operation Procedure (WHO, 1991) as follows.

1. About half a teaspoonful of the preserved stool specimen was placed inside the designated leak proof stool container mentioned above and emulsified with 4mls of 10% formol water and transferred into a screw-cap tube.
2. About 3-4mls of the 10% formol water was added and shaken for about 20seconds.
3. The contents of the tube were sieved and collected into a beaker.
4. The suspension was transferred into a glass conical centrifuge tube and an equal volume of ether (3-4) mls added.
5. The tubes were stoppered and mixed for 1 minute.

6. The stopper was loosened and tubes centrifuged at 3000 revolutions per minute (rpm) for 1minute.
7. By the use of an applicator stick, the layer of faecal debris was loosened from the side of the tube and rapidly inverted, discarding the ether, faecal debris and formol water.
8. The tube was positioned upright and the remaining fluid drained down the bottom.
9. The bottom of the tube was gently tapped by a finger to mix the sediments, which by the use of a Pasteur pipette were transferred onto two different slides.
10. A cover slip was applied to one preparation and a thick smear made out of the second preparation.
11. The preparation under the cover slip was examined immediately under the microscope for parasites.
12. A drop of 1% neutral red solution was run under the cover slip , mixed with the preparation and examined further to detect cysts of protozoa which were difficult to find in unstained preparations.
13. The second slide smear preparation was air-dried, fixed in methanol and stained by the modified Zeihl-Neelsen Acid Fast staining technique as follows.

The Modified Zeihl-Neelsen staining technique was performed on both fresh stool smears and those prepared from the concentrated specimen.

MODIFIED ZEIHL-NEELSEN STAINING TECHNIQUE

Thick smears were prepared from both saline and concentrated stool samples. These were air dried, fixed in 70% methanol and stained by the Modified Ziehl-Neelsen staining procedure (Cheesbrough, 2005). The stained preparations were examined under the immersion oil lens (x100) of the microscope for parasites such as *Cryptosporidium parvum* (*C. parvum*), *Isospora belli* (*I. belli*) and *Cyclospora cayetanensis* (*C. cayetanensis*).

1. As mentioned above, the air-dried smears of stool samples from both direct and concentrated preparations were fixed in methanol for about 3 minutes.
2. The fixed slides were stained with cold Carbol Fushin for about 10minutes and washed under clean slowly running tap water in a sink.
3. The smear was decolourised using 1% acid-alcohol until no more colour appeared on the smear.
4. The smear was rinsed under clean slowly running tap water in a sink.
5. It was then counterstained with 0.3% methylene blue solution for 30 seconds.

6. The stained slides were rinsed in slowly running tap water, air-dried and examined under the high power lens (X100 objective eye piece) of the microscope after the application of a drop of immersion oil on the smear.
7. Smears were all thoroughly examined for the presence of oocysts of protozoa. (Cheesbrough, 1992).

All parasites found were recorded and reports given to the Infections Diseases Unit (IDU) of the study facility for prompt treatment of the infected patients.

3.1.4 COLLECTION OF URINE SAMPLES FOR URINALYSIS, MICROSCOPY, CULTURE AND SENSITIVITY

About 10-15 millilitres of midstream urine samples were collected each into a sterile, dry, wide necked, leak-proof screw capped container for urinalysis, microscopy, and culture. Each urine sample was well mixed and about 3mls aseptically poured into a clean dry glass centrifuge tube. The second sample was reserved for culture. A urine strip with a combination of eleven parameters was dipped into the urine sample in each centrifuge tube. This was used to detect both qualitatively and quantitatively the parameters such as; Leucocytes, nitrite, Urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin, glucose and microalbumin on each strip used. This was detected using an automated urine analyzer (DIRUI H-100 URINE ANALYSER, DIRUI INDUSTRIAL COMPANY LIMITED, CHINA). The centrifuge tubes containing the urine samples were placed in a centrifuge and spun at 3000 revolutions per minute (rpm) for 3-5 minutes. The supernatant fluid was decanted into a container of disinfectant. The urine deposits were mixed and the last drops of urine in the tube, poured onto a slide and a cover slip applied on it.

The urine deposits were examined microscopically for parasites such as *Schistosoma haematobium*, *Trichomonas vaginalis* and Candida species. Other abnormalities such as the presence of pus cells and their numerical concentration, red blood cells, casts; both granular and hyaline, crystals of elements and debris were noted. Samples containing significant abnormalities were all documented.

For qualitative analysis, colour was observed and reported as follows; straw to amber was normal specimen, orange colour indicates a concentrated specimen, orange-brown specimen indicated the presence of urobilin , greenish orange urine indicated the presence of bilirubin, smokey specimen indicated the presence of red blood cells, almost colourless urine showed a

dilute specimen, turbid or cloudy specimen indicate the presence of numerous bacteria or cells whiles a milky specimen indicates the presence of lymph fluid chyluria.

Urine samples in which pus cells and or casts were detected to be more than (5-6) per high power field (Pus cells > 5/HPF) were cultured on Cysteine Lactose Electrolyte Deficient (CLED) agar. A standard calibrated loop was used to fetch a loopful (0.002 ml) of well mixed urine sample and inoculated on CLED agar. This was incubated aerobically at 37^{o}C for 18-24 hours in an incubator (IPF 400 Precision, Memmert, Germany).

Bacterial colonies were identified based on colonial morphology (color, growth size, and growth pattern). Standard biochemical tests: Triple Sugar Iron agar (T.S.I), citrate, urease, indole, catalase, and coagulase tests were used for further identification of isolates. A pure single colony of the isolate was inoculated into Peptone water and incubated at 37^{o}C for 3hours. Bacterial count was estimated from the product of the loop volume and the colony count on CLED. Bacterial counts >1x10^{5}CFU/ml was considered significant whilst bacterial counts between 1 x10^{4}-10^{5}CFU/ml was considered doubtfully significant. Bacterial count <1 x10^{4} CFU/ml was considered insignificant (Harding *et al.*, 2002). The isolates from this study were tested against the following antibiotics: Ampicillin (10µg), Cefuroxime (30µg), Cotrimoxazole (25 µg), Ciprofloxacin (5µg), Gentamicin (10µg), Tetracycline (30µg), Nalidixic acid (30µg) and Nitrofurantoin (300 µg). Inhibition zone sizes were measured according to CLSI standards. The average turnaround time was 2 days.

All significant findings were reported to the clinician at the Infectious Disease Clinic for treatment to be effected.

3.1.5 BLOOD SAMPLE COLLECTION AND ESTIMATION OF CD4 T-CELL COUNT

Two millilitres (2mls) of venous blood sample was collected into a four millilitre (4mls) plastic test tube containing crystals of Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant. The samples collected were labelled with the patients' clinic identification numbers.

IMMUNOPHENOTYPING (CD4 T-CELL COUNTS)

Immunophenotyping of lymphocytes was carried out using the principle of FACS Flow Cytometry. The FACS Count (Becton Dickinson Immunocytomery system, Singapore (BD) was used, strictly following the prescribed procedures outlined for sample analysis.

The CD4 T-cell counts of the patients were carried out within 48 hours of collection of the blood samples as prescribed under the National Aids Control Program (NACP) regulations.

TEST PROCEDURE FOR CELL COUNTS

1. The CD4 reagents tubes were labelled and vortexed upside down for 5 seconds and upright for 5 seconds.
2. The reagent tubes were opened with the coring station.
3. BD vacutainer tubes containing blood samples were inverted 5 to 10 times to adequately mix the sample.
4. 50µl of whole blood was pipetted into the reagent tubes.
5. Each tube was capped and vortexed for 5 seconds.
6. The tubes were incubated for 60 to 120 minutes at room temperature in the dark.
7. The tubes were uncapped and 50µl of fixative solution pipetted into the tubes.
8. The tubes were recapped and vortexed upright for 5 seconds before they were analysed on the FACS Count instrument (BD Bioscience, 2005).
9. Results were printed out from the in –built printer in the cytometer.

Patients were categorised by their immune status according to the 1993- revised classification system for the HIV infection by CD4 T-cell categories (Castro *et al.*, 1993) as follows; <200cells/ul, (200-500)cells/ul, and >500cells/ul.

STATISTICAL ANALYSIS

Continuous variables are expressed as their mean ± SEM, while categorical variables were expressed as proportion. Comparisons of the infection (Intestinal parasite infected and UTI infected) against the control group (those without infection) were performed using Mann Whitney tests, $\chi 2$ tests, or Fisher exact tests where appropriate. Kruskal Wallis test coupled with Dunnet multiple comparison tests were used in comparison between more than two median. SPSS software version 16 (Chicago Inc) and GraphPad Prism version 5.00 for windows where appropriate were used for these statistical analyses (GraphPad software, San Diego California USA, www.graphpad.com). $P<0.05$ was considered statistically significant.

Chapter 4

RESULTS

A total of 256 patients were recruited out of which 71 (27.7%) were males and 185 (72.3%) females. Most of the participants were urban settlers 207 (80.9%) and were within age group 36-45 (41.0%). A higher proportion of them cooked in open space (71.9%), used KVIP as a source of toilet facility (78.9%) and drunk pipe borne water (84.0%). Age and gender did not have any significant effect on the intestinal parasite infection ($p>0.05$) though higher prevalence were observed among females (12.9%) and age group 36-45 (6.6%). Conversely, place of abode, source of drinking water, food preparation area and the type of toilet facility used had a significant ($p<0.05$) effect in association to intestinal parasite infection. Out of 28 participants with diarrhoea, higher prevalence were observed among those with parasite infection (8.2%) than those without parasite infection (2.7%) ($p<0.0001$). Participants with CD_4 counts <200 cells/μL had higher and significant ($p<0.0001$) prevalence of parasitic infection (8.6%) than those without infection (4.3%). The overall prevalence of parasite infection was 48 (18.8%). **(Table 4.1)**

Table 4.1 Sociodemographic, Clinical and Prevalence of Intestinal Parasite in HIV positive HAART naïve patients

Variables		Intestinal parasite infection			
		Infected N=48	Non-infected N=208		
	Total N=256	(18.8%)	(81.2%)	p-value	χ^2 value
Age range (years)	N (%)				
0-25	5 (2.0)	2 (0.8)	3 (1.2)	0.681	2.299
26-35	70 (27.3)	15 (5.9)	55 (21.5)		
36-45	105 (41.0)	17 (6.6)	88 (34.4)		
46-55	61 (23.8)	11 (4.3)	50 (19.5)		
>56	15 (5.9)	3 (1.2)	12 (4.7)		
Gender					
Male	71 (27.7)	15 (5.9)	56 (21.9)	0.546	0.364
Female	185 (72.3)	33 (12.9)	152 (59.4)		

Place of Abode					
Urban	207 (80.9)	27 (10.6)	180 (70.3)	<0.0001	23.117
Rural	49 (19.1)	21 (8.2)	28 (10.9)		
Food preparation area					
designated kitchen	72 (28.1)	7 (2.7)	65 (25.4)	0.021	5.359
open space	184 (71.9)	41 (16.0)	143 (55.9)		
Source of drinking water					
Pipe borne	215 (84.0)	29 (11.3)	186 (72.7)	<0.0001	24.396
Borehole	41 (16.0)	19 (7.4)	22 (8.6)		
Type of toilet					
water closet	51 (19.9)	2 (0.8)	49 (19.1)	<0.0001	21.215
KVIP	202 (78.9)	43 (16.8)	159 (62.1)		
Pit latrine	3 (1.2)	3 (1.2)	0 (0.0)		
Diarrhoeal status					
Diarrheic	28 (10.9)	21 (8.2)	7 (2.7)	<0.0001	65.296
nondiarrhoeic	228 (89.1)	27 (10.5)	201 (78.5)		
Dewormer knowledge					
Yes	115 (44.9)	11 (4.3)	104 (40.6)	0.001	11.562
No	141 (55.1)	37 (14.5)	104 (40.6)		
CD_4 counts (cells/μL)					
>500	95 (37.1)	8 (3.1)	87 (34.0)	<0.0001	58.234
200-500	128 (50.0)	18 (7.0)	110 (43.0)		
<200	33 (12.9)	22 (8.6)	11 (4.3)		

Table 4.2 Prevalence of bacterial isolate in HIV positive HAART naïve patients

		bacterial infection		
	Total	Infected	Non-infected	
	N=256	N=34 (13.3%)	N=222 (86.7%)	χ^2 value
Age range (years)				
0-25	5 (2.0)	0 (0.0)	5 (2.0)	1.512
26-35	70 (27.3)	10 (3.9)	60 (23.4)	
36-45	105 (41.0)	14 (5.5)	91 (35.5)	
46-55	61 (23.8)	9 (3.5)	52 (20.3)	
>56	15 (5.9)	1 (0.4)	14 (5.5)	
Gender* **				
Male	71 (27.7)	2 (0.8)	69 (27.0)	9.341
Female	185 (72.3)	32 (12.5)	153 (59.8)	
Type of toilet				
water closet	51 (19.9)	3 (1.2)	48 (18.8)	3.904
KVIP	202 (78.9)	30 (11.7)	172 (67.2)	
Pit latrine	3 (1.2)	1 (0.4)	2 (0.8)	

Values are in frequency (percentage). *** Shows statistical significance compared to control (p<0.05)

Prevalence of bacterial infection in HIV positive subjects are shown in Table 2. The highest prevalence of infection was among age group 36-45 (5.5%) followed by 26-35 (3.9%). A significant (p<0.05) and higher proportion of females (12.5%) than males (0.8%) had bacterial infections. Most of the infections were from KVIP toilet facility (11.7%). The overall prevalence of bacterial infection was 34 (13.3%). **(Table 4.2).**

Distribution of Intestinal Parasites in the HIV seropositive subjects

Figure 4.1 shows the distribution of intestinal parasite infection among HIV seropositive subjects. The most common intestinal parasites were *Giardia lamblia* 19 (39.6%) and *Entamoeba histolytica* 11 (22.9%). Other parasites detected in this study were *Ascaris lumbricoides* 3 *(6.3%), Strongyloides stercoralis* 2 *(4.2%), Taenia spp* 2 *(4.2%), Cryptosporidium parvum* 1 *(2.1%), Trichuris trichiura* 1 *(2.1%), Isospora belli* 1 *(2.1%)* and

8 (16.7%) for those with mixed infection. The most prevalent protozoan parasite was *Giardia lamblia* and the most prevalent *helminth* was *Ascaris lumbricoides.* **(Figure 4.1).**

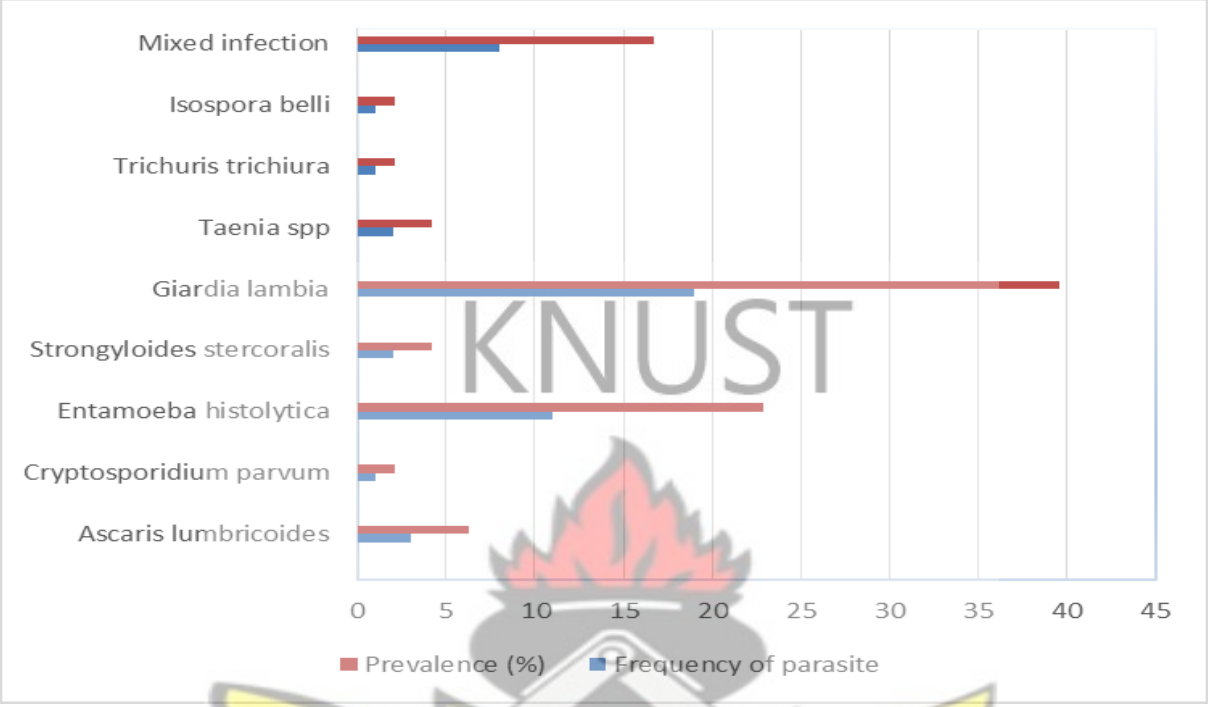


Figure 4.1: Prevalence and distribution of intestinal parasites among HIV positive HAART naïve patients

Prevalence of bacteria infections in HIV positive HAART naïve participants

Prevalence of bacterial infections among HIV positive HAART naïve participants are shown in Figure 4.2. Bacteria and yeast cells were isolated from 34 samples. *Escherichia coli* was the most frequently isolated pathogen (29.4%) followed by *Staphylococcus aureus* (20.6%) and *Candida albicans* (20.6%). A prevalence of 8.8% was recorded for *Klebsiella pneumonia*, *Proteus vulgaris* and *Salmonella typhi* isolates while *Shigella spp* were the least isolated pathogen (2.9%). **(Figure 4.2).**

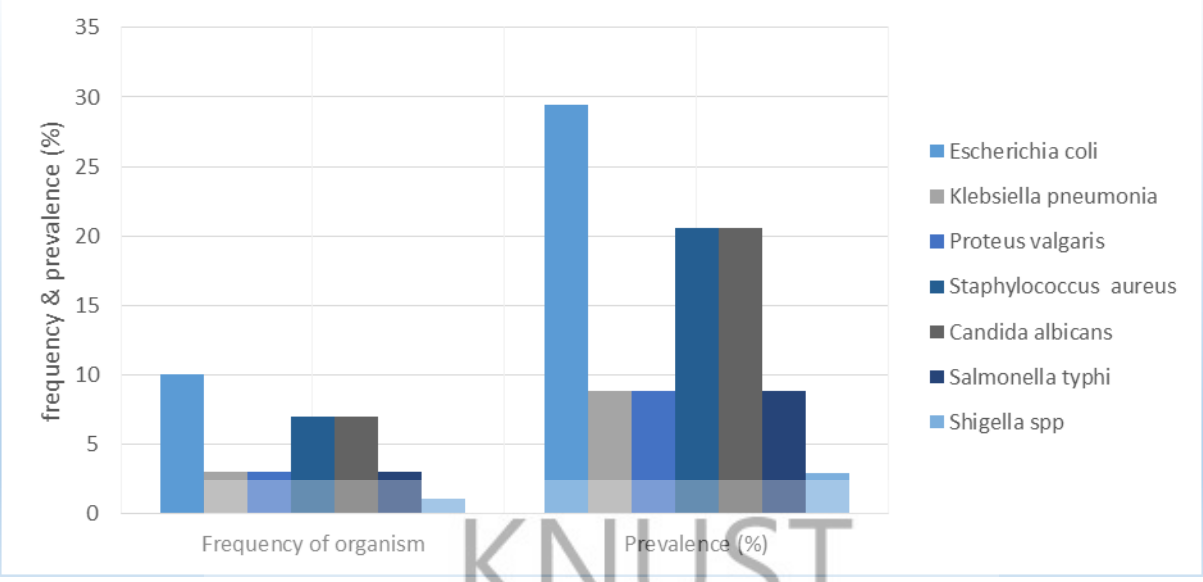


Figure 4.2. Prevalence of bacterial infections among HIV positive HAART naïve participants

Distribution of CD4 counts among age groups and gender

In relation to CD4 severity, subjects with age group 36-45 had highest prevalence and most of them had CD4 count <200 (48.5%). More females than male recorded high proportion of CD4 count >500 (80.0%) than those with <200 (69.2%). Participants with CD4 counts <200 cells/μL had higher and significant ($p<0.0001$) incidence of UTI infection (54.5%) than those with 200-500 cells/μL (8.6%). Twelve (36.4%) subjects with IPI coexisting UTI had CD4 counts <200 cells/μL. Two (1.6%) subjects with IPI coexisting UTI had CD4 counts between 200-500 cells/μL. A significant and higher proportion of rural (54.5%) than urban settlers (45.5%) had CD4 counts <200 cells/μL ($p<0.0001$). HIV positive participants in this study recorded an overall prevalence of 5.5% of GPI coexisting with UTI **(Table 4.3).**

Table 4.3 Demographic and clinical characteristic in relation to the severity of CD4+ count in HIV positive HAART naïve patients

Variable	CD_4 count (cells/μL)			
	>500 N=95	200-500 N=128	<200 N=33	p-value (χ^2 value)
Age (Mean ±SD)	40.15 ± 9.01	40.92±9.30	42.06±6.99	0.559
Age range (years)				
0-25	3 (3.2)	2 (1.6)	0 (0.0)	0.533 (7.031)
26-35	26 (27.4)	37 (28.9)	7 (21.2)	
36-45	41 (43.2)	48 (37.5)	16 (48.5)	
46-55	18 (18.9)	33 (25.8)	10 (30.3)	
>56	7 (7.4)	8 (6.2)	0 (0.0)	
Gender				
Male	19 (20.0)	42 (32.8)	10 (30.3)	0.101 (4.591)
Female	76 (80.0)	86 (67.2)	23 (69.7)	
Urinary tract infection				
Uninfected	90 (94.7)	117 (91.4)	15 (45.5)	<0.0001 (56.532)
Infected	5 (5.3)	11 (8.6)	18 (54.5)	
IPI coexisting UTI				
Uninfected	95 (100.0)	126 (98.4)	21 (63.6)	<0.0001(70.20)
Infected (5.5% of population)	0 (0.0)	2 (1.6)	12 (36.4)	
Place of abode				
Urban	88 (92.6)	104 (81.2)	15 (45.5)	<0.0001 (35.246)
Rural	7 (7.4)	24 (18.8)	18 (54.5)	

Values are in frequency (percentage); Intestinal parasite infection (IPI); χ2: chi-square

Table 4.4 Levels of CD4+ count, effect of Dewormer knowledge and diarrhoeal

	Without parasite infection N=208	*A. lumbricoides* N=3	*E. histolytica* N=11	*S. stercoralis* N=2	*G. lamblia* N=19	*Taenia spp.* N=2	Mixed parasite infection N=8
CD4 count (cells/μL)							
median (95% CI of median)	456.5 (456.1-514.6)	483 (180-593)	304 (250.9-507.1)	206 (108-304)	327*** (245-405.7)	94.5 (85-104.0)	85*** (54.7-121.3)
Dewormer knowledge ***n (%)***							
yes	104 (50.0)	0 (0.0)	4 (36.4)	1 (50.0)	5 (26.3)	0 (0.0)	1 (12.5)
no	104 (50.0)	3 (100.0)	7 (63.4)	1 (50.0)	14 (73.7)	2 (100.0)	7 (87.5)
Diarrhoeal status* **							
diarrheic	7 (3.4)	1 (33.3)	4 (36.4)	0(0.0)	13 (68.4)	0(0.0)	7 (87.5)
nondiarrheic	201 (96.6)	2 (66.7)	7 (63.6)	2 (100.0)	6 (31.6)	2 (100.0)	1 (12.5)

status in HIV-infected individuals, stratified by Intestinal parasitic infection ***N=Frequency; Kruskal Wallis test coupled with Dunnet multiple comparison test was applied in paired comparism between group and the group without parasite infection was considered as control. *** Shows statistical significance compared to control (p<0.05).***

In relation to diarrhoea, *Giardia lamblia* had the highest prevalence (68.4%), followed by *Entamoeba histolytica* (36.4%), and *A. lumbricoides* (33.3%) in that order. A higher and statistically non-significant (p>0.05) median CD_4 count 483 (95% CI 180-593) was observed in participants with A. *lumbricoides* infection than in non-parasitic infection 456.5 (95% CI 456.1-514.6). Meanwhile the median CD_4 count revealed among *G. lamblia* infected patients 327(95% CI 245-405.7) was statistically significant compared to those without parasitic infection **(Table 4.4).**

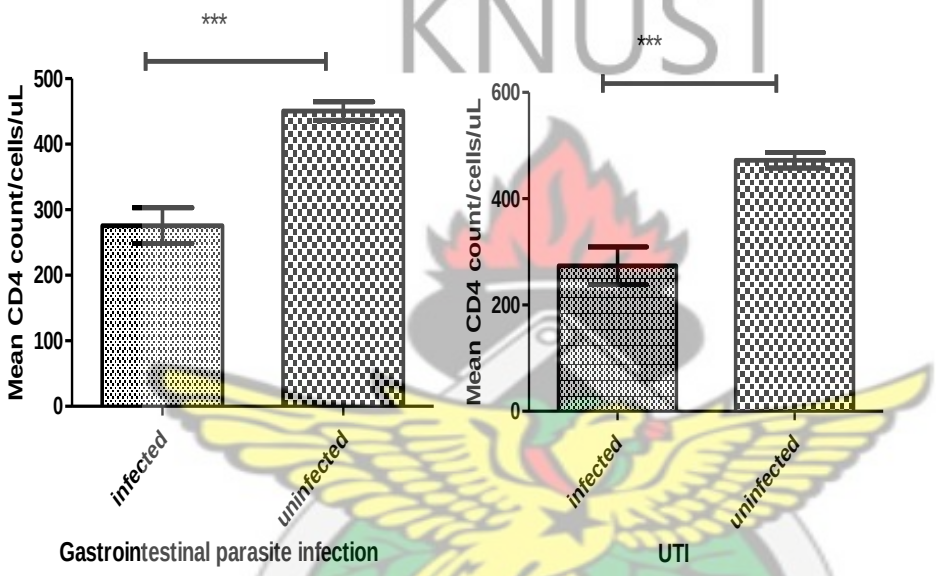


Figure 4.3. Mean CD4 count of intestinal parasite and urinary tract infection in HIV seropositive respondents.

Mean CD4 count was lower (275.8 ± 18.0) and statistically significant (p<0.0001) among participants with intestinal parasite infections than those without the infections (485.3 ± 21.5).

Similarly, UTI infected participants recorded a lower level of CD4 (273.2 ± 20.1) than those without the infections (472.5 ± 14.4) (p<0.0001).

Chapter 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATION

5.1 DISCUSSION

Boaitey *et al.*, (2012) in an unpublished paper at the Komfo Anokye Teaching Hospital (KATH) in Kumasi documented that, Gastrointestinal Parasitic Infections (GPI) and Urinary Tract Infections (UTI) contribute to the progression of HIV infection, by further disturbing the immune system. This has raised a public health burden especially in developing countries resulting in morbidity and mortality effect of coexisting infection. We estimated the prevalence of GPI and UTI among HIV seropositive HAART naïve patients at the Bomso Specialist Hospital, Kumasi, Ghana.

The overall prevalence of GPI among HIV seropositive HAART naïve patients was 18.8% which is similar to Akinbo *et al.,* (2011) who reported a prevalence of 18.0% and 19.0% respectively. This prevalence though lower compared to previous studies (Berenji *et al.*, 2010; Missaye *et al.*, 2013) is higher than recent work done by Nkenfou *et al.,* (2013). The discrepancies in results could be due to difference in sample size coupled with difference in HIV positive individual immunity levels, and geographical location as well as difference in the endemicity of parasites. Our data showed that factors associated with intestinal parasitosis were socio-economic activities and environmental sanitation ($p<0.05$). The most common intestinal protozoan parasites were *G. lamblia* and *Entamoeba histolytica* (**Figure 4.1**). A Study conducted by Teklemariam *et al.*, (2013) in Ethiopia observed that *Entamoeba histolytica* was the most prevalent intestinal parasite which is similar to the findings in this present study. The predominance of *Giardia lamblia* in this study is in agreement with the findings of Feitosa *et al.*, (2001). However, the pattern is different from other studies (Awole *et al.*, 2003; Al-Megrin, 2010; Akinbo *et al.*, 2011). Though the rate of a specific parasite may vary from country to country, geographical location and even from different regions in the same country *G. lamblia* among other parasite infection have been associated with immunological dysfunction and not only HIV infection (Feitosa *et al.*, 2001). Prevalence of GPI among female (12.9%) was higher compared to male (5.9%). This finding is consistent with several studies (Abaver *et al.*, 2012; Inabo *et al.*, 2012; Nkenfou *et al.*, 2013) but not others (Akinbo *et al.*, 2011; Tian *et*

al., 2012) who reported high prevalence in male than in female counterparts. High prevalence in females is due to the fact that more females were recruited than males in this study and who might have harboured more infections. It was found that GPI was prevalent in age group between (26-35) and (36-45), and these age categories are mostly at risk and affected by HIV (Ibrahim *et al.*, 2007). There was no statistical significant association between respondent's age and gender with parasitic infection (P>0.05). This could be explained that all participants had the HIV infection, hence the reduced immunity which makes them susceptible to intestinal parasitic infection (Ibrahim *et al.*, 2007). GPI was more prevalent among poor urban dwellers in this study which is not in agreement with previous studies (Rukmanee *et al.*, 2010; Kipyegen *et al.*, 2013). It has been shown that treated water could still harbour parasites, especially cysts of *G. lamblia* which is resistant to standard water treatment (Azim *et al.*, 2008). This has probably accounted for the high prevalence of infection among respondents who drink pipe borne water in our study. Rivers, lakes and ponds are the sources of these pipe borne water, which contain a vast variety of microbial and chemical contaminants (Rukmanee *et al.*, 2010). Ineffective treatment such as without filtration before being channelled for household use could have also accounted for the high prevalence. Sources of drinking water was associated with GPI (P<0.05). Previous studies have reviewed that increased risk of intestinal parasitic infections are associated with CD4 count less than <200 cells/μL. This report is consistent with our study where high and significant (p<0.05) prevalence of GPI **(Table 4.1)** and UTI **(Table 4.3)** was associated with CD4 count of <200 cells/μL. This study seems to report for the first time an association between GIs, UTI and the severity of CD4 count in Ghana.

Though most urinary tract infected respondents used KVIP facility, the type of toilet facility did not have any significant association with UTI (P>0.05) **(Table 4.3).** This means that the UTI was not contracted from the toilet facilities, but the immune suppression of the HIV patients could have caused opportunistic parasites from the gastrointestinal tract to evoke this infection. The highest bacteria isolate was *Escherichia coli* (29.4%) followed by Staphylococcus *aureus* (20.6%) **(Figure 4.2).** This does not agree with what Omoregie and Eghafona, (2008) found in Benin where *Staphylococcus aureus* (27.2%) was the most prevalent urine isolate. However, previous studies (Jombo *et al.*, 2004; Samuel *et al.*, 2012) elsewhere have shown

Escherichia coli to be the most frequently isolated bacteria. This indicates that this bacterium is an opportunistic pathogen, causing infection in those in a weak immunological condition. *Escherichia coli* is known to cause 85% of community acquired UTI infection.

In this study the most prevalent causative parasite for diarrhoeal symptoms was *Giardia lamblia* followed by *E. histolytica***. (Table 4.4).** This agrees with findings of Alakpa and Fagbenro-Beyioku, (2002) but not with Oguntibeju, (2006). *Giardia lamblia* among other intestinal parasite can last for months in HIV and AIDS patients causing severe dehydration incidence. Diarrhoea was significantly associated with GIT infection ($p<0.05$). Administering metronidazole and mebendazole to protozoan and helminth infected HIV patients respectively have shown to reduce diarrhoea and GPI risk (Kipyegen *et al.*, 2013).

5.2 CONCLUSION

Prevalence of GPI and UTI was high in this study and thus implies that these infections are a major public health burden to HIV seropositive patients at the Bomso Specialist Hospital. Female than male, aged group 36-45 years, urban dwellers, drinking from unfiltered pipe borne water and CD4 count of <200 cells/μL are significant risk factors for these infections. *Giardia lambia* and *Entamoeba histolytica* were mostly associated with GPI while *Escherichia coli* and *Staphylococcus aureus* were the most prevalent UTI causative organisms among HIV positive HAART naïve subjects.

5.3 RECOMMENDATION

Previous work (Boaitey *et al.*, 2012) in Ghana at the Komfo Anokye teaching hospital, Kumasi, reported a prevalence GPI which is approximately equal to our study. This signifies that the campaign on the awareness of intestinal parasitic infections among HIV seropositive has not been effective and that the infection is still on the increase. Therefore, HIV seropositive patients aside antiretroviral therapy (ART) must also be examined for intestinal parasite and urinary tract infection.

Further studies must look at the antimicrobial susceptibility pattern of these bacterial isolates and should be based on local experience of sensitivity and resistance patterns since the sensitivity varies over time at different geographical locations. Intestinal parasites like *Cryptosporidium parvum*, *A. lumbricoides* and *Taenia species* have been associated with anaemia. Future studies should look at the anaemia intestinal causative parasites in HIV seropositive patients. Our study revealed 5.5% prevalence of mixed infections (GPI coexisting UTI infections) among HIV seropositive patients. A study on the prevalence of GPI coexisting UTI infection needs to be elucidated.

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APPENDIX

PREPARATION OF REAGENTS

Physiological Saline 8.5g/L (0.85% w/v)

Sodium Chloride – 8.5g

Distilled water – 1 Litre

1. Sodium Chloride was weighed and transferred into a leak-proof bottle premarked to hold 1 litre.
2. Distilled water was added up to the half litre mark and mixed to dissolve the salt. Then the distilled water was added up to the 1litre mark.
3. The bottle was labelled and stored at room temperature.

Formol Saline 10%

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

Sodium chloride …….. 8.5g

Distilled water ……. 100ml

To make 500ml of 10% v/v Formol Saline:

Physiological saline …… 450ml

Formaldehyde solution, concentrated ……... 50ml

1. The physiological saline was measured and transferred into a leak-proof bottle.
2. The formaldehyde solution was measured and added to the saline and well mixed.
3. The bottle was labelled and stored at room temperature in a safe place.

Acid-Alcohol 3% v/v

This is a 3% v/v of hydrochloric acid in 70% v/v alcohol. To make 1Litre

Ethanol absolute - 680ml

Distilled water - 290ml

Hydrochloric acid – 30ml

1. A 1L volumetric flask was filled up to the 680mls mark with absolute ethanol and distilled water added up to the 970mls mark.
2. 30mls of the concentrated hydrochloric acid was added to the 1L mark.
3. The solution was transferred into a clean bottle and mixed.
4. The bottle was labelled and marked flammable.

Acetone-alcohol decolourizer

To make 1L

Acetone – 500mls

Ethanol – 475mls

Distilled water – 25mls

1. The absolute ethanol was measured and transferred into a clean 1L bottle. The distilled water was then added.
2. Acetone was measured and added immediately to the alcohol solution and mixed.
3. The bottle was labelled and marked Highly flammable and stored at room temperature.

Carbon Fuschin Stain

To make about 115mls

Basic Fuschin – 10g

Ethanol -100ml

Phenol – 50g

Distilled water – 1L

1. Basic fuschin was weighed on a clean piece of paper and transferred into a 2 litre round bottom flask.
2. Ethanol was measured and added at intervals whiles mixing until the basic fuschin was completely dissolved.
3. Phenol was continuously weighed in a beaker. Water was measured and some added into the beaker enough to dissolve the phenol.
4. The remainder o the water was added and mixed.
5. The bottle was labelled and stored at room temperature.

Crystal Violet (Gram Stain)

To make 1Litre

Crystal violet – 20g

Ethanol (absolute) – 95mls

Distilled water – up to 1itre

1. Crystal violet was weighed in a clean piece of paper and transferred into a brown bottle premarked to hold 1 litre.
2. The absolute ethanol was measured and added to the crystal violet. The bottle was shaken to dissolve the stain.

3. The bottle was labelled and stored at room temperature.

Eosin, 5g/L (0.5% w/v) for Faecal Preparation

To make 100ml

Eosin powder – 0.5g

Distilled water – 100mls

1. Eosin powder was weighed on a clean piece of paper and transformed into a brown bottle premarked 100ml.
2. 100mls of distilled water was added and mixed to dissolve the stain.
3. The bottle was labelled and stored at room temperature.

Lugol's Iodine Solution

To make 1L

Potassium iodide – 20g

Iodine – 10g

Distilled water up to 1L

1. The potassium iodide was weighed transferred into a clean brown bottle premarked to hold 1 litre.
2. About 250ml s of distilled water was added and mixed until the potassium iodide crystals were dissolved completely.
3. The iodine was weighed and added to potassium iodide solution and mixed to dissolve.
4. The distilled water was then added and made up to the 1 litre mark and mixed.
5. The bottle was labelled and stirred at room temperature.

Neutral Red 1g/L (0.1% w/v)

To make 1 litre

Neutral red – 1g

Distilled water – 1 litre

1. Neutral red was weighed on a clean piece of paper and transferred into a bottle of 1 litre capacity.
2. About 250mls of distilled water was added and mixed to dissolve the stain dye completely.
3. The remainder of the water was added and mixed.
4. The bottle was labelled and stored at room temperature.

Loeffler (alkaline) methylene blue

To make about 130mls

Methylene blue – approximately 0.5g

Ethanol (absolute) – 30.0 mls

20% Potassium hydroxide – 0.1ml

Distilled water – 100mls

1. The methylene blue powder was weighed on a clean piece of (pre-weighed) paper and dissolved in about 30mls of distilled water.
2. The stain was transferred into a clean brown bottle.
3. The alcohol, potassium hydroxide solution and the rest of the distilled water was added and mixed.
4. The bottle was labelled and stored in a dark place at room temperature.

EQUIPMENTS AND REAGENTS

1. Binocular light microscope
2. Normal saline (0.85%)
3. Slide file
4. Slides
5. Marker
6. Stool specimen containers
7. Giemsa stain
8. Modified Field Stain A
9. Modified Field Stain B
10. Carbol Fuchsin
11. 3% Acid Alcohol
12. 0.3% Methylene blue
13. Oral Quick HIV 1&2 rapid test kit
14. First Response HIV 1&2 rapid test kit
15. CD4 reagent
16. CD4 control beads
17. FacsFlow
18. Cell clean
19. Distilled water
20. Electronic pipette

21. Pipette tips
22. Stop watch
23. BD EDTA vacutainer tubes
24. BD vacutainer needles
25. FacsCount Machine
26. Vortex mixer
27. Coring station
28. Methylated spirit
29. Examination gloves
30. 10% formol saline
31. Iodine solution
32. Methanol