KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

INTESTINAL PARASITIC INFECTION IN HIV- INFECTED PATIENTS AT DIFFERENT CD4 T-CELL COUNTS IN AN AFRICAN RURAL AND PERIURBAN SETTING

BY:

ARYEE ERIC NII OKAI

AUGUST, 2012

INTESTINAL PARASITIC INFECTION IN HIV- INFECTED PATIENTS AT DIFFERENT CD4 T-CELL COUNTS IN AN AFRICAN RURAL AND PERIURBAN SETTING

By

Eric Nii Okai Aryee (BSc. Medical Laboratory Technology (Hons.)

A Thesis submitted to the Department of Clinical Microbiology, Kwame Nkrumah University of Science and Technology in partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY

School of Medical Sciences, College of Health Sciences

AUGUST, 2012

DECLARATION

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

Eric Nii Okai Aryee (20129229) …….. ……

Signature Date

Certified by:

Dr. S.C.K. Tay ……. …….

Signature Date

Certified by:

Prof. E.H Frimpong …….. …….

Signature Date

ACKNOWLEDGEMENTS

I am very grateful to God for his strength, grace and favour that has brought me this far.

My sincere appreciation goes to my wife Francisca Aryee for her immense support and encouragement towards my education.

I am also grateful to my supervisor Dr. S.C.K Tay for taking some time off his busy schedule to supervise this work and for his immense contribution, support and encouragements.

My sincere gratitude goes to The Medical Superintendent of Atwima Mponua District Hospital, Dr. Yusuf Adamu Al-Hassan, the Nurses in the VCT clinic at St. Patrick's Hospital, Offinso, all in the Ashanti Region.

Special thanks also go to Mr. Dennis Yar, Community Health, KNUST and Dr. Richard Ephraim Dadzie of the Department of Laboratory Technology, University of Cape Coast.

May God bless you all.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background: Intestinal parasites especially coccidian parasites are related to gastrointestinal symptoms causing severe diarrhoea in HIV/AIDS patients. These parasitic infections have further complicated the problem of morbidity and mortality in HIV/AIDS patients especially in the sub Saharan Africa. Hence, this study investigated the occurrence of intestinal parasites in HIV/AIDS at different CD4 T-cell counts.

Method: A cross sectional study was conducted on six hundred and seventy two (672) participants aged from 8 to 72 years of both sexes from April to July, 2011. Examination of stool by wet mount, formol-ether concentration including staining techniques; Field's stain, Modified Field's stain, and modified Ziehl Neelsen staining procedures were performed. Immunophenotyping was employed for CD4 T-cell counts determination.

Results: The overall total prevalence of intestinal parasitic infections among the study participants was 19.3% with a significant difference ($p<0.001$) between HIV positive and negative participants giving a prevalence of 25.2% and 13.3% respectively. Coccidian parasites (*Isospora belli* ($p<0.001$), *Cryptosporidium* ($p=0.032$)) and the helminth *Strongyloides stercoralis* ($p<0.001$) infections were exclusive to HIV positive participants. The prevalence of *Giardia lamblia* was common among both study groups having prevalence of 11.4% and 11.8% in HIV positive and negative participants ($p=0.905$) respectively reaffirming the unopportunistic nature of the parasite. Infections with *Cryptosporidium* was common with participants in rural dwellings ($p=0.039$). *I. belli* ($p<0.001$), *Cryptosporidium* ($p<0.05$), *Giardia lamblia* ($p<0.001$) and *Strongyloides stercoralis* ($p<0.05$) were mostly found in diarrhoea stools. *Isospora belli* and Microsporidia infections were associated with CD4 T-cell count of 200 cells/µl and below. Diarrhoea was associated with participants with CD4 T-cell count of ≤50cells/µl.

Conclusion: This finding showed that intestinal parasitic infections have a higher prevalence in HIV positive patients than HIV negative patients with coccidian parasites and *S. stercoralis* infections occurred exclusively in HIV positive patients. As HIV/AIDS disease coexists with intestinal parasitic infections in the sub-saharan region it is important to provide the necessary logistics required to diagnose important parasites to include PCR, Isoenzyme Analysis and Antigen detection which has proven to be a very effective means of diagnosing intestinal parasites.

CHAPTER ONE- INTRODUCTION

1.1 Background

Even though immunosuppressive diseases existed long before the emergence of Human Immunodeficiency Virus/Acquired Imunodeficiency Syndrome (HIV/AIDS), immunosuppression has become widespread in the last two decades because of the devastating effects of the HIV virus on the CD4 T- cell. A major health problem among HIV infected patients is superimposed infections (**Soave and Johnson, 1988; Smith *et al*., 1998**). HIV is a worldwide infection with the highest number of cases occurring in the sub-Saharan Africa (**UNAIDS/WHO, 2002**). Infected patients are usually burdened with parasitic infections, a major health problem, in this region (**Hunter *et al*., 1992; Same-Ekobo *et al*., 1997; Nworkediuko *et al*., 2002**). Among the parasitic infections are enteric parasites of helminths and protozoa. Poverty and malnutrition are some of the factors that contribute to concomitant infections of both HIV and parasitic infections in the sub region (**Assefa *et al*., 2009**). Unfortunately, available data on the prevalence of intestinal parasitic infections are mostly centred on school-aged children (**WHO, 2006**).

Diarrhoea, both acute and chronic can cause high mortality rates in HIV/AIDS patients (**Cheesbrough, 2005**). Diarrhoea affects 90% of people living with HIV/AIDS, causing significant morbidity and mortality (**Lekha *et al*., 2008**). Intestinal parasitic infections, both protozoa and helminths, are known to cause diarrhoea in patients (**Gomez *et al*., 1995**). Moreover, parasites that are opportunistic in nature have been documented as a significant cause of diarrhoea in HIV/AIDS patients (**Mohandas *et al*., 2002**).

It is known that helminths cause T- cell dysfunction, worsening the already devastated immune system of HIV patients (**Borkow and Bentwich, 2004**). Concomitant infections of HIV/AIDS and intestinal parasites among HIV/AIDS patients will lead to increasing morbidity and mortality and therefore the Millennium Development Goal that is aimed at achieving health for all by year 2015 would not be realised.

Studies conducted in most African countries and elsewhere have demonstrated the presence of intestinal parasites is the cause of severe diarrhoea in HIV/AIDS patients (**Gupta *et al.*, 2008; Assefa *et al.*, 2009; Kelly *et al.*, 2009**). The HIV/AIDS epidemic is a serious problem and has already claimed more than 25million lives, another 40 million people are living with HIV/AIDS worldwide (**UNAIDS, 2005**). The proportion of this devastation occurs in developing countries and the number of people living with HIV has been rising in every region of Africa (**Kam *et al.*, 1998**). Seventy percent (70%) of deaths from HIV occur in sub-Saharan Africa (**Nwachukwu and Okebe, 2008**) and diarrheoa is one of the commonest complaints among these patients, causing significant morbidity and mortality (**Lekha *et al.*, 2008**). Quality of life in both those receiving antiretroviral therapy (ART) and the ART naïve are compromised (**Nwachukwu and Okebe, 2008**) since diarrhoea reduces antiretroviral medicaments and nutrient absorption.

HIV/AIDS infection manifests in individuals as serious gastrointestinal symptoms at various stages of the disease (**Awadh and Anazi, 2009**) and it is known that most opportunistic infections take place when the CD4 T- cell count falls below 200cells/ul (**Assefa *et al.*, 2009**). Moreover, it has been established that 80% of T- cell population is found in the gastrointestinal tract making it an important site for HIV-induced immunodeficiency (**Douek, 2007; Brenchley**

and Douek, 2008). The devastative effect of HIV on the CD4 T- cell coupled with intestinal parasites especially diarrhoea- causing parasites such as *Cryptosporidium* will probably burden patients since conditions of diarrhoea will reduce ART and nutrient absorption. The infectious etiological agents include opportunistic and non- opportunistic agents that cause diarrhoea, a common presenting complaints in HIV infected individuals (**Smith *et al.*, 1998**). Chronic diarrhoea, defined as persistence of diarrhoea beyond four weeks (**Thomas *et al.*, 2003**) is a common symptom in HIV- infected patients in the tropics (**Modjarrad *et al.*, 2005; Sarfati *et al.*, 2006**). This may result in weight loss and wasting syndrome leading to profound morbidity and mortality.

The presence of non-opprotunistic parasites such as *Entamoeba histolytica, Giardia lamblia, Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis* and *Ancyclostoma duodenale* in developing countries infect HIV/AIDS patients (**Lucas, 1990**). Moreover, opportunistic parasites play a major role in causing chronic diarrhoea accompanied by weight loss (**Hammouda *et al.*, 1996**). Among the species of opportunistic protozoa associated with diarrhoea in HIV/AIDS patients are; *C. parvum, I. belli, Microsporidium* species, and *Cyclospora* species (**Gupta *et al.*, 2008; Assefa *et al.*, 2009; Adamu and Petros, 2009**). *Strongyloides stercoralis,* a nematode can cause diarrhoea and overwhelming infestation in patients with immunosuppressive disorders (**Gupta *et al.*, 2008**). Although many studies of diarrhoeal disease in AIDS patients living in Africa were done in the pre-Anti Retroviral Therapy (ART) era, there is little community-based information on the changes in susceptibility to intestinal infections and diarrhoea in relation to stage of HIV disease (**Kalinkovich *et al.*, 2001**).

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

1.2 Problem statement

Over 70% of the world's 40 million people living with HIV/AIDS are in Africa (**UNAIDS, 2005**). The high cost of treatment and the negative impact of HIV/AIDS have affected Africa's economic development. In Ghana the prevalence of HIV is 2.9% with the most affected age group being 20-39 years (**HIV Sentinel Survey Report, 2009**). Mortality and morbidity in this group will have an impact on labour, thereby affecting productivity. The most productive age group coincidentally is the most affected by the HIV pandemic in Ghana. Due to the endemicity of intestinal parasites among HIV infected patients in tropical Africa HIV patients are exposed to these parasitic infections (**Modjarrad *et al.*, 2005; Sarfati *et al.*, 2006**). It is therefore imperative to curb the occurrence of concomitant infections with these pathogens and appropriate measures by public health experts must be put in place to stop the spread of HIV/AIDS.

Apparently, common opportunistic pathogens such as *Cryptosporidium*, *I. belli*, *Cyclospora cayetanensis* and the helminth *S. stercoralis* constitute a major secondary aggravating factor of HIV/AIDS disease (**Blans *et al.*, 2005; Chacin-Bonilla *et al.*, 2008 Mannheimer and Soave,**

1994, Keiser and Nutman, 2004; Ignatius *et al*., 1997). These infections frequently cause severe diarrhoea which is often responsible for the gravity of the disease leading to fatal conditions (**Lekha *et al*., 2008**).

1.3 Rationale and justification

Since intestinal parasitic pathogens responsible for infection and diarrhoea in different geographic areas are not similar, laboratory diagnostic evaluations are needed to determine disease prevalence in a specific population, so that it can provide guidelines for empirical treatment for the prevention and treatment of treatable etiologic agents as well as the prevention of untreatable opportunistic parasites including generation of the necessary data for planning and evaluation of HIV/AIDS patients'care.

Furthermore, previous study in the Department of Clinical Microbiology, School of Medical Sciences in Kwame Nkrumah University of Science and Technology was general and not specific for demographic and centred only on parasites not considering CD4 T-cell count in HIV/AIDS patients. It is therefore important to conduct this study among rural and periurban HIV/AIDS patients, correlating the patterns of infections to the levels of CD4 T-cell and compare parasitic infections to HIV negative subjects (control group). Successful outcome of this study will engender appropriate care by management as a short term control procedure since there are no specific treatment and vaccine for HIV/AIDS patients but drugs are widely available for the treatment of most intestinal protozoa and helminths and moreover preventive and control measures can be put in place to prevent the occurrence of such infections among HIV/AIDS patients. Also, this research is likely to yield other therapeutic approaches which will facilitate

the management of diarrhoea and contribute to further improvement in the quality of life of HIV/AIDS patients.

1.4 General objective

The main objective of the study is to assess the prevalence and proportions of intestinal parasites in people with HIV/AIDS infection and measure their relationship with diarrhoea and CD4 T-cell count.

1.5 Specific objectives

1. Determine the prevalence of intestinal parasites among HIV positive and negative patients in a periurban and rural area.
2. Determine the proportion of the various parasitic infections in HIV positive and negative patients.
3. Determine the distribution of parasites and their correlation with CD4 T-cell count.
4. Determine parasites in diarrhoea stools of HIV negative and positive patients.

CHAPTER TWO – LITERATURE REVIEW

2.1. Human Immunodeficiency Virus

Human Immunodeficiency Virus (HIV) is a lentivirus and, like all viruses of this type, it attacks the immune system (**Harindra, 2008)**. Lentiviruses are a part of a larger group of viruses known as retroviruses (**Abass and Lichtman, 2003**). They have been found in a number of different animals, including cats, sheep, horses and cattle (**Harindra, 2008**). However as far as the origin of HIV is concerned, the most interesting lentivirus is the simian immunodeficiency virus (SIV) that affects monkeys (**Harindra, 2008**). According to the hunter theory, SIV was transferred to humans when hunters ate the flesh of infected chimpanzees or when the blood of the chimpanzees contaminated cuts or wounds of the body of the hunter (**Harindra, 2008**). The fact that each time the virus was passed from a chimpanzee to man, a slightly different strain of the virus was produced supports this theory (**Wolfe *et al.*, 2004**). Another school of thought believes that during the 19th and early 20th century, much of Africa was ruled by colonial forces and the poor conditions (e.g overcrowding and poor sanitation) and the physical demands of the inmates at the labour camps were extreme, weakening their immune system and paving the way for the SIV to become HIV (**Harindra, 2008**). This theory was first proposed by an American Specialist Jim Moore (**Harindra, 2008**).

The initial human infection occurred in Africa in the 1930s but went unnoticed in rural areas (**Chitnis *et al.*, 2000**). The migration of infected people to the cities after the 1960s brought the virus into population centres, and cultural acceptance of prostitution promoted its transmission throughout the world's population (**Hunter, 2006**). Although HIV came to light in the early 1980s, there is evidence that HIV infection was prevalent much earlier (**Harindra, 2008**).

According to Dr. Bette Korba of Los Alamos National Laboratory (**Harindra, 2008**) the first case of HIV-1 infection occurred around 1930 in West Africa (**Harindra, 2008**). The first recognized cases of AIDS occurred in the USA in 1981 (**Sharp and Hahn, 2010)**. Since and before the disease was discovered, HIV has devastated families, communities, and whole continent (**Weiss *et al*., 2001**; **UNAIDS, 2008**).

AIDS is the disease caused by infection with HIV and characterized by profound immunosuppression causing malignant tumours, wasting, and central nervous system degeneration with associated opportunistic infections (**Abass and Lichtman, 2003**). HIV infects a variety of cells of the immune system, including CD4-expressing helper T cells, macrophages, and dentritic cells (**Cunningham *et al*., 2010**). The degree of morbidity and mortality caused by HIV and the global impact of HIV infection on health care resources and economics are enormous and continue to grow (**UNAIDS, 2008; Weiss *et al*., 2001**). The disease has infected 50 to 60 million people and has caused the death of nearly 20 million adults and children (**UNAIDS, 2008; UNAIDS, 2005**). Majority of people infected with HIV are in Africa, forming about 70% of infected people in the world followed by Asia which forms 15% (**UN report on global AIDS epidemic, 2010**).

2.1.1 History of HIV

The story of human immunodeficiency virus (HIV) is perhaps the most exciting one in the medical sciences (**Harindra, 2010**). It began in June 1981 when the Centers for Disease Control and Prevention (CDC) reported five young male homosexuals from Los Angeles having HIV/AIDS with *Pneumocystis carinii* opportunistic infection (**Basavapathruni and Anderson, 2007**). Since then Acquired Immunodeficiency Syndrome (AIDS) has spread like wildfire far

and wide (**Murthy, 2008**). The pace of scientific investigations has matched that of the epidemic (**Cohen and Enserink, 2008**). Perhaps no other virus has been studied so extensively in such a short time **(Cohen and Enserink, 2008)**. Within two years of the detection of the disease the causative agent was identified, in the next year it was cultured and cloned and its nucleotide sequence was established soon after (**Gelderblom, 1997; Cohen and Enserink, 2008**).

In the late 1970s and early 1980s, an unusual number of young homosexual men, Haitians, heroin addicts, and haemophiliacs in the United States were noted to be dying of normal benign opportunistic infections (**Hunter, 2006**). Their symptoms defined a new disease, Acquired Immunodeficiency Syndrome (AIDS) **(Hunter, 2006)**. In 1999 it was estimated 15000 HIV infections occurred per day, with 95% occurring in developing countries (**UNAIDS-WHO, 1999**).

2.1.2 Molecular and Biologic Features of HIV

HIV is a member of the lentivirus family of animal retroviruses (**Weiss, 1993**). Lentiviruses, including Visna and Bovine, Feline, and Simian Immunodeficiency Viruses (SIV), are capable of long-term cytopathic effects, and they all produce slowly progressive, fatal diseases that include wasting syndrome and CNS degeneration (**Abass and Lichtman, 2003**). Two closely related types of HIV, designated HIV-1 and HIV-2, have been identified (**Gilbert *et al.*, 2003**). HIV-1 is by far the most common cause of AIDS, but HIV-2, which differs in genomic structure and antigenicity, causes a similar clinical syndrome (**Gilbert *et al.*, 2003**). An infectious HIV particle consist of two strands of RNA packed within a core of viral proteins and surrounded by a phospholipid bilayer envelope derived from the host cell membrane but including virally

encoded membrane proteins (**Kuilen *et al*, 2008**). The RNA genome of HIV is 9.2kb long and has the basic arrangement of nucleic acid sequences characteristic of all non retroviruses (**Kuilen *et al*, 2008)**. Long terminal repeats (LTRs) at each end of the genome regulate viral integration into the host genome, viral gene expression, and viral replication (**Pollard and Malim, 1998**).

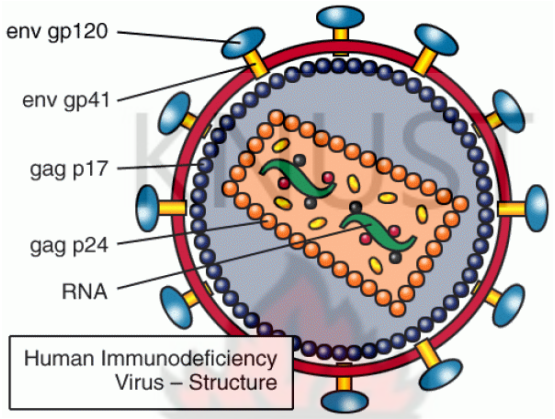

Figure 2.1: HIV structure

The *gag* sequences encode core structural proteins (**Kuilen *et al*, 2008)**. The *env* sequences encode the envelope glycoproteins gp120 and gp41, which are required for infection of cells (**Chan and Kim, 1998; Wyatt and Sodroski, 1998**). The *pol* sequences encode reverse transcriptase, integrase, and viral protease enzymes required for viral replication (**Zheng *et al*., 2005**). In addition to these typical retrovirus genes, HIV-1 also includes other regulatory genes, namely, the *tat*, *rev*, *vif*, *vpr*, and *vpu* genes, whose products regulate viral production in various ways (**Kuilen *et al*., 2008**).

HIV infection of cells begins when the envelope glycoprotein (*Env*) of a viral particle 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**).

The viral particles that initiate infection are usually in the blood, semen, or other body fluids of one individual and are introduced into another individual by sexual contact, needle stick or transplacental passage (**Donegan *et al*., 1990; Kuplan and Heimer, 1995; Varghese *et al*., 2002; Coovadia, 2004**). *Env* is a complex composed of transmembrane gp41 subunit and an external, non-covalently associated gp120 subunit. These subunits are produced by proteolytic cleavage of a gp 160 precursor (**Kuilen *et al*., 2008**). The *Env* complex is expressed as a trimetric structure of three gp120/gp41 pairs (**McGovern *et al*., 2002**). This complex mediates a multistep process of fusion of the virion envelope with the membrane of the target cell (**Kuilen *et al*., 2008**). The first step of this process is the binding of gp120 subunits to CD4 molecules, which induces a conformational change that promotes secondary gp120 binding to a chemokine co receptor (**Abass and Lichtman, 2003**). Co receptor binding induces a conformational change in gp41 that exposes a hydrophobic region, called the fusion peptide that inserts into the cell membrane and enables the viral membrane to fuse with the target cell membrane (**Abass and Lichtman, 2003**). After the virus completes its life cycle in the infected cell, free viral particles are released from one infected cell and bind to an uninfected cell, thus propagating the infection (**Abasss and Lichtman, 2003**). In addition, gp120 and gp41, which are expressed on the plasma membrane of infected cells before virus is released, can mediate cell-cell fusion with an uninfected CD4 and coreceptor-expressing cell, and HIV genomes can then be passed between the fused cells directly (**Abass and Lichtman, 2003**).

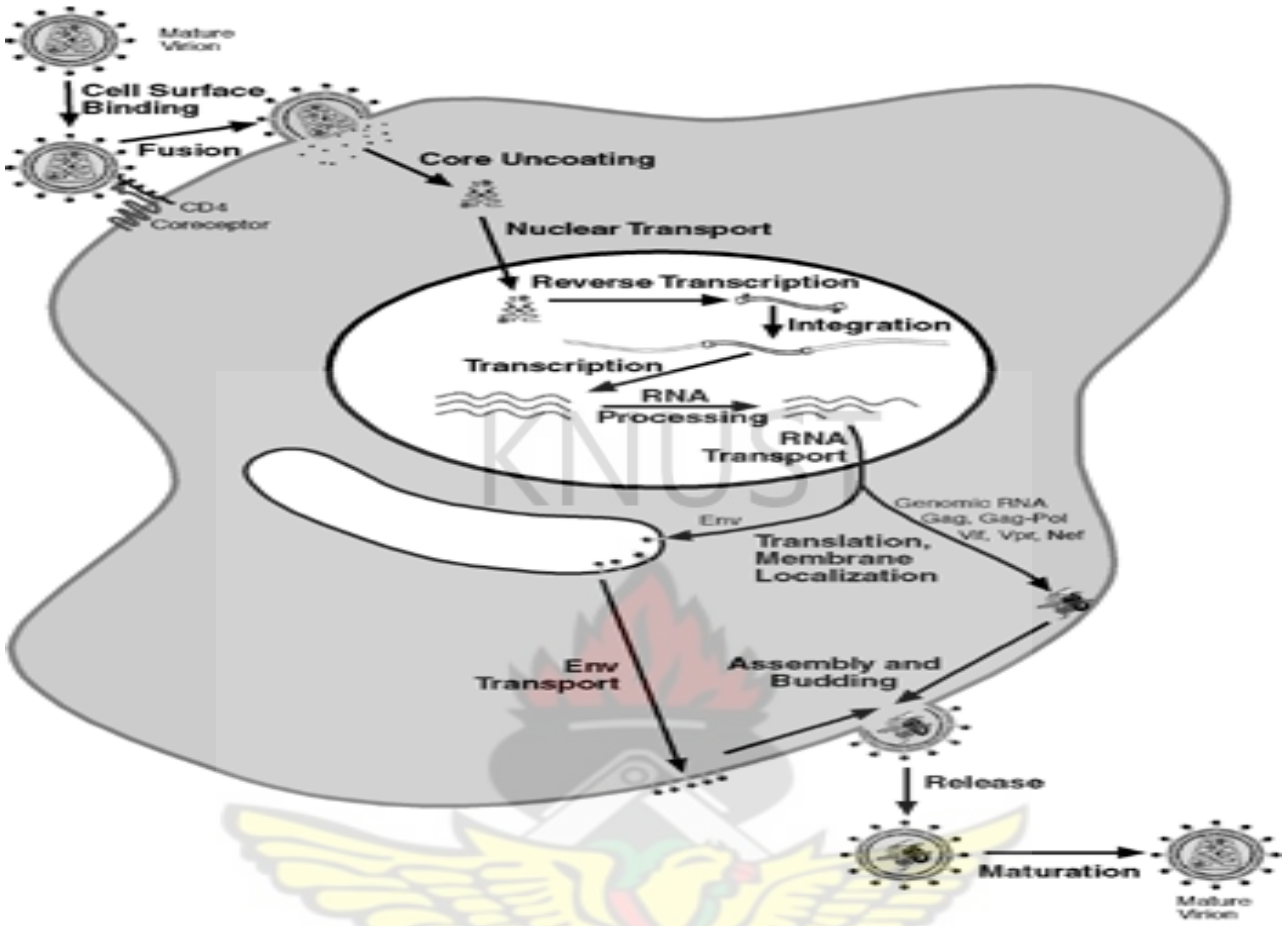

Figure 2.2: Life cycle of HIV

2.1.3 Clinical Syndromes of HIV/AIDS

AIDS is one of the most devastating epidemics ever recorded (**Greener, 2002**). Most HIV infected people will become symptomatic and the overwhelming majority of these will ultimately succumb to the disease (**Lawn, 2004**). HIV disease progresses from an asymptomatic infection to profound immunosuppression, referred to as full blown AIDS (**Buchbinder *et al.*, 1994**). The diseases related to AIDS mainly consist of opportunistic infections, cancers, and the direct effects of HIV on the nervous system (**Abass and Lichtman, 2003**). Although rare, there are cases of long term survivors (**Graber *et al.*, 2009; Blankson, 2010**). Some of these result from infection with HIV strains that lack a functional *nef* protein (**Papkalla *et al.*, 2002**).

Resistance to the virus correlates with a lack of expression of the chemokine co-receptor for the virus (**Papkalla *et al.*, 2002**).

The initial symptoms following HIV infection (2-4 weeks after infection) may resemble those of influenza or infectious mononucleosis, with 'aseptic' meningitis or a rash occurring up to 3 months after infection (**Khan and Walker, 1998**). As in mononucleosis, the symptom stem from immune responses triggered by a widespread infection of lymphoid cells (**Abass and Lichtman, 2003**). These symptoms subside spontaneously after 2 to 3 weeks and are followed by a period of asymptomtic infection or a persistent generalized lymphadenopathy that may last for several years (**Abass and Lichtman, 2003; Burton *et al.*, 2002)**. During this period, the virus is replicating in the lymph nodes (**Burton *et al.*, 2002)**.

Deterioration of the immune response is indicated by increased susceptibility to opportunistic pathogens, especially those controlled by CD4 T–cell Delayed Type Hypersensitivity Responses (DTH) (e.g. yeast, herpesvirus, or intracellular bacteria) (**Abass and Lichtman, 2003**). The onset of symptoms correlates with a reduction in the number of CD4 T cells to less than 450cells/ul and increased levels of virus and protein p24 in the blood (**Pentaleo *et al.*, 1993**). Full blown AIDS occurs when the CD4 T-cell counts are less than 200 cells/µl and involves the onset of more significant diseases, including HIV wasting syndrome (weight loss and diarrhoea for more than one month) and the occurrence of indicator diseases such as Kaposi sarcoma or specific opportunistic diseases especially *Pneumocystis carini* pneumonia, *Mycobacterium avium-intracellare* complex infection, and severe cytomegalovirus disease (**Awadh and Anazi, 2009**).

2.1.4 Management of HIV/AIDS

An extensive effort to develop antiviral drugs and vaccines effective against HIV has been initiated worldwide (**Kirk *et al.*, 2004**). There are currently five main classes of drugs, operating at different points in the HIV cycle; nucleoside analogue reverse transcriptase inhibitors, non nucleoside reverse transcriptase inhibitors, protease inhibitors, entry inhibitors and integrase inhibitors (**Harindra, 2008**).

Azidothymidine (AZT), dideoxyinosine (ddI), and dideoxycytidine (ddC) and the other nucleotide analogues are phosphorylated by cellular enzymes, inhibit the reverse transcriptase, and after incorporation into DNA cause chain termination (**Hammer *et al.*, 1994**). Non-nucleoside reverse transcriptase inhibitors (nevirapine) inhibit the enzyme by other mechanisms (**Pathel and Benfield, 1996**). Protease inhibitors block the morphogenesis of the virion by inhibiting the cleavage of the *gag* and *gag-pol* polyproteins (**Kohl *et al.*, 1998**). This prevents activation of the virion (**Kohl *et al.*, 1998**; **McQuade *et al.*, 1990**). Other anti-HIV drugs being developed include different nucleotide analogues and other inhibitors of reverse transcriptase, receptor antagonists (CD4 and gp120 analogues), inhibitors of *tat* function, glycoprotein glycosylation inhibitors, interferon and interferon inducers, and antisense DNA to essential genome sequences (**Madhu and Lalit., 1999**).

The clinical benefit of antiretroviral drugs is not well indicated by CD4 counts (unlike viral RNA load) and it is less effective in monitoring therapy (**Gupta and Gupta, 2004**). Antiretroviral therapy is indicated when the CD4 T-cell count falls below 500cells/ul (**Gupta and Gupta, 2004**). All effective forms of antiretroviral therapy to date have been associated with at least a

transient increase in either CD4 T-cell count or CD4 T-cell proportion (**Gupta and Gupta, 2004)**. Use of zidovudine in symptom free HIV infected subjects with CD4 T-cell counts below 0.5 x 10^9/litre has been reported to slow the rate of progression to AIDS or advanced AIDS related complex (**Gupta and Gupta, 2004**).

2.2. CD4 T-cell

A number of non HIV specific cellular markers, have been used for staging, monitoring progression of HIV infection and assessing response to therapy but the most commonly used cellular marker 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, and cells expressing this protein usually decline in number with progressive HIV infection (**Gupta and Gupta, 2004**). The median interval between HIV 1 infection and the development of 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 microbes has been suggested as one of the probable factors leading to AIDS progression (**Webster *et al.*, 1989**). The Centres for Disease Control (CDC), Atlanta , USA suggest a classification system using CD4 T-cell as markers of relative risk of developing HIV related Opportunistic Infections (OIs)(**WHO, 1990**) i.e. Stage I: Acute (primary) infection (seroconversion), stage II: early disease (assymptomatic) CD4 T-cell usually >500cells/ul, stage III: intermediate HIV infection (symptomatic) CD4 T-cell usually 200-500cells/ul, stage IV: late stage HIV disease (symptomatic) CD4 T-cell count is 50-

200cells/ul and stage-V: Advanced HIV disease (symptomatic) $CD4^+$ T-cell <50 cells/ul (**WHO, 1990**).

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 lymphocyte, 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 immune system (**Abass and Lichtman, 2003**).

2.3 Gastrointestinal Tract Infection

Many of the infections of the gastrointestinal tract (GI) are caused by parasites that are cosmopoloitan in distribution (**Cheesbrough, 2005**). Human intestinal parasites are either one cell organism (protozoa) or intestinal worms (helminths) that live in the small or large intestine and use the stool or blood from the intestine as its source of food (**Ryan and Ray, 2004**). Protozoa have only one cell, and can multiply inside the human body and this can allow serious infections to develop and can be directly infectious to man when they are passed in the faeces into the environment. However, helminths require a period of maturation while in the soil (ova or larvae), where they become infectious and transmission occurs when one comes into contact with infected faeces (for example, through contaminated soil, food, or water) (**Cheesbrough, 2005**).

Others such as *Taenia saginata* require the involvement of an intermediate host during their life cycle (**Robert *et al*., 2005**).

Infections of the GI tract account for a high proportion of deaths in infants where the standards of hygiene and nutrition are low (**Stoltzfus, 2003**). In the wake of HIV/AIDS, threatening diarrhoeal diseases caused by infections of the GI also exist (**DeHovitz *et al*., 1986**). Faecal-oral transmission of the pathogens is the most common mode of GI infections, whereby water, food and hands become contaminated with faecal material which then come into contact with the mouth (**Cheesbrough, 2005**). A number of GI infections can reach epidemic proportion 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**).

2.3.1. Intestinal Protozoa

Protozoa are single celled organism (**Cheesbrough, 2005**). There are four classes of Protozoa commonly found in concentrated faecal samples of infected patients. They include *Sarcodina*, *Mastigophora*, *Sporozoa*, and *Ciliophora*. Common intestinal protozoa of medical importance include the Genera *Entamoeba*, *Giardia*, *Trichomonas*, *Cryptosporidium*, *Isospora*, *Cyclospora* and *Balantidium* (**Ryan and Ray, 2004**).

2.3.1.1 *Coccidia*

Gastrointestinal (GI) opportunistic infections are commonly encountered at various stages of human immunodeficiency virus (HIV) disease (**Gupta *et al.*, 2008**). In view of the suppressive nature of the virus and direct contact with the environment, the GI tract is readily accessible and a common site for the clinical expression of HIV (**Awadh and Anazi, 2009**). Common coccidian parasites that have emerged as a result of immunosuppression due to HIV include *Isospora belli*, *Cryptosporidium spp.*, *Cyclospora cayetanenesis* (**Assefa *et al.*, 2009**).

2.3.1.1.1 Isosporiasis

Isospora belli was first recognized as a human pathogen among military personnel during World War I (**Woodcock, 1915**). *Isospora belli* is an *Apicomplexa* belonging to the class *Sporozoa* and subclass *Coccidia* and family *Eimeriidae* and it causes a self-limiting diarrhoeal illness in immunocompetent hosts (**Tier, 1974**). In individuals who are immunocompromised, it may cause chronic life-threatening diarrhoea and dehydration (**DeHovitz *et al.*, 1986**). Other species of *Isospora* are known to infect reptiles, birds and mammals (**Kirkpatrick, 1988**). Only *I.belli* is known to infect humans (**Lindsay *et al.*, 1997**).

2.3.1.1.2 Epidemiology

Isospora belli infection is distinctly rarely serious among immunocompetent individuals (**Cheesbrough, 2005**). Infection with *Isospora* is endemic in tropical regions, particularly of Central and South America, Africa, and Southeast Asia (**Chessbrough, 2005; Lindsay *et al.*, 1997**).

Prevalence of *I. belli* among HIV patients in Thailand is 6.0% and 0.02% in non- HIV patients with 86.8% of *I. belli* positive patients showing immunosuppression (**Somachai *et al.*, 2007**). Similar cross sectional studies in Venezuela showed a higher prevalence of *I. belli* (**Certad *et al.*, 2003**). Zero point two to one percent (0.2-1%) of patients with AIDS in the United States of America have *I. belli* infection (**Robert *et al.*, 2002**). Between the periods of 1985 to 1992, 1% of AIDS patients in Los Angeles were known to carry oocyst of *I. belli* in their stools (**Sorvillo *et al.*, 1995**). The infection is less common among patients on trimethoprim-sulfamethoxazole prophylaxis administration (**Sorvillo *et al.*, 1995**). In developing countries 8-40% of patients with AIDS have *I. belli* infections and occurs in up to 15% of Hiatians infected with AIDS (**Dehovitz *et al.*, 1986**). Eleven point seven percent (11.7%) out of 111 HIV positive patients in Southern India carried oocyst of *I. belli* (**Mukhopadhya *et al.*, 1999**). *Isospora belli* is frequently identified parasites in HIV infected individuals with diarrhoea in India and other parts of the world (**Prasad, 2000**).

2.3.1.1.3 Life Cycle

Isospora belli is ingested in contaminated food or water and its life cycle requires a stage outside the host (**Cheesbrough, 2005**). Oocysts liberate sporozoites (possibly in response to bile in the small intestine), which invade the enterocytes of the proximal small intestine (**Somachai *et al.*, 2007**). Here, they become trophozoites, and asexual multiplication (schizogony) produces merozoites that invade previously uninfected cells (**Rosiere *et al.*, 2003**). Shortly thereafter, a sexual multiplication cycle (sporogony) begins, generating oocysts that may pass into the environment (**Cheesbrough, 2005**). Outside the host, oocysts mature and become infectious 2-3 days later (**Morakote *et al.*, 1987**). Oocysts may persist for months in the environment (**Robert**

***et al*., 2002**). The parasite is acquired after ingestion of sporulated oocyst, which excyst in the small intestinal epithelial cells, and undergoes asexual schizogony to form merozoites (**Cheesbrough, 2005**). The merozoites may undergo further asexual replication or sexual replication, forming oocyst that are excreted in the stool, sporulated outside the body in 24 to 48hrs, and again become infectious (**Morakote *et al*., 1987; Zaman, 1968**).

Isospora belli infection usually causes a 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**). In normal adults the disease is self limiting (**Certad *et al*., 2003**). However, in patients with AIDS, and other immunodeficient states the illness is chronic and may be associated with severe dehydration and debilitating effects (**DeHovitz *et al*., 1986**). Isosporiasis is mainly found in the tropical and subtropical areas and has been associated with cholecystitis in patients with AIDS (**Debra *et al*., 1994**).

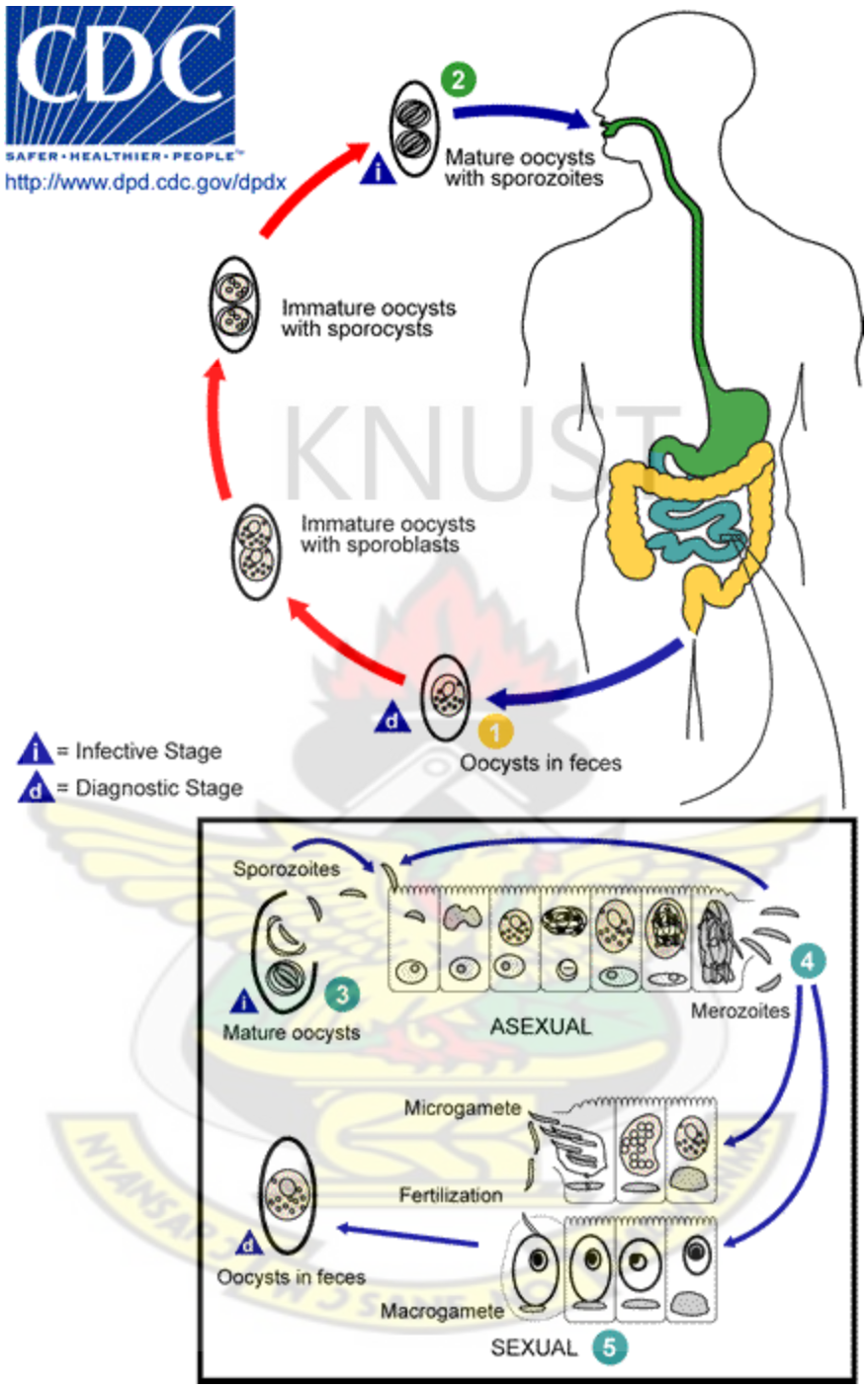

Figure 2.3 Life cycle of *Isospora belli*

Source: Centres for Disease Control and Prevention

2.3.1.1.4 Clinical Manisfestation

Typically, patients with isosporiasis present with mild crampy abdominal pain and profuse, watery, extremely foul-smelling diarrhoea (**Robet *et al.*, 2002**). Symptoms begin approximately 1 week after ingesting the oocysts and last 2-3 weeks, with gradual improvement (**Lindsay *et al.*, 1997**). Infection in people who are immunocompromised may continue indefinitely (**Certad *et al.*, 2003**). Clinical symptoms include: foul smelling flatulence, anorexia, low grade fever, and Steatorrhoea may occur (**Stark *et al.*, 2009**). Headache is a rare symptom (**Robert *et al.*, 2002**). In immunocompromised individuals with severe or long-lasting disease, dehydration may be evident (**Robert *et al.*, 2002**). Otherwise, minimal abdominal tenderness may be present (**Robert *et al.*, 2002**).

2.3.1.1.5 Laboratory Diagnosis

Stool examination for oocyst is the test of choice for isosporiasis (**Cheesbrough, 1992**). Mature oocysts measure 30 X 12 µm and have a thin translucent wall and 2 round sporocysts, each of which has 4 crescentic sporozoites (**Somachai *et al.*, 2007**). Auramine-rhodamine fluorescent, modified Kinyoun acid-fast, hematoxylin/eosin, Giemsa, and/or carbol fuchsin staining may be helpful in identifying the translucent oocysts (**Somachai *et al.*, 2007; Robert *et al.*, 2002)**. Charcot-Leyden crystals and high fat content are often observed (**Robert *et al*, 2002**). Full blood count may reveal mild peripheral eosinophilia in one half of patients (**Robert *et al.*, 2002**). Serologic testing is not presently available but detection of oocyst using PCR has been developed recently (**Ten Hove *et al.*, 2008; Robert *et al.*, 2002; Muller *et al.*, 2000**). Electron microscopy may be helpful to find the organism on colonic biopsy, but it is labour intensive and lacks specificity (**Robert *et al.*, 2002**).

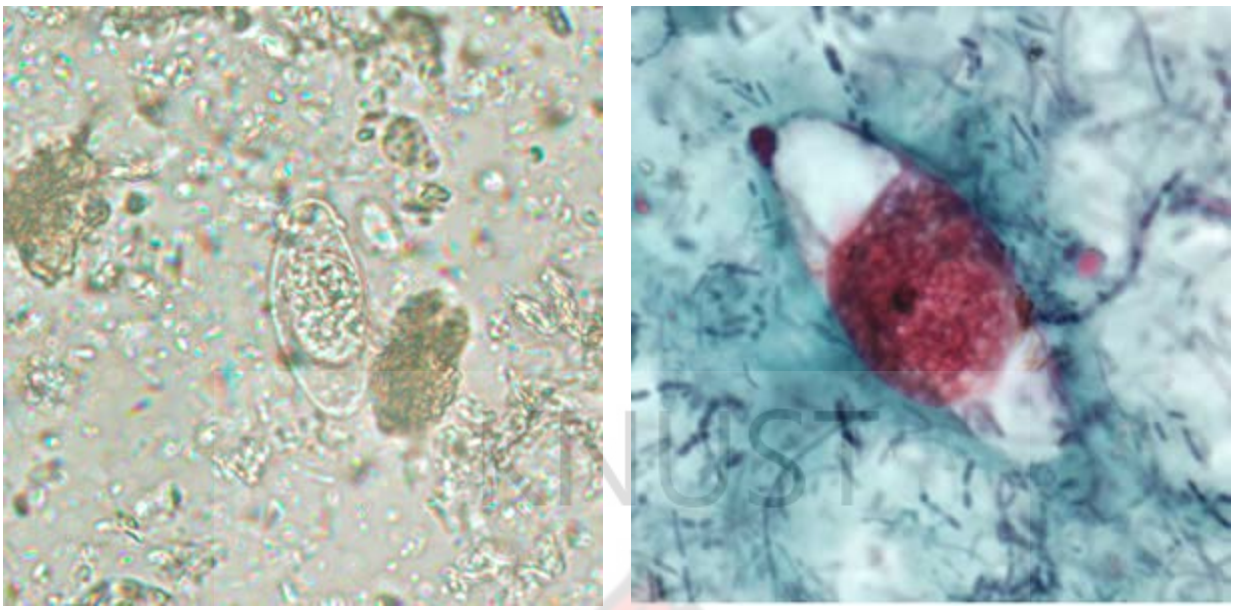

Figure 2.4: *Isospora belli* oocyst in faeces. Left: oocyst in saline preparation and right: Modified Z-N stained oocyst.

2.3.1.1.6 Treatment

Although generally infection is self-limiting, patients with isosporiasis who are treated tend to improve in 2-3 days, whereas those who are not treated remain sick considerably longer but dosage normally is for 10 days with 160 mg of Trimethoprim-sulfamethoxazole (TMP-SMZ) (**Robert *et al*.,2002**). Therapy for dehydration may be the most urgent intervention in children and severely immunocompromised patients (**Ryan and Ray, 2004; Washington *et al*., 2006**). Immunocompetent hosts generally respond very rapidly to therapy, with symptomatic improvement within 7 days (**Washington *et al*., 2002**). The immunocompromised host also responds well, although less rapidly taking as long as 4 weeks (**Robert *et al*., 2002**).

Trimethoprim-sulfamethoxazole (TMP-SMZ) is the drug of choice because it is the best studied and most readily available agent (**Ryan and Ray, 2004**). Many patients with AIDS are already taking this agent as prophylaxis for *Pneumocystis* infection (**Ryan and Ray, 2004**). An

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

2.3.1.2.1. *Cyclospora cayetanensis*

Cyclospora cayetanensis is a coccidian parasite that infects the GI tract of both immunocompetent and immunocompromised hosts (**Cheesbrough, 2005**). The organism now included in the genus *Cyclospora* was originally thought to be cyanobacteria or coccidian-like body (**Ashford *et al.*, 1993**). This organism was first described in human faeces in 1979 (**Ashford, 1979**). Since the advent of the acquired immunodeficiency syndrome (AIDS) epidemic, *C. cayetanensis* has been increasingly recognized as an enteric pathogen (**Mannheimer and Soave, 1994**). The organism is a protozoan parasite of the small intestine causing flu like symptoms accompanied by nausea, vomiting and explosive diarrhoea normally lasting for 3 weeks in immunocompetent people (**Washington *et al.*, 2006)**. In immunoincompetent people, particularly those with AIDS who are commonly infected, diarrhoea may be prolonged lasting 4-6 weeks stimulating tropical sprue and causing biliary disease sometimes (**Washington *et al.*, 2006**). Transmission is by ingestion of oocyst in contaminated food or water (**Cheesbrough, 2005**). First described as the cause of gastrointestinal illness in 1979, has since gained recognition internationally especially among travelers (**Blans *et al.*, 2005**).

2.3.1.2.2 Epidemiology

Cyclosporiasis is endemic in Haiti, Nepal, and Peru, with a strong seasonal predominance during rainy spring and summer months (**Hoge *et al.*, 1993; Madico *et al.*, 1997; Lopez *et al.*, 2003**). Cyclosporiasis has also been reported in travelers returning from Mexico, Southeast Asia, Puerto

Rico, Indonesia (**Blans *et al,* 2005**). *C. cayetanensis* has been recognized as a waterbourne pathogen and report suggests that it is associated with waterbourne outbreaks worldwide (**Ortega *et al.,* 1993**).

2.3.1.2.3 Life Cycle

Cyclospora cayetanensis is like the majority of eimerid coccidians. The end result of infection in its host is the production of an oocyst that undergoes sporogony outside the host's body (**Sterling and Ortega, 2004**). Under favourable laboratory conditions, the parasite completes sporogony in 8-14 days (**Ortega *et al.,* 1993**). The oocyst shed in faeces of infected person must mature (sporulate) outside the host, within 3-5 days in the environment and seasonality patterns suggests that oocyst may survive for extended periods in the environment (**Sterling and Ortega, 2004**). Direct person- to- person (faecal oral) transmission of *Cyclospora* is most unlikely (**Forsythe, 2010**). However, indirect transmission can occur if an infected person contaminates the environment and oocysts have sufficient time thereafter, under favourable conditions, to become infective (**Forsythe, 2010**). People living in the tropics and subtropics are at increased risk of infection because of the endemicity of cyclosporiasis in some developing countries (**Soave *et al.,* 1998**). The disease is also taught to be at its peak in certain seasons but is not well understood (**Sterling and Ortega, 2004**). Humans are the only known host of *C.cayetanenesis* (**Eberhard *et al.,* 2000**). Ingestion of sporulated and infectious oocysts leads to parasite colonization of the jejunum by sporozoites (**Ortega *et al.,* 1997**). The incubation period between acquisition of infection and the onset of symptoms averages approximately 1 week (**Soave, 1996**) and the oocyts is shed in the faeces for more than 3 weeks (**Forsythe, 2010**). It has been established that the presence of distinctive intracellular asexual merozoites and sexual

gametocytes stages, are requisite forms for completion of the life cycle within a single host (**Sterling and Ortega, 2004**).

Cyclospora species are variably acid-fast (**Garcia *et al.*, 1983**), round-to-ovoid organisms that measure 8-10 µm in diameter (**Cheesbrough, 2005**). *Cyclospora* species exogenously sporulate and have 2 sporocysts per oocyst (**Levine, 1973**). The disease manifests as protracted and relapsing enteritis in patients who are immunosupressed (**Cheesbrough, 2005**). *Cyclospora* species are characterized by an anterior polar complex that allows penetration into host cells, but the life cycle of the parasite and the mechanisms by which it interacts with human host target cells to cause disease are poorly understood (**Forsythe, 2010**).

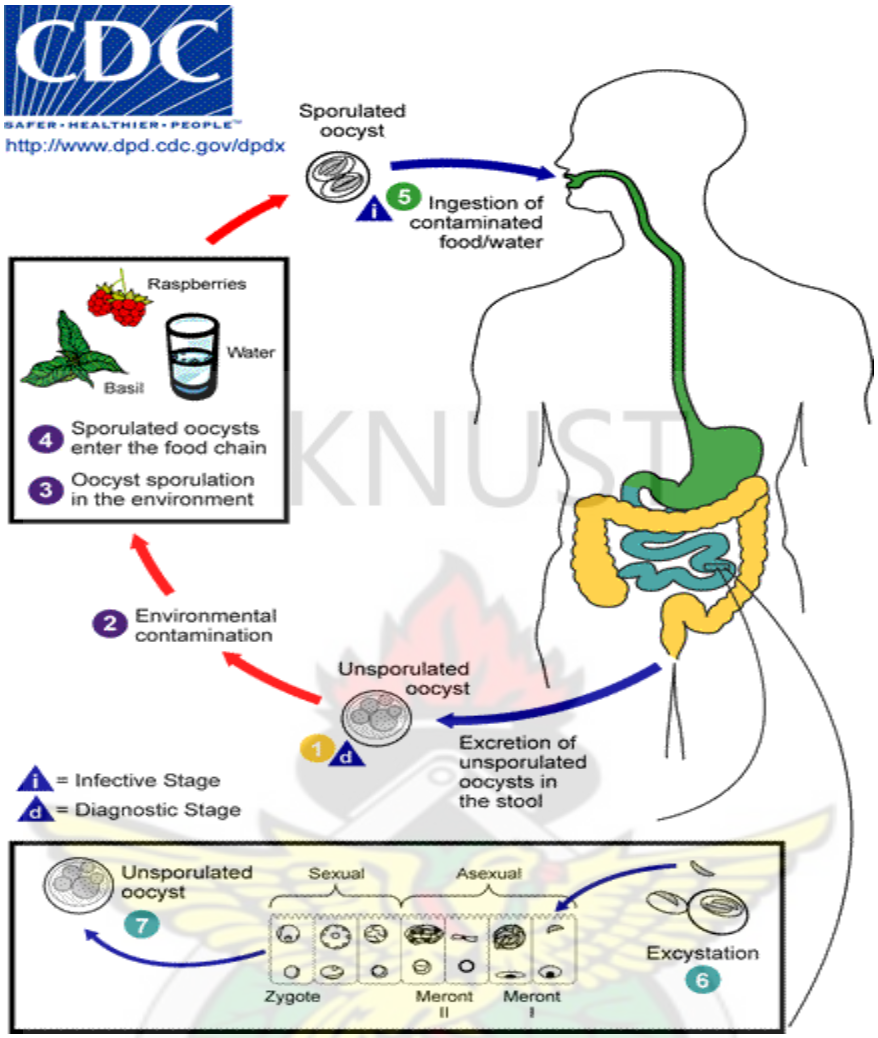

Figure 2.5 Life cycle of *Cyclospora cayetanensis*

Source: Centres for Disease Contol and Prevention

2.3.1.2.4 Clinical Manisfestation

Symptoms may develop abruptly or gradually and may be of relative short duration or last an average of 7 weeks in immunocompetent individuals (**Sterling and Ortega, 2004**). The parasite causes watery diarrhoea, with frequent, sometimes explosive stools (**Forsythe, 2010**). The diarrhoea is described as profuse, malodorous, and watery and can cause dehydration and weight loss (**Forsythe, 2010).** Diarrhoea may be associated with one or more nonspecific symptoms, including intermittent crampy abdominal pain, nausea, vomiting, low-grade fever, malaise,

myalgias, anorexia, bloating, flatulence, and/or profound fatigue (**Forsythe, 2010; Washington *et al.*, 2006**). Biliary disease with right upper quadrant pain, increased alkaline phosphatase, and thickened gallbladder on ultrasound findings have been reported in an immunocompromised host infected with *Cyclospora* (**Debra *et al.*, 1994**). Small-bowel biopsy reveals pathologic changes, including blunting and atrophy of 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 assymptomatic carriers of oocyst are available in literature, particularly in communities where cyclosporiasis is endemic (**Eberhard *et al.*, 1999; Ortega *et al.*, 1993; Chacin-Bonilla *et al.*, 1993; Reinthaler, 1989**).

2.3.1.2.5 Laboratory Diagnosis

Microscopic examination of faecal specimens with acid-fast staining are indicated in cyclosporiasis (**Garcia *et al.*, 1983**). Diagnosis is based on the microscopic detection of oocysts in faecal specimens (**Cheesbrogh, 2005**). Oocysts are round and resemble those of *Cryptosporidium*, but they are twice the size measuring 8-10 µm (**Ashford, 1979**). They are autofluorescent, appearing green with 450-490nm and blue with 365 dichroic filter when examined with ultraviolet fluorescence microscopy (**Berlin *et al.*, 1998**). This property is not specific for *Cyclospora* species; moreover, it wanes as the specimen ages (**Berlin *et al.*, 1998**). Staining *Cyclospora* species using modified Ziehl-Neelsen or Kinyoun acid-fast stains is appropriate (**Cheesbrough, 2005**). Polymerase chain reaction (PCR) tests for detection of

Cyclospora DNA in stool specimens are now commercially available and may become the test of choice for making the diagnosis(**Van Lieshout and Verweij, 2010**). A PCR assay for detecting oocysts in foodstuffs has recently been described (**Relman *et al.*, 1996**).

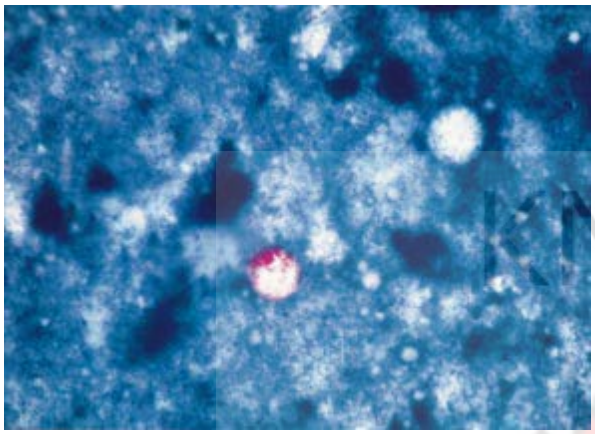

Figure 2.6 *Cyclospora cayetanenesis* in stool (Modified Z-N stain)

2.3.1.2.6 Treatment

Cyclosporiasis appears to be self-limiting in an immunocompetent host, lasting several days to 2 weeks (**Sterling and Ortega, 2004**). Infection in an immunocompromised host or in a child may require parenteral rehydration (**Sterling and Ortega, 2004**). Trimethoprim-sulfamethoxazole (TMP-SMZ) has proven effective in managing cyclosporiasis in immunocompetent and immunocompromised hosts (**Bouree *et al*, 2007**). TMP-SMZ administration can reduce shedding of oocysts to 1.3 days (from 9 days) and stops diarrhoea within 2 days (**Bouree *et al*, 2007)**. An immunocompromised host requires Trimethoprim-sulfamethoxazole (TMP 160mg & SMX 800mg bid) for 7-10 days therapy and may continue for longer periods, followed by prophylaxis to prevent recurrence (**Sterling and Ortega, 2004**). One study has indicated that if the patient is allergic to or does not tolerate sulfa-containing medications, especially AIDS patients, ciprofloxacin are an alternative treatment (**Verdier *et al*., 2000**).

2.3.1.3.1 Cryptosporidiosis

The genus *Cryptosporidium* consists of a group of protozoan parasites within the protist subphylum Apicomplexa, class Sporozasida and subclass Coccidiasina (**Robert *et al.*, 2002**). There are 10 recognized *Cryptosporidium* species based on host specificity, morphology, and molecular biology studies. Besides humans, the parasite can infect many different species of animals (eg, mammals, birds, reptiles) (**White, 2005**) and is pathogenic to immunocompetent and immunocompromised hosts (**Robert *et al.*, 2002**). Two species mainly infect humans: *Cryptosporidium hominis* (previously *Cryptosporidium parvum* genotype 1), which infects only humans, and *C. parvum* (previously *C parvum* genotype 2), which infects humans and animals (**Flynn, 1997)**. *Cryptosporidium canis* infects dogs and humans (**White, 2005**). Additional subspecies of *Cryptosporidium parvum* have been identified in stools of AIDS patients (**Robert *et al.*, 2002**).

The name *Cryptosporidium* was proposed in 1912 (**Keush *et al.*, 1995**). *Cryptosporidium* was first associated with human GI disease in 1976 (**Flanigan and Soave, 1993)** but was first identified in stomachs of mice in 1907 (**Keush *et al.*, 1995**). In early 1980, with the advent of the acquired immunodeficiency syndrome (AIDS) epidemic, *Cryptosporidium* infections became increasingly recognized as a cause of diarrhoeal illness in imunocompromised patients and widely distributed in HIV/AIDS patients (**Fisseha *et al.*, 1999; Adamu *et al.*, 2006; Gupta *et al.*, 2008; Adamu and Petros, 2009**). The disease is transmitted via the faecal-oral route from infected humans or animals (**Forsythe, 2010)**. Infection usually occurs following ingestion of contaminated water, but transmission can also occur through food and person-to-person contact (**Cheesbrough, 2005**). Extensive waterborne outbreaks have occurred from contamination of

municipal water and recreational waters (**Kwakye-Nuako *et al*., 2007)**. Although less common, transmission through certain sexual practices involving oro-anal contact has been documented (**Meinhardt *et al*., 1996**).

A high prevalence of *Cryptosporidium parvum* has been reported in assymptomatic HIV positive patients without diarrhoea (**Cardoso *et al*., 2004**). Oocysts of *Cryptosporidium* are infective when passed in faeces (**Cheesbrough, 2005**).

2.3.1.3.2 Epidemiology

Prevalence appears to be greater in less developed countries, possibly as a result of lack of clean water and sanitary facilities, crowding, and animal reservoirs in close proximity to residences (**Ryan and Ray, 2010; Brett *et al*., 2003**). In the developed world sporadic outbreak and epidemics of cryptosporidiosis occurs in the USA (**Brett *et al*., 2003**). Children younger than 2 years may be more susceptible to infection, possibly because of increased fecal-oral transmission in this age group and because of a lack of protective immunity (**Cheesbrough, 2005**; **Clark, 1999**). Oocyst excretion is found in 4-11% of the population in less developed countries, and seroprevalence of antibodies may be as high as 50% in rural China and nearly all children living in urban slum in Brazil (**Ryan and Ray, 2004; Brett *et al*., 2003**). The largest outbreak of cryptosporidiosis occurred in Milwaukee, USA around 1993 (**MacKenzie *et al*., 1995**).

More than 50% of individuals with AIDS in Africa and Hiati develop chronic cryptosporidiosis, and about 10% have a fulminant course (**Ryan and Ray, 2004**). *Cryptosporidium parvum* is a frequently identified parasite in HIV infected individuals with diarrhoea in India and other parts of the world (**Prasad *et al*., 2000**).

2.3.1.3.3 Life Cycle

Cryptosporidium species infect a wide range of animals and human infections may be zoonotic but person to person infection is thought to occur (**Cheesbrough, 1992**). Infection is caused by ingesting oocyst from hands, food, or water contaminated with faeces containing infective oocysts (**Cheesbrough, 1992**). *Cryptosporidium* can complete its life cycle within a single host, including both asexual (merogony) and sexual (sporogony) reproductive cycles (**Ryan and Ray, 2004**). Infection is initiated by ingestion of oocysts, which are activated in the stomach and upper intestines and release 4 infective sporozoites (**Ryan and Ray, 2004**). These motile sporozoites bind 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 GI and respiratory tracts without causing cytopathic effects (**Ryan and Ray,2004**). Two morphologic forms of the oocysts have been described: thin-walled oocysts (asexual stage) excyst within the same host (causing self-infection), whereas the thick-walled oocysts (sexual stage) are shed into the environment (**Robert *et al*., 2002**).

In immunocompetent individuals, the organism is primarily localized to the distal small intestines and proximal colon, whereas in immunocompromised hosts, the parasites have been identified throughout the gut, biliary tract, and respiratory tract (**Robert *et al*., 2002**). Infected persons have been reported to

shed 10^8 -10^9 oocysts in a single bowel movement and to excrete oocysts for as long as 50 days after diarrhoea has ceased (**Dillingham *et al.*, 2002**).

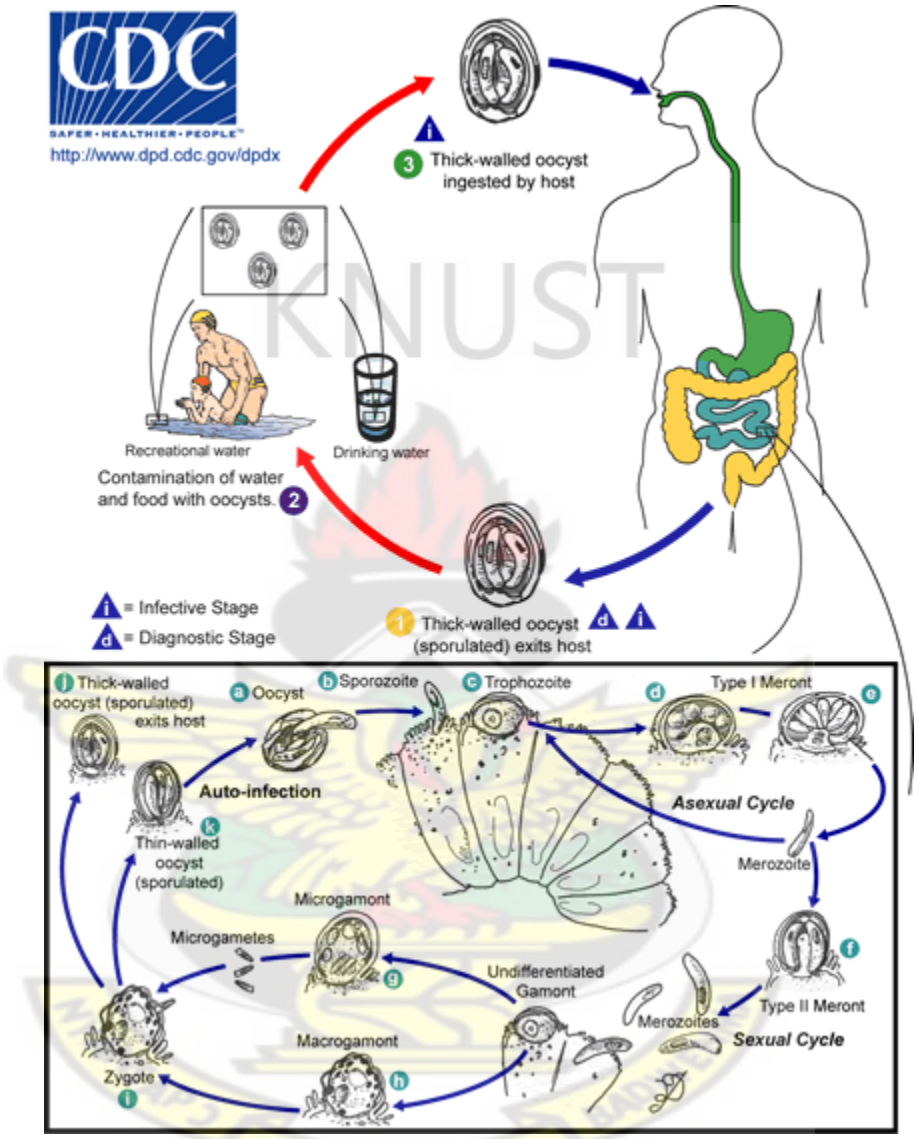

Figure 2.7 Life cycle of *Cryptosporidium* (Source: Centres of Disease Control and Prevention)

2.3.1.3.4 Clinical Manifestation

The incubation period is 2-14 days and symptoms begin 2-10 days after being infected by the parasite (**Brett *et al.*, 2003**). The duration in immunocompetent host is variable lasting several days to 5 weeks (**Jokipii and Jokipii, 1986**). The main symptoms are related to the GI tract, but respiratory symptoms like shortness of breath may also develop in immunocompromised patients

(**Hunter and Nicolas, 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**). In individuals who are immunocompetent, the median duration of diarrhoea ranges from 5-10 days (**Ryan and Ray, 2004**). Diarrhoea can persist longer in individuals who are immunosuppressed (**Brett *et al*., 2003**). Oocyst shedding can continue for as long as 2 weeks after clinical improvement (**Ryan and Ray, 2004**). The volume of fluid losses through diarrhoea may be as high as 25 litres per day, particularly in individuals with AIDS (**Ryan and Ray, 2004**).

In sporadic cases, fever may be low grade or nonexistent; however, during outbreaks, fever may occur in 30-60% of patients (**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 AIDS may present with fever, right upper-quadrant pain, jaundice, nausea, and vomiting but not necessarily with concomitant diarrhoea (**Wolska-Kusnierz *et al*., 2007**). The clinical manifestations of cryptosporidiosis in HIV patients vary (**Abubakar *et al*., 2007**). In patients with CD4 T-cell counts of more than 200, most infections are self-limited, similar to those in immunocompetent hosts (**Wolska-Kusnierz *et al*., 2007**). Other patients develop chronic diarrhoeal illness with frequent, foul-smelling, bulky stools associated with significant weight loss (**Ryan and Ray, 2004**). A minority of them have cholera-like symptoms (**Brett *et al*., 2003**). Biliary involvement is correlated with significantly low CD4 T-cell counts, and patients present with acalculus cholecystitis, sclerosing, cholangitis, or pancreatitis (**Ryan and Ray, 2004**). Secondary malabsorption of fat, D-xylose, and vitamin B-12 has been noted in some HIV patients (**Cooke, 2009**).

In waterborne outbreaks, immunocompetent patients present with subclinical or milder illness that lasts for less than 5 days (**Craun, 1990; Brett *et al*., 2003**). Although healthy individuals can become ill from outbreaks of infections due to water contamination, immunodeficiency places an individual at increased risk for cryptosporidiosis, particularly for more severe and disseminated disease (**Brett *et al*., 2003**). Immunodeficiency or immunosuppression may be congenital or may be secondary to HIV, cancer chemotherapy, diabetes mellitus, or bone marrow or solid organ transplantation (**Hunter and Nicholas, 2002**).

2.3.1.3.5 Laboratory Diagnosis

The detection of oocysts upon microscopic examination of stool specimens is diagnostic (**Brett *et al*., 2003; Cheesbrough, 2005**). Patients may be asked to submit several stool samples over several days as detection of *Cryptosporidium* can be difficult (**Bronsdon, 1984**). Most often, stool specimens are examined microscopically using different techniques (eg, acid-fast staining, direct fluorescent antibody (DFA), and/or enzyme immunoassays for detection of *Cryptosporidium* species antigens (**Garcia *et al*., 1983; Bronsdon, 1984; Weber *et al*., 1991**). If immediate examination is not possible, one of several preservatives such as 5-10% buffered Formalin, Merthiolate-Iodine-Formalin (MIF), or (Sodium acetate, Acetic acid, Formaldehyde) SAF is recommended (**Cheesbrough, 2005**). Refrigeration of unpreserved specimens to delay deterioration is helpful (**Cheesbrough, 2005**). Even though oocyst can be detected in unconcentrated faecal specimens due to its numerous numbers in acute infections, others are of the view that concentration by the Formalin ethyl acetate method is preferable (**Garcia *et al*., 1983; Casemore *et al*., 1985; Cheesbrough, 1992**). Optimal centrifugation time and speed, 10 minutes at 500g, are critical for concentrating *Cryptosporidium* oocysts (**Cheesbrough, 1992**).

Modified acid-fast staining technique is useful for the identification of oocysts of the coccidian species (*Cryptosporidium, I. belli* and *Cyclospora*), which may be difficult to detect with routine stains such as trichrome (**Weber *et al.*, 1991; Garcia *et al.*, 1983, Bronsdon, 1984)**. *Cryptosporidium* species stains a pinkish-red colour and the background should stain blue-green (**Cheesbrough, 2005**). Unlike the Ziehl-Neelsen, Modified Acid-Fast Stain does not require the heating of reagents for staining (**Cheesbrough, 2005**). Concentrated sediment of fresh (within 30 min after passage of stools) or formalin-preserved stool may be used (**Garcia *et al.*, 1983; Cheesbrough, 1992**). Other types of clinical specimens such as duodenal fluid, bile, pulmonary samples (induced sputum, bronchial wash, and biopsies) may also be stained **(Weber *et al.*, 1991; Garcia *et al.*, 1983, Bronsdon, 1984)**. An alternative is the sucrose flotation method or formalin ethyl acetate method used to concentrate stool before staining with a modified Kinyoun acid-fast stain (**Garcia *et al.*, 1983**). This technique stains oocysts pink or red, whereas faecal debris or yeast assumes the color of blue or green counterstain (**Weber *et al.*, 1991; Garcia *et al.*, 1983)**.

A monoclonal antibody-based fluorescein conjugated stain for oocysts in stool is not as specific as an enzyme immunoassay (EIA) in detecting antigen in stool. The latter is the most specific, reliable test that is widely available commercially (**Weintraub, 2006**). Because shedding may be intermittent, at least 3 stool specimen collected on separate days must be examined before considering the test results negative (**Casemore *et al.*, 1985**). Faecal leukocytes are not found in stool specimens because it does not invade below the epithelial layer of the mucosa (**Cheesbrough, 1992**). Oocysts are small (4-6 μm in diameter) and can be missed without a very

careful examination of the slide (**Cheesbrough, 2005**). Electron microscopy of stool or biopsy specimens can also be performed for direct visualization of oocysts (**Casemore *et al*., 1985**). For research purposes and for species identification, polymerase chain reaction assays are used and molecular epidemiological studies have classified the parasite into heterogenous species with most being associated with zoonotic transmission (**Morgan *et al*., 2000**).

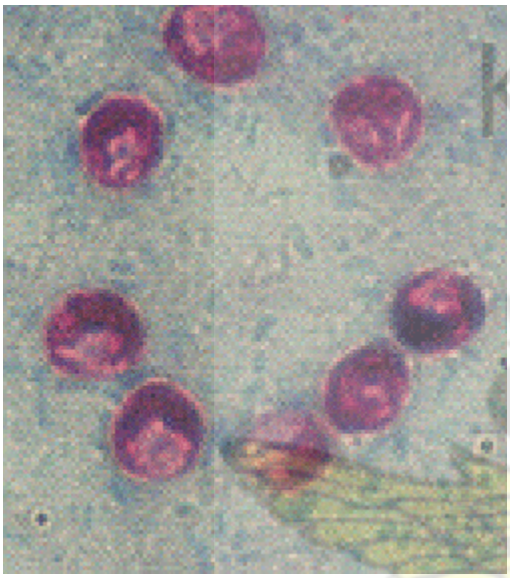

Figure 2.8 *Cryptosporidium* oocyst in stool (Modified Z-N stain)

2.3.1.3.6 Treatment

No reliable curative treatment for cryptosporidiosis is available (**Xiao and Ryan, 2004; Soave, 1990; Clark, 1999**). A compartment established within the host cell, a unique parasitophorous vacuole shelter the parasite from antimicrobial drugs (**Griffiths *et al*., 1998**). Supportive therapy is the key component in the management of cryptosporidiosis (**Clark, 1999**). Fluid and electrolyte management is critical; particularly in AIDS patients where infection causes protracted diarrhoea causing loss of large volumes of water (**Fayer, 1997; Clark, 1999**). Nonspecific antidiarrheal agents may provide relief (**Clark, 1999**). Octreotide, a somatostatin analogue and substance pantagonist, suppresses diarrhoea in chronic cryptosporidiosis (**Clark,**

1999). Biliary involvement in cryptosporidiosis which is found in some HIV infected patients requires specific intervention such as cholecystectomy, retrograde cholangiopancreatography (RECP) (**Blumberg *et al*., 1984**). Most people who have healthy immune systems will recover without treatment (**Clark, 1999**). Mature epithelial cells at the tips of the villi are preferentially lost; hence, enzymes expressed on these cells, including lactase are lost; these losses lead to secondary lactose intolerance (**Downs, 2010**).

Nitazoxanide has been approved to treat children with diarrhoea caused by *Cryptosporidium* (**Clark, 1999; Fox and Saravolatz, 2005**). In clinical trials, the agent significantly reduced the duration of diarrhoea caused by *Cryptosporidium* infections (**Fox and Saravolatz, 2005)**. It also reduced the rate of death in malnourished children in Africa with *Cryptosporidium* infection (**Fox and Saravolatz, 2005**). In patients with AIDS, antiretroviral treatment has been associated with improvement, possibly because of general improvement of immune function (**Smith N. *et al*., 1998; Mofenson *et al*., 2009**). Combination therapy with paromomycin and azithromycin (**Hicks *et al*., 1996**) for 4 weeks followed by paromomycin monotherapy for 8 weeks has been successfully used in adult patients with AIDS (**Clark, 1999**). The best approach to the prevention of cryptosporidiosis in HIV infected patients is the maintenance of the immune system through the administration of ART since available data suggests that cryptosporidiosis occurs mostly in immunocompromised individuals (**Clark, 1999**). However the administration of Claritomycin aimed at preventing *Mycobacterium* infections in severely immunocompromised individuals has a protective effect against *Cryptosporidium* infection (**Clark, 1999**). This drug has also shown activity in vitro against *Cryptosporidium* and in animal trials (**Clark, 1999**).

2.4. Other Gastrointestinal Parasites

2.4.1 Microsporidia

Microsporidia are small, obligate intracellular protozoan parasites (**Franzen and Muller, 2001**). It was first recognized as a pathogen of silkworms in 1857 with the first human disease occurring in 1959 (**Matsubayashi *et al*., 1959)**. Long before microsporidia was recognised as pathogenic protozoan in humans, they were recognised as cause of disease in many non-human host including a wide range of vertebrates and non vertebrates (**Franzen and Muller, 2001**). In 1985 a new species of microsporidia, *Enterocytozoan bineusis* was found in an HIV infected patient and since then many species of the parasite has now been recognized as an etiological agents of opportunistic infections in persons with AIDS (**Bryan and Shwartz, 1999**). The prevalence of the protozoan in non-HIV infected individuals, travelers and organ transplant recipients being treated with immunosuppressive drugs is well documented (**Bryan *et al*., 1997; Raymond *et al*., 1998; Sax *et al*., 1995**).

The phylum Microsporidia consist of nearly 150 genera with more than 1000 species, but only 7 genera as well as unclassified Microsporidia have been described as pathogens in humans (**Franzen and Muller, 2001**). The Microsporidia classified as pathogens in humans include; *Enterocytozoon*, *Encephalitozoon*, *Pleistophora*, *Trachipleistophora*, *Vittaforma*, *Brachiola* and *Nosema* (**Franzen and Muller, 2001).** *Enterocytozoon bieneusis*, *Encephalitozoon intestinalis* and *Encephalitozoon hellem* are frequently associated with AIDS (**Cheesbrough, 2005**). Microsporidia infections of the gastrointestinal tract, liver, eye, sinus, respiratory tract, kidney, muscle and cerebral region are documented (**Cheesbrough, 2005; Cali *et al*., 1993**). In this review, emphasis is on microsporidia that are involved in gastrointestinal infections. The

commonest microsporidia involved in gastrointestinal infections is *E. bieneusis* (**Cheesbrough, 2005; Franzen and Muller, 2001**) and less frequently *E. intestinalis* (**Franzen and Muller, 2001**).

2.4.1.1 Intestinal tract infections caused by microsporidia.

Intestinal infections with Microsporidia have been found mainly in HIV-infected patients with most infections due *E.bieneusis* or less frequently to *E. intestinalis* (**Franzen and Muller, 2001**). Infections are most common in HIV- infected patients with severe immunodeficiency and a CD4 T-cell count below 100 cells/µl (**Franzen and Mulle,2001**). Parasites often cause severe, non bloody, non mucoid diarrhoea with up to ten or even more bowel movements per day.There is also slow progressive weight loss, malabsorption of fat, D-xylose, and vitamin B12 (**Franzen and Muller, 2001)**. Intestinal infections is associated with lactase deficiency, a reduced activity of alkaline phosphatase and α glucosidase at the basal part of the villus, and with reduced villus level and a villus surface reduction (**Franzen and Muller, 2001)**. Diarrhoea appears gradually and may continue for months (**Franzen and Muller, 2001)**. The patients are often reluctant to eat and may complain of nausea (**Franzen and Muller, 2001)**. Some patients have intermittent diarrhoea, but only a few excrete microporidial spores without having diarrhoea (**Franzen and Muller, 2001)**. In groups of patients with chronic diarrhoea the prevalence of *E. bienusis* solely varied between 7 and 50%, depending on the study population and method of diagnosis (**Kortler and Orienstein, 1999**).

2.4.1.2 Epidemiology

Microsporidia belongs to a huge phylum with about 150 genera and over 1000 species, infecting almost every kind of insect and animal (**Franzen and Muller, 2001**). *Enterocytozoon, Encephalitozoon, Pleistophora, Trachispleisphora, Vittaforma, Brachiola* and *Nosema* have been reported in humans (**Franzen and Muller, 2001**). The most commonly reported Microsporidia which infects humans is *E. bieneusis* (**Weiss and Lindsay, 2004)**. Other natural hosts of *E. bieneusis* include pigs, cats, farm dogs, chickens, and non-human primates (**Weiss and Lindsay, 2004)**. Infection usually remains localized to the small intestine to cause persistent diarrhoea and weight loss, and some infections will spread to the gall bladder to cause cholangitis and cholecystitis (**Weiss and Lindsay, 2004**).

Infection of an AIDS patient with *Encephalitozoon cunniculi* III which is identical to that isolated from domestic dogs (**Didier *et al*., 1996**) provides evidence of zoonotic potential of the organism (**Didier *et al*., 1996)**. Microsporidia infections are commonly seen in AIDS and reports suggests that patients who received organ transplant developed microsporidia infection (**Gumbo *et al*.,1999**), probably due to immunosuppression secondary to drug administration. Fulminant hepatic failure caused by *Encephalitozoon* has been reported in an AIDS patient (**Franzen and Muller, 2001**). This patient suffered from microsporidia diarrhoea which later precipitated into fulminant hepatitis resulting in the death of the patient (**Franzen and Muller, 2001)**. Disseminated microsporidia infection of the liver, gall bladder wall, and a mediastinal lymph node are documented complications of Microsporidia (**Sheth *et al*., 1997**). Enteric microsporidial infections are the commonest and are suspected aetiological agents of diarrhoea in up to 30% of AIDS patients, although studies from the UK have found prevalence rates of only about half this value (**Curry and Smith, 1998)**. Studies using conventional microsporidial techniques may

underestimate the true prevalence in AIDS patients with diarrhoea (**Curry and Smith, 1998)**. The use of PCR has yielded higher prevalence (**Curry and Smith, 1998**). **Safarti *et al*. (2006)** using PCR as the method of diagnosis, discovered that, 5.2% of HIV patients were infected with *E. bieneusis* with diarrhoea occurring in half the number of these patients (**Sarfarti *et al*., 2006**).

2.4.1.3 Life cycle

The infective form of microsporidia is the resistant spore and it can survive for a long time in the environment. The spore extrudes its polar tubule and infects the host cell. The spore injects the infective sporoplasm into the eukaryotic host cell through the polar tubule. Inside the cell, the sporoplasm undergoes extensive multiplication either by merogony (binary fission) or schizogony (multiple fission). This development can occur either in direct contact with the host cell cytoplasm (e.g., *E. bieneusi*) or inside a vacuole termed parasitophorous vacuole (e.g., *E. intestinalis*). Either free in the cytoplasm or inside a parasitophorous vacuole, microsporidia develop by sporogony to mature spores. During sporogony, a thick wall is formed around the spore, which provides resistance to adverse environmental conditions. When the spores increase in number and completely fill the host cell cytoplasm, the cell membrane is disrupted and releases the spores to the surroundings. These free mature spores can infect new cells thus continuing the cycle (**Wittner and Weiss, 1999**).

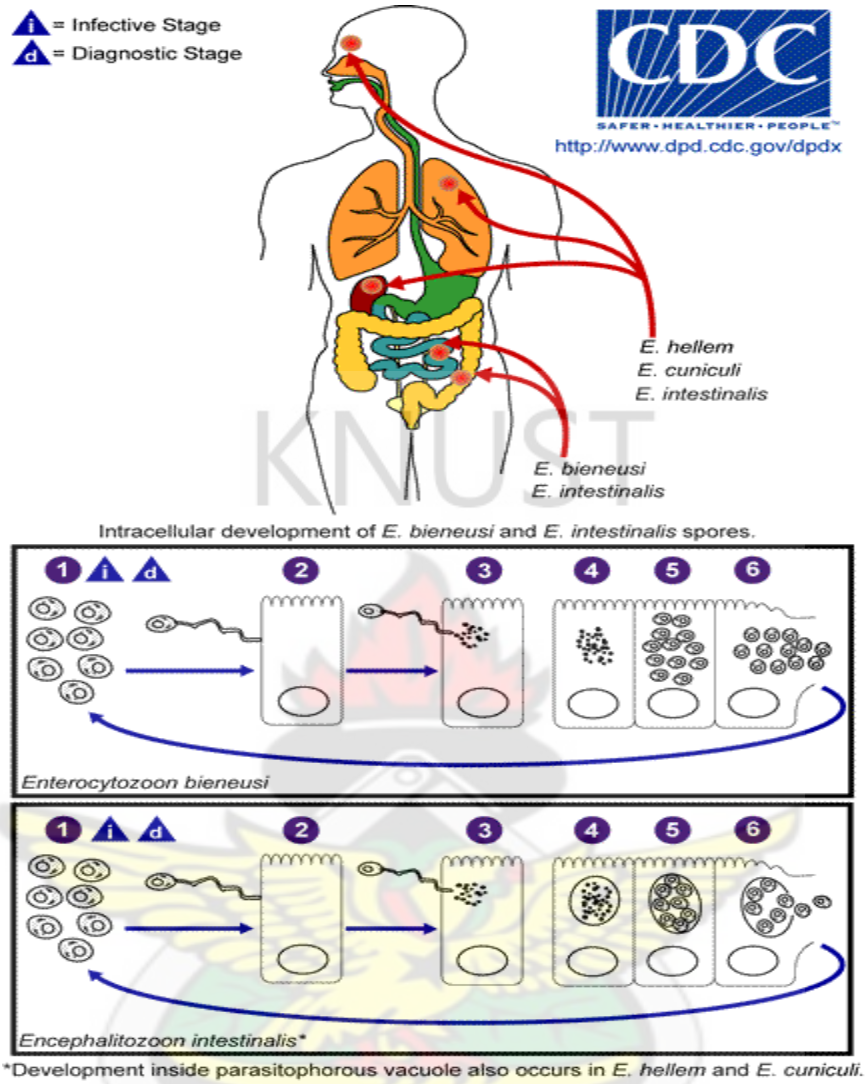

Figure 2.9 Life cycle of Microsporidia

Source: Centres of Disease Control and Prevention

2.4.1.4 Laboratory Diagnosis

The recommendations for the use of current, less-sensitive methodology suggest that multiple diagnostic methods may be required to diagnose a microsporidial infection, particularly when fecal specimens are examined (**Garcia, 2002**). Since microsporidial infections often involve multiple body sites, detection of organisms from any clinical specimen should be followed by examination of other body tissues and fluids (**Garcia, 2002; Franzen and Muller, 2001**). In

patients for whom disseminated microsporidiosis is suspected, urine specimens should always be examined (**Garcia, 2002**). It is important to remember that microsporidial spores are quite resistant to environmental conditions and can remain infectious for several years, particularly if they are protected from desiccation (**Cheesbrough, 2005; Garcia, 2002)**. Stool or duodenal drainage specimens can be submitted fresh or preserved in 5 or 10% formalin, or sodium acetate-acetic acid-formalin (**Cheesbrough, 2005**). Biopsy specimens are also acceptable (**Garcia, 2002**). In cases of disseminated infection, it is recommended that urine (fresh or preserved) be submitted for analysis (**Garcia, 2002**).

2.4.1.4.1 Modified trichrome staining

Smears are prepared using 10 to 20 µl of concentrated specimen (stool specimen or urine or other fluid) that is thinly spread onto the slides (**Garcia, 2002**). Some laboratories use concave well slides; these are very helpful when examining the stained preparations (**Garcia, 2002**). It is important to remember to centrifuge the specimen for 10 min at 500 g prior to smear preparation (**Garcia, 2002**). There is also some evidence to indicate that pretreatment of faecal specimens (1:1) with 10% KOH may provide a better-quality smear when modified trichrome stains are used (**Garcia,2002**). Specimens can be fresh or preserved (with 5 or 10% formalin or with sodium acetate-acetic acid-formalin or by using one of the newer single-vial systems) (**Garcia, 2002**). The modified trichrome stain methods are based on the fact that stain penetration of the microsporidial spore is very difficult; thus, the dye content in the chromotrope 2R component of the formula (**Garcia, 2002**). The use of positive control material is highly recommended (**Cheesbrough, 2005**). Spore detection requires adequate illumination and magnification (oil immersion; total magnification: x1000) (**Garcia, 2002**). The spore wall should stain pinkish to

red, with the interior of the spore being clear or perhaps showing a horizontal or diagonal stripe, which represents the polar tube (**Garcia, 2002**). The background will appear green or blue, depending on the method (**Garcia, 2002**). Other bacteria, some yeast cells, and some debris will also be evident (stained shades of pink and red); the shapes and sizes of the artifacts may be helpful in differentiating the spores from other structures (**Garcia, 2002**). Results should be reported only if the positive control smears are acceptable (**Garcia, 2002**). The choice of counterstain, with either fast green or aniline blue dyes in the stain formulation, depends on laboratory preference and does not change the colour results of the actual microsporidial spores, which stain pink (**Cheesbrough, 2005**). In addition to the counterstain (green or blue), several modifications of the original chromotrope staining solution have been proposed, including changes in temperature of the staining solution and staining time (**Garcia, 2002**). Results indicate that staining at 50°C for 10 min or staining at 37°C for 30 min may improve the detection of spores; the background appears to contain less debris, and the spores may stain more intensely (**Garcia, 2002**).

2.4.1.4.2 Giemsa stain

Although Giemsa staining of stool can be performed and results in a light-blue staining of microsporidial spores, the spores can be very difficult to differentiate from debris in the smear (**Garcia, 2002**). However, body fluid cytology preparations or intestinal biopsy specimens containing less debris and artifact material can be stained with Giemsa stain; identification of spores will be much easier with these types of specimens than with preparations from stool (**Garcia, 2002**).

2.4.2 Giardiasis

Giardia intestinalis also called *Giardia lamblia* and *G. duodenalis* was described in 1681 by Van Leeuwenhoek from own stool (**Hill, 2005**). *G. intestinalis* has a worldwide distribution and is particularly common in the tropics and subtropics, in places where water supplies and the environment become faecally contaminated (**Cheesbrough, 2005**). It is a gastrointestinal protozoan most often identified in individuals with asymptomatic colonization of the parasite in the intestinal tract or in individuals with acute or chronic diarrhoeal illness (**Moore *et al*., 1969**). In endemic areas, young children are more frequently infected than adults (**Cheesbrough, 2005; Huston, 2006**). It is associated with diarrhoea with at least the presence of one enteric pathogen in HIV/AIDS positive patients (**Concalve *et al*., 2009**). *Giardia lamblia* occurs in humans and the infection may be passed from primates, dogs, or cat to humans (**Ryan and Ray, 2004**). HIV patients who have a defect in immunity against diseases are at risk of infections if they become exposed to dogs and cat which are kept as pets in homes (**Ryan and Ray, 2004**). *Giardia* species are endemic in areas of the world that have poor sanitation (**Ryan and Ray, 2004**). In developing countries, the disease is an important cause of morbidity, and water-borne and food-borne outbreaks are common (**Ryan and Ray, 2004; Craun, 1990; Cheesbrough, 1992**). *Giardia lamblia* is a particularly significant pathogen for people with malnutrition, immunodeficiency and cystic fibrosis (**Flannagan, 1992; Monis, 2003**).

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

Although many infections, particularly in adults are without symptoms, *G. lamblia* can cause abdominal pain, severe diarrhoea, flatulence, vomiting, weight loss, and malabsorption with lactose intolerance and in children less than 3 years of age, and in the undernourished (**Cheesbrough**, **2005**). Those with reduced immune responses, gastrointestinal disorders or intestinal bacterial infections, tend to be more susceptible to *Giardia* infection (**Cheesbrough**, **2005**). The mechanism by which *G. lamblia* causes diarrhoea and low intestinal absorption is not clearly understood. However one possible explanation is a number of multiple factors such as age of cyst, host immunity and parasite genetic variability (**Caccio and Ryan, 2008**). The mechanisms by which *G. lamblia* causes diarrhoea and intestinal malabsorption are probably multifactorial and not yet fully elucidated (**Ryan and Ray, 2004**). Although the parasite appears to alter epithelial structure and function, leading to malabsorption, diarrhoea can occur in individuals in the absence of obvious light microscopic changes in small intestinal structure (**Buret, 2008**). Also, marked or moderate partial villous atrophy in the jejunum can be observed in histologic sections from asymptomatic individuals who are infected (**Buret, 2008**). In addition to disrupting the mucosal epithelium, effects in the luminal may contribute to malabsorption and the production of diarrhoea (**Ryan and Ray, 2004; Buret, 2008**).

2.4.2.1 Morphology

Motile *G. lamblia* flagellates measure 9-20μm in length (usual size is 10-12μm) (**Cheesbrough, 1992**). They have a characteristic shape with concavity at the front end (**Cheesbrough, 1992**). There are 8 flagella (**Cheesbrough, 1992**). The flagellates have a rotating and twisting movement (**Cheesbrough, 1992**). The cyst is small, oval in shape, measuring about 10x6μm and contains 4 nuclei which are difficult to see under light microscope (**Cheesbrough, 1992**). It

contains the remains of axonemes and parabasal bodies which stain with iodine (**Cheesbrough, 1992**). The thread like remains of flagella may also be seen (**Cheesbrough, 1992**).

2.4.2.2 Epidemiology

Giardia is found in other mammals, including domestic and farm animals (**Ryan and Ray, 2004**). *Giardia* has been recovered from intestines of a broad range of hosts, including livestock, cats, dogs, beavers, and guinea pigs (**Ryan and Ray, 2004; Meyer, 1994**). Several studies have also found these organisms in treated sewage effluent and wildlife (**Dykes, 1980**). The organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically (**Farthing, 1994**).

About 70% of HIV- infected individuals contract at least one pathogen causing diarrhoea (**Nwachukwu and Okeke, 2008**). Chronic giardiasis is one of the most common causes of diarrhoea in subjects with HIV (**Tosones, 1993)**. Prevalence of *G. lamblia* among HIV patients was 30% **(Tosones, 1993)**. Patients in stage IV had a prevalence of 36.8%, and 18.1% in patients in stages II-III (**Tosones, 1993)**. Ninety four point four percent (94.4%) of *G. lamblia* positive patients were symptomatic (**Tosones, 1993)**. The mean absolute CD4 T-cell count was 84.2/ccm in *Giardia* infected patients (**Tosones, 1993)**. In conclusion it was stipulated in this study that *Giardia* infection is higher in HIV infected patients than HIV negative in age matched general population with prevalence of 30% and 9.7% respectively (**Tosones, 1993**).

2.4.2.3 Life cycle

Giardia lamblia is transmitted by faecal oral route (**Cheesbrough, 2005**). Cysts can also be ingested in food, water or from hands contaminated with faeces (**Cheesbrough, 2005**). The

parasite is shed with the faeces as an environmentally robust cyst, which can then be transmitted to a new host (**Cheesbrough, 2005**). In the duodenum of the new host, the trophozoite emerges from the cyst and undergoes a mitotic division (**Cheesbrough, 2005**). Each of the two trophozoites produced in this way attaches to the epithelial cells by means of an adhesive disc, and then feeds on the epithelial cells (**Cheesbrough, 2005**). The trophozoites detach from the epithelial cells, probably because of the rapid turnover (72 hours) of these cells, and undergo mitotic division in the intestinal lumen (**Cheesbrough, 2005**). During periods of diarrhoea, these trophozoites may be transported with the intestinal contents and excreted, but do not survive long outside the host (**Cheesbrough, 2005**). Some of the trophozoites encyst during the passage through the intestine and leave the host with the faeces as cysts (**Cheesbrough, 2005**). In formed stools, cysts are encountered more often than trophozoites (**Cheesbrough, 2005**). *G. lamblia* cysts are elliptical, 8–12mm long and 7–10mm wide. The cyst wall is 0.3–0.5mm thick and has a fibrillous structure (**Cheesbrough, 2005**). Two to four nuclei are found in each cyst, together with axonemes of the flagella of the trophozoite (**Cheesbrough, 2005**). Cysts are infective directly they are passed in faeces (**Cheesbrough, 2005**).

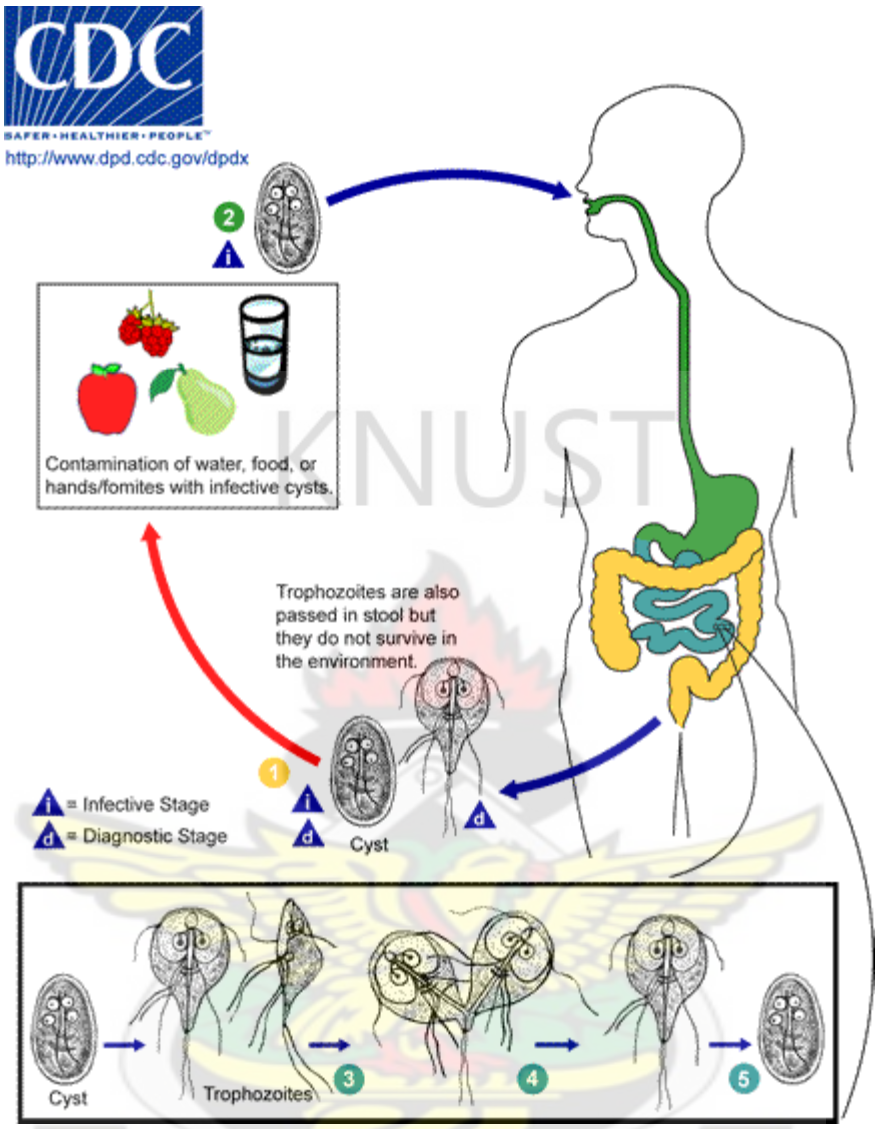

Figure 2.10 Life cycle of *Giardia lamblia*

Source: Centres of Disease Control and Prevention

2.4.3 Amoebiasis

Amoebic infection was first described by Fedor Losch in 1875 in St. Petersburg, Russia (**Losch, 1875**). In 1890, Sir William Osler reported the first North American case of amebiasis, when he observed amoebae in stool and abscess fluid from a physician who previously resided in Panama (**Bhanu *et al.*, 2011**). The species name *Entamoeba histolytica* was first coined by Fritz

Schaudin in 1903 (**Saklavalva, 1993**). In 1913, in the Philippines, Walker and Sellards documented the cyst as the infective form of *E. histolytica* (**Bhanu *et al*., 2011**). The life cycle was then established by Dobell in 1925 (**Bhanu *et al*., 2011)**. *E. histolytica* amoebiasis is caused by *E. histolytica*, a protozoan found worldwide (**Bhanu *et al*., 2011**). 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**).

Although most cases of amoebiasis are asymptomatic, dysentery and invasive extraintestinal disease can occur (**Cheesbrough, 1987**). Amoebic liver abscess is the most common manifestation of invasive amoebiasis, but other organs can also be involved, including pleuropulmonary, cardiac, cerebral, renal, genitourinary, and cutaneous sites (**Ryan and Ray, 2004**). In developed countries, amoebiasis primarily affects migrants from and travelers to endemic regions, men who have sex with men, and immunosuppressed or institutionalized individuals (**Bhanu *et al*., 2011**). Excystation then occurs in the terminal ileum or colon, resulting in trophozoites (**Cheesbrough, 1987**). The trophozoites can penetrate and invade the colonic mucosal barrier, leading to tissue destruction, secretory bloody diarrhoea, and colitis resembling inflammatory bowel disease (**Cheesbrough, 2005**). In addition, the trophozoites can spread haematogenously via the portal circulation to the liver or even to more distant organs (**Cheesbrough, 1987**).

2.4.3.1 Epidemiology

Entamoeba histolytica is endemic in many parts of tropical and subtropical Africa, Asia, Mexico, South America and China (**Cheesbrough, 1987**). Distribution is related more to inadequate

environmental sanitation and poor personal hygiene than to climate (**Cheesbrough, 1987**). *Entamoeba* species infect approximately 10% of the world's population (**Cheesbrough, 1987**). The prevalence of *Entamoeba* infection 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 to an outpatient clinic were found to have amoebic colitis (**Stanley, 2003**). *Entamoeba histolytica* seroprevalence studies in Mexico revealed that more than 8% of the populations were positive (**Caballero-Salcedo, 1994**). Asymptomatic *E. histolytica* infections seem to be region-dependent, as high as 11% in Brazil (**Fotedar *et al.*, 2007**). Since the introduction of molecular techniques, it is estimated that 500 million individuals with *Entamoeba* infection are colonized as *E. dispar* (**Fotedar *et al.*, 2007**). In Western countries, approximately 20%-30% of men who have sex with men are colonized with *E. dispar* (**Fotedar *et al.*, 2007**).

Amoebiasis is second only to malaria in terms of protozoa-associated mortality (**Bhanu *et al.*, 2011**). The combined prevalence of amoebic colitis and amoebic liver abscess is estimated at 40-50 million cases annually worldwide, resulting in 40,000-100,000 deaths (**Li and Stanley, 1996; Stanley, 2003**). Asymptomatic intestinal amoebiasis occurs in 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 extraintestinal disease (**Fotedar, 2007**). Case fatality rates associated with amoebic colitis range from 1.9%-9.1% (**Aristizabal *et al.*, 1991**). Amoebic colitis evolves to fulminant necrotizing colitis or rupture in approximately 0.5% of cases; in such cases, the mortality rate jumps to greater than 40% (**Aristizabal *et al.*, 1991**). The mortality rate due to amoebic liver abscess has fallen to 1-3% in the last century following the introduction of effective medical 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, HIV seropositivity is a risk factor for invasive extraintestinal amoebiasis (**Hung *et al.*, 2005**). In India, *E. histolytica* recorded a prevalence rate of 7.1% in HIV positive patients with a higher prevalence of 23% in HIV 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 to 5% of the population habour *Entamoeba*, most of which are nonpathogenic *E.dispar* (**Ryan and Ray, 2004**).

2.4.3.2 Life cycle

It is transmitted by the faecal oral route with infective cyst being ingested in food, water, or from hands contaminated with faeces (**Cheesbrough, 2005**). Faecal-oral transmission can also occur in the setting of anal sexual practices or direct rectal inoculation through colonic irrigation devices (**Ryan and Ray, 2004**). Following ingestion each cyst excysts in the large intestine to produce amoeba which multiply repeatedly (**Cheesbrough, 1987**). The amoeba form single-nucleated cysts which develop into infective cyst which have 4 nuclei (**Cheesbrough, 1987**). Once cysts are formed they do not become amoeba again in the same host (**Cheesbrough, 1987)**. The infective cysts are excreted in faeces (**Ryan and Ray, 2004**). They can survive and remain infective several weeks in sewage and water (**Cheesbrough, 2005**). Trophozoites passed in faeces are not infective to other people and die rapidly (**Cheesbrough, 1987**). Formerly a pathogenic invasive and non–pathogenic strain of *E.histolytica* was thought to exist

(**Cheesbrough, 2005**). Using isoenzyme-electrophoretic techniques, these two strains have now been recognized as separate species (**Cheesbrough, 2005**). *Entamoeba histolytica* is the invasive pathogenic species and *E. dispar* has been designated the non-invasive non pathogenic species (**Cheesbrough, 2005**). The two species are morphologically identical (**Cheesbrough, 2005**). *Entamoeba histolytica* causes amoebic dysentery and amoebic liver abscess (**Cheesbrough, 2005**). The cyst of *E.histolytica* and *E.dispar* are indistinguishable microspically (**Cheesbrough, 2005**). However, the trophozoite of *E.histolytica* can be identified by the presence of red blood cells within the the amoeba (**Cheesbrough, 2005**).

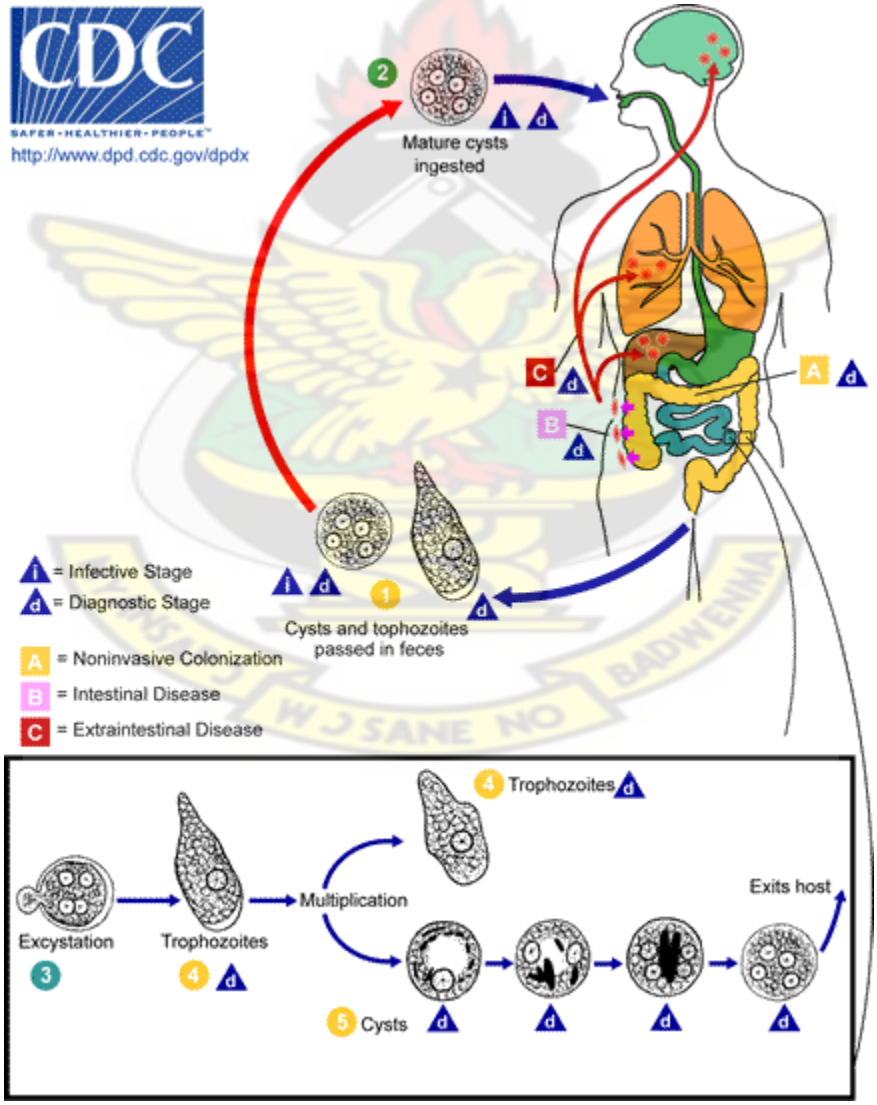

Figure 2.11 Life cycle of *Entamoeba histolytica*

Source: Centres for Disease Contol and Prevention

2.4.3.3 Clinical Manisfestation

The most common presentation of amoebic colitis is gradual onset of bloody diarrhoea, abdominal pain, and tenderness spanning several weeks' duration (**Bhanu *et al*, 2011**). Rectal bleeding without diarrhoea can occur, especially in children (**Cheesbrough, 1987**). Only approximately 10-30% of patients with amoebic colitis develop fever (**Adams and MacLeod, 1977**). Weight loss and anorexia may occur (**Ryan and Ray, 2004**). Fulminant or necrotizing colitis usually manifests as severe bloody diarrhoea and widespread abdominal pain with evidence of peritonitis and fever (**Ryan and Ray, 2004**). Predisposing factors for fulminant colitis include poor nutrition, pregnancy, corticosteroid use, and very young age (**Mondal *et al*., 2006**).

The most typical presentation of amoebic liver abscess is fever, right upper quadrant pain, and tenderness of less than 10 days' duration (**Ryan and Ray, 2004**). Unlike amoebic colitis, amoebic liver abscess is associated with fever in 85-90% of cases (**Ryan and Ray, 2004**). A more sub acute presentation can be seen, with concomitant weight loss and anorexia (**Gillepsie and Pearson, 2001**). Cough can occur but jaundice is unusal (**Gillepsie and Pearson, 2001**). Acute abdominal symptoms and signs should prompt rapid investigation for intraperitoneal rupture (**Gillepsie and Pearson, 2001**). Sixty to seventy percent (60 to 70%) of patients with amoebic liver abscess do not have concomitant colitis, although a history of dysentery within the

previous year may be obtained (**Gillepsie and Pearson, 2001**). Amoebic liver abscess may manifest years after travel to or residency in an endemic area (**Ryan and Ray, 2004**).

2.4.3.4 Laboratory Diagnosis

Microscopic stool examination for trophozoites from a single stool sample in amoebic colitis is only 33-50% sensitive (**Fotedar *et al.*, 2007)**. Examination of 3 stool samples over not more than 10 days can improve the detection rate to 85-95% (**Fotedar *et al.*, 2007**). Stool leukocytes may be found, but in fewer numbers than in shigellosis (**Cheesbrough, 1987**). Stool examination findings in patients with amoebic liver abscess are usually negative (**Ryan and Ray, 2001**). Repeated stool sampling in patients with proven amoebic liver abscess is positive in 8-40% of cases (**Fotedar *et al.*, 2007)**. Identification of the parasite in a liver abscess aspirate is only 20% sensitive (**Fotedar *et al.*, 2007)**. The presence of intracytoplasmic red blood cells in trophozoites is diagnostic of *E. histolytica* infection, although recent studies demonstrated the same phenomenon with *E. dispar* (**Fotedar *et al.*, 2007).** The World Health Organization (WHO) recommends that intestinal amoebiasis be diagnosed with an *E. histolytica* -specific test, thus rendering the classic stool ova and parasite examination obsolete (**Fotedar *et al.*, 2007**).

2.4.3.4.1 Antigen detection

Enzyme-linked immunosorbent assay (ELISA) is used to detect antigens from *E. histolytica* in stool samples (**Fotedar *et al.*, 2007)**. Antigen-based ELISA kits using monoclonal antibodies against the GAL/GalNAc–specific lectin of *E. histolytica* (*E. histolytica* II, TechLab, Blacksburg, VA) yield an overall sensitivity of 71%-100% and specificity of 93%-100% (**Fotedar *et al.*, 2007**). One study showed a much lower sensitivity (14.2%). In patients with

amoebic liver abscess, serum and liver aspirate antigen detection using the same kit was shown to yield a sensitivity of 96% and 100%, respectively (**Fotedar *et al*., 2007)**. Other stool detection kits use monoclonal antibodies against the serine-rich antigen of *E. histolytica* (Optimum S kit, Merlin Diagniostika, Bornheim-Hersel, Germany) or against other specific antigens (*Entamoeba* CELISA-PATH, Cellabs, Brookvale, Australia; ProSpecT EIA, Remle Inc, Lenexa, KY). No specific antigen tests are available for the detection of *E .dispar* and *E. moshkovskii* from clinical samples (**Fotedar *et al*., 2007**).

2.4.3.4.2 Serology

Multiple serologic assays are available for the diagnosis of amoebiasis (**Fotedar *et al*., 2007**). ELISA is the most used assay throughout the world and is used to measure the presence of serum antilectin antibodies (IgG) (**Fotedar *et al*., 2007**). The sensitivity for detection of antibodies to *E. 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 7-10 days following infection (**Fotedar *et al*., 2007**). Immunofluorescent assay (IFA) is also rapid, reliable, and reproducible. In the setting of amoebic liver abscess, the sensitivity and specificity of IFA was shown to be 93.6% and 96.7%, respectively (**Fotedar *et al*., 2007**). Indirect hemagglutination (IHA) is very specific (99.1%) but is less sensitive than ELISA (**Fotedar *et al*., 2007)**. Immunoelectrophoresis, counter-immunoelectrophoresis (CIE), and immunodiffusion tests use the precipitation property of antigen-antibody complexes in agar (**Fotedar *et al*., 2007**). CIE is time-consuming but has shown a sensitivity of 100% in invasive amoebiasis (**Fotedar *et al*., 2007**). Complement fixation (CF) is less sensitive than other techniques (**Fotedar *et al*., 2007**). The seropositivity prevalence is very high in endemic areas, limiting antibody-based testing for

diagnosing currently active disease, since antibodies can persist for years after infection (**Fotedar *et al.*, 2007**).

2.4.3.4.3 Polymerase Chain Reaction (PCR)

Entamoeba histolytica can be identified in various types of clinical specimens, including faeces, tissues, and liver abscess aspirates (**Fotedar *et al.*, 2007**). A wide variety of polymerase chain reaction (PCR) methods targeting different genes, including a small-subunit rRNA gene (18S rDNA), 30-kDa antigen gene, serine-rich protein gene, chitinase gene, hemolysin gene, and extrachromosomal circular DNA, have been described for the detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii* (**Fotedar *et al.*, 2007**). Sensitivities can vary according to sampling and the specific target gene used. Performed on faeces, PCR yields a sensitivity that is similar to that of stool antigen-based assay (**Fotedar *et al.*, 2007**). PCR-based tests have been strongly endorsed by the WHO (**Fotedar *et al.*, 2007**). Application of PCR-based methods in routine diagnosis is still very limited, as the generation of nonspecific DNA fragments from environmental and clinical samples often leads to false-positive result (**Fotedar *et al.*, 2007**).

2.4.3.5 Treatment

Asymptomatic amoebiasis should be treated with a luminal agent (iodoquinol, paromomycin, diloxanide furoate) to eradicate infection (**Stanley, 2003**). This recommendation is based on two arguments: First, invasive disease may develop; second, shedding of *E. histolytica* cysts in the environment is a public health concern (**Stanley, 2003**). Asymptomatic *E. dispar* infections should not be treated, but education should be pursued since it is a marker of faecal-oral contamination. Amoebic colitis is first treated with a nitroimidazole derivative, followed by a

luminal agent to eradicate colonization (**Bhanu *et al*., 2011**; **Stanley, 2003**). Amoebic liver abscess can be cured without drainage and even by one dose of metronidazole (**Stanley, 2003**). Clinical defervescence should occur during the first 3-4 days of treatment (**Stanley, 2003**). Metronidazole failure may be an indication for surgical intervention (**Stanley, 2003**). Treatment with a luminal agent should also follow (**Stanley, 2003**). Disseminated amoebiasis should be treated with metronidazole, which can cross the brain-blood barrier (**Stanley, 2003**). Empirical antibacterial agents should be used concomitantly if perforated viscus is a concern (**Stanley, 2003**).

2.5. Helminthiasis

Helminths of medical importance in many parts of Africa can be divided into three groups: cestodes (tapeworms), trematodes (flukes) and nematodes (roundworms) (**Cheesbrough, 1992**). However, according to their shape, worms are divided into two groups: flat or round (**Ryan and Ray, 2004**). The flukes and the tapeworms are included in the group of the flatworms or *Platyhelminthes* (**Cheesbrough, 1992**). This is because they are usually flattened, bilaterally symmetrical, have no true body cavities, and are hermaphrodite (**Cheesbrough, 1992**). On the other hand, roundworms are zoologically distinct, more tubular and simple but the sexes are distinct (**Ryan and Ray, 2004**). Adult tapeworms have no digestive systems (**Ryan and Ray, 2004**). They absorb nutrients directly from the host's gut contents through their surfaces (**Ryan and Ray, 2004**). An adult tapeworm has a small head (scolex) with two or four suckers and usually a circle of hooks by which it attaches to the host's intestinal wall (**Cheesbrough, 1992**). It also has a body (strobila) which is generally long and tape like and consists of many units also referred to as proglottids (**Cheesbrough, 1992**). It is from the tape like structure of its body that

the tapeworm gets its name (**Ryan and Ray, 2004**). The larval stages occur in various organs and tissues of vertebrate intermediate hosts (**Ryan and Ray, 2004**).

The roundworms have a complete digestive system with a mouth and anus. Hookworms, *Ascaris*, *Trichuris* and *Strongyloides* are all soil-transmitted helminthes (**Cheesbrough, 1992**). *Enterobius* is transmitted directly by the faecal–oral route (**Cheesbrough, 1992**). The final host of all the worms is man except for the dog tapeworm for which the human is an accidental intermediate host (**Cheesbrough, 1992**). The eggs of all the intestinal worms are excreted in stools (**Cheesbrough, 1992**).

Sanitary disposal of human faeces is the preventive measure of choice because it will control most of these worm diseases with the exception of the hydatid disease, *enterobius* and schistosomiasis (**Cheesbrough, 2005**).

2.5.1 Ascariasis

Ascariasis is one of the major helminth diseases in developing countries (**Ryan and Ray, 2004**). The evidence of effectiveness of public health interventions to relieve its disease burden is worth attention (**Adams *et al*, 2006**). Ascariasis is an infection caused by *Ascaris lumbricoides*, the largest intestinal roundworm, reaching up to 40cm in length (**Cheesbrough, 1992**). It lives in the small intestine and the female produces up to 200,000 eggs which pass with feaces daily (**Cheesbrough, 1992**). It is one of the commonest nematode infestations of the small intestine (**Cheesbrough, 1992**). It does not appear to depend so much on the climate, although it is more

perennial in the damp and humid areas of the tropics (**Cheesbrough, 1992**). This explains why ascariasis is common in all areas of Africa (**Cheesbrough, 1992**).

2.5.1.1 Epidemiology

Ascaris lumbricoides infects an estimated population of about 1.3 billion people worldwide (**Ryan and Ray,2004**) and is a major cause of disease burden especially in developing countries, with an estimated loss of from 1.2 to 10.5 diability-adjusted life year per infected person (**Bethony *et al.*, 2006**). The parasite has a worldwide distribution and particularly in the tropics and sub tropics where environmental sanitation is inadequate and untreated human faeces are used as fertilizer (**Cheesbrough, 2005**). In 2002, WHO estimated that there were 1450 million persons infected with *A.lumbricoides* and annually 60000 dying from ascariasis (**Cheesbrough, 2005**). Heavy *Ascaris* infections can be found in children of 3-8years whose fingers become contaminated while playing on open ground (**Cheesbrough, 2005**).

2.5.1.2 Life cycle

Infection with the *Ascaris* parasite results when a person swallows food containing eggs of this parasite (**Cheesbroug, 1992**). The eggs have to be embryonated in soil before they are infective for a period of 8-50 days (**Cheesbrough, 1992**). The soil must be loose and not too dry (**Cheesbrough, 1992**). Oxygen must be available and the temperatures over 15 °C (**Ryan and Ray, 2004**). The embryonated eggs hatch when swallowed by a human being (**Cheesbrough, 1992**). The eggs can also pass through the gastrointestinal tract of animals and remain infective (**Ryan and Ray, 2004**). The usual vehicle is fruit or other food eaten raw (**Ryan and Ray, 2004**). Unwashed hands and children picking up things from the floor or ground and putting

them into the mouth are common ways of acquiring *Ascaris* (**Ryan and Ray, 2004**). In communities that use human faeces as manure, the possibility of ingesting the eggs from foodstuffs grown using this manure is very high (**Ryan and Ray, 2004**). This is especially after consumption of vegetables which are sometimes eaten raw or half cooked (**Cheesbrough, 1992**). Eggs can survive adverse circumstances for a long time, and embryonated eggs can be carried away from the contaminated place into houses by feet, footwear or in the dust by wind (**Ryan and Ray, 2004**). Temperatures above 60°C are necessary to destroy the eggs (**Cheesbrough, 1992**).

To reach maturity the larvae need to pass through the lungs (**Ryan and Ray, 2004**). The larvae penetrate the intestinal wall and reach the liver via the portal system (**Cheesbrough, 2005**). From the liver they are carried in the blood through the right side of the heart into the lungs (**Cheesbrough, 2005**). Here they penetrate into the airways and pass via the bronchiole, bronchi and trachea to the pharynx (**Cheesbrough, 1992**). Then they are swallowed, return to the gastrointestinal tract and settle in the jejunum where they develop into adult worms (**Cheesbrough, 1992**). During the lung phase, eosinophilia develops (**Ryan and Ray, 2004**). This eosinophilia is temporal if no new infestation occurs (**Ryan and Ray, 2004**). The migration phase may be associated with fever, cough, wheezing shortness of breath and allergic dermatitis (**Ryan and Ray, 2004**). Lung migration may also cause pneumonia (**Cheesbrough, 1992**). The life span of an adult worm is about 1 to 2 years (**Ryan and Ray, 2004**).

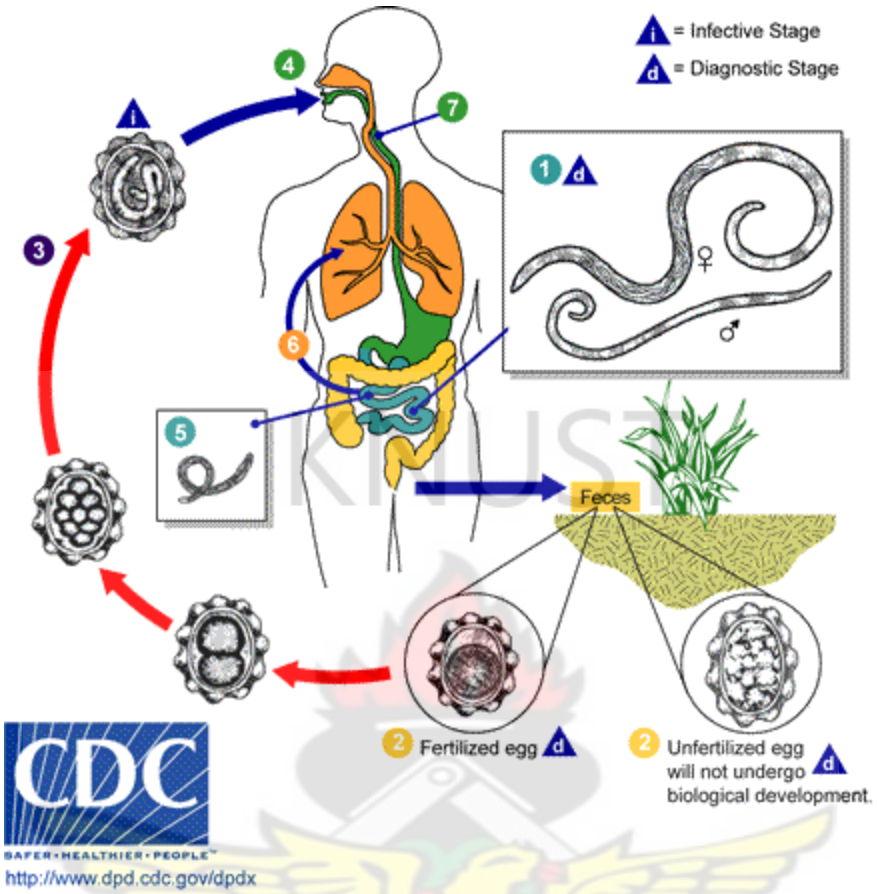

Figure 2.12 Life cycle of *Ascaris lumbricoides*

Source: Centres of Disease Control and Prevention

2.5.1.3 Clinical Manisfestation

Except for the temporary symptoms during larval migration through the lungs, infection with a few *Ascaris* is usually asymptomatic or if symptoms are present, they are not characteristic (**Ryan and Ray, 2004**). There may be vague abdominal discomfort (**Ray and Ryan, 2004**). Occasionally a worm may leave the body (in vomitus or stools) upsetting the patient and the family (**Cheesbrough, 1992**). Complications may occur in very heavy infections or due to wandering worms (**Ryan and Ray, 2004**). In some instances young *Ascaris* larvae migrate to the

lungs via hepatic blood vessels causing tissue destruction and if their numbers are large, enlargement and tenderness of the liver results (**Ryan and Ray, 1987**). This hepatitis is usually short-lived (**Ryan and Ray, 2004**). The diagnosis cannot be established until a few weeks later when the worms are mature and their eggs can be found in the host's faeces (**Cheesbrough, 2005**).

Intestinal obstructions may occur at the illio-caecal junction by a large ball of worms (**Cheesbrough, 2005**). Wandering worms may be provoked by tetra-chloroethylene, a drug used previously for hookworm treatment (**Ryan and Ray, 2004**). Wandering *Ascaris* may reach abnormal foci and cause acute symptoms (**Ryan and Ray, 2004**). For instance, if a person vomits worms, this may cause swelling of the glottis and larynx resulting in difficulties in breathing (**Ryan and Ray, 2004**). The wandering worms can also cause blockage of bile ducts resulting in obstructive jaundice (**Cheesbrough, 2005**). The worms can migrate into the liver tissue resulting in the formation of a liver abscess (**Cheesbrough, 1992**). The worms feed on the nutrients consumed by the host (**Cheesbrough, 1992**). Ascariasis may contribute to malnutrition states such as kwashiorkor and vitamin A deficiency (**Cheesbrough, 2005**).

2.5.1.4 Laboratory Diagnosis

Diagnosis is by stool microscopy which should show the characteristic *Ascaris* eggs (**Cheesbrough, 2005**). During the early lung phase, when eosinophilic pneumonia occurs, larvae can be found in sputum or gastric aspirates before diagnostic eggs appear in the stool (**Ryan and Ray, 2004**). A plain abdominal radiograph may reveal masses of worms in gas filled loops of bowel in patients with intestinal obstruction (**Ryan and Ray, 2004**).

2.5.1.5 Treatment

Ascariasis should always be treated to prevent potentially serious complications (**Ryan and Ray, 2004**). Both Mebendazole and Albendazole are effective, but are contraindicated in pregnancy and in heavy infections in which they may provoke ectopic migration (**Ryan and Ray, 2004**). Piperazine is safe in 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 100 mg twice daily for 3 days. Alternatively, Levamisole at 5mg/kg can be given as a single dose or Albendazole 400 mg stat (**Steinmann *et al.*, 2008**). Piperazine 150mg/kg can be given as a single dose and should not exceed 4g. Pyrantel pamoate is also highly effective (**Steinmann *et al.*, 2008**).

2.5.2 Hookworm Disease

Hookworm infection in humans is caused by two types of worms: *Necator americanus* and *Ancylostoma duodenale* (**Cheesbrough, 2005**). The two species overlap in many tropical regions (**Ryan and Ray, 2004**). In most areas, older children have the greatest incidence and intensity of hookworm infection (**Ryan and Ray, 2004**). Hookworms need a hot humid climate for their development (**Cheesbrough, 1992**). A minimum temperature of 18°C is required and a soil temperature of 20-32°C is optimal (**Ryan and Ray, 2004**). Hookworm infection is therefore, most common in the hot, humid areas of Africa (**Cheesbrough, 1992**).

Infection with hookworm disease may vary from asymptomatic infection to a chronic debilitating disease caused by severe iron deficiency anaemia, and in some cases loss of protein from the

bowel leading to oedema (**Ryan and Ray, 2004**). Heavy worm burden, a prolonged duration of infection and poor iron intake all contribute to the development of hookworm disease (**Cheesbrough, 1992**). Many people (hookworm carriers) harbour the worms without any ill effects (**Ryan and Ray, 2004**). Nutrition, daily iron intake and total worm burden determine whether a carrier develops anaemia (**Ryan and Ray, 2004**).

2.5 .2.2 Epidemiology

An estimated population of 1.3 billion is affected (**Ryan and Ray, 2004**). *N. americanus* is the common hookworm infecting man in the Far East, South Asia, Pacific Islands, Tropical Africa, Central and South America (**Cheesbrough, 1992**). *A. duodenale* is found in the Middle East, in countries around the Mediterranean, and North China but can also be found with *N. americanus* in Africa, South East Asia, the Pacific Islands and South America (**Cheesbrough, 1992**). As of 1990, an estimated 7% of the world's preschoolchildren (41 million), 26% of school age children (239million), and 44.3 million of the developing world are infected with Hookworm. One hundred and twenty four point point three (124.3) million pregnant women haboured hookworm infection (**Holland and Kennedy, 2002**).

2.5.2 .1 Life cycle

The eggs are already embryonated when passed out with faeces (**Kayser *et al*., 2001**). The eggs hatch into *rhabditiform* larvae which leave the faeces and bury themselves in moist damp soil where they change into the infective sheathed *filariform* stage (**Kayser *et al*., 2001**). The development from the non-infective *rhabditiform* larvae to the infective *filariform* stage occurs

over a period of 1-week (**Cheesbrough, 1992**). The *filariform* larvae may attach themselves to grass or hide in the soil (**Ryan and Ray, 2004**).

An infective *filariform* larva then penetrates the skin and reaches the lungs through the venous system and the right side of the heart (**Ryan and Ray, 2004**). There, they invade the alveoli and ascend the airways before being swallowed back and reaching the small intestine 3-5 days after penetrating the skin (**Kayser *et al.*, 2001**). The larvae develop into adults in the small intestine and stay attached onto the mucosa by hooks in their buccal cavity (**Cheesbrogh, 1992**). The period from skin penetration to appearance of eggs in the faeces is about 6 to 8 weeks, but it may be longer with *Ancylostoma duodenale* (**Ryan and Ray, 2004**). Larvae of *Ancylostoma duodenale,* if swallowed, can survive and develop directly in the intestinal mucosa into adults (**Cheesbrough, 1992**). Adult hookworms may survive over 10years but usually live 6 to 8 years for *A.duodenale* and 2 to 5 years for *Necator americanus* (**Ryan and Ray, 2004**).

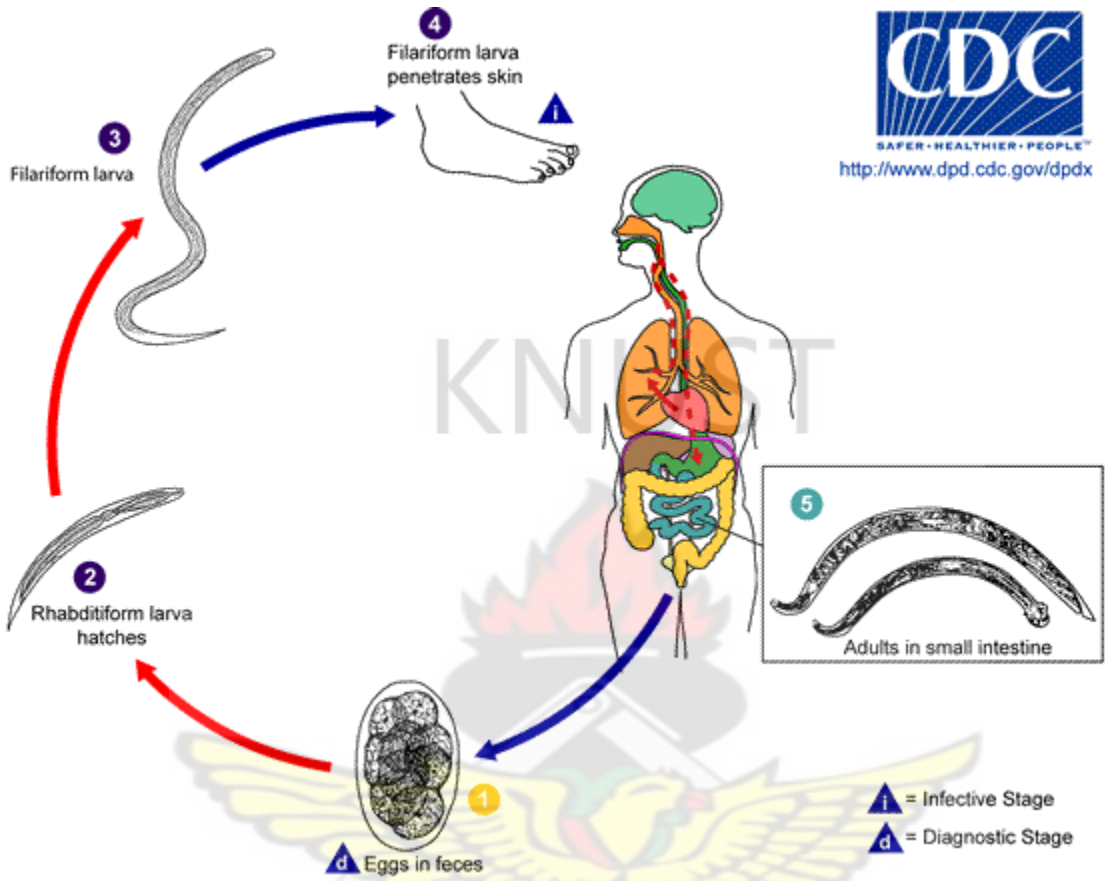

Figure 2.13 Life cycle of Hookworm

Source: Centres of Disease Control and Prevention

2.5.2 .3 Clinical manisfestation

Hookworm infestation is asymptomatic in the vast majority of cases (**Ryan and Ray, 2004**). Infective larvae may provoke an itch "ground itch" at the site of skin penetration (**Cheesbrough, 1992**). Itchy erythematous papules appear at the site as well as tracts of subcutaneous migration (similar to *cutaneous larva migrans*) (**Ryan and Ray, 2004**). This is most common between the toes and on the sole of the feet (**Holland and Kennedy, 2002**). The lung passage is also affected,

and there may be some coughing, wheezing, eosinophilia and occasionally mild transient pneumonia, but this condition develops less frequently in hookworm infection than in ascariasis (**Cheesbrough, 1992**).

In the digestive tract, there is dyspepsia, abdominal pain, distension, sometimes diarrhoea (**Cheesbrough, 1992**). In heavy infections, diarrhoea is mixed with blood (**Holland and Kennedy, 2002**). The symptoms may be mistaken for those of duodenal or gastric ulcers (**Ryan and Ray, 2004**). Iron deficiency anaemia, however, develops when a heavy hookworm load overtaxes the iron reserves (**Holland and Kennedy, 2002**). The hookworm suck blood from the intestines and this leads to loss of red blood cells, iron and protein, especially albumin, from the host (**Holland and Kennedy, 2002**). When the host's iron stores are depleted, iron deficiency anaemia sets in and symptoms related to anaemia appear (**Holland and Kennedy, 2002**). Symptoms are minimal if iron intake is adequate (**Ryan and Ray, 2004**). The evolution of this anaemia is slow and because of the physiological adjustments it evokes, the patient can continue to be up and about with a surprisingly low haemoglobin level, the so called "walking anaemia of hookworm" (**Ryan and Ray, 2004**).

2.5 .2.4 Laboratory Diagnosis

Diagnosis is established by the finding of the typical oval hookworm eggs in the stool (**Cheesbrough, 1992**). Differentiation of the two species from the eggs is not possible, but the adult worms can be distinguished (**Cheesbrough, 1992**). Stool concentration techniques may be required to detect light infections (**Cheesbrough, 1992**). In a stool sample that is not fresh, the eggs may have hatched to release *rhabditiform* larvae which need to be differentiated from those of *Strongyloides* (**Ryan and Ray, 2004**) Features of iron deficiency anaemia (hypochromic

microcytic anaemia), occasionally with eosinophilia or hypoalbuminemia are common in blood profile (**Holland and Kennedy, 2002**).

2.5 .2.5 Treatment and Prevention

Re-infection is very likely if the community as a whole does not improve its methods of faecal disposal (**Ryan and Ray, 2004**). Where anaemia is present, treatment should aim at eliminating the worms and correcting the anaemia (**Ryan and Ray, 2004**). Iron deficiency anaemia is treated with oral iron for at least 3 months (**Holland and Kennedy, 2002**). A high protein diet is necessary to replace protein loss (**Holland and Kennedy, 2002**). Even when the anaemia is severe, patients respond quickly and well to 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**).

According to **Steinmann *et al*. (2008)** hookworms can be eradicated by the use of several safe and highly effective antihelminthic drugs including:

- Mebendazole 100mg twice daily for 3 days;
- Pyrantel Pamoate 10mg/kg body weight daily for 3 days;
- Levamisole 3 tablets stat. This can be used in mixed infections. The disadvantage with it is that it is expensive and not very effective against *Necator Americanus*, the more common hookworm;
- Bephenium – This drug is more expensive than Levamisole and less effective with *Necato rAmericanus.*

Prevention requires improved sanitation.

2.5.3 Strongyloidiasis

Strongyloidiasis is an infection caused by *Strongyloides stercoralis*, a nematode which is distinguished by its ability to replicate in the human host (**Ryan and Ray, 2004**). This unusual behaviour among helminthes enables ongoing cycles of autoinfection due to the internal production of infective larvae (**Ryan and Ray, 2004**). The infection thus can persist for ages without further exposure of the host to external infective larvae (**Cheesbrough, 2005**). *Strongyloides stercoralis* is distributed in tropical areas and other hot humid regions and is particularly common in sub-Sahara Africa (**Cheesbrough, 1992**).

The adult female worms live in the mucosa of the duodenum and jejunum (**Cheesbrough, 2005**). Most infections are asymptomatic (**Cheesbrough, 1992**). *Strongyloides* infection may remain quiescent for many years and become reactivated during immunosuppressive chemotherapy or when the host is immunocompromised (**Ryan and Ray, 2004**). This can cause severe and even fatal disease (**Cheesbrough, 1992**).

S. stercoralis has been shown to be endemic in developing countries, where it is associated with situations that lead to immunodeficiency including HIV infections (**Gomez *et al.*, 1995**). The transformation of rhabditiform larvae into infective filariform larvae, in the intestine of the carrier, could also favour interpersonal transmission of the parasite; these larvae present in the anorectal region of an individual, man or woman could penetrate the penis of the partner during anal sexual intercourse (**Dias *et al*, 1992**). Thus, men would have a greater chance of being and/or reinfected by having sodomy relations with partners of both sexes (**Dias *et al.*, 1992**).

2.5 .3.1 Epidemiology

It is known that tens of millions of people are infected with *Strongyloides* worldwide (**Keiser and Nutman, 2004**) and mostly found in the Tropical and Sub tropical region but cases have been reported in the temperate zones (**Walzer *et al*., 1982**). Occupations that increase contact with soil contaminated waste which may include farming and coal mining depending on local practices, increase the risk of infections (**Walzer *et al*., 1982**). Different prevalences among ethnic groups may simply reflect behavioral or socioeconomic factors but some have suggested that different skin types may be more or less resistant to larva penetration (**Keiser and Nutman, 2004**). Hyperinfection syndrome of *Strongyloides* has a mortality rate ranging from 15% to as high as 87% (**Marcos *et al*., 2008**).

2.5.3.2 Life Cycle

Eggs hatch in the small intestinal mucosa, releasing rhabditiform larvae that migrate to the lumen and pass with the stool into the soil (**Cheesbrough, 1992**). *Rhabditiform* larvae in the bowel lumen can also develop directly into *filariform* larvae that penetrate the colonic wall or perianal skin and enter the circulation to repeat the migration that establishes ongoing internal re-infection (**Ryan and Ray, 2004**). This autoinfection cycle allows the infection to persist long after the host has left the endemic area (**Ryan and Ray, 2004**).

Rhabditiform larvae passed onto the soil with faeces transform into infective *filariform* larvae directly which penetrates the skin or may develop into free-living adults which continue to reproduce outside the body (**Cheesbrough, 2005**). The larvae then travel through the venous

circulation to the lungs where they break into the alveolar spaces, ascend the bronchial tree, are swallowed and thereby reach the small intestines (**Ryan and Ray, 2004**). Here the larva matures into the adult worm that penetrates the mucosa and they can then hatch eggs to perpetuate the cycle of infection (**Cheesbrough, 2005**).

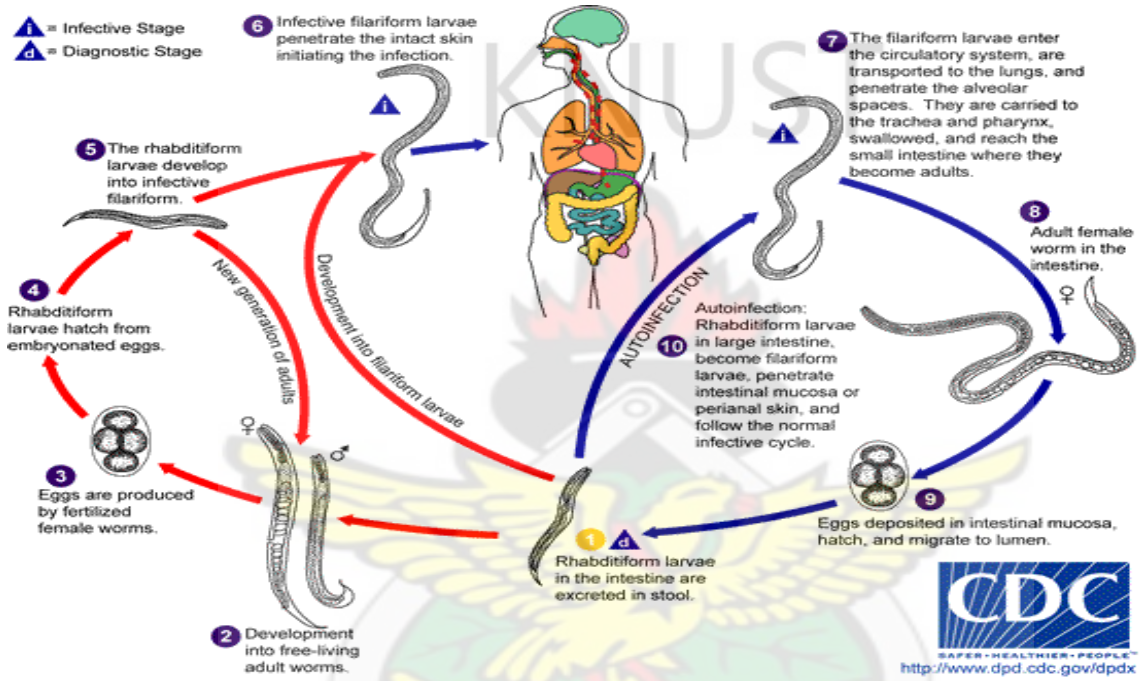

Figure 2.14 Life cycle of *Strongyloides stercoralis*

Source: Centres of Disease Control and Prevention

2.5.3.3 Clinical Manisfestation

Usually the strongyloidiasis infection is asymptomatic, or has 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**). Migrating larvae can elicit a pruritic raised erythematous lesion that advances rapidly along the course of larval migration (**Ryan and Ray, 2004**). This is known as l*arva currens (*running larva) (**Ryan and Ray, 2004**).

Pulmonary symptoms are rare in light infections (**Ryan and Ray, 2004**). Adult worms residing in the small intestine mucosa can cause abdominal pain which is worsened with food ingestion (**Ryan and Ray, 2004**). Nausea, diarrhoea, gastrointestinal bleeding and weight loss can occur (**Cheesbrough, 2005**).

In disseminated infection, apart from the gastrointestinal and lung tissues, larvae may also invade the central nervous system, peritoneum, liver and kidney (**Cheesbrough, 2005**). Bacteremia may also develop due to the entry of enteric flora through disrupted mucosal barriers (**Ryan and Ray, 2004**). In immunocompromised patients, transformation to filariform stage occurs within the gut itself, producing marked autoinfections and hyperinfection (**Ryan and Ray, 2004**)

2.5.3.4 Laboratory Diagnosis

Diagnosis is by observing the larvae in a fresh stool specimen. The eggs are almost never detectable because they hatch in the intestines (**Cheesbrough, 2005**). Single stool examination will detect about one-third of uncomplicated infections, in which there are usually few larvae passed (**Ryan and Ray, 2004**). Serial stool examination is thus encouraged or advocated (**Cheesbrough, 1992**). An ELISA for antibodies to excretory-secretory antigens of *Strongyloides* is a sensitive method of diagnosing uncomplicated infections (**Cheesbrough, 2005**).

2.5.3.5 Treatment

Even in the asymptomatic state, treatment must be given because of the potential for fatal hyper infection (**Steinmann *et al*., 2008**). The drugs of choice are Mebendazole 100mg twice daily for 3 days or Thiabendazole 25mg/kg body weight twice daily for 2 days (**Steinmann *et al*., 2008**).

However, in disseminated disease, treatment should be extended for 5-7 days (**Steinmann *et al*., 2008**). Albendazole 400mg/kg for 3 days is also effective. Levamisole is only effective in about 50% of the cases and is therefore not the drug of choice (**Steinmann *et al*., 2008**).

2.5.4 *Enterobius vermicularis*

The adult female is a 10mm long, cream-colured worm with a sharply pointed tail, characteristics that have given rise to the common name pinworm (**Cheesbrough, 1992**). Running longitudinally down both sides of the body are small rigges that widen anteriorly to fin-like algae (**Ryan and Ray, 2004**). The seldom-seen male is smaller (3mm) and possesses a ventrally curved tail and copulatory spicule (**Cheesbrough, 1992**). The clear thin shelled, ovoid eggs are flattened on one side and measure 25 by 50 µm (**Ryan and Ray, 2004**).

2.5.4.1 Epidemiology

The pinworm is the oldest and the most widespread of the helminths (**Ryan and Ray, 2004**). Eggs have been found in 10000-year-old coprolith, making this nematode the oldest demonstrated infectious agent of humans (**Ryan and Ray, 2004**). It has been estimated to infect at least 200 million people,

particularly children, worldwide (**Ryan and Ray, 2004**). Infection is more common among the young and poor, but may be found in any age or economic class (**Ryan and Ray, 2004**).

The eggs are relatively resistant to desiccation and may remain viable in the linens, bed-clothes, or house dust for several days (**Ryan and Ray, 2004**). Once infection is introduced into a household, other family members are rapidly infected (**Ryan and Ray, 2004**).

2.5.4.2 Life cycle

The adult worms lie attached to the mucosa of the caecum (**Cheesbrough, 1992**). As its period of gravidity draws to a close, the female migrates down the colon, slips unobserved through the anal canal in the dark of the night, and deposit as many as 20000 sticky eggs on the host's perianal skin, bedclothes, and linens (**Ryan and Ray, 2004**). The eggs are near maturity at the time of deposition and become infectious shortly after (**Cheesbrough, 1992**). Handling of bedclothes or scratching of the perianal area to relieve the associated itching results in adhesion of the eggs to the fingers and subsequent transfer to the oral cavity during eating or other finger-mouth maneuvers (**Ryan and Ray, 2004**). Alternatively, the eggs may be taken into the air (eg, during making of the bed), inhaled, and swallowed (**Cheesbrough, 1992**). The eggs subsequently hatch in the upper intestine and the larvae migrate to the caecum, maturing to adults and mating in the process (**Cheesbrough, 1992**). The entire adult-to-adult cycle is completed in 2 weeks (**Ryan and Ray, 2004**).

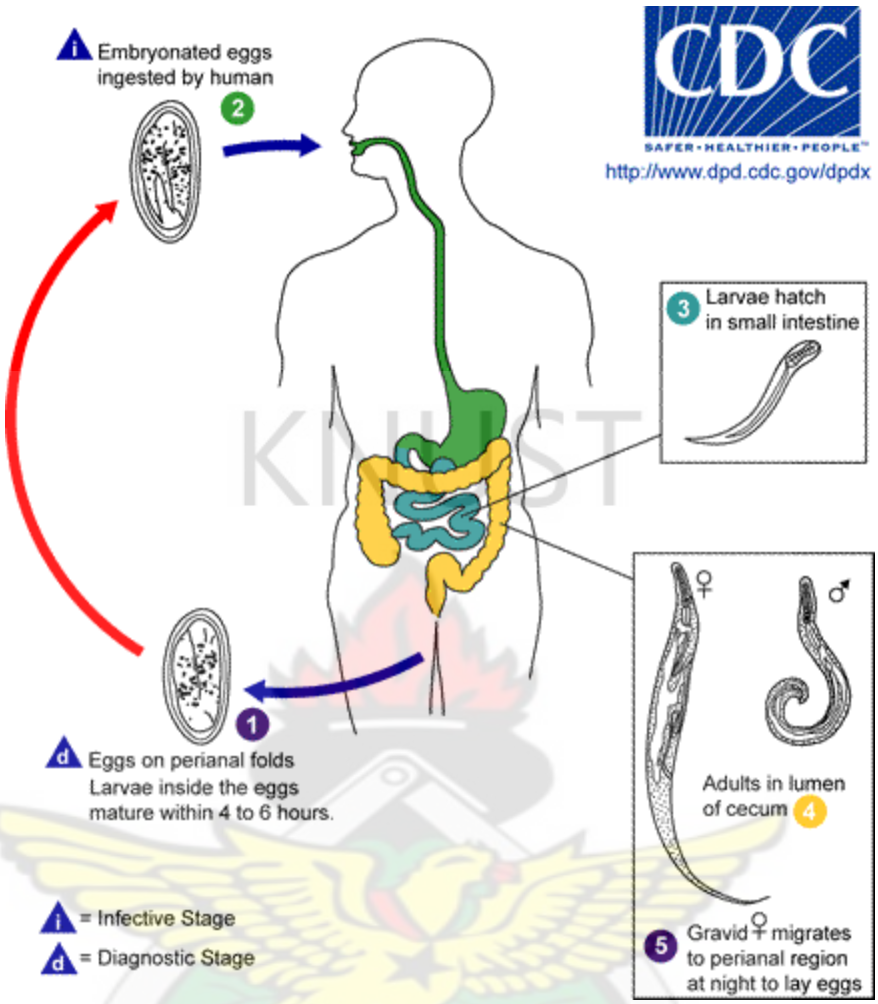

Figure 2.15 Life cycle of *Enteriobius vermicularis*

Source: Centres of Disease Control and Prevention

2.5.4.3 Clinical Manifestation

Enteriobius vermicularis seldom produces serious disease (**Ryan and Ray, 2004**). The most frequent symptom is pruritis ani (anal itching) (**Ryan and Ray, 2004**). This symptom is most severe at night and has been attributed to the migration of the gravid female (**Ryan and Ray, 2004**). It may lead to irritability and other minor complaints (**Ryan and Ray, 2004**). In severe infections, the intense itching may lead to scratching, excoriation, and secondary bacterial

infections (**Ryan and Ray, 2004**). In female patients, the worm may enter the genital tract, producing vaginitis, granulomatous endometritis, or even salpingitis (**Ryan and Ray, 2004**). It has also been suggested that migrating worms might carry enteric bacteria into the the urinary bladder in young women, inducing an acute bacterial infection of the urinary tract (**Ryan and Ray, 2004**). Although this worm is frequently found in the lumen of the resected appendix, it is doubtful that it plays a causal role in appendicitis (**Ryan and Ray, 2004**).

2.5.4.4 Laboratory Diagnosis

Eosinophilia is usually absent (**Ryan and Ray, 2004**). The diagnosis is suggested by the clinical manifestations and confirmed by the recovery of the characteristic eggs from the anal mucosa (**Ryan and Ray, 2004**). Identification is accomplished by applying the sticky side of cellophane tape to the mucocutaneous junction, then transferring the tape to a glass slide and examining the slide under the low power lens of a microscope (**Ryan and Ray, 2004**). Occasionally, the adult female is seen by a parent of an infected child or recovered with the cellophane tape procedure (**Cheesbrough, 2005**).

2.5.4.5 Treatment and Prevention

Several highly satisfactory agents, including pyrantel pamoate and mebendazole, are available 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, retreatment after 2 weeks is recommended (**Ryan and Ray, 2004**). Although cure rate are high, reinfection is extremely common (**Ryan and Ray, 2004**).

2.5.5 *Trichiuris trichura*

The adult whipworm is 30 to 50 mm in length (**Ryan and Ray, 2004**). The anterior two thirds is thin and threadlike, whereas the posterior end is bulbulous, giving the worm the appearance of a tiny whip (**Ryan and Ray, 2004**). The tail of the male is coiled; that of the female is straight. The female produces 3000 to 10000 oval eggs each a day (**Ryan and Ray, 2004**). They are of the same size as pinworm eggs but have a distinctive thick brown shell with translucent knobs on both ends (**Ryan and Ray, 2004**).

2.5.5.1 Epidemiology

Although it is less widespread than pinworm, the worm is cosmopolitan parasite, infecting approximately 1 billion people throughout the world (**Ryan and Ray, 2004**). It is concentrated in areas where indiscriminate defaecation and warm, humid environment produce extensive seeding of soil with infectious eggs (**Ryan and Ray, 2004**). In tropical climates, infection rates may be as high as 80% (**Ryan and Ray, 2004**). Though the intensity of infection is generally low, adult worms may live 4 to 8 years (**Ryan and Ray, 2004**).

Attachment of the worms to the colonic mucosa and their subsequent feeding activities produce localized ulceration and haemorrhage (0.005ml blood per worm per day) (**Ryan and Ray, 2004**). The ulcers provide enteric bacteria with a portal of entry to the blood stream, and occasionally a sustained bacteremia results (**Ryan and Ray, 2004**). A decrease in the prevalence of trichuriasis in the postadolescent period and demonstration of acquired immunity in experimental animal infections suggest that immunity may develop in naturally occurred human infections (**Ryan and**

Ray, 2004). An Ig E-mediated immune mucosal response is demonstrated in humans, but is insufficient to cause appreciable parasite expulsion **(Ryan and Ray, 2004**)

2.5.5.2 Life cycle

Trichuris trichura has a life cycle that differs from that of the pinworm only in its external phase (**Ryan and Ray, 2004**). The adults live attached to the colonic mucosa by their thin anterior end (**Ryan and Ray, 2004**). While retaining its position in the caecum, the gravid female releases its eggs into the lumen of the gut (**Ryan and Ray, 2004**). These pass out of the body with the faeces and, in poorly sanitized areas of the world, are deposited on soil (**Ryan and Ray, 2004**). The eggs are immature at the time of passage and must incubate for at least 10 days before they become fully embryonated and infectious (**Ryan and Ray, 2004**). Once mature, they are picked up on the hands of children at play or of agricultural workers and passed to the mouth (**Ryan and Ray, 2004**). In areas where human faeces are used as fertilizer, raw fruits and vegetables may be contaminated and later ingested (**Ryan and Ray, 2004**). Following ingestion, the eggs hatch in the duodenum, and the released larvae mature for approximately one month in the small bowell before migrating to their adult habitat in the caecum (**Ryan and Ray, 2004**).

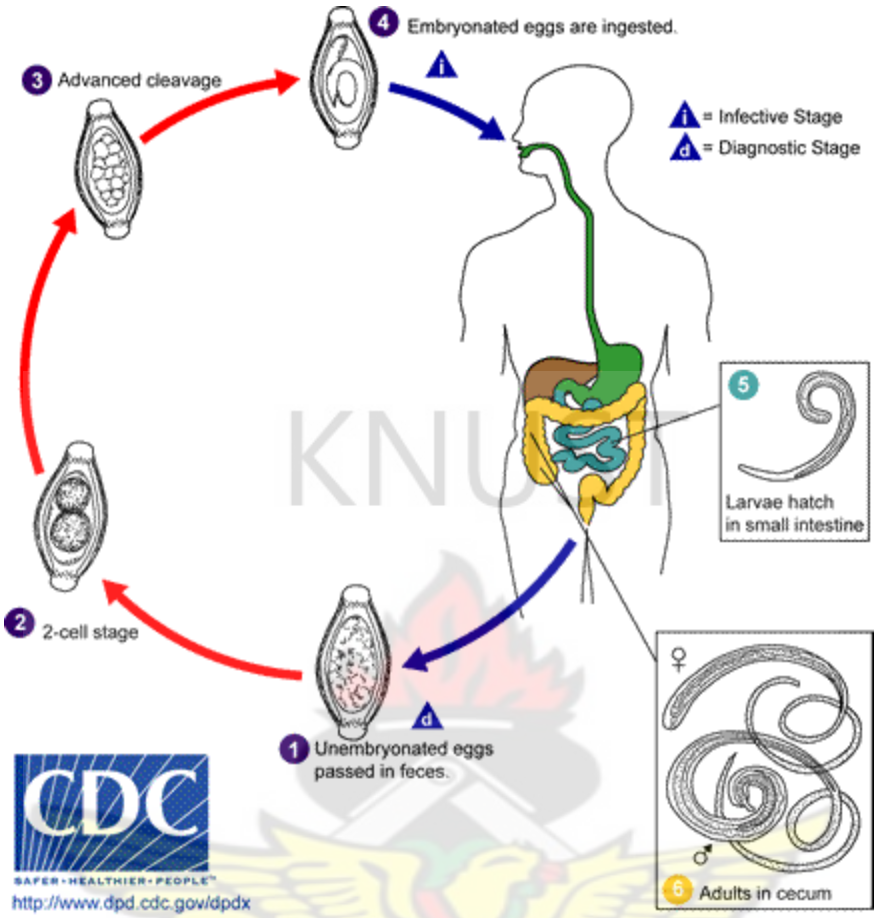

Figure 2.16 Life cycle of *Trichuris trichura*

Source: Centres of Disease Control and Prevention

2.5.5.3 Clinical Manifestations

Light infections are assymptomatic (**Ryan and Ray, 2004**). With moderate worm loads, damage to the intestinal mucosa may induce nausea, abdominal pain, diarrhoea, and stunting growth (**Ryan and Ray, 2004**). Occasionally, a child may harbor 800 worms or more. In these situations, the entire colonic mucosa is parasitized, with significant mucosal damage, blood loss, and anaemia (**Ryan and Ray, 2004**). The sheer force of the faecal stream on the bodies of the worms may produce prolapsed of the clonic or rectal mucosa through the anus, particularly when the host is straining at defaecation or during child birth (**Ryan and Ray, 2004**).

2.5.5.4 Laboratory Diagnosis

In light infections, stool concentration methods may be required to recover the eggs (**Ryan and Ray, 2004**). Such procedures are almost never necessary in symptomatic infections, as they inevitably produce more than 10000 eggs per gram of faeces, a density readily detected by examining 1 to 2 mg of emulsified stool with the lower-power lens of a microscope (**Ryan and Ray, 2004**). A moderate eosinophilia is common in such infections (**Ryan and Ray, 2004**).

2.5.5.5 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 to 70%, more than 90% of the adult worms are usually expelled, rendering the patient asymptomatic (**Ryan and Ray, 2004**). Prevention requires improvement of sanitary facilities (**Ryan and Ray, 2004).**

2.6 Overview of enteric parasites in HIV/AIDS

A survey on intestinal parasites in a rural area of Tanzania revealed the presence of eight protozoa and seven helminths in 287 (81.8%) subjects (**Gomez *et al*., 1995**). The prevalence of *E. histolytica* in HIV negative and positive patients was 25.1% and 12.5% respectively ($p<0.01$). *Ascaris lumbricoides* was also higher in HIV-negative (10.5%) than in HIV-positive patients (3.7%) ($p<0.04$) (**Gomez *et al*., 1995**). On the other hand, *C. parvum, I. belli* and *S. stercoralis* prevalence were higher in HIV-positive than in HIV-negative patients ($P < 0.01$) (**Gomez *et al*., 1995**). The prevalence of these two opportunistic protozoa was also higher in AIDS patients than

in HIV-positive patients without AIDS. Specific anti-*C. parvum* IgG were detected by ELISA in 18 % and 56 % of HIV-negative and positive patients, respectively, confirming the high number of contacts between this parasite and humans (**Gomez *et al.*, 1995**). Specific anti-*Encephalitozoon cuniculi* and anti-*Encephalitozoon hellem* IgG were detected by IFA in 18 % and 19 % of subjects, respectively, without any correlation with HIV and malaria infections (**Gomez *et al.*, 1995**).

In Zambia, prevalence of intestinal helminth was 24.9% in HIV-infected adults (**Modjarrad *et al.*, 2005**). Thirty-nine (52.7%) were infected with *A. lumbricoides*, and 29 (39.2%) were infected with hookworm predominantly, even though *S. stercoralis, S. mansoni, Hyemenolepsis nana* and *Taenia* sp. were on the low side (**Modjarrad *et al.*,2005**).

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 *Enterocytozoon bineusis* recorded a prevalence of 5.6% followed by *Blastocystis, C. cayetanensis* and *I. belli* having a prevalence of 2.2, 1.1 and 1.1% respectively (**Saksirisampant *et al.*, 2009**). The only helminth infection detected was *Opistorchis sinensis* which recorded a prevalence of 2.2% (**Saksirisampant *et al.*, 2009**). In an earlier study carried out among HIV patients with diarrhoea, *Cryptosporidium* (19.2%) was the predominant parasite followed by *I. belli* (14.5%), *G. lamblia* (3.8%), *E. histolytica* (0.9%) and *Iodamoeba butschlii* (0.3%) (**Saksirisampant *et al.*, 2009**).

In a study carried out among HIV patients in Northern India, out of 36 patients (30%) who tested positive for intestinal parasites, *Cryptosporidium* was the most common parasite (10.8%) followed by *G. lamblia* (8.3%) (**Mohandas *et al.*, 2002**). *C. cayetanensis* and *Blastocystis hominis* each were detected in 3.3% of the patients, while *I. belli* and *Enterocytozoon bieneusi* were each detected in 2.5% of the patients (**Mohandas *et al.*, 2002**). The other parasites observed were *E. histolytica*/*E. dispar* in two cases and hookworm ova in one patient (**Mohandas *et al.*, 2002**). Of the 36 patients who tested positive for intestinal parasites, 27 (75%) had diarrhoea (**Mohandas *et al.*, 2002**). The most common parasite, which was associated with diarrhoea, was *C. parvum* (**Mohandas *et al.*, 2002**). Previous study in Northern India centred on HIV patients presenting with diarrhoea reported *I. belli* as the most frequent parasite, followed by *Cryptosporidium* (**Prasad *et al.*, 2000**).

Sarfati *et al.* (2006) studied the prevalence of intestinal parasites in 154 HIV-infected adults in Cameroon. They found a prevalence rate of 33%. Opportunistic protozoa were found in 9.7% in patients although 53% showed diarrhoea. Of the protozoa spp. found *Enterocytozoon bineusis* was found in 8(5.1%) patients, *C. parvum* in 6(3.9%) patients and *I. belli* in 3(1.9%) patients (**Safarti *et al.*, 2006**). A higher prevalence (32%) of opportunistic protozoa among patients with CD4 T- cell count less than 50/mm^3 was recorded (**Safarti *et al.*, 2006**). Half of the patients that had *Cryptosporidium* infection had diarrhoea (**Safarti *et al.*, 2006**).

There is a significant reduction of *S. stercoralis*, *A. lumbricoides*, Hookworm, *Trichuris trichiura*, *G. lamblia*, *E. histolytica*, *I. belli* and *Cryptosporidium* in the advent of Anti-Retroviral Therapy (**Bachur *et al.*, 2008**). Anti-Retroviral Therapy helps in the control of HIV infection and

in 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 (**Willemot and Klein, 2004**). This was clearly demonstrated by **Bachur *et al*.(2008)** when his work revealed a remarkable reduction in the number of parasitic infections among HIV patients in this Anti-Retroviral Therapy era (**Bachur *et al*.,2008**).

CHAPTER THREE-MATERIALS AND METHODS

3.1 Parasitological survey

A pre-survey was made to prospective study sites (Hospitals) within the Ashanti Region during which consultations and discussions were held with the medical superintendents and the heads of the Laboratories of the hospitals to help in mobilization of patients for sample collection and administration of questionnaires. Written permission was sought from the heads of the selected Hospitals.

3.2 Study Type

This study was designed to determine the patterns of intestinal parasitic infections in HIV/AIDS individuals and its relationship with diarrhoea and CD4 T- cell counts. Hence a cross sectional study was conducted in selected Voluntary Counseling and Testing centres (VCT) of Nyinahin Government and St. Patrick's Hospital located in a rural and periuban areas respectively within the Ashanti Region of Ghana from April - July, 2011.

3.3 The Study area/population

Ashanti Region lies approximately between longitude 0.15' to 2.25' west and latitude 5.50' to 7.40' north. It has common boundaries with Brong Ahafo Region in the north, Central Region in the south, Eastern Region in the east and Western Region in the west. The Region has a land size of 24,390sq km representing about 10.2% of the land area of Ghana.

Ashanti is the most heavily populated region in Ghana, with a population of 4,881,738 in 2009 (Projection from the 2000 Housing and Population Census, Ghana Statistical Service). It has a population density of 169.3 per sq. km. The region has 27 districts and 132 sub-districts. Kumasi has the highest population of 1,559,807 (32.4%) of the regional total. About 47% of the population is in the rural areas. About 51.3% of the people live in urban settlements. The region has abundant food supplies. These include plantain, maize, cassava, cocoyam, yam, vegetables and other cereals and legumes. The cash crops grown include cocoa, oil palm, tobacco, cotton, citrus and cashew. It is also endowed with large deposits of gold and bauxite.

Ashanti Regional Map showing Metro Municipal and Districts

Figure 3.1 Source: Regional Health Directorate, Ashanti Region

The study population was made up of HIV positive patients of the following ART centres and HIV negative patients (control group) attending Nyinahin Govenment Hospital located in the Atwima Mponua District (rural area) and St. Patrick's Hospital located in Offinso Municipal (periurban). The selected sites were chosen based on their demographic characteristics. Atwima Mponua District is situated in the Western part of Ashanti Region and has Nyinahin as its capital. It shares borders with Ahafo Ano South in the North, Amansie West and Atwima Nwabiagya in the West, Bibiani Anhwiaso – Bekwai of the western Region, and Asunafo South

in Brong Ahafo Region in the East. The District lies between longitude 2.00' and 2.23' west, and latitude 6.32' and 6.75' north. The District lies within the wet-semi equatorial zone marked by double maxima rainfall. The rainfall ranges between 170 and 185 cm. Temperatures are fairly uniform ranging between 27ºC (August) and 31ºC (March). Relative humidity is generally high throughout the year. The predominant vegetation found in the district is of the semi – deciduous forest type. The vegetation has largely been disturbed by human activities depriving the district of its valuable tree species and other forest products. There are, however, large areas of forest reserves. These include Asanayo Forest Reserve, Gyemara Forest Reserve and Tano Offin Forest Reserve.

Offinso Municipality is bounded by four districts; to the north, Offinso North, on the south Afigya Kwabre; on the east, Atwima Nwabiagya; and on the west, Ahafo Ano South. Politically, Offinso Municipality is divided into three (3) sub districts namely; Abofour, Bonsua and Offinso Central. The Municipal is one of the (27) districts in Ashanti Region, located along the main Kumasi – Techiman trunk road. Formally, it was the old Offinso District, which presently has its Northern part carved out to be the new Offinso north district. The old district had a land total area of 1254 square km. The climate is typically wet equatorial with the major rainy season running from late February to early July and the minor from mid September to early November. The dry season is at its peak in the months of December and January to 30°C in March. The vegetation can be described as mostly semi-deciduous forest with several trees. Majority of the populace are engaged in farming and trading. Main crops cultivated are; cocoa, plantain, yam, cocoyam, maize and tomatoes. The main occupation of the citizens are trading and farming.

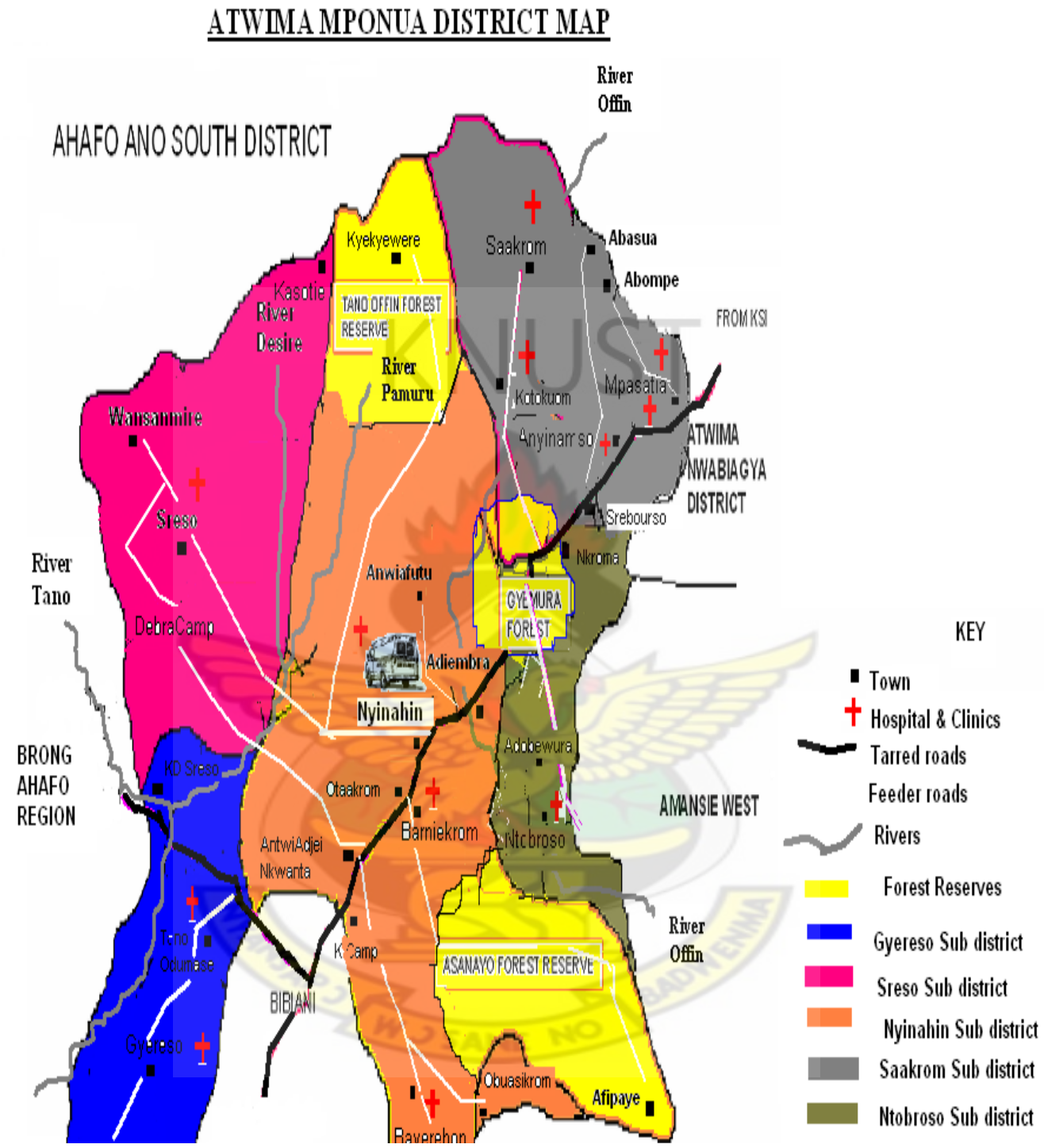

Figure 3.2 Source: Atwima Mponua District Health Directorate, Nyinahin

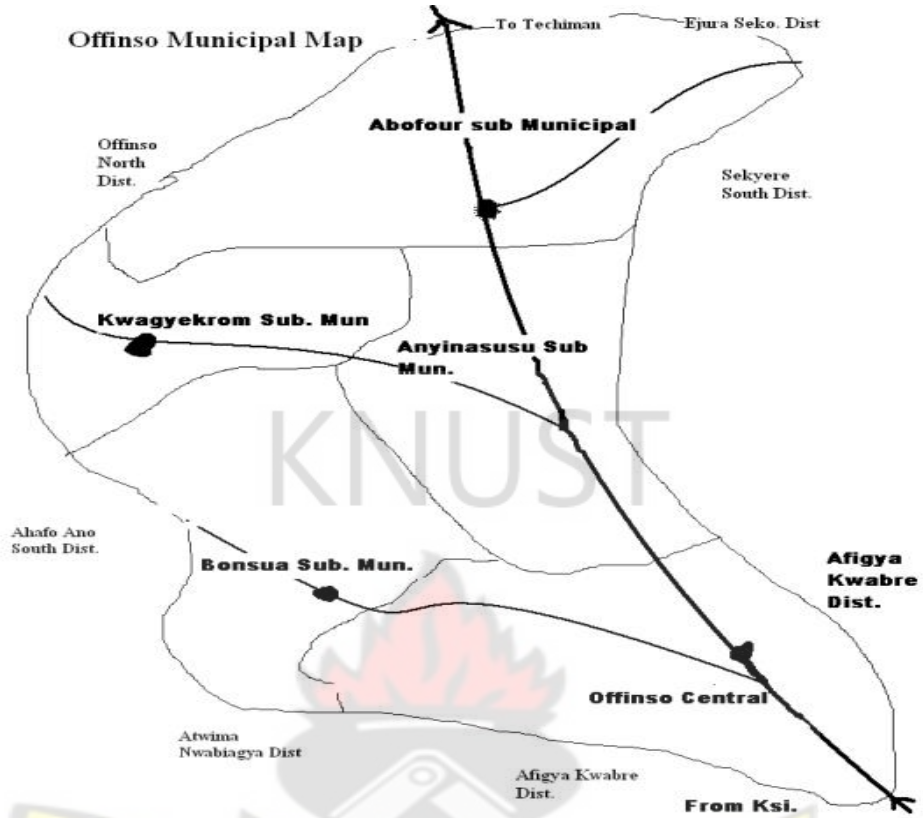

Figure 3.3 Source: Offinso Municipal Health Directorate

3.4 Sample size

A sample size of 343 participants had been estimated for data collection in HIV patients. An overall prevalence of 33% intestinal parasites was chosen based on a cross sectional study of HIV patients in Cameroon (**Sarfati *et al.*, 2006**). The sample size was determined using the formula:

$$N=z^2 (p*q)/d^2$$

Where n = sample size, z = Statistical certainty chosen = (1.96), p= Estimated prevalence of intestinal parasites among HIV positive patients, q = 1- p, d = precision desired.

The following assumptions were made:

Z (Statistical certainty chosen) = 1.96

d (precision desired) = 0.05

p (estimated prevalence) = Prevalence of intestinal parasites among HIV patients in Cameroon is 33% (0.33)(**Sarfati *et al*., 2006**)

Substituting into the formula:

$N = (1.96)^2(0.33)(0.67)/(0.05)^2$

= 0.84937776/0.0025

=339.75

Assuming a response rate of 99%

Sample size = 339.75/0.99

=343.18

=343 subjects to the nearest whole number

To assess if a trend of intestinal parasitic infection occurrence is evident among HIV positive patients, an equal sample size of 343 was chosen for HIV negative participants which served as a control group. Therefore a total sample size of 686 participants was selected. The ratio of cases to control was therefore 1:1.

3.5 Data collection tools and technique

Questionnaire was administered to retrieve information on age, sex, history of antiparasitic and Anti-Retroviral Therapy and toilet facilities.

3.6 Ethical issues

The study was conducted with the approval of the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Sciences, Kwame Nkrumah University of Science

and Technology. An informed written consent of each participant prior to inclusion in the study was also obtained. Subsequently proxy consent forms for participants of 17years and below were administered seeking parental consent. Participants were also informed that they were free to withdraw consent anytime and their medical records and specimens were examined and treated with strict confidentiality. Study participants who had parasites in their stool samples and those that had episodes of diarrhoea were treated free of charge based on the Ghana Health Service treatment guidelines. The drugs were administered by the prescribers working at the study sites. The full cost of treatment of each participant was absorbed by the research team.

3.7 Sample collection

Stool samples voided in the morning by participants were collected in a clean, wide mouth, and well capped container. Blood collected in EDTA anticoagulant tubes was used to estimate the CD4 T-cell count on the FACS Count machine and to screen for HIV I&II using First Response and Oral Quick.

3.8 Experimental Design

This cross sectional study was conducted on 672 participants (HIV positive and HIV negative) who accepted to be enrolled during their visit to Nyinahin Hospital and ST. Patrick's Hospital, Offinso between April and July, 2011. Participants aged between 8 and 72 years previously enrolled in the ART clinic and all other new patients who were admitted to the clinic upon a Voluntary Counselling and Testing (VCT) were asked to volunteer for this study and to provide 2 stool samples on 2 consecutive days for the detection of ova, larvae, flagellates and cyst of parasites, regardless of the presence of diarrhoea, during their scheduled visit in the cohort. For

each patient, data regarding age, sex, use of antiretroviral drug and cotrimoxazole and CD4 T-cell counts were estimated. The control group consisted of participants attending the general Out Patient Department (OPD) of the respective Hospitals who were selected using random sampling. Participants in this group were enrolled after laboratory investigations had confirmed that they were HIV negative. About 1g of two (2) consecutive stool samples voided in the morning by participants was collected in clean screw capped containers. For all prospective HIV positive participants, after stool specimen collection, blood specimens were taken for CD4 T-cell count.

All participants who tested positive for parasites or other abnormalities in stool that suggested a disease condition were treated based on consultations with the Specialists in the study centres. The drugs of choice included; albendazole, mebendazole, metronidazole, paromomycin and co-trimoxazole depending on the type of parasitic infection.

3.9 Laboratory tests

3.9.1 Stool examination- Direct Microscopy

A total of 1,334 stool samples collected from 672 participants (which included 341 and 331 HIV positive and HIV negative participants respectively) irrespective of diarrhoea in Atwima Mponua District Hospital, Nyinahin and ST. Patrick's Hospital, Offinso were subjected to routine stool examination for helminthic ova and larvae; protozoan cyst and oocyst of *I. belli*; and trophozoites. Direct wet mount of stool in normal saline (0.85% NaCl) were prepared immediately upon arrival at the laboratory and examined for the presence of vegetative forms, larvae, and ova of helminthes under light microscopy (x10 and x 40 objectives). For the

diagnosis of *G. lamblia*, Field's staining method was used to confirm the identity of *G. lamblia* trophozoites. Lugol's iodine staining was used to detect cysts of protozoa. Diarrhoea was defined by laboratory staff on the basis of the appearance of stool.

3.9.2 Stool examination- Formalin-ether concentration

With the aid of an applicator stick, about 1g of stool sample was emulsified in 3- 4 ml of 10% formalin and the contents transferred into 10mls centrifuge tube. The contents were mixed by shaking for 20 seconds and then sieved, collecting the sieved suspension in a beaker. The sieved suspensions were poured back into the centrifuge tube and the debris discarded. Equal volumes of ether (3-4ml) were added, mixed well and the contents centrifuged at 3000rpm for 1 minute. The supernatants were decanted and the tubes placed in a rack. The sediments were transferred onto a slide, stained with iodine, covered with a cover slip and the entire area under the cover slip examined using the x10 and x40 objective.

3.9.3 Stool examination- Modified Ziehl Neelsen Method

Modified- Ziehl Neelsen staining was performed on both the direct stool smears and stool samples concentrated by formol ether concentration technique; and screening was done for *Cryptosporidium* oocyst, which was spherical to oval, measuring 4µm to 6µm in diameter, stained bright pink against a blue background. *C. cayetanensis* oocyst appeared spherical; measuring about 8µm-10µm stained pink resembling a wrinkled raisin with granules in the interior against a blue background.

3.9.3.1 Staining Procedure

1. A thin smear of fresh faecal specimen was prepared.
2. The smear was air dried and fixed in absolute methanol for 3 minutes
3. The smear was stained with cold carbol fuchsin for 5-10 minutes and washed with clean tap water.
4. The smear was then decolourised using 1% acid alcohol until no more colour floods from the smear.
5. The smear was rinsed with clean tap water.
6. It was then counterstained with 0.3% methylene blue for 30 seconds.
7. Finally washed off with clean tap water, allowed to air dry on a draining rack and finally examined microscopically for oocyst, using X40 and X100 objective (**Cheesbrough, 2005**).

3.9.4 Stool examination- Modified Field's technique

Modified Fields Technique was employed to diagnose intestinal microsporidiosis. Smears were prepared from fresh formalin fixed stool specimens and stained using the modified Fields Technique. The stained smears were then examined using 40X and 100X objective of the binocular light microscope. Microsporidia stains blue with a pale palor

Procedure:

1. One (1) volume of unformed or fluid faeces was mixed with three (3) volumes of 10% formalin solution. With the aid of a wooden applicator stick, a smear was made on the slide, allowed to dry and fixed with absolute methanol for two (2) minutes.

2. The smear was later covered with 0.5ml diluted Field stain B and immediately equal volumes of Field's stain A were added, mixed with Field's stain B on the slide.
3. The smear was allowed to stain for 3 minutes at room temperature.
4. The stain was gently washed off with clean tap water and then counterstained with 0.25% malachite green, washed and allowed to dry.
5. Examination was done under the microscope using the 40X and 100 X objectives (**Cheesbrough, 2005**).

3.9.5 Immunophenotyping

Immunophenotype of lymphocytes was carried out using the principle of flow cytometry. The FACS count (Becton Dickinson Immunocytometry system, Singapore (BD)) was used, following strictly the procedures outlined for sample analysis. The instrument is a compact cell counter with a built in computer. When whole blood is added to the reagent, fluorochrome labeled antibodies in the reagent bind specifically to lymphocyte surface antigen. After a fixative solution is added to the reagent tubes, the sample is run in the instrument. The cell comes in contact with the laser beam, which causes the fluorochrome labeled cells to fluoresce. The fluorescent light provides the information necessary for the instrument to count the cells. The software identifies T-lymphocyte subpopulations and correlates with the absolute count. Results provide absolute counts of CD4+ and CD3+ and CD4+/CD3+ ratio.

For this study, each time an HIV positive participant provided stool samples their blood sample were taken into EDTA anticoagulated tube for CD4 T-cell count estimation.

Procedure:

1. The CD4 reagent tubes were labeled 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 were inverted 5 to 10 times to adequately mix the blood.
4. 50 ul of whole blood was pipetted into the reagent tubes
5. Each tube was caped and vortexed upright 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 50 ul of fixative solution were pipetted into the tubes.
8. The tubes were recapped and vortexed for 5 seconds.
9. The samples were vortexed upright for 5 seconds before they were analysed on the FACS Count instruments. (**BD Bioscience, 2005**)

3.9.6 HIV1&2 Screening and confirmation

Selection of HIV negative participants was based on laboratory evidence. Therefore the First Response rapid test kit was used to screen prospective participants. Enrollment of HIV positive participants consisted of clients of the ART clinics of the prospective study sites.

Procedure:

First Response HIV card test (Premier Medical Corporation Ltd, 32-35, Shree Indl.Estate, Kachigam, Nani Daman, Daman-396 215. INDIA).

1. The test device and the dropper were removed from the foil pouch and placed on a flat, dry surface.
2. 10µl of serum was added to the sample well and added 35ul of Assay Diluent.
3. Results were interpreted within 5-15minutes.
4. The presence of only one band within the result window at the control line region indicated a negative result.
5. Two colour bands, one control and the other for HIV-1 indicated reactivity for antibodies to HIV-1.
6. Two colour bands, one control and the other for HIV-2 indicated reactivity for antibodies to HIV-2.
7. All three colour bands, one control and the other 2 for HIV-1 and HIV-2, indicated reactivity for antibodies to HIV1&2.

OralQuick Rapid HIV-1/2 Antibody Test (OraSure Technologies, Inc., Bethlehem, PA 18015, USA):

1. The test device was carefully removed from the foil.
2. The outside of the upper and lower gums was swabbed completely.
3. The pad end of the test device was inserted into the developer vial.
4. The test result was interpreted after 20 minutes.

3.10 Data Analysis

Data was entered into a computer using excel. Statistical analysis was carried out using SPSS. Data were summarized using frequency tables and bar charts. The proportions of parasitic

pathogens were compared between the CD4 T- cell groups. The relationship between the CD4 T-cell count and diarrhoeal stools were assessed using the chi- square analysis. A two-tail Pearson correlation was also performed to test relationship of parasites and diarrhoeal stools. Finally the association between organisms isolated and participant's CD4 T-cell counts were also analysed using chi-square.

CHAPTER FIVE- DISCUSSION

This study reports the prevalence of intestinal parasites among HIV positive and HIV negative individuals with special emphasis on HIV positive individuals in two hospitals in the Ashanti region of Ghana; one located in a rural (Atwima Mponua Hospital in Nyinahin) area and the other in a peri-urban (ST. Patrick's Hospital, Offinso) area. We looked for protozoa and helminths intestinal parasites in 672 participants, who submitted their stool samples to St. Patrick's hospital in Offinso and Atwima Mponua District Hospital in Nyinahin between the period of April to July, 2011. An overall prevalence of intestinal parasites among the study population was 19.3% with a significant difference ($p<0.05$) in prevalence between HIV positive (25.2%) and HIV negative (13.3%) participants. This observation is similar to results found in Zambia (**Kelly *et al*., 1996**) which reported a prevalence of 25%. Similar studies carried out in North India, Ethiopia and Tanzania (**Gupta *et al*., 2008**; **Fontanet *et al*., 2000; Tarimo *et al*., 1996**) reported higher prevalence rate of 30% and above. Conversely, a lower prevalence rate of 10.6% was reported from Senegal among HIV positive patients (**Faye *et al*., 2010**). The occurrence of intestinal parasitic infection in general in the rural and peri-urban areas recorded similar values of 18.8% and 19.6% prevalence respectively ($p>0.05$).

This study showed that opportunistic parasitic infections occurred exclusively in HIV/AIDS patients with a corresponding depletion of CD4 T- lymphocyte count. Compared to HIV-negative participants group, the prevalence of parasitic infections belonging to the opportunistic group were absent. The prevalence rate of *G. lamblia*, even though higher among HIV positive participants than HIV negative participants was not significantly high ($p=0.804$). This observation is rather inconclusive but has reemphasized the unopportunistic nature of the parasite as was reported in a previous study in Southwestern Ethiopia (**Awole *et al*., 2003**).

Helminthic infections generally were low among the study groups compared to results obtained in Ethiopia which reported 37.04% prevalence rate (**Assefa *et al.*, 2009**). The helminths recorded were *A. lumbricoides*, *E. vermicularis*, Hookworm and *S. stercoralis*. *Strongyloides stercoralis* nevertheless was only associated with HIV positive participants while *A. lumbricoides* occurred only in one HIV negative participant.

Coccidian parasites according to our findings are important components of HIV related diarrhoea especially among patients with devastated state of CD4 T-cell count. Among the coccidian parasites encountered in this study are *Cryptosporidium*, *I. belli*, Microsporidium, *and C. cayetanensis*. The most widely encountered coccidian parasite was *I. belli* and its strong association with diarrhoea seems to be associated with patients who were ART naïve ($p<0.001$). These patients presented very late to the facilities with conditions of wasting, general weakness and diarrhoea at the time of HIV diagnosis and subsequent enrolment into the study. Diarrhoea generally occurred in about 32.2% of participants with or without parasites. However coccidian parasites were mostly found in diarrhoea stools among HIV/AIDS patients (Table 4.5). One hundred and ninety five (195) participants were on ART and one hundred and forty six (146) were not on ART. The prevalence of parasitic infection in the ART naïve (24.66%) compared to participants on ART (24.10%), though slightly higher, was not statistically significant ($p=0.424$).

The prevalence of coccidian parasites was 6.2% (21/341) (Table 4.1). This is lower than results obtained by **Gupta *et al.*** (**2008**) in North India who recorded a prevalence of 25.6% (29/113) (**Gupta *et al.*, 2008**). Among the coccidian parasites in HIV positive participants which were mostly opportunistic, *I. belli* (3.5%) was the most predominant followed by *Cryptosporidium*

(2.1%). Microsporidia and *Cyclospora cayetanensis* had a prevalence of 0.9% and 0.3% respectively occurring exclusively among HIV positive participants (Table 4.1). This is in contradiction to results of **Assefa *et al*.** (**2009**) who recorded a higher prevalence of *I. belli* (12.2%) and *Cryptosporidium* (20.1%) which was the predominant coccidian parasite (**Assefa *et al*., 2009**) compared to our findings. Even though the prevalence of intestinal parasites in HIV positive participants in this study was relatively lower than that reported by other researchers (**Assefa *et al*., 2009**; **Gupta *et al*., 2008**), it is also possible that the prevalence of *I. belli* in our research may be underestimated because oocyst of *I. belli* are usually excreted in small numbers or may not be found in spite of actual infection (**Brandborg *et al*., 1970**) especially in diarrhoeal participants. The wide spread administration of chemoprophylaxis among the study participants also might have contributed to this low prevalence. *C. cayetanensis* which is an emerging parasite was found in only one participant with diarrhoea. Even though there is not much information in the literature, a prevalence of 2- 4 % has been reported (**Awole *et al*., 2003; Tarimo *et al*., 1993**).

This study recorded 0.3% prevalence of Microsporidia only. Higher prevalence of *I. belli*, *Cryptosporidium*, and *C. cayetanensis* were reported by **Gupta *et al*.** (**2008**). Microsporidia however was not recorded by Gupta *et al*. (2008). The high and low prevalence of *Cryptosporidium* and *I. belli* respectively reported in this study is similar to studies carried out in Cameroun (**Sarfati *et al*., 2006**). The similar findings could be due to the location of the study sites reflecting the existing prevalence of these parasites in the West Africa region. Microsporidia on the other hand was higher (5.2%) compared to our findings. Higher *I. belli* and *Cryptosporidium* were recorded in Ethiopia (**Adamu and Petros, 2009**). This study reveals a

correlation of coccidian parasites with HIV infection ($p<0.001$) which is statistically significant. This is in line with studies carried out elsewhere (**Wiwanitkit, 2006**).

All participants with *I. belli* infections presented with diarrhoea. Ten (10) out of eleven (11) of these participants representing 90.9% were females. About 6.1% prevalence of *Cryptosporidium* were found in diarrhoea stools of HIV positive participants. Other literature confirms the resolution of *Cryptosporidium* infections by the reconstitution of the immune system following ART administration only without specific treatment for the parasite (**Schmidt *et al.*, 2001; Oldfield, 2002; Gupta *et al*, 2010**). This perhaps accounted for the 1% prevalence of *Cryptosporidium* in non diarrhoea stools of HIV positive participants (Table 4.5). The prevalence of *Cryptosporidium* (6.1%; $p=0.04$) in diarrhoea stools of HIV positive participants was found to be much lower than the prevalence of *I. belli* (16.3%; $p<0.001$) in diarrhoea stools of HIV positive participants. More than 56% of participants were already on ART at the time of stool collection and examination. *Isospora belli* infection was significantly higher in HIV positive participants who were not enrolled on ART (100%) compared to those enrolled on ART (0%). *Cryptosporidium* however was higher in HIV positive participants enrolled on ART (57.1%) compared to HIV positive participants not on ART (42.9%). The higher prevalence rate of *Cryptosporidium* in these patients is so because their inadequate CD4 T-cell counts warranted their enrollment on ART. Other factors such as period of ART administration needs to be brought to bear in its protective role against *Cryptosporidium*. On the other hand the lower prevalence of *Cryptosporidium* infection compared to other studies (**Assefa *et al.*, 2009**), may be due to the administration of ART in 56% of HIV positive patients because of its protective role against cryptosporidiosis in patients on ART which acts on aspartyl-protease of the parasite

(**Gomez, 2004; Schmidt *et al.*, 2001**). The study also shows that *I. belli, Cryptosporidium, C. cayetanensis, and* Microsporidia were common parasites in HIV positive participants with diarrhoea. This is consistent with studies done in India and elsewhere (**Gupta *et al.*, 2008; Adamu and Petros, 2009**). Even though HIV predisposes patients to opportunistic parasitic infections, the infections are common among participants with CD4 T- cell count of 200cells/µl and below (Table 4.3). This suggests that opportunistic infections are not established with increasing CD4 T-cell counts and to prevent HIV disease from taking a fulminant course, steps should be taking to prevent this, by advocating early HIV testing and early management including comprehensive screening of enteric pathogenic bacteria, protozoa, helminthes, virus, etc.

The predominant protozoan parasite was *G. lamblia*, occurring in both HIV positive (11.4%) and negative (11.8%) participants. Since there is no evidence for an increased prevalence of *G. lamblia* in HIV positive participants, as observed in this study, despite the fact that important immune defenses against them may be disrupted by HIV infections, affirms the unopportunistic nature of the parasite (**Awole *et al.*, 2003**). *E. histolytica* was higher in HIV positive (2.3%) participants than HIV negative (0.30%) participants in this study. However lower values of these two protozoa were reported among HIV positive participants and higher values among HIV negative participants elsewhere (**Gupta *et al.*, 2008; Norhayati *et al.*, 1998**). **Adamu and Petros (2009)** reported higher prevalence of *G. lamblia* (16%) and *E. histolytica* (13%) among HIV positive participants in India. Furthermore a higher prevalence of 26.5% was reported in Hawassa City, Southern Ethiopia (**Assefa *et al.*, 2009**). Sixty nine point two percent (69.2%) of *G. lamblia* positive HIV positive participants in this study were on ART similar to what was

reported by Adamu and Petros, who demonstrated that the incidence of *G. lamblia* among HIV patients on ART was 63.6%.

Helminthic infections were higher in HIV positive participants (5.3%) than in HIV negative participants (1.2%) with *S. stercoralis* (3.8%) and Hookworm (0.9%) being the predominant helminths among HIV positive participants. *Strongyloides stercoralis* occurred exclusively among HIV positive participants ($p<0.001$) and only one HIV negative participant (0.3%) had *A. lumbricoides* in stool. Modjarrad *et al*. (2005) reported relatively higher prevalence of intestinal helminths (24.9%) with *A. lumbricoides* and hookworm in predominance with prevalence of 52.7% and 39.2% respectively among HIV-1 patients in an urban African setting (**Modjarrad *et al.*, 2005**). From our findings; with the exception of *S. stercoralis*, the other helminths had a lower prevalence compared to studies carried out in India (**Gupta *et al.*, 2008**).

The outcome of this cross-sectional study with respect to *S. stercoralis* is consistent with other studies elsewhere (**Cheesbrough, 2005; Brown *et al.*, 2006**) which shows an association between *S. stercoralis* infection and HIV disease. *Strongyloides stercoralis* however was not found in any HIV negative participant in this study, suggesting that HIV infection predisposes patients to *S. stercoralis* infections probably due to the defect in immunity. The detection of increased numbers of larvae in stool is the hallmark of hyperinfection (**Keiser and Nutman, 2004**). This study did not quantify *S. stercoralis* larvae present in stool of infected participants. The manifestation of hyperinfection is accompanied by large numbers of filariform larvae in stool increasing its effortless detection among HIV positive participants whose immune system is disrupted by the virus thereby promoting hyperinfection. In HIV negative patients however, limitation of hyperinfection as a result of competent immunity relative to HIV positive

participants may be assigned as reasons for our inability to detect larvae due to probable scanty numbers in these participants.

The low prevalence of parasites especially helminths in this study suggest that these parasites might not commonly circulate in the study groups or probably the wide spread administration of antihelminth in the populace may have contributed to the low prevalence of helminthiasis among the study group. Moreover this low prevalence could be related to the location in which the study was conducted and the study population was selected regardless of diarrhoea and other gastrointestinal manifestations.

Multiple infections were found in three participants; Microsporidium and *I. belli* were found in two participants, *S. stercoralis* and *I. belli* were found in one participant and *E. histolytica* and *G. lamblia* were found in three participants. With the exception of one of the participants with *E. histolytica* and *G. lamblia* having a CD4 T-cell count of more than 200 cells/µl, the rest had a CD4 T-cell count of less than 200cells/µl. This result is consistent with works carried out in Southern Ethiopia (**Assefa *et al.*, 2009**). Furthermore diarrhoea was found in all HIV positive participants that had mixed infections consisting of at least one coccidian parasite and also participants who had mixed infections consisting of *S. stercoralis* or/and *I. belli*. Mixed infections with at least one opportunistic parasite were found exclusively in HIV positive participants especially in patients in the advance stage of the disease and this poses a lot of health risk to patients.

Diarrhoea is a life threatening complication of infection with HIV causing wasting and well known as the presenting symptom of full blown AIDS (**Smith *et al*., 1998; Cranendonk *et al*., 2003**). The prevalence of diarrhoea among HIV positive participants irrespective of parasitic infection was 110 out of 341 participants representing 32.2% (Table 4.5). In all 9.97% (33/331) of HIV negative participants had episodes of diarrhoea at the time of stool examination. The incidence of diarrhoea among HIV participants was significantly higher than HIV negative participants ($p<0.01$). Diarrhoea among HIV participants increased with decreasing CD4 T-cell count with the highest number of diarrhoea participants occurring at the CD4 T-cell count of less than 50cells/ul ($p<0.001$) (Table 4.4). The presence of diarrhoea without parasites in stool could be from bacteria etiology, lactose intolerance (**Downs, 2010**) or sensitivity of method of parasite diagnosis among HIV positive participants (**Downs, 2010**). To further add to this, studies have shown that no etiological agent is found in 15-50% of HIV positive patients with chronic diarrhoea (**Awole *et al*., 2003; Grant and De Cock, 2001**). It has also been suggested that HIV has a direct "virotoxic" effect on the enterocyte (**Downs, 2010**) which could also cause diarrhoea. These factors may explain the significantly higher prevalence of diarrhoea in HIV positive participants than HIV negative participants. Therefore interpretation of diarrhoea association with parasitic infection in this study must be made cautiously. Parasites that were significantly found in diarrhoeal stools of HIV positive participants included; *G. lamblia*, *I. belli*, *Cryptosporidium*, and *S. stercoralis* (Table 4.5). Even though this study did not exclude other etiological agents of diarrhoea in HIV positive participants, it seems to be in agreement with studies conducted in neighbouring West African countries and other developing countries which have implicated protozoan parasites as a leading cause of diarrhoea and wasting in HIV infected patients (**Maiga *et al*., 1997; Dieng *et al*., 1998; Lebbad *et al*., 2001; Assoumou *et al*., 1993;**

Nwokediuko *et al*., 2002; Cranendonk *et al*., 2003; Cegielski *et al*., 1999). There was a statistically significant presence of *G. lamblia* in diarrhoeal stools of both HIV positive and negative participants ($p<0.001$). The increase prevalence of *G. lamblia* infection without diarrhoea relative to diarrhoea in HIV positive participants is likely to be due to the delayed clearance of the parasite in immunocompromised patients even when symptoms of diarrhoea has subsided (**Awole *et al*., 2003**). In this study, infections with *E. histolytica* only were found in non-diarrhoeic stools. More advance methods of *E. histolytica* diagnosis has differentiated pathogenic *E. histolytica* from non pathogenic *E. histolytica* now known as *E. dispar* (**Cheesbrough, 2005**). It is therefore in order to suggest that the *E. histolytica* identified in this study could actually be *E. dispar* since the method of parasite diagnosis in this study has its limitations. According to our findings; all participants that had Microsporidia presented with diarrhoea at the time of stool collection. Even though Sarfati *et al*. (2006) reported a higher prevalence of 5.2%, diarrhoea however was less than 50% among Microsporidia positive patients (**Safarti *et al*., 2006**).

Based on the classifications systems of Centres of Disease Control and Prevention (CDC), using CD4 T-cells as a marker of relative risk of developing HIV related Opportunistic infections (OIs), we encountered 100(29.3%), 151(44.3%), 67(19.6%) and 23(6.7%) of HIV positive participants having CD4 T-cell count in the ranges of >500, 200-500, 50-200 and less than 50 cells/µl respectively. Based on this method of classification it was evident that among the HIV positive participants enrolled in our study, 29.3% were in the acute (primary) infection stage or early disease (asymptomatic stage), 44.3% were in the intermediate stage (symptomatic), 19.6% were in the late stage HIV disease (symptomatic), and 6.7% were in the advance HIV disease

stage. The study revealed that the prevalence of intestinal parasitic infection among HIV positive participants was 25.2% with the highest prevalence of infection occurring among participants in the CD4 T-cell range of less than 50 cells/µl forming 56.5% of participants in the advance stage of HIV disease. The predominant parasites recovered among this group of participants belong to the coccidian groups (47.8%) which are well known as opportunistic parasites in HIV disease. Chronic diarrhoea was strongly associated with CD4 T-cell count of 200 cells/ul and below ($P<0.001$). This is consistent with studies carried out in North India and elsewhere (**Gupta *et al*., 2008; Singh *et al*., 2007**). Higher prevalence of opportunistic parasites might be found in those in the advance stage of HIV disease who are mostly bed ridden and report to the Health facilities when they are at the verge of death. This means that opportunistic parasite are more likely to be encountered in bed ridden HIV patients who hardly report for medical attention because of certain misconceptions, beliefs and stigma associated with HIV disease. Only 6.7% of participants in the advance stage of the disease were encountered. However stigmatization and marginalization has forced relatives and patients in the advance stage to keep patients indoors and fail to report to the health centres early enough for diagnosis and management. In view of this it is highly predictive that a large proportion of patients in this stage are likely to harbour parasites in their stools which go unnoticed due to failure to report to Health facilities.

The distribution of parasites at different immunity status in HIV positive participants revealed that the highest prevalence of *G. lamblia* (16.4%) occurred in patients in the CD4 T-cell range of 50-200 cells/ul. However, the occurrence of *G. lamblia* was recorded at all CD4 T-cell categories and seems not to be associated with stage of HIV disease or level of CD4 T-cell ($p=0.852$) buttressing our point on the unopportunistic nature of the parasite. In line with our findings,

opportunistic protozoa are higher in HIV positive participants with CD4 T-cell count less than 200cells/ul and trends of infections reduces with increasing CD4 T-cell count (**Adamu and Petros, 2009; Sarfati *et al.*, 2006**). Furthermore, it is most likely that as patients CD4 T-cell count increases with the administration of ART, opportunistic infections are not established even if they are exposed to infection.

The highest number of diarrhoea cases was 78.3% of participants with CD4 T-cell count less than 50cells/µl and the lowest (2%) occurring in patients with CD4 T-cell count of 500 cells/µl or more showing a significant association of diarrhoea with CD4 T-cell less than 200 cells/µl ($p<0.001$).

Sociodemographic differences between the rural and periurban areas are not determinants to the risk of intestinal infection in general as the rural and periurban areas reported similar values of 18.8% and 19.6% respectively ($p>0.05$). However infections with *Cryptosporidium* were rather common with participants in rural dwellings ($p=0.019$). The major sources of drinking water in the rural area are from Rivers and streams, boreholes and hand-dug wells. Most water bodies in the district are contaminated by farming and household waste. Refuse disposal is largely unorganized, as people tend to dump refuse anywhere in the communities. These factors may be considered as some contributing factors of the higher prevalence of *Cryptosporidium* infections. In the year 2000, United Nations unanimously pledged to meet eight Millenium Development Goals by the year 2015. These goals cannot be fully realized without integrated strategy to prevent, control and eliminate disease causing parasites. In this study however, lack of basic social amenities in both study areas such as potable drinking water has given rise to a high prevalence of *G. lamblia* infection as most residents resort to purchasing sachet water as their

source of drinking water. Unfortunately producers of sachet water in these communities are not properly monitored and therefore may serve as a source of *G. lamblia* infection.

5.1 Conclusion

The overall prevalence of intestinal parasites in this study is 19.3% with a statistically significant difference in the rate of infection among HIV positive and negative participants. *G. lamblia*, the predominant parasite occurred in both study groups (cases and controls) without any significant difference between the groups reaffirming the unopportunistic nature of *G. lamblia* ($p>0.05$). Coccidian parasites and the helminth *S. stercoralis* occurred exclusively among HIV positive participants. The exclusive occurrence of *S. stercoralis* among HIV positive participants in this study is a potential for adverse interactions with HIV and its associated situations that lead to immunodeficiency can thwart efforts to reduce mortality and morbidity associated with HIV disease.

The lower prevalence of the helminths, in this study compared to findings in studies elsewhere may be an indication of the low prevalence or the wide spread administration of antihelminth. It is possible that, if participant populations were selected based on gastrointestinal symptoms a higher prevalence would have been recorded. We agree with **Adamu and Petros (2009)** on the protective role cotrimoxazole may confer on opportunistic parasites.

5.2 Recommendations

The importance of testing for intestinal parasites in patients who are HIV-positive must be highlighted, and emphasize the necessity of increasing awareness among clinicians regarding the

occurrence of these parasites in this population. All diagnostic techniques for parasites especially opportunistic parasites should be made widely available since these parasites are potential threats to HIV patients. The results of this research even though relatively lower than other researchers results, should prompt physicians caring for HIV patients to request stool examination for the specific diagnosis of *Cryptosporidium*, *I. belli*, and microsporidia, especially in patients at the symptomatic stage (CD4 T-cell:50-200 cells/μl) of HIV disease.

Intensifying campaign against HIV and education on HIV disease would encourage people to willingly make themselves available to be screened for the presence of the disease. The need to create awareness to the populace to know their HIV status early in the course of infection for prompt enrollment on Anti-Retroviral Therapy and subsequent screening for opportunistic parasite to curb the occurrence of the parasite and diarrhoea related conditions due to immunosuppression would be of great benefit to patients.

Training programs should be held for laboratory professionals to improve their knowledge on the special diagnostic procedures aimed at diagnosing specific opportunistic intestinal parasites.

The high prevalence of *G. lamblia* suggests a potential contamination of water used for drinking purpose with the cyst of the parasite. To curb the occurrence of infection with this parasite, public health division of the health sector should emphasize the importance of environmental hygiene and collaborate with the Government to provide social amenities such as quality drinking water, good waste management systems and monitor the safety of sachet water sold on the market.

As far as HIV/AIDS disease coexist with intestinal parasitic infections in the sub-Saharan region it is imperative that the National AIDS control Programme provide the necessary logistics required to diagnose important parasites to include PCR, Isoenzyme Analysis and Antigen detection which has proven to be a very effective means of diagnosing intestinal parasites.

5.3 Limitations

1. The calculation of the sample size was based on the presence of intestinal parasites in HIV patients in Cameroun because of lack of available data in Ghana.
2. The prevalence of coccidian parasites may have been underestimated because oocyst of *I. belli* are excreted in small numbers and may not be found inspite of actual infection. A well designed study employing PCR is recommended to determine the true prevalence of *I. bell*.
3. This study did not exclude other causative agents of diarrhoea and therefore the association of parasites identified with diarrhoea should be made cautiously. A well designed study to consider other causes of diarrhoea to determine the association of specific intestinal parasites with diarrhoea is recommended.
4. Available data on intestinal parasites among HIV patients elsewhere were general and was not specific on demographics, hence our inability to compare our findings on rural and periurban areas.

References

Abass, K. and Lichtman, H. (2003). Cellular and Molecular Immunology. 5th ed., Elsevier Science, USA. p.115

Abubakari, I., Aliyu, S.H., Arumugam, C., Osman, N.K. and Hunter, P.R. (2007). Treatment of cryptosporidiosis in immunocompromised individuals; Systematic review and metaanalysis. BrJ Clin Pharmacol; **63** (No. 4): 387-393.

Adams, E.B. and MacLead, I.N. (1977). Invasive amoebiasis II.Amoebic Liver abscess and its complications. Medicine; **56**: 315-323.

Adams, V.J., Markus, M.B., Kwitshana, L.Z, Dhansay, M.A., Merwe, L., Walzl, G. and Fincham, J.E. (2006). Recall of intestinal helminthiasis by HIV-infected South Africans and avoidance of possible misinterpretation of egg excretion in worm/ HIV coinfection analysis.

Adamu, H., Endeshaw, T., Teka, T., Kifle, A. and Petros, B. (2006). The Prevalence of Intestinal Parasites in Paediatric Diarrhoeal and Non-Diarrhoeal Patients in Addis Ababa Hospitals, With Special Emphasis On Opportunistic Parasitic Infections and with Insight into The Demographic and Socio-Economic Factors. Ethiop. J. Health Dev.; **20** (No. 1): 39-46.

Adamu, H. and Petros, B. (2009). Intestinal protozoan infections among HIV positive persons with and without Antiretroviral Treatment (ART) in selected ART centers in Adama and Dire-Dawa, Ethiopia. Ethiop. J. Health Dev.; **23** (No. 2): 133-139.

Amadi, B., Mniya, M. and Musuku, J. (2002). Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis; a randomized controlled trail. Lancet; **360**: 1375-80.

Ambroise-Thomas, P. (2000). Emerging parasite zoonosis: the role of host-parasite relationship. International Journal of Parasitology; **30**: 1361-1367.

Aristizábal, H., Acevedo, J. and Botero, M. (1991). Fulminant amoebic colitis. *World J Surg*.; **15**(No. 2): 216-21.

Ashford, R.W. (1979). Occurrence of an undescribed coccidian in man in Paupa New Guinea. Ann Trop Med Parasitol; **73**: 497-500.

Ashford, R.W., Warhurst, D.C. and Reid, D.F. (1993). Human infections with cyanobacterium like bodies. Lancet; 341: 1034.

Assefa, S., Erko, B., Medhin, G., Assefa, Z. and Shemilis, T. (2009). Intestinal parasitic infections in relation to HIV/AIDS status, diarrhoea and CD4 T-cell count. BioMed Central Infectious Disease; **9**:155.

Assoumou, A., Kone, M., Penali, L.K., Coulibaly, M. and N'Draman, A.A. (1993). Cryptosporidiosis and HIV in Abidjan (Ivory Coast). Bull Soc Pathol Exot; **86**: 85-86.

Awadh, R. and Anazi, A. (2009). Gastrointestinal Opportunistic Infections in Human Immunodeficiency Virus Disease. The Saudi Journal of Gastroenterology; **15** (No. 2): 95-9.

Awole, M., Gebre-Selassie, S., Kassa, T. and Kibru, G. (2003). Prevalence of intestinal parasites in HIV-infected adult Patients in Southwestern Ethiopia. Ethiop J Health Dev.; **17**: 71-78.

Bachur, T.P.R., Vale, J.M., Coelho, I.C.B., Queiroz, T.R.B. and Chaves, C.D. (2008). Enteric Parasitic Infections in HIV/AIDS Patients Before and After the Highly Active Antiretroviral Therapy. The Brazilian Journal of Infectious Diseases; **12** (No.2):115-122.

Basavapathruni, A. and Anderson, K. (2007). Reverse transcriptase of HIV-1 pandemic. The FASEB Journal; **21**(No. 14): 3795-3808.

Berlin, G.W., Peter, J.B., Cagne, C., Conteas, C.N. and Ash, L.R. (1998). Autofluorescence and the destruction of *Cyclospora* oocyst. Emerging Infectious Disease; **1**: 127-128.

Bethony, J., Brooker, S., Albonico, M., Geiger, S., Loukas, A. and Dietmert, D. (2006). Soil transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet; **367**: 1521-1532.

BD Biosciences. (2005). BD FACSCount System Users Guide for use with CD4/CD3 Reagent Kit.

Bhanu, S., Vandana, S. and Archana, S. (2011). In vitro study of *Entamoeba histolytica* causative agent of Amoebiasis with lemon juice at different concentration showed antiamoebic properties. IRJP; **2**(No. 9): 88-89.

Blankson, J. (2010). Control of HIV-1 replication in elite suppressors. Discovery Medicine; **9** (No. l46): 261-266

Blans, M.C., Ridwan, B.U. and Verweij, J.J. (2005). Cyclosporiasis outbreak, Indonesia. Emerg Infect Dis.; **11**(No. 9): 1453-1455.

Blumberg, R.S., Kelsey, P., Peppone, T., Dickersin, R., Laquaglia, M. and Ferruci, J. (1984). Cytomegalovirus and *Cryptosporidium* associated alcalculus gangrenous cholecystitis. Am J Med; **76**: 1118-1123.

Borkow, G. and Bentwich, Z. (2004). Chronic immune activation associated with chronic helminthic and human immunodeficiency virus infections: Role of hypo-responsiveness and anergy. Clin Microbiol Rev; **17**(No. 4): 1012-1030.

Bouree, P., Lancon, A., Bisaro, F. and Bonnot, G. (2007). Six human cyclosporiasis: with general review. J Egypt Soc Parasitol; **37**(No. 2): 349-360.

Brandborg, L., Goldberg, S. and Breidenbach, W. (1970). Human coccidiosis: a possible cause of malabsorption. N Engl J Med; **283**: 1306-1313.

Brenchley, J.M. and Douek, D.C. (2008). HIV infection and gastrointestinal immune system. Mucosal Immunology; **1**(No. 1): 23-30.

Brett, A., Melanie, M. and Honorine, D. (2003). *Cryptosporidium* species: New Insights and Old Challenges. Food Safety; CID; **36**: 903-907.

Bronsdon, M. (1984). Rapid Dimethyl Sulfoxide- Modified Acid-Fast Stain of *Cryptosporidium* oocyst in stool specimens. Journal of Clinical Microbiology; **19** (No. 6): 952-953.

Brown, M., Mawa, P., Kaleebu, P. and Elliot, A. (2006). Helminths and HIV infection: epidemiological observation on immunological hypotheses. Parasite Immunol; **28**: 613-623.

Bryan, R.T., Schwartz, D.A. and Weber, R. (1997). Microsporidiosis in patients who are not infected with human immunodeficiency virus. Clin. Infect. Dis.; **24**: 534-535.

Bryan, R. and Schwartz, D. (1999). Epidemiology of microsporidiosis, American Society for Microbiology, Washington, D.C. pp502-516.

Buchbinder, S., Katz, M., Hessol, N., O'Malley P. and Holmberg, S. (1994). Long term HIV-1 infection without immunologic progression. AIDS **8**(No. 8): 1123-1128.

Buret, A.G. (2007). Mechanisms of epithelial dysfunction in giardiasis. Gut; **56**(No. 3): 316-317.

Buret, A.G. (2008). Pathophysiology of enteric infections with *Giardia duodenalis*. Parasite; **15**(No. 3): 261-265.

Burton, G., Keele, B., Esthers, J., Thacker, T. and Gartner S. (2002). Follicular dendritic cell contributes to HIV pathogenesis. Semin Immunol.; **14**(No. 4):275-284.

Caballero-Salcedo, A., Viveros-Rogel, M. and Salvatierra, B. (1994). Seroepidemiology of amebiasis in Mexico. Am J Trop Med Hyg; **50**(No. 4): 412-419.

Caccio, S.M. and Ryan, U. (2008). Molecular epidemiology of giardiasis. Mol Biochem Parasitol; **160**: 75-80.

Cali, A., Kotler, D.P. and Orenstein J.M. (1993). *Septata intestinalis*: an intestinal Microsporidium associated with chronic diarrhoea and dissemination in AIDS patients. J. Protozool; **40**: 101-112.

Cannor, B.A., Shlim, D.R., Scholes, J.V., Rayburn, J.L., Reidy, J. and Rajah, R. (1993). Pathological changes in the small bowel in nine patients with diarrhoea associated with coccidia like body. Ann Intern Med; 119: 377-382.

Cardoso, L.V., Marques, F.R., Cavasini, C.E., Almeida, M.C., Bassi, N.A., Gongora, D.V., Maia, I.L., Rossit, A.R. and Machado, R.L. (2004). Correlation of intestinal parasitic pathogens in HIV-seropositive adults with or without diarrhoea in Northeast region of Sao Paulo State, Brazil. Rev Pana Infect; **6**: 8-11.

Casemore, D.P., Amstrong, M. and Sands, R.L. (1985). Laboratory diagnosis of crytosporidiosis. J. Clin Pathol; **38**: 1337-1341.

CDC. (1992). Morbidity and mortality Weekly reports from 1992. (http://www.cdc.gov/hiv/resources/reports/mmwr/1992.htm) (accessed 2011 May 5)

Cegielski, J.P., Ortega, Y.R., Mckee, S., Madden, J.F. and Shwartz, D.A.(1999). *Cryptosporidium*, *Enterocytozoon*, and *Cyclospora* infections in paediatric and adult patients with diarrhoea in Tanzania. Clin Infect Dis; **28**:314-321.

Cello, J. and Day, L. (2009). Idiopathic AIDS Enteropathy and Treatment of Gastrointestinal Opportunistic Pathogens. Gastroenterology; **136**(No. 6): 1952-1965.

Centers for Disease Control and Prevention. (1992). Guidelines for the performance of CD4+T-cell determination in persons with human immunodeficiency virus infection. Morbid Mortal Wkly; 44: 1-17.

Certad, G., Arenas-Pinto, A., Pocaterra, L., Ferrara, G., Castro, J., Bello, A. and Nunez, L. (2003). Isosporiasis in Venezuelan adults infected with human immunodeficiency virus: clinical characterization. Am J Trop. Med. Hyg; **69**: 217-222.

Chacin-Bonilla, L., de Mejia, Y.M., Cano, G., Guampa, N., Estevez, J. and Bonilla, E. (1993). *Cryptosporidium* infection in sub community in Maracaibo, Venezuela. Am J Trop Med Hyg; **49**: 63-67.

Chacin-Bonilla, L. (2008). Transmission of *Cyclospora cayetanesis* infection: a review focusing on soil-bourne cyclosporiasis. Trans R Soc Trop Med Hyg; **102**(No. 3): 215-216.

Chan, D. and Kim, P. (1998). HIV entry and its inhibition. Cell; **93**(No. 5): 681-684.

Cheesbrough, M. (1992). Medical Laboratory Manual for Tropical Countries, 2nd ed. Volume 1, pp.164- 359. Cambridge University Press, United Kingdom.

Cheesbrough, M. (2005). District Laboratory Practice in Tropical Countries, 3rd ed., part 1, pp. 178-215. Cambridge University Press, United Kingdom.

Chitnis, A., Rawls, D. and Moore, J. (2000). Origin of HIV type 1 in colonial French Equitorial Africa. AIDS Res. Hum. Retrovirus; **16**: 5-8.

Clark, D.P. (1999). New Insights into Human Cryptosporidiosis. Clinical Microbiology Reviews; **12** (No. 4): 554-563.

Cohen, J. and Enserink, M. (2008). Nobel Prize in Physiology, or medicine: HIV, HPV researchers honoured, but one scientist left out. Science; **322** (No. 5899): 149-175.

Cooke, M.L.(2009). Endoscopy findings in HIV infected children from sub-Saharan African. Journal of Tropical Paediatrics; **55**(No. 4) 238-243.

Cookley, E., Petropoulous, C. and Whitcomb, J. (2005). Assesing ch vbge-mokine co-receptor usage in HIV. Current Opin Infect. Dis; **18** (No. 1) 9-15.

Coovadia, A. (2004). Antiretroviral agents-how best to protect infants from HIV and save their mothers from AIDS. N Engl. J. Med.; **351**(No. 3): 289-292.

Cranendonk, R.J., Kodde, C.J., Chipeta, D., Zijlstra, E.E. and Sluisters, J.F. (2003). *Crytosporidium parvum* and *Isospora belli* infections among patients with and without diarrhoea. East Africa Med J; **80**: 398-401.

Craun, G.F. (1990). Waterbourne giardiasis. In: Meyer EA ed. Human parasitic diseases. Giardiasis. Amsterdam, Elsevier; 3: 267-293.

Cryptosporidiosis surveillance--United States, 2003-2005 (2007). Division of Parasitic Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases, CDC, Atlanta, GA 30333, USA. Yoder JS, Beach MJ; Centers for Disease Control and Prevention (CDC).

Cunningham, A., Donaghy, H., Harman, A., Kim, M. and Turville, S. (2010). Manipulation of dendritic cell function by virus. Current Opinion in Microbiology; **13**(No. 4): 524-529.

Curry, A. and Smith, H.V. (1998). Emerging pathogens: *Isospora*, *Cyclospora* and Microsporidia. Parasitology; **117**:143-144.

Dar, L. and Singh, Y. (1999). Laboratory tests for monitoring stage and progression of HIV infection. In HIV testing manual by NICD and NACO; 114-125.

Debra, A., Benator, M.D., Audry, L., French, M.D., Lisa, M., Beaudet, M.D., Charles, S., Levy, M.D. and Jan Orenstein, M.D. (1994). *Isospora belli* Infection Associated with Acalculous Cholecystitis in a Patient with AIDS. Ann Intern. Med; **121**: 663-664.

DeHovitz, J.A., Pape, J.W., Boncy, M. and Johnson, W.D. (1986) .Clinical manifestation and therapy of *Isospora belli* infection in patients with the Acquired Immunodeficiency Syndrome. N. Engl J Med; **315**: 87-90.

Dias, R., Mangini, A. and Torres, D. (1992). Ocorrencia de *Strongyloides stercoralis* em pacientes portadores da syndrome de immunodeficiencia adquirida (AIDS). Rev Inst Med Trop S Paulo; **34**: 15-17.

Didier, E., Visvesvara, G., Baker, M., Rogers, L., Bertucci, D. and Vossbrinck, C. (1996). A microsporidian isolated from an AIDS patient corresponds to *Encephalitozoon cuniculi* III, originally isolated from domestic dogs. Journal of Clinical Microbiolgy; **34**:2835-2837.

Dieng, Y., Dieng, T. H., Diouf, G., Coll-Seck, A.M. and Diallo, S. (1998). Recherche des spores de microsporidies chez des patients sideens au CHU de Fann a Dakar (Senegal). Resultats preliminaries. Med Mal Infect; **28**: 265-267.

Dillingham, R., Lima, A. and Guerrant, R. (2002). Cryptosporidiosis epidemiology and impacts. Microbes Infect; **4**: 1059-1066.

Doller, P.C., Dietrich, K. and Filipp, N. (2002). Cyclosporiasis outbreak in Germany associated with consumption of salad. Emerg Infect Dis; **8**(No. 9): 992-994.

Donegan, E., Stuart, M. and Niland J. (1990). Infection with immunodeficiency virus type1 (HIV-1) among recipients of body- positive blood donors. Ann. Intern Med.; **113**(No.10): 733-739.

Douek, D. (2007). HIV Disease Progression: Immune Activation, Microbes and Leaky Gut. International AIDS Society- USA; **15**(No. 4), 114-117.

Downs, J.H. (2010). The gastrointestinal tract and HIV pathogenesis. S. Afr J Clin Nutr; **23** (No. 1): 65-68.

Dykes., K. (1980). Municpal waterborne giardiasis: an epidemiological investigation. Annals of Internal Medicine; **92**: 105-120.

Eberhard, M.L., Nace, E.K., Freeman, A.R., Streit, T.G., da Silver, A.J. and Lammie P.J. (1999). *Cyclospora cayetanensis* infection in Hiati. Journal of Parsitology; **86**: 577-582.

Eberhard, M.L., Ortega, Y.R., Hanes, D.E., Nace, E.K., Do, R.Q., Robl, M.G., Won, K.Y., Gavidia, C., Sass, N.L., Mansfield, K., Gozalo, A., Griffiths, J., Gilman, R., Sterling, C.R. and Arrowood, M.J.(2000). Attempts to establish experimental *Cyclospora cayetanensis* infection in laboratory animals. Journal of Parsitology; **86**: 577-582.

Faye, B., Tine, R.C., Ndiaye, J.L., Kintega, C., Manga, N.M., Sow P.S. and Gaye, O. (2010). Impact of Intestinal Parasites on Intensity of HIV Infection in Senegal. J Antivir Antiretrovir; **1**: 11-12.

Fayer, R. (1997). *Cryptosporidium* and cryptosporidiosis. CRC Press. Boca Raton; **251**: 24.

Farthing, M.G. (1994). Giardiasis as a disease. In: Thompson RCA, Reynolds JA., Lymbery AJ eds- Giardia: from molecules to disease. Wallingford, England, CAB International; pp.15-37.

Feregriuno, G.M., Higuera, R.F., Rossingnol, J.F, Cavier, R., Villarreal, C., Padierna, O.J. and Hidalgo, H. (1996). Extraordinary potency of the nitozoxanida, a new antiparasitary against the *Cryptosporidium* infection in advanced AIDS. In XI International Conference on AIDS.

Fisseha, B., Petros, B., Woldemivhael, T. and Mohammed, H. (1999). Diarrhoea-associated Parasitic Infectious Agents in AIDS Patients within selected Addis Ababa Hospitals. Ethiop. J. Health Dev; **13**(No. 3): 169-173.

Flanigan, T.P. and Soave, R. (1993). Cryptosporidiosis programme Clin Parasitol, 1-20.

Flannagan, P.A. (1992). *Giardia* diagnosis, clinical course and epidemiology-a review. Epidemiology and Infection, **109**: 1-22.

Flynn, P.M. (1997). *Cryptosporidium parvum*. In Long SS, Pickering LK, Prober CG eds. Principles and practice of paediatrics. New York, NY: Churchill Livingstone

Fontanet, A., Sahlu, T., Rinke de Wit, T., Messele, T., Masho, W., Woldermichael, T., Yeneneh, H. and Coutinho, R. (2000). Epidemiology of infections with intestinal parasites and human immunodeficiency virus (HIV) among sugar estate residents in Ethiopia. Ann Trop Med Parasitol; **94**: 269-278.

Forsythe, S. (2010). Microbiology of safe food, 2nd ed., Garsington Road, Oxford, OX42DQ, United Kingdom, 212 State. p.221

Fotedar, R., Stark, D. and Beebe, N. (2007). Laboratory diagnostic techniques for *Entamoeba* species. Clin Microbiol Rev; **20**(No. 3): 511-532.

Fox, L.M., and Saravolatz, L.D. (2005). Nitazoxanide: a new thiazolide antiparasitic agent. Clin Infect Dis; **40** (No. 8): 1173-1180.

Franzen, C. and Muller, A. (2001). Microsporidiosis: human diseases and diagnosis. Microbes and Infection; **3**: 389-400.

Fryauff, D.J., Krippner, R., Prodjodipuro, P., Exald, C., Kawengian, S., Pegelow, K., Yun T., von Heydwolff-Wehnert, C., Oyofo, B. and Gross, R.(1999). *Cyclospora cayetanenesis* among expatriate and indigenous populations of West Java, Indonesia. Emerging Infectious Disease **5**: 585-588.

Garcia, L.S, Bruckner, D., Brewer, T. and Shimizu, R. (1983). Techniques for the Recovery and Identification of *Cryptosporidium* oocyst from stool specimen. Journal of Clinical Microbiology; **18**(No. 1): 185-190.

Garcia, S.L. (2002). Laboratory Identification of the Microsporidia. Journal of Clinical Microbiology, **40**(No. 6): 1892-1901

Garthiram, V. and Jackson, T.F. (1987). A longitudinal study of assymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. S. Afr. Med J.; **72**: 669-672.

Gassama, A., Sow, P., Fall, F., Camara, P., Philippe, H., Gueye-N'diaye, Seng, R., Samb, B., M'Boup, S., Germani, Y. and Aidara-kane, A. (2001). Ordinary and opportunistic

enteropathogens associated with diarrhoea in Senegalese adults in relation to human immunodeficiency virus serostatus. International Journal of Infectious Disease; **5**: 192-198.

Gelderblom, H. (1997). Fine structure of HIV and SIV. HIV sequence Compendium. Los Alamos, New Mexico: Los Alamos National Laboratory pp 31-44.

Gilbert, P., McKeage, I., Eisen, G., Mullins, C., Gueye-Ndiaye, H., Mboup, S. and Kanki P. (2003). Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. Statistics in Medicine; **22**(No. 4): 573-593.

Gillepsie, S.H. and Pearson, R.D. (2001). Principles and practices of Clinical Parasitology. Baffins Lane, Chichester, West Sussex PO19 IUD, England; pp197-214.

Goodgame, R.W. (1996). Understanding intestinal spore forming protozoa: *Cryptosporidia*, Microsporidia, *Isospora* and *Cyclospora*. Ann Intern Med; **124**: 429-441.

Gomez, M., Atzoric, L., Rossi, P., Scalgia, M. and Pozio, E. (1995). Opportunistic and non-opportunistic parasites among HIV positive and negative patients with diarrhoea in Tanzania. Tropical Medicine and Parasitology; **46**(No. 2), 109-114.

Gomez, M.A. (2004). La highly active antiretroviral Therapy e la cryptosporidiosi. Parassitologia; **46**: 95-99.

Goncalves, M., Gabbay, B., Mascarenhas, J., Yassaka, B., Moran, C., Fraga, D., Castro, E., Franco, C., Machado, D. and Rossit, B. (2009). Calicivirus and *Giardia lamblia* are Associated with Diarrhoea in Human Immunodeficiency Virus- Seropositive Patients from Southeast Brazil. Am. J. Trop. Med.Hyg; **81**(No. 3): 463-466.

Graber, S., Selinger-Leneman, H., Abgrall, S., Pialoux, G., Weiss, L. and Costagliola, D. (2009). Prevalence and comparative characteristics of long-term nonprogressors and HIV controller patients in the French Hospital Database on HIV. AIDS **23**(No. 9): 1163-1169.

Grant, A. and De Cock, K. (2001). HIV infection and AIDS in developing world. British Medical Journal; **322**: 1475-1478.

Greener, R. (2002). AIDS and macroeconomic Impact. In S.Forsythe (ed). State of the Art AIDS and economics. IAEN pp.49-55.

Griffiths, J.K., Balakri, S., Widmer, G. and Tzipori, S. (1998). Paromomycin and geneticin inhibit intracellular *Cryptosporidium parvum* without trafficking through the host cell cytoplasm: implications for drug delivery. Infect. Immun; **66**: 3874-3883.

Gumbo, T., Hobbs, R., Carlyn, C., Hall, G. and Isada, C. (1999). Microsporidia infection in transplant patients. Transplantation; **67**: 482-484.

Gupta, S., Narang, V. and Singh, S. (2008). Chronic diarrhoea in HIV patients. Prevalence of coccidian parasites. Indian Journal of Medical Microbiology; **26**(No. 2): 172-175.

Gupta, V. and Gupta, S. (2004). Laboratory markers associated with progression of HIV infection. Indian Journal of Medical Microbiology; **22**(No. 1): 7-15.

Hammer, S., Kessler, H. and Saag, M. (1994). Issues in combination antiretroviral therapy. J Acquir Immune Defic SynDr; **7**: 124-135.

Hammouda, N., Sadaka, H., El-Gebaly, W. and El-Nassery, S. (1996). Opportunistic intestinal protozoa in chronic diarrheic immunosuppressed patients. J Egypt Soc Parasitol; **26**: 143-153.

Haque, R., Mondal, D. and Duggal, P. (2006). *Entamoeba histolytica* infection in children and protection from subsequent amoebiasis. Infect Immun; **74**(No. 2): 904-909.

Hardiman, M. (2003). Konkonuru: Life in a West African Village. The impact of Socio-Economic change on Rural Communitities: Ghana Universities Press, Accra. pp.12-60.

Harindra, V. (2008). HIV: Past, Present and Future. Indian J Sex Transm Dis; **29**: 1-6.

Herwaldt, B.L. and Ackers, M.L. (1997). An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The *Cyclospora* working Group. N. Engl J Med; **336**(No. 22): 1546-1548.

Herwaldt, B.L. (2000). *Cyclospora cayetanensis*. Focusing on the outbreaks of cyclosporiasis in the 1990s. Clin. Infect. Dis. **31**:1040-1057.

Hicks, P., Zwiener, R.J., Squires, J. and Savell, V. (1996). Azithromycin therapy for *Cryptosporidium parvum* infection in four children infected with human immunodeficiency virus. J Pediatr; **129**(No. 2): 297-300.

HIV Sentinel Survey Report. (2009). Republic of Ghana and Ghana Health Service. Accra

Hill, D.R. (2005). Giardia lamblia. In: Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. Philadelphia, Pennsylvania: Churchill Livingstone, An Imprint of Elsevier Inc; 3198-3203 / 277.

Hoge, C.W., Shlim, D.R., Rajah, R., Triplett, J., Shear, M., Rabold, J.G. and Esheverria, P. (1993). Epidemiology of diarrhoeal illness associated with coccidian-like organism among travelers and foreign residents in Nepal. Lancet; **341**: 1175-1179.

Holland, V.C. and Kennedy, M.W. (2002). World Class Parasites. The Geohelminths: *Ascaris*, *Trichuris* and hookworm; Vol 2, pp. 43-119.

Hung, C.C., Deng, H.Y., Hsiao, W.H., Hsieh, S.M., Hsiao, C.F. and Chen, M.Y. (2005). Invasive amoebiasis as an emerging parasitic disease in patients with human immunodeficiency virus type 1 infection in Taiwan. Arch Intern Med; **165**(No. 4):409-415.

Hunter, G., Bagshawe, A., Baboo, K., Luke, R. and Prociv, P. (1992). Intestinal parasites in Zambian patients with AIDS. Trans R Soc Trop Med Hyg; **86**: 543-545.

Hunter, S. (2006). AIDS in America. New York, NY: Palgrave Macmillan.p. 140

Hunter P.R. and Nicholas G. (2002). Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. Clin Microbiol Rev; **15**:145-154.

Huston, C.D. (2006). Intestinal Protozoa. In: Feldman, M. Friedman, L.S., Brandt, L.J., Sleisenger and Fordtran's Gastrointestinal and Liver Disease. Vol. 2, 8th ed. Philadelphia PA: Saunders, An imprint of Elsevier Inc. 2420-2423.

Ignatius, R., Lehmann, M., Miksits, R.M., Engelmann, E., Futh, U., Hahn, H. and Wagner, J. (1997). A new Acid Fast Trichrome Stain for simultaneous Detection of *Cryptosporidium parvum* and Microsporidial species in stool specimens. Journal of Clinical Microbiology; **1**: 446-449.

IDLO. (2006). Water Tenure Reform and Pubic Access to Water as a Basic Need, International Development Law Organization Voice of Development Jurists Series. p. 1

Jinnerman, K.C., Wetherington, J.H., Hill, W.E., Omiescinski, C.J., Adams, A.M., Johnson, J.M., Tenge, B.J., Dang, N.L. and Wekell, M.M. (1999). An oligonucleotide-ligation assay for the differentiation between *Cyclospora* and *Eimeria* spp. Polymerase chain reaction amplification products. Journal of food Protection; **62**: 682-685.

Joint united Nations Programme on HIV/AIDS. (2010). Overview of the global AIDS epidemic UN report on the global AIDS epidemic (2010).

Jokipii, L. and Jokippi, A. (1986). Timing of symptoms and oocyst excretion in human cryptosporidiosis. N Eng J Med; **315**: 1643-1647.

Kalinkovich, A., Borkow, G., Weisman, Z., Tsimanis, A., Stein, M. and Bentwich, Z. (2001). Increased CCR5 and CXCR4 Expression in Ethiopians Living in Israel: Environmental and constitutive factors. Clin Immunol; **100**(No. 1): 107-117.

Kam, K.M., Wang, K.H., Li, P.C., Lee, S.S., Leung, W.L. and Kwok, M.Y. (1998). Proposed CD4+ T- cell criteria for staging human immunodeficiency virus infected Chinese adults. Clin Immunol Immunopathol; **89**: 11-22.

Kaushal, K., Ganga, P., Sanjeev, S., Surbhi, M. and Usha, K. (2007). Enteric Opportunistic Parasites among HIV Infected Infected Individuals: Associated Risk Factors and Immune Status. Jpn. J. Infect. Dis; **60**: 76-81.

Kayser, F.H., Bienz, K.A., Eckert, J. and Zinkernages, R.M. (2001). Medical Microbiology. Thieme New York, 333 Seventh Avenue, New York, NY 10001, USA. p. 7

Kelly, P., Baboo, K., Wolff, M., Ngwenya, B., Luo, N. and Farthing, M. (1996). The prevalence and aetiology of persistent diarrhoea in adults in urban Zambia. Acta Trop; **61**: 183-190.

Kelly, P., Todd, J., Sianongo, S., James, M., Sinsungwe, H., Max, K., Farthing, M. and Feldman, R. (2009). Susceptibility to intestinal infection and diarrhoea in Zambian adults in relation to HIV status and CD4 count. BMC Gastroenterology; **9**:7.

Keiser, B. and Nutman B. (2004). *Strongyloides stercoralis* in Immunocompromised Population. Clinical Microbiology Reviews; **17**: 208-217.

Keush, G.T., Hamer, D., Joe, A., Kelly, M., Griffiths, J. and Ward, H. (1995) *Cryptosporidia*-who is at risk? Schweiz Med Wochem Schr. **125(** No.18): 899-908.

Khan, J. and Walker, B. (1998). Acute Human Immunodeficiency virus type 1 infection N. Engl J. Med; **331**(No. 1):33-39.

Kirk, O., Macroft, A. and Lundgren J. (2004). Decline in AIDS and death rates in the EuroSIDA study: An observable study. Ugeskr Laeger; **127**: 123-128.

Kirkpatrick, C.E. (1988). Animal reserviour of *Cryptosporidium* and *I. belli*. J Infect Dis; **158**: 909-910.

Kohl, N.E., Emini, E.A. and Schleif, W.A.(1988). Active human immunodeficiency virus protease is required for viral infectivity. Proc Natl Acad Sci, USA; **85**: 4689-4690.

Kortler, D.and Orenstein, J.M. (1999). The Microsporidia and Microsporidiosis, American Society for Microbiology; 1:258-292.

Kuilen, C., Leitner, T., Foley, B. and Hahn B. (2008). HIV Sequence Compendium. (http://www.hiv.lanl.gov) (accessed 2011 February 3).

Kuplan, E. and Heimer, R. (1995). HIV incidence among new Haven needle exchange participants: updated estimates from syringe tracking and testing data. J. Acquir. Immune Defi Syndr. Hum. Retrovirol; **10**(No. 2): 175-176.

Kwakye-Nuako, G., Borketey, B., Mensah-Attipoe, I., Asmah, H. and Ayeh-Kumi, F. (2007). Sachet Drinking Water in Accra: The Potential Threats of Transmission of Enteric Pathogenic Protozoan Organisms. Ghana Med J.; **41**(No. 2):62–67.

Lawn S. (2004). AIDS in Africa: the impact of coinfections on the pathogenesis of HIV-1 infections. J. Infect. Dis; **48**(No. 1): 1-12.

Lebbad, M., Norrgren, H., Naucler, A., Dias, F., Anderson, S. and Linder, E. (2001). Intestinal parasites in HIV-2 associated AIDS cases with chronic diarrhoea in Guinea-Bissau. Acta Trop; **80**: 45-49.

Lekha, T., Anil, K.G., Shyam, S. and Tribhuban, M. (2008). Correlation between CD4 counts of HIV patients and enteric protozoans in different seasons- An experience of a tertiary care hospital in Varanasia (India). BMC Gastroenterology; **8**:36.

Lemey, P., Pybus, O., Wang, B., Saksena, N., Salemi, M. and Vandamme, M. (2003). Tracing the origin and history of the HIV-2 epidemic.

Levine, N.D. (1973). Introduction, history and taxonomy In: Hammond DM, Long PL, ed, the coccidia: *Eimeria*, *Isospora*, *Toxoplasma* and related genera. Baltimore, MD, University Park Press: pp1-22.

Li, E. and Stanley, S.L. (1992). Protozoa. Amebiasis. Gastroenterol Clin North Am.; **25**(3):471-492.

Lindsay, D.S., Dubey, J.P. and Blagburn, B.L. (1997). Biology of *Isospora* spp from humans, non human primates and domestic animals. Clin Microbiol Rev; **10**:19-34.

Lopez, A.S., Bendik, T.M., Alliance, J.Y., Roberts, M.J., da Silva, J.A., Moura, N.S., Arrowood, J.M., Eberhard, L.M. and Herwadt, B.L. (2003). Epidemiology of *Cyclospora cayetanensis* and other intestinal parasites in a community in Hiati. Ann Intern Med; 377-382

Losch, F. (1875). Massenhafte Entwickelung von Amoeben im Dickdam. Virchow's Archiv; **65**: 196-221.

Lucas, S.B. (1990). Missing infections in AIDS. Trans R Soc Trop Med Hyg; **86**: 353-354.

Mahel, A. and Rao B. (2005). HIV-AIDS epidemic in India. An economic perspective. Indian J Med Res; **121**:582-600.

MacKenzie, W.R., Schell, W.L. and Blair, K.A. (1995). Massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin. Clin Infect Dis; **21** (No. 1): 57-62.

Madhu, V. and Lalit, D. (1999). Antiretroviral Drugs in HIV infections. Indian Journal of Pharmacology; **31**: 96-103.

Madico, G., McDonald, J., Gilman, R.H., Cabrera, L. and Sterling, E.R. (1997). Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children; Clin Infect Dis; **24**: 977-981.

Maiga, I., Doumbo, O., Dembele, M., Traore, H. and Desportes-Livage, I. (1997). Human intestinal microsporidiosis in Bamako (Mali): the presence of *Enterocytozoon bieneusis* in HIV seropositive patients. Sante; **7**:257-262.

Mannheimer, S.B. and Soave, R. (1994). Protozoan infections in patients with AIDS, cryptosporidiosis, isoporiasis, cycloporaisis, microsporidiosis. Infect Dis Clin North; **8** (No. 2): 483-498.

Marcos, L., Terashima, A., Dupont, H. and Gottuzo, E. (2008). *Strongyloides* hyperinfection syndrome: an emerging global infectious disease. Trans R Soc Trop Med Hyg; **102**:314-8.

Matsubayashi, H., Koike, T., Mikata, T. and Hagiwara, S. (1959). A case of Encephalitozoon-like-body infection in man. Arch.Pathol; **67**:181-187.

May, G.R., Gill, M.J., Church, D.L. and Sutherland, L.R. (1993). Gastrointestinal symptoms in ambulatory HIV- infected patients. Diag Dis Sci; **138**: 1388-1394.

McGovern, S., Caselli, E., Grigorieff, N. and Shoilet, B. (2002). A common mechanism underlying promiscuous inhibitors from virtual and high throughput screening. J Med Chem; **45**(No. 8): 1721-1722.

McQuade, T.J., Tomasseli, A.G. and Liu, L. (1990). A synthetic HIV-1 protease inhibitor with antiviral activity arrest HIV like particle maturation. Science **247**: 454-456.

Meinhardt, P.L., Casemore, D.P. and Miller, K.B. (1996). Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. Epidemiol Rev; **18**(No. 2):118-36.

Meyer, E.A. (1994). *Giardia* as an organism. In Thompson RCA, Reynolds JA, Lymberg AJ eds. *Giardia*: from molecules to disease. Wallingford, England, CAB International; pp. 3-14.

Minvielle, M.C., Molina, N.B., Polverino, D. and Basualdo, J.A. (2008). First genotyping of *Giardia lamblia* from human and animal faeces in Argentina, South America. Mem Inst Oswaldo Cruz; **103**(No. 1): 98-103.

Modjarrad, K., Zulu, I., Redden, D., Lungowe, N., Freedman, D. and Sten, H. (2005). Prevalence and predictors of intestinal helminth infections among human immunodeficiency virus type 1-infected adults in an urban African setting. Am J. Trop. Med. Hyg; **73**(No. 4): 777-782.

Mofenson, L.M., Brady, M.T., Danner, S.P., Dominguez, K.L., Hazra, R., Handelsman, E. (2009). Guidelines for the Prevention and Treatment of Opportunistic Infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Paediatric Infectious Diseases Society, and the American Academy of Pediatrics. MMWR Recomm Rep; **58** (No. 11):1-166.

Mohandas, K., Sehgal, R., Sud, A. and Malla, N. (2002). Prevalence of intestinal parasitic pathogens in HIV seropositive patients in Northern India, Jpn J Infect Dis; **55**(No. 3): 83-84.

Monis, P.T. and Thompson, R.C. (2003). *Cryptosporidium* and *Giardia*-zoonoses: fact or fiction? Infect Genet Evol; **3**(No. 4): 233-244.

Mondal, D., Petri, J., Sack, R.B., Kirkpatrick, B.D. and Haque R. (2006). *Entamoeba histolytica* associated diarrhoeal illness is negatively associated with growth of preschool children: evidence from a propective study. Trans. R. Soc. Trop. Med. Hyg; **100**: 1032-1038.

Morakote, N., Muangyineong, Y., Somboun, P. and Khambounruang, C. (1987). Acute human isosporiasis in Thailand: a case report. South East Asian J Trop Med Public Health; **18**: 107-111.

Moore, G.T., Cross W.H. and McGuine, D. (1969). Epidemic giardiasis at a ski resort. N Engl J Med; **281**: 402-407.

Morgan, U. (1994). Random Amplified Polymorphic DNA analysis of *Giardia* DNA and correlation with isoenzyme data. In: Thompson RCA., Raynolds JA, Lymberg AJ (Ed) *Giardia* from molecules to disease. Wallinford, England, CAB International: 61-63.

Morgan, U., Weber, R., Xiao, L., Sulaiman, I., Thompson, R.C., Ndiritu, W., Lal, A., Moore, A. and Deplazes, P. (2000). Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya and the United States. J Clin Microbiol; **38**: 1180-1183.

Mukhopadhya, A., Ramakrishna, B.S., Kang, G., Pulimood, A.B., Mathan, M.M. and Zacharian, A. (1999). Enteric pathogens in Southern India HIV-infected patients with and without diarrhoea. Indian J Med Res; **109**: 85-89.

Muller, A., Bialek, R., Fatkenheuer, G., Salzberger, B., Diehl, V. and Franzen. (2006). Detection of *Isospora belli* by polymerase chain reaction using primers based on small units' ribosome RNA sequences. Euro. Clin J Microbiol Infect Dis; **19**: 631-634.

Munoz, A., Wang, M., Bass, S., Taylor, J. and Kingsley, L. (1989). Acquired immunodeficiency syndrome (AIDS) free time after human immunodeficiency virus type 1(HIV 1) seroconversion in homosexual men. Am J Epidemiol; **130**: 530-539.

Murthy, G. (2008). The socioeconomic impact of human immunodeficiency virus/acquired immune deficiency Syndromme in India and its relevance to eye care. Indian J Ophtalmol; **56**: 395-397.

Navin, T.R. and Hardy, A.M. (1987). Cryptosporidiosis in patients with AIDS. J Infect Dis; **155**(No. 1):150.

Norhayati, M., Pengabean, M., Oothhuman, P. and Fatimah, M.S. (1998). Prevalence and some risk factors of *Giardia duodenalis* infection in rural communities in Malaysia. Southeast Asian Journal of Tropical Medicine and Public Health; **29**: 735-738.

Nwachukwu, C.E. and Okebe, J.U. (2008). Antimotility agents for chronic diarrhoea in people with HIV/AIDS. Cochrane Database of Systemic Reviews; **4**: 5644.

Nworkediuko, S.C., Bojuwoye, B.J. and Onyenekwe, B. (2002). Apparent rarity of cryptosporidiosis in human immunodeficiency virus (HIV)-related diarrhoea in Enugu, south eastern Nigeria. Niger Postgrad Med J; **9**: 70-73.

Oldfield, E.C. (2002). Evaluation of chronic diarrhoea in patients with human immunodeficiency virus infection. Rev Gastroenterol Disord; 176-88.

Ortega, Y.R., Sterling, C.R., Gilman, R.H., Cama, V. and Diaz, F. (1993). *Cyclospora* species: a new protozoan pathogen of humans. N Engl J Med.; **328**: 1308-1312.

Ortega, Y.R., Gilman, R.H. and Sterling, C.R. (1994). A new coccidian parasite (Ampicomplexa: Eimeriidae) from humans. Journal of Parasitology; **80**: 625-629.

Ortega, Y.R., Nagle, R., Gilman, R.H., Watanabe, J., Miyagui, J., Quispe, H., Kanagusuku, P., Rojas, C. and Sterling, C.R. (1997). Pathologic and Clinical findings in patients with cyclosporiasis and a description of intracellular parasite life cycle stages. The Journal of Infectious Diseases; **176**: 1584-1589.

Ortega, Y.R., Sterling, C.R. and Gilman, R.H.(1998). *Cyclospora cayetanenesis*. Advances in Parasitology; **40**: 399-418.

Pape, J.W., Verdier, R.L., Boncy, M., Boncy, J. and Johnson, W.D. (1994). *Cyclospora* infection in adults infected with HIV. Clinical manifestation, treatment and prophylaxis. Annals of Internal medicine; **121**:654-657.

Papkalla, A., Munch, J.O. and Kirchoff, F. (2002). Nef enhances Human Immunodeficiency Virus Type 1 infectivity and replication Independently of viral coreceptor tropism. Journal of Virology; **76**: 8455-8459.

Pathel, S. and Benfield, P. (1996). New drug profile: nevirapine. Clinical Immunotherapeutics; **6** (No. 4): 307-317.

Pentaleo, G., Graziosis, C. and Fauci, A. (1993). The immunopathogenesis of human immunodeficiency virus infection. N Engl J Med; **328**:327-335.

Petri, W.A. Jnr., Mann, B.J. and Haque, R. (2002). The bittersweet interface of parasite and host lectin-carbohydrate interaction during human invasion by the parasite *Entamoeba histolytica*. Annu Rev. Microbiol; 39-64.

Phair, J., Jacobson, L., Detels, R., Rinaldo, C. and Saah, A. (1992). Acquired immunodeficiency syndrome occurring 5 years of infection with immunodeficiency virus type 1. The multicenter AIDS cohort study. J. AIDS; **5**: 490-496.

Pollard, V. and Malim, M. (1998). The HIV-1 Rev Protein. Annu Rev. Microbiol; **52**: 491-532.

Prasad, K., Nag, V., Dhole, T. and Ayyagari, A. (2000). Identification of enteric pathogens in HIV-positive patients with diarrhoea in Northern India. J Health Popul Nutr; **18**(No. 1):23-6.

Que, X. and Reed, S.L. (2000). Cysteine proteinases and the pathogenesis of amebiasis. Clin Microbiol Rev; **13**(No. 2):196-206.

Rachel, M., Chalmers, K.E., Hadfield, S.J. and Robinson, G. (2011). Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus*, United Kingdom, 2007-2008. Emerging Infectious Disease; **17** (No. 3): 563-538.

Raymond, L., Delbac, F., Brousselle, V., Rabodoriirina, M., Giraut, V., Walon, M., Cozon, G., Vivares, C.P. and Peyron, F. (1998). Identification of *Encephalitozoon intestinalis* in travelers with chronic diarrhoea by specific PCR amplification, J. Clin. Microbiol; **36**: 37-40.

Reinthaler, F. (1989). Epidemiology of cryptosporidiosis in children in tropical countries. J. Hyg Epidemiol Microbiol Immuniol; **33**: 503-513.

Relman, D.A., Schmidt, T.S., Gajadhar, A., Sogin, M., Cross, J., Yoder, K., Sethabutr, O. and Echeverria, P. (1996). Molecular phylogenetic analysis of *Cyclospora*, the human intestinal pathogen, suggests that it is closely related Eimeria species. Journal of Infectious Diseases **173**: 440-445.

Rosiere, D., Vantelon, M., Bouree, P., Chachaty, E., Nitenberg, G. and Blot F. (2003). *Isospora belli* infection in a patient with non-Hodgkin's lymphoma. Clin. Microbiol. Infect; **9**: 1065-1067.

Robert, H., Robin, M., Nowells, S. and Young, M.C. (2002). Approach to infections in the compromised host. 4th ed., Kluwer Academic/Plenum Publishers, New York.p.306.

Roberts, L., Janovy, Jnr. J. and Schmidt, G.D. (2005). Foundations of Parasitology. 8th ed., McGraw-Hill Companies, Inc., New York. pp.100-150

Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology. 4th ed., McGraw-Hill Medical Publishing Division, New York .pp.693-766

Saklatvalva, T. (1993). Milestones in Parasitology. Parasite Today; **9**: 347-348.

Same-Ekobo, A., Lohoue, J. and Mbassi, A. (1997). A clinical and biological study of parasitic and fungal diarrhoea in immunosupressed patients in an urban and suburban area of Yaounde. Sante; **7**: 349-354.

Sarfati, C., Bourgeois, A., Menotti, J., Liegeois, F., Moyou-Somo, R., Delaporte, E., Dero uin, F., Ngole, M. and Molina, J. (2006). Prevalence of intestinal parasites including Microsporidia in Human Immunodeficiency virus-infected adults in Cameroon: A cross-sectional study. Am. J. Trop. Med. Hyg; **74**(No. 1): 162-164.

Saksirisampant, W., Prownebon, J., Saksirisampant, P., Mungthin, M., Siripatanapipong, S. and LeelavoOva, S. (2009). Intestinal Parasitic Infections: Prevalences in HIV/AIDS patients in a Thai AIDS-care centre,Ann Trop Med Parasitol; **103**(No. 7), 573-581.

Sax, P.E., Rich, J.D., Piaciak W.S. and Trnka Y.M. (1995). Intestinal microsporidiosis occurring in a liver transplant recipient. Transplantation; **60**:617-618.

Schmidt, W., Wahnschaffe, U. and Schafer, M. (2001). Rapid increase of mucosal CD4 T-cells followed by clearance of intestinal cryptosporidiosis in an AIDS patients receiving highly active antiretroviral therapy. Gastroenterology; **120**: 984-7.

Scull, M., Castro, O., Nunez, F. and Capo, V. (2006). Estrongiloidiosis diseminada en pacientes con SIDA: a proposito de 2 casos. Rev Cubana Med Trop; 58.

Seydel, K.B. and Stanley, S.L. (1998). *Entamoeba histolytica* induces host cell death in amoebic liver abscess by a non-Fas-dependent, non-tumor necrosis factor alpha-dependent pathway of apoptosis. Infect Immun; **66**(No. 6):2980-2983.

Sharp, M. and Hahn, H. (2010). The evolution of HIV/AIDS. Phil. Trans R. Soc. B; **365**:2487-2494.

Sheppard, H., Lang, W., Ascher, M., Vittinghoff, E. and Winkelstein, W. (1998). The characteristics of non progressors: long term HIV-1 infection with stable CD4+ T cell levels. AIDS; **7**: 1159-1166.

Sheth, S., Bates, C., Federman, M. and Chopra, S. (1997). Fulminant hepatic failure caused by microsporidia infection in a patient with AIDS, AIDS; **11**: 553-554.

Shlim, D.R. (1991). An algae-like organism associated with an outbreak of prolonged diarrhoea among foreigners in Nepal. American Journal of Tropical Medicine and Hygiene; **45**: 383-389.

Silva, C.V., Ferreira, M.S., Goncalves-PiresMdo, R., Costa-Cruz JM. (2003). Detection of *Cryptosporidium*-specific coproantigen in human immunodeficiency virus/acquired immunodeficiency syndrome patients by using a commercially available Immunoenzymatic assay. Mem Inst Owaldo Cruz; **98**: 1097-99.

Singh, H.R., Singh, N.G. and Singh, T.B. (2007). CD4+ and CD8+ T-lymphocytes in HIV Infection and AIDS. Indian Journal of Medical Microbiology; **25**(No. 2): 126-32.

Smith, N.H., Cron, S. and Valdez, L.M. (1998). Combination drug therapy for cryptosporidiosis in AIDS. J Infect Dis; **178**(No. 3):900-903.

Smith, P., Lane, H., Gill, V., Manischewitz, J., Quinnan, G. and Fauci, A. (1998). Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS). Etiology and response to therapy. Ann Intern Med; **108**:328-333.

Smith, R.D. (1990). The pathopysiology of HIV infection. Arch Pathol Lab Med; **114**: 235-239.

Soave, R. and Johnson, W. (1988). *Cryptosporidium* and *Isospora belli* infections. J Infect Dis; 225-229.

Soave, R. (1996). *Cyclospora*: an overview. Clinical infectious Diseases, **23**: 429-437.

Soave, R., Herwaldt, B.L. and Relman, D.A. (1998). *Cyclospora* Infect Dis Clin North Am; **12**: 1-12.

Somachai, J., Chaturong, P., Malee, C., Takuya, I. and Takuro, E. (2007). Morphologic and Molecular Characterization of *Isospora belli* oocysts from Patients. Am J. Trop. Med. Hyg; **77**(No. 1): 107-112.

Sorvillo, F.J., Lieb, L.E. and Seidel, J.(1995). Epidemiology of isosporiasis among persons with acquired immunodeficiency syndrome in Los Angeles County. Am J Trop Med Hyg; **53**(No. 6): 656-659.

Stanley, S.L. (2003). Amoebiasis. Lancet; **361**(No. 9362):1025-1034.

Stark, D., Barratt, J.L., van Hal, S., Marriott, D., Harkness, J. and Ellis, J.T. (2009). Clinical significance of enteric protozoa in the immunosuppressed human population. Clin Microbiol Rev; **22**(No. 4):634-650.

Stein, D.S., Korvick, J.A. and Vermund, S.H. (1992). CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: A review. J Infect Dis; **165**: 352-363.

Steinmann, P., Zhou, X.N. and Duz, W. (2008). Tribendimidine and Albendazole for Treating soil- transmitted Helminths, *Strongyloides stercoralis* and Taenia spp: Open lebel Randomised Trail. PLOS Negl Trop Dis; **2**(No. 10):322.

Sterling, C.R. and Ortega, Y.R. (1999). *Cyclospora*; an enigma worth unraveling. Emerging Infectious Disease; **5**: 48-53.

Sterling, C.R. and Ortega, Y.R. (2004). *Cyclospora cayetanensis*: An emergent and still perplexing coccidian parasite In: World Class Parasites: The Pathogenic Enteric Protoazoan: *Giardia*, *Entamoeba*, *Cryptosporidium* and *Cyclospora*. Vol.8, pp.43-52. Kluwer Academic Publishers, 3300 AA Dordrecht, The Netherlands.

St Georgiev, V. (1993). Opportunistic infections: treatment and developmental therapeutics of cryptosporidiosis and isosporiasis. Drug development and research; **28**: 445-459.

Stoltzfus, R.J. (2003). Low Dose Daily iron Supplementation improves iron status and Appetite but not Anaemia, whereas Quaterly Antihelminthic Treatment Improves growth, appetite, and anaemia in Zanzibari Preschool children. The Journal of Nutrition **134**(No. 2): 348-356.

Sturbaum, G.D., Ortega, Y.R., Gilman, R.H., Sterling, C.R., Cabrera, L. and Klein, D.A. (1998). Detection of *Cyclospora cayetanensis* in wastewater. Applied and Environmental Microbiology. **64**: 2284-2286.

Sun, T., Illard, C.F., Asnis, D., Bresciani, A.R., Goldenberg, S., Roberts, B. and Teichberg, S. (1996). Light and electron microscopic identification of *Cyclospora* species in the small intestine. Evidence of the presence of asexual life cycle in the human host. American Journal of Clinical Pathology; **105**: 216-220.

Tarimo, D., Killewo, J., Mnjas, J. and Msamanga, G. (1996). Prevalence of intestinal parasites in patients with enteropathic AIDS in North Eastern Tanzania. E Afr Med J.; **73**(No. 6), 397-399.

Ten Hove, R.J., Van Lieshout, L., Brienen, E.A., Perez, M.A. and Verwaij, J.J.(2008). Real time Polymerase chain reaction for the detection *Isospora belli* in stool samples. Diag Microbiol Infect; **61**: 280-283.

Thomas, P.D., Forbes, A., Green, J., Howdle, P. and Playford, E. (2003). Guidelines for the investigation of chronic diarrhoea. Gut; **52**: 1-15.

Tosones, G., Bonadies, G., Liuzzi, G., Tullio, P., Santoro, G. and Piazza, M. (1993). International Conference on AIDS. Int Conf AIDS; **9**:388.

Tier, J.S., Moxey, P.C., Schemmel, E.M. and Robles, E.(1974). Chronic intestinal coccidiosis in man: intestinal morphology and response to treatment. Gastroenterology; 66: 923-925.

UN report on global AIDS epidemic. (2010). (http://www.unaids.org/documents/20101123_GlobalReport_em.pdf.) (accessed 2010 May 10).

UNAIDS/WHO. (2002). HIV Epidemic Update. Geneva.

UNAIDS. (2005). AIDS Epidemic Update 2005.

UNAIDS. (2006). AIDS epidemic update: Special report on HIV/AIDS. Geneva (Switzerland): UNAIDS.

UNAIDS. (2008). Report on Global AIDS epidemic 2008.

Van Lieshout, L. and Verweij, J.J. (2010). Newer diagnostic approaches to intestinal protozoa. Curr Opin Infect Dis; **23**(No. 5): 488-493

Vajpayee, M. and Dar, L. (1999). Antiretroviral Drugs in HIV infection. Indian Journal of Pharmacology; **31**: 96-103.

Varghese, B., Maher, J., Peterman, T., Branson, B. and Steketee, R. (2002). Reducing risk of sexual HIV transmission: quantifying the per act risk for HIV on the basis of choice of partner, sex act, and condom use. Sex. Transm. Dis.; **29**(No. 1): 38-43.

Verdier, R.I., Fitzgerald, D.W., Johnson Jnr., D.W. and Pape, J.W. (2000). Trimethoprim-Sulfamethoxazole compared with aprofloxacin for treatment and prophylaxis of *Isospora belli* and *Cyclospora cayetanensis* infection in HIV infected patients. A randomized controlled trial. Annals of Internal Medicine; **132**:885-888.

Vignesh, R., Balakrishman, P., Shankar, E., Murugavel, K. and Hanas, S. (2007). Short Report: High Proportion of Isosporiasis among HIV-infected Patients with Diarrhoea in Southern India. Am. J. Trop. Med. Hyg; **77** (No. 5): 823-824.

Visvesvara, G.S. (1997). Uniform staining of *Cyclospora* oocyst in faecal smears by modified saffranin technique with microwave heating. Journal of Clinical Microbiology; **35**: 730-733.

Walzer, P., Milder, J., Banwell, G., Kilgore, G., Klein and M., Parker, R.(1982). Epidemiological features of *Strongyloides stercoralis* infection in an endemic area in the United States. Am. J. Trop. Med. Hyg; **31**: 313-319.

Washington, W., Allen, S. Jnr., Jander, W., Procop, G., Schreckenberger, P. and Woods, G. (2006). Colour atlas and

Wurtz, R.M. (1994). *Cyclospora*: a newly identified intestinal pathogen of humans. Clinical Infectious Diseases; **18**: 620-623.

Weber, R., Ralph, T.B., Henry, S.B., Susanne, P.W., James, J.S. and Dennis, D.J. (1991). Threshold of detection of *Cryptosporidium* oocyst in Human stool specimens: Evidence for low sensitivity of current Diagnostic methods. J. Clin Microbiol; **29** (No. 7):1324-1327.

Webster, A., Lee, C., Cook, D., Grundy, J. and Emery V. (1989). Cytomegalovirus infection and progression towards AIDS in haemophiliacs with human immunodeficiency virus infection. Lancet; **2**: 63-66.

Weintraub, J.M. (2006). Improving *Cryptosporidium* testing methods: a public health perspective. J Water Health; **4**(No. 1): 23-6.

Weiss, R. (1993). How does HIV cause AIDS. Science; **200** (No. 5112): 1273-1279.

Weiss, R.A., Alder, M.W. and Roland-Jones, S.L. (2001). The changing phase of HIV and AIDS. British Medical Bulletin; **58**: 1-2.

Weiss, M.L. and Lindsay, D.S. (2004). World Class Parasites: Opportunistic infections: *Toxoplasma*, *Sarcocystis* and Microsporidia.vol.9, p.138. Kluwer Academic Publishers, Boston.

White, C. (2005). Cryptosporidiosis. In: Mandell, G.L., Bennett, J.E., Doilin, R. Principles and practice of Infectious Diseases. Vol. 2, 6th ed., p.280. Philadelphia, Pennsylvania: Elsivier Churchill Livingstone.

WHO. (1990). Interim Proposal for a WHO staging system for HIV infection and Disease. WHO Wkly Epidem. Rev; **65**(No. 29):221-228.

WHO. (1987). Prevention and control of I intestinal parasitic infections. WHO Technical Report; **749**: 1-86.

WHO. (2006). Schistosomiasis and soil-transmitted helminth infection-preliminary estimates of the number of children treated with albendazole and mebendazole. Wkly Epidemiol Rec; **16**: 145-164.

Willemot, P. and Klein, M. (2004). Prevention of HIV associated opportunistic infections and diseases in the age of highly active antiretroviral therapy. Expert Rev Anti Infect Ther; **2**: 521-32.

Wittner, M. and Weiss, L.M. (1999). The Microsporidia and Microsporidiosis. American Society for Microbiology, 1325 Massachusetts Avenue NW, Washington DC.

Wiwanitkit, V. (2006). Intestinal parasitic infection in HIV infected patients. Curr. HIV Rev; **4**: 87-96.

Wolfe, U., Switzer, W., Carr, J., Bhullar, V., Shanmugam, V. and Tamoufe, U. (2004). Naturally acquired simian retrovirus infections in Central African Hunters. Lancet; **363**: 932-937.

Wolska-Kusnierz, B., Bajer, A., Cassio, S., Heropolitan. K. E., Bernatouska, E. and Socha, P. (2007). **45**(No. 4): 458-464.

Woodcock, H.M. (1915). Notes on the protozoan parasite in the excreta. British Medical Journal; 1: 704.

Wyatt, R. and Sodroski, J. (1998). The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science; **280** (No. 5371): 1884-1888.

Xiao, L. and Ryan, U.M. (2004). Cryptosporidiosis: an update in molecular epidemiology. Curr Opin Infect Dis; **17**: 257-264.

Zaman, V. (1968). Observation on human *Isospora*. Trans R Soc Trop Med Hyg; **62**: 556-557.

Zheng, Y., Lovsin, N. and Peterlin, B. (2005). Newly identified host factors modulate HIV replication. Immunol. Lett; **97**(No. 2): 225-234.

APPENDIX

Participant Information Leaflet and Consent Form

What every prospective participant should know before deciding to or not to participate

Title of Research:

Intestinal parasitic infection in relation to CD4+ T- lymphocyte count and diarrhoea among HIV patients in a rural and urban setting.

Name and Affiliation of Researchers:

This study is being conducted by Dr. S.C.K Tay of the Department of Clinical Microbiology, KNUST.

Background

Human immunodeficiency virus (HIV) infection, a worldwide infection is a serious problem in this present day. One of the major health problems among HIV patients is the possibility of infectious organisms that may take advantage of the prevailing defect in immunity and cause infection. Furthermore, intestinal parasite infection, which is also one of the basic health problems in the tropical region, is common in these patients . Sub Saharan Africa is among the regions where intestinal parasitic infection is established and the largest burden of Acquired Immunodeficiency Syndrome (AIDS) cases also exist. Intestinal parasitic infection causes illness and death in HIV infected individuals.

Apparently, stool examination is not a routine test among HIV patients in our Hospitals. In developing countries, intestinal tract diseases caused by intestinal parasites may be complicated and is a major cause of death, in general and kills millions of AIDS patients annually. Thus the consequence of parasitic diseases is among the major health problems in tropical developing countries. This study is therefore aimed at studying the pattern of intestinal parasitic infection among HIV participants in our surrounding.

Purpose of Research:

The purpose of this research is to study the pattern of intestinal parasitic infection among HIV patients and find out their association with CD4 count and diarrhoea. A control group which will consist of HIV negative participants would also be studied to ascertain whether the rate of infection and diarrhoea are exclusive to HIV patients.

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

There are two different groups of participants in this study. For Group 1, participants of the Antiretroviral Therapy Clinic would be enrolled. For Group 2, participants would consist of HIV negative individuals based on a confirmed HIV test. All participants would have to respond to a standard questionnaire and records of their most CD4+ T-lymphocyte count would be taken. Stool specimens would be collected from participants and observed for parasites. In total we expect to recruit 686 participants into this study in two (2) selected

Ghana Health Service institutions (Nyinahin Government and St. Patricks Hospital) within Ashanti Region of Ghana.

Risk(s):
It is not risky to participate in this research. However blood samples may be taken to estimate CD4+ T-lymphocyte count and also rule out HIV positive participants for Group 2 participants.

Benefits:
The goal of this research is to study the pattern of intestinal parasitic infections among HIV patients, their relationship with diarrhoea and $CD4^+$ T-lymphocyte count. This will contribute to our knowledge in the prevalence of intestinal parasitic infection HIV patients, the risk of diarrhoea and their relationship with CD4+ T-lymphocyte count. Even though some parasites that cause disease in HIV/AIDS patients are considered AIDS- defining disease causing agents according to Centres for Disease Control and Prevention (CDC), their screening is not done even in known HIV patients in most routine laboratories at the primary care level, due to lack of knowledge, expertise and technique. Successful outcome of the research will bring to light to care takers of HIV patients to include stool examination as a routine test for clients and specific request for parasites such as microsporidia and coccidian parasites that occur in HIV/AIDS patients. It would also engender health care managers to organise training workshops on the special laboratory procedures required to diagnose intestinal microsporidiosis and coccidiosis. Knowledge about the pattern of pathogens can guide appropriate therapy.

Confidentiality:
All participants' information collected in this research will be identified by code numbers. No name will be recorded. Data collected cannot be linked to you in anyway. No name or identifier will be used in any publication or reports from this study. However, as part of our responsibility to conduct this research properly, we may allow the ethics committee to have access to your records.

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

Alternatives to participation:
If you choose not to participate, this will not affect your treatment in this hospital. However if you participate and parasites are identified in your stool, you would be treated accordingly based on Ghana Health Service treatment guidelines.

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

Consequence of Withdrawal:
There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study.

Costs/Compensation:
Contacts: If you have any question concerning this study, please do not hesitate to contact Dr. S.C.K Tay of the Department of Clinical Microbiology, Kwame Nkrumah Universty Of Science and Technology KNUST) (0208186142)

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

The Chairman
Committee on Human Research and Publication Ethics
Kumasi
Tel 03220-63248 or 0205453785

CONSENT FORM

Statement of person obtaining informed consent:

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

DATE: _____ SIGNATURE: _____

NAME: _____

Statement of person giving consent:

I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction. I understand that my participation is voluntary (optional). I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this information leaflet and consent form to keep for myself.
Name_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

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

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

WITNESS' NAME: _____

MOTHER'S SIGNATURE (maintain if participants could be under 18 years): _____

MOTHER'S NAME:_____

FATHER'S SIGNATURE (maintain if participants could be under 18 years): _____

FATHER'S NAME:_____

QUESTIONNAIRE

This project is being conducted at the Department of Clinical Microbiology, KNUST to study the pattern of intestinal parasitic infections among HIV positive individuals, their relationship with diarrhoea and $CD4^+$ T-lymphocyte count. The information that you provide will contribute to our knowledge in how susceptible an HIV patient is to intestinal parasitic infection, the risk of diarrhoea and its relationship with CD4+ T-lymphocyte.

Participation in this study would involve completing this questionnaire

Participant's No.…….
Date of Interview:…….
Study site:……
Name of community where you live:……..

Socio economic data

1. Age: 1. <14yrs ☐ 2.15-25yrs ☐ 3. 26-35yrs ☐ 4.36-45 ☐ 5.46 and above ☐
2. Sex: 1. Male ☐ 2.Female ☐
3. Marital status : 1.Single ☐ 2.Married ☐ 3.Divorced ☐ 4.Widowed ☐ 5.Seperated ☐ 6.Concubine ☐

4. Are you pregnant: 1.Yes ☐ 2.No ☐ 3.Non applicable(NA) ☐

5. Current occupation: 1.Farmer ☐ 2.Trader ☐ 3.Public servant ☐ 4.others(specify)……..

6. Monthly income: 1. less than GH¢45 ☐ 2.GH¢50 - 100 ☐ 3.GH¢101- 200 ☐ 4.GH¢201 - 400 ☐ 5.GH¢ 401 – 500 ☐ 6.more than GH¢ 500

7. Educational level: 1.Primary ☐ 2.Secondary ☐ 3.Tertiary ☐ 4. No school ☐ 5.Others(specify)……..

General hygiene

8. Type of water used for drinking and other domestic purposes? 1.Pipe ☐ 2.Bore hole ☐ 3.Well ☐ 4.River ☐ 5.Others(specify)……

9. Toilet facilities: 1.Private WC ☐ 2.Private pit latrine ☐ 3. Public pit latrine ☐ 4. Public WC ☐ 5.Public KVIP ☐ 6. Others (specify)…….

Personal hygiene

10. Do you wash hands with soap before eating?: 1.Yes ☐ 2.No ☐

11. Do you wash hands with soap after visiting the toilet? : 1.Yes ☐ 2.No ☐

12. Do you buy prepared meals?: 1.Yes ☐ 2.No. ☐ If yes go to Q 13. If no go to Q 14

13. How often?: 1.sometimes ☐ 2.always ☐

14. Do you have a pet in your home?: 1.Yes ☐ 2.No. ☐ If yes go to Q 15. If no go to Q 16

15. If yes, what animal?: 1.Cat ☐ 2.dog ☐ 3. Others(specify)……

16. Do you rear animals in the compound where you live?: 1.Yes ☐ 2.No. ☐ If yes go to Q 17. If no go to Q 18

17. What animal? 1. Goat ☐ 2. Sheep ☐ 3. Fowl ☐ 4. Cattle ☐ 5.Others (specify)……..

Gastrointestinal tract manifestation

18. How many times do you defecate in a day?: 1.once ☐ 2.two times ☐ 3.three times ☐ 4. Four times or more ☐ If three times or more go to Q19. If no go to Q 20.

19. What is the period of diarrhoea?: 1.Less than 3wks ☐ 2.Three wks or more ☐

20. What is the consistency of your stool? 1. Semi formed ☐ 2. Formed ☐ 3. Loose ☐ 4. Watery ☐

21. Any GIT disease symptoms? 1. Yes ☐ 2.No. ☐ If yes go to Q 21.If no go to Q 22.

22. Specify symptom or symptoms

1. Abdominal pain ☐ 2.Nausea or vomiting ☐ 3.Gas or bloating ☐ 4.Stomach pain or tenderness ☐

History of therapy

23. Antiretroviral Therapy? 1. Yes ☐ 2.No. ☐ If yes go to Q 23. If no go to Q 24

24. If yes how long have you been on the drug?: 1.1-3months ☐ 2.3-6months ☐ 3.6-12months ☐ 4.more than 1yr ☐

25. Have you taken antihelminth or antiprotozoa drug in the past 3 months?: 1.Yes ☐ 2.No ☐

26. Are you on prophylaxis i.e. cotrimoxazole? 1. Yes ☐ 2. No ☐

27. Any other medical complains (specify)?.....

QUESTIONAIRE
CONTROL GROUP

Participant number…….
Date of Interview:…….
Study site:……
Name of community where you live:……..

Socio economic data

Name:……

28. Age: 1. <14yrs ☐ 2.15-25yrs ☐ 3. 26-35yrs ☐ 4.36-45 ☐ 5.46 and above ☐

29. Sex: 1. Male ☐ 2.Female ☐

30. Marital status : 1.Single ☐ 2.Married ☐ 3.Divorced ☐ 4.Widowed ☐ 5.Seperated ☐ 6.Concubine ☐

31. Are you pregnant: 1.Yes ☐ 2.No ☐ 3.Non applicable(NA) ☐

32. Current occupation: 1.Farmer ☐ 2.Trader ☐ 3.Public servant ☐ 4.others(specify)……..

33. Monthly income: 1. less than GH¢45 ☐ 2.GH¢50 - 100 ☐ 3.GH¢101- 200 ☐ 4.GH¢201 - 400 ☐ 5.GH¢ 401 – 500 ☐ 6.more than GH¢ 500 ☐

34. Educational level: 1.Primary ☐ 2.Secondary ☐ 3.Tertiary ☐ 4. No school ☐ 5.Others(specify)……..

General hygiene

35. Type of water used for drinking and other domestic purposes? 1.Pipe ☐ 2.Bore hole ☐ 3.Well ☐ 4.River ☐ 5.Others(specify)……

36. Toilet facilities: 1.Private WC ☐ 2.Private pit latrine ☐ 3. Public pit latrine ☐ 4. Public WC ☐ 5.Public KVIP ☐ 6. Others (specify)…….

37. **Personal hygiene**

38. Do you wash hands with soap before eating?: 1.Yes ☐ 2.No ☐

39. Do you wash hands with soap after visiting the toilet? : 1.Yes ☐ 2.No ☐

40. Do you have a pet in your home?: 1.Yes ☐ 2.No.If yes go to Q 15. If no go to Q 16

41. If yes, what animal?: 1.Cat ☐ 2.dog ☐ 3. Others(specify)……

42. Do you rear animals in the compound where you live?: 1.Yes ☐ 2.No. ☐ If yes go to Q 17. If no go to Q 18

☐ ☐ ☐ ☐

43. What animal? 1. Goat 2. Sheep 3. Fowl 4. Cattle 5.Others (specify)……..

Gastrointestinal tract manifestation

☐ ☐ ☐

44. How many times do you defecate in a day?: 1.once 2.two times 3.three times 4. Four times or more ☐ If three times or more go to Q19. If no go to Q 20.

☐ ☐

45. What is the period of diarrhoea?: 1.Less than 3wks 2.Three wks or more

☐ ☐ ☐

46. What is the consistency of your stool? 1. Semi formed 2. Formed 3. Loose 4. Watery ☐

☐ ☐

47. Any GIT disease symptoms? 1. Yes 2.No. If yes go to Q 21.If no go to Q 22.

48. Specify symptom or symptoms

☐ ☐ ☐

1. Abdominal pain 2.Nausea or vomiting 3.Gas or bloating 4.Stomach pain or tenderness ☐

History of therapy

☐ ☐

49. Have you taken antihelminth drug in the past 3 months?: 1.Yes 2.No

50. Any other medical complains (specify)?.....

For Laboratory use only

☐ ☐

51. Retroviral screen: 1.Pos 2.Neg

☐ ☐ ☐

52. CD4+ T- cell count: 1. >500cells/ul 2.200-500cells/ul 3. 50-200cells/ul 4) <50cells/ul. ☐

☐ ☐ ☐ ☐ ☐ ☐

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

54. Stool R/E(Micro):1.other helminth ☐ 2.cryptosporidium ☐ 3.Isospora belli ☐
4.cyclospora ☐ 5.Giardia intestinalis ☐ 6.other flagellates ☐ 7.Microsporidia ☐
8.Strongyloides ☐ 9.Hookworm ☐
10. H.nana ☐ 11.Isospora belli + cryptosporidium ☐

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

PREPARATION OF REAGENTS

Formol Saline, 10% v/v

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

Sodium chloride…….8.5 g

Distilled water……1000ml

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

Physiological saline……..450ml

Formaldehyde solution, concentrated……50ml

1. Measure the physiological saline and transfer it to a leak-proof bottle.
2. Measure the formaldehyde solution and add to the saline. Mix well.
3. Label the bottle and store at room temperature in a safe place. The reagent is stable indefinitely.

Carbol Fuchsin stain

To make 1115ml:

Basic Fuchsin	10g
Ethanol or methanol,absolute	100g
Phenol	50g
Distilled water	1litre

1. Weigh the basic fuchsin on a piece of clean paper (preweighed), and transfer to a bottle of at least 1.5 litre capacity
2. Measure the ethanol (ethyl alcohol) or methanol (methyl alcohol), and add to the bottle. Mix at intervals until the basic fuhsin is dissolved

3. With great care, weigh the phenol in a beaker. Measure the water, and add some of it to the beaker to dissolve the phenol. Transfer to the bottle of stain, and mix well.
4. Add the remainder of the water, and mix well.
5. Label, and store at room temperature. The stain is stable indefinitely.

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

To make 1 litre

Methylene blue	3g
Distilled water	1litre

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

Field's stain A

To make 500ml:

Field's stain A powder	6g
Distilled water (Hot)	500ml

1. Weigh the powder on a piece of clean paper (pre-weighed), and transfer it to a large Pyrex beaker or high density polyethylene reagent bottle.
2. Measure the water and heat to boiling.

3. Add the hot water to the stain and mix to dissolve the powder
4. When cool, filter the stain into a clean bottle.
5. Label the bottle and store it at room temperature.

Field's stain B
To make 500ml:

Field's stain powder B	5g
Distilled water (Hot)	500ml

1. Weigh the powder on a piece of clean paper (pre-weighed), and transfer it to a large Pyrex beaker or high density polyethylene reagent bottle.
2. Measure the water and heat to boiling.
3. Add the hot water to the stain and mix to dissolve the powder
4. When cool, filter the stain into a clean bottle.
5. Label the bottle and store it at room temperature.