PREVALENCE OF CRYPTOSPORIDIUM INFECTION AND ITS CORRELATION WITH CD4$^+$ COUNT AMONG HUMAN IMMUNODEFICIENCY VIRUS SEROPOSITIVE PATIENTS WITHIN KADUNA METROPOLIS, NIGERIA

BY

NDAKS, COMFORT MAMMAN B.Sc (ABU 1995)
M. Sc/SCIE/00159/2008-2009

A THESIS SUBMITED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE,
AHMADU BELLO UNIVERSITY,
ZARIA- NIGERIA

FEBRUARY, 2013

DECLARATION

I hereby declare that the work reported in this thesis entitled 'Prevalence of *Cryptosporidium* Infection and its correlation with $CD4^+$ Count among Human immunodeficiency virus Seropositive Patients within Kaduna Metropolis, Nigeria' has been performed by me in the Department of Microbiology under the supervision of Dr. M. Aminu- Mukhtar and Dr. E.E. Ella. The information derived from the literature has been duly acknowledged in the text and list of references provided. No part of this thesis was previously presented for another degree or at any university.

_____	_____	_____
Name	Signature	Date

CERTIFICATION

This thesis entitled "PREVALENCE OF CRYPTOSPORIDIUM INFECTION AND ITS CORRELATION WITH $CD4^+$ COUNT AMONG HUMAN IMMUNODEFICIENCY VIRUS SEROPOSITIVE PATIENTS WITHIN KADUNA METROPOLIS, NIGERIA" meets the regulations governing the award of degree of Master of Science of Ahmadu Bello University for its contribution to scientific knowledge and literacy presentation

_____ _____

Dr. M. Aminu- Mukhtar Date
Chairperson, Supervisory Committee
Department of Microbiology
Faculty of Science
Ahmadu Bello University Zaria

_____ _____

Dr. E. E. Ella Date
Member, Supervisory Committee
Department of Microbiolgy Faculty of Science
Ahmadu Bello University Zaria

_____ _____

Dr.S.A. Ado Date
Head,
Department of Microbiology
Faculty of Science
Ahmadu Bello University Zaria

_____ _____

Professor A.A. Joshua Date
Dean, Postgraduate School
Ahmadu Bello University, Zaria.

DEDICATION

This thesis is dedicated to God Almighty whose grace has seen me through all my endeavors in life.

ACKNOWLEGEMENT

My appreciation goes to several persons who contributed in making this research work a success. I wish to first of all thank the Lord Almighty who has brought me this far. To my supervisors, Dr Mrs Aminu-Mukhtar and Dr E.E. Ella, words are not enough to express my gratitude, for your encouragement support, guidance and hardwork that anchored this thesis.

My thanks also goes to all staff of FCTA most especially the Deputy Director Clinical and Diagnostic, Mrs Doris Oriaku for the opportunity given to me to acquire more knowledge.

I wish to also acknowledge the support and cooperation given to me by Kaduna State Ministry of Health, Barau Dikko Specialist Hospital, Gwamna Awan General Hospital and Yusuf Dantsoho General Hospital. Special thanks to Mrs Felicia Kurah for her encouragement and assistance. I want to also thank all HIV seropositive patients in these hospitals; without whom this work will not be possible.

I sincerely thank the post graduate coordinator (Dr H.I. Inabo), all Staff of the Department of Microbiology, Professor A.J. Nok and Professor I.S. Ndams for their support and assistance.

My special thanks goes to my family, Major (Rtd) and Mrs D.M. Ndaks and my other siblings, Lawrence, Vincent, Nancy, Grace, Gideon and their families for their prayers and encouragement.

My gratitude also goes to The Tekus, Rhoda Goshie, and Jeremiah Kyom who made my stay, in Zaria worthwhile. I want to also thank The Handas, Madglene Augustine, Mary Haruna Maryam Ocheme , Moses Kujiyat, Joy Maina, Becky Yisa, Julius Okojokwu, Joy Gofwan, Jacob Ukoduah, Ubandoma Ande, Micheal Yero and all my classmate for assistance and encouragement.

ABSTRACT

Cryptosporidiosis being an opportunistic infection is becoming more prevalent in HIV seropositive patients. This study was carried out to determine the prevalence of *Cryptosporidium* infection and $CD4^+$ count among HIV seropositive patients within Kaduna metropolis. Stool specimens were collected from 300 HIV seropositive patients and 30 controls (HIV seronegative patients) between October 2011 and January 2012, and were examined for oocyst and antigen of *Cryptosporidium* by microscopy and ELISA method respectively. Microscopy was used as the Gold standard and its sensitivity was compared with that of ELISA. Patients' blood were also analysed for $CD4^+$ T-cell count by flow cytometry. Prevalence of 15% (45/300) was obtained using microscopy. Sensitivity of microscopy when compared to ELISA was found to be 24.9%. For the control, a prevalence of 3.3% was obtained. Association between the infection and socio-demographic characteristic of patients shows a higher prevalence among those who defecate in open layouts (50%: 3/6) and those whose source of drinking water was river (50%: 1/2). There was a significant association between *Cryptosporidium* infection and diarrhoea (χ^2 = 104.669, df=1, p=0.000) and the duration of diarrhoea (χ^2 = 117.073, df=4, p=0.000). There was no significant difference in relation to occupation, marital status, age, sex, education, animal contact, overseas travel and swimming (p>0.05). There was a decrease in prevalence with increase in duration of being on HAART. The study showed a strong association between Cryptosporidiosis and $CD4^+$T-cell count (χ^2 = 58.478, df=10, p=0.000). Mean $CD4^+$ count was 409.86±14.1 while the median was 382.

TABLE OF CONTENT

Page

Title page - - - - - - - - - - i

Declaration - - - - - - - - - - ii

Certification - - - - - - - - - - iii

Dedication - - - - - - - - - - iv

Acknowledgement - - - - - - - - - v

Abstract - - - - - - - - - - vi

Table of content - - - - - - - - - vii

List of tables - - - - - - - - - xi

List of figures - - - - - - - - - xii

Appendixes - - - - - - - - - - 83

CHAPTER ONE

1.0 **INTRODUCTION-** - - - - - - - - 1

1.1 Statement of problem - - - - - - - - - 3

1.2 Justification - - - - - - - - - - 4

1.3 Aim - - - - - - - - - - 5

1.4 Specific objectives - - - - - - - - - 5

1.5 Research hypothesis - - - - - - - - - 6

1.6 Research question - - - - - - - - - 6

CHAPTER TWO

2.0 **LITERATURE REVIEW** - - - - - - - - 7

2.1.0 Taxonomy of *Cryptosporidium* - - - - - - - 7

2.1.1 History of *Cryptosporidium* - - - - - - - 8

2.1.2 Transmission - - - - - - - - - 9

2.1.3 Life Cycle - - - - - - - - - 10

2.1.4 Epidemiology - - - - - - - - 12

2.1.5 Immunity - - - - - - - - - 13

2.1.6 Diagnosis - - - - - - - - - 14

2.1.7 Treatment - - - - - - - - - 17

2.1.8 Clinical manifestation - - - - - - - 18

2.1.9 Prevention and control - - - - - - - 19

2.2.0 Human immunodeficiency virus/Acquired immune syndrome - - 20

2.2.1 History of HIV/AIDS - - - - - - - - 20

2.2.2 Transmission - - - - - - - - - 21

2.2.3 Pathogenesis - - - - - - - - - 22

2.2.4 Epidemiology - - - - - - - - 24

2.2.5 Immunity - - - - - - - - - 25

2.2.6 Treatment - - - - - - - - - 26

2.2.7 Diagnosis - - - - - - - - - 26

2.2.8 Symptoms - - - - - - - - - 28

2.2.8.1 Chronic infection - - - - - - - - 29

2.2.8.2 Acquired immune deficiency syndrome - - - - - 29

2.2.9 Prevention - - - - - - - - - 31

2.3.0 CD4 T cells - - - - - - - - - 32

CHAPTER THREE

3.0 **MATERIALS AND METHODS** - - - - - - 35

3.1 Study area - - - - - - - - - 35

3.2 Study population - - - - - - - - 35

3.3 Control population - - - - - - - - 35

3.4 Inclusion criteria - - - - - - - - 36

3.5 Exclusion criteria - - - - - - - - 36

3.6 Ethical approval and consent - - - - - - - 36

3.7 Sample size - - - - - - - - - 36

3.8 Study design - - - - - - - - - 37

3.9 Data collection using questionnaires - - - - - - 37

3.10 Sample collection - - - - - - - - 37

3.11 Sample analysis - - - - - - - - 38

3.11.1 Formol ether concentration and Modified Ziehl-Neelsen (ZN) method - 38

3.11.2 ELISA method for detection of *Cryptosporidium* - - - - 39

3.11.2.1 Principle of test - - - - - - - - 39

3.11.2.2 Test Procedure - - - - - - - - 39

3.11.2.3 Interpretation of result - - - - - - - 40

3.11.3 CD_4^+ T cell count - - - - - - - - 40

3.11.3.1 Principle of test - - - - - - - - 40

3.11.3.2 Test procedure - - - - - - - - 41

3.12 Sensitivity and Specificity - - - - - - - 41

3.13 Data analysis - - - - - - - - - 41

CHAPTER FOUR

4.0 **RESULT** - - - - - - - - - 42

CHAPTER FIVE

5.0 **DISCUSSION** - - - - - - - - 61

CHAPTER SIX

6.0 **CONCLUSION** - - - - - - - - 66

6.1 Recommendation - - - - - - - - 66

6.2 Limitations of study - - - - - - - - 67

References - - - - - - - - - 68

Appendix I - - - - - - - - - 82

Appendix II - - - - - - - - - 83

Appendix III - - - - - - - - - 84

Appendix IV - - - - - - - - - 85

LIST OF TABLES

Table 4.1 Prevalence of *Cryptosporidium* infection among HIV seropositive patients attending some selected hospitals within Kaduna metropolis, Nigeria - - - - - - 43

Table 4.2 Distribution of *Cryptosporidium* infection according to demographic factors among HIV seropositive patients within metropolis, Nigeria - - - - - - - 47

Table 4.3 Distribution of *Cryptosporidium* infection in relation to risk factors among HIV seropositive patients within Kaduna metropolis, Nigeria - - - - - - - 49

Table 4.4 Distribution of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis, Nigeria in relation to clinical symptoms - - - - - - 51

Table 4.5 Prevalence of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis by their HAART status- - - - - - - 53

Table 4.6 Prevalence of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis, Nigeria in relation to $CD4^+$ T cell count - - - - - 57

Table 4.7 Determination of sensitivity and specificity of microscopy using ELISA test - - - - - - - 60

LIST OF FIGURES

Figure 2.1 Life cycle of *Cryptosporidium parvum* - - - - 11

Figure 2.2 *Cryptosporidium* oocysts in modified Ziehl-Neelsen-stained faecal smear 16

Figure 2.3 Computer generated image of a human $CD4^+$ white blood cell - 34

Figure 4.1 *Cryptosporidium* oocyst in the stool of patient under the Microscope - - - - - - - - 44

Figure 4.2 Venn diagram of the number of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis by ELISA and Microscopy - - - - - - - 45

Figure 4.3 Prevalence of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis in relation to duration on HAART 55

Figure 4.4 Prevalence of *Cryptosporidium* infection among HIV seropositive patients in relation to $CD4^+$ count. - - - - 58

-

CHAPTER ONE

1.0 INTRODUCTION

Human immunodeficiency virus (HIV) infection is a global health emergency. According to World Health Organisation (WHO) progress report of 2010, 33.3 million people are living with HIV worldwide, 2.6 million people were infected in 2009 and 1.8 million people died of acquired immune-deficiency syndrome (AIDS) in that same year. Acquired immune-deficiency syndrome is a disease of the human immune system caused by HIV (Weiss, 1993; Septowits, 2001). The condition progressively reduces the effectiveness of the immune system and leaves the individual susceptible to opportunistic infections and tumors. Opportunistic infections caused as a result of loss of immunity due to HIV are called opportunistic HIV infections (Kaplan *et al.,* 2002). Diarrhoea is a common clinical manifestation of HIV regardless of whether or not patients have AIDS (Joshi *et al.,* 2002). The etiology of such diarrhoea could either be parasitic, bacterial, fungal, enteroviral or HIV itself (Framm and Soave, 1997). Several species of intestinal parasites have been associated with diarrhoea which occurs with HIV infection. The commonly reported parasites include *Cryptosporidium parvum, Isospora belli, Microsporidium* spp, *Giardia lamblia, Entamoeba histolytica* and *Cyclospora* spp. Besides these, the nematode, *Strongyloides stercoralis* can cause an overwhelming infection in patients with immunosuppressive disorders (Arora and Arora, 2009).

Cryptosporidium is a coccidian protozoan, belonging to the phylum Apicomplexa, class Sporozoa, and sub-class Coccidian (Juranek *et al*., 2001). Protozoan parasites belonging to the genus *Cryptosporidium* have been described as a cause of diarrhoeal disease in immunodeficient and immunocompetent individual (Martin *et al.,* 2009). Cryptosporidiosis is the disease caused by *Cryptosporidium* and is due to infection by one or more species of the genus.

Cryptosporidium hominis is reported as being largely restricted to humans while *Cryptosporidium parvum* is found in a wide range of animals (particularly cattle and sheep) including humans. Mixed infections of *Cryptosporidium hominis* and *Cryptosporidium parvum* do occur and have been reported among Nigerians by Molley *et al.* (2010).

The *Cryptosporidium* parasite is transmitted by environmentally hardy microscopy cyst (oocyst) which excyst in the small intestine once ingested and results in an infection of intestinal epithelial tissue. A major problem of public health concern is the fact that the oocyst are only 4 to 6 μm in diameter, much too small to be easily removed by sand filters used to purify drinking water (Willey *et al.,* 2011). In addition the oocyst may remain viable for 3 to 6 months in moist environment and is extremely resistant to disinfectants such as chlorine (Willey *et al.,* 2011). Infection is generally self-limiting followed by variable protective immunity involving humoral and cell-mediated responses, except in the immuno-suppressed, when infection may be prolonged and fatal (Kosek *et al.,* 2001).

Most of the risk factors for *Cryptosporidium* infection were determined from outbreaks investigations. Outbreaks have been associated with drinking water from public and private supplies, swimming in pools, consumption of unpasteurised milk and contact with farm animals, especially during farm visits (Goh *et al.,* 2004). Other sources of infection are child care workers, parents of infected children, people who take care of other people with cryptosporidiosis and international travelers. Most cases of cryptosporidiosis are sporadic rather than outbreaks associated infections. Outbreaks represent 40% of all cases of *Cryptosporidium* infection (Djuretic *et al.,* 1995), although a further proportion of all cases may likely be associated with undetected outbreaks (Hunter *et al.,* 2001).

There is no reliable treatment for *Cryptosporidium* enteritis, but certain chemotherapeutic agent such as paramycin, atavaquone, nitazoxanide (Smith and Corroran, 2004) and azithromycin are sometimes used in its treatment which have only temporary effects. Treatment is primarily supportive and involves continuous replacement of fluids.

1.1 Statement of Problem

Human immunodeficiency virus infection has a worldwide distribution and is a serious health problem in the present day. The National Agency for the Control of AIDS (NACA) estimates that there are about 2.95 million people living with HIV (PLHIV) in Nigeria in 2009, ranking the country third among countries with the highest burden of HIV infection in the world, next only to India and South Africa. According to the 2010 National sentinel survey report, the prevalence rate for HIV in Nigeria is 4.1% (FMOH, 2010). In HIV infected patients, progressive decline in their immunological responses makes them extremely susceptible to a variety of common opportunistic infections. Cryptosporidiosis being an opportunistic HIV infection is becoming more prevalent in HIV seropositive patients. Person with compromised immune system can suffer life threatening chronic diarrhoea especially when their CD_4^+ lymphocyte count falls below 200 cell/µl (Cama *et al.,* 2006). Reports indicate that diarrhoea occurs in 30 – 60% of AIDS patients in developed countries and up to 90% in AIDS patients in developing countries (Framm and Soave, 1997).

Cryptosporidiosis in patient with HIV/AIDS who also have severely impaired immunity may be a devastating disease as it causes chronic severe and intractable diarrhoea that greatly reduces the patient's quality of life or in many people shorten their life expectancy. There are no reproducible reliable palliative or curative therapies for cryptosporidiosis and HIV infected persons are not educated and counselled about the modes of transmission and preventive

measure. High prevalence of cryptosporidiosis have been reported in HIV/AIDS patients though prevalence varies depending on location, age of study, stage of disease and laboratory method used for diagnosis (Zaidah *et al*., 2008). Reports in Nigeria have also shown varying results, Aminu and Yakubu (2008) reported a prevalence of 25% among non-diarrhoeic HIV-positive children in Zaria, Ibrahim *et al.* (2007) reported a prevalence of 16.83% among adults with chronic diarrhoea at Jos University Teaching Hospital and Akinbo *et al.* (2010) reported a prevalence of 22.2% among HIV patients in Benin City.

Microscopy method of diagnosis of *Cryptosporidium* is laborious, time consuming and requires high level of technical skill thus the oocyst of the organism can be easily missed. Also the intact organism has to be present before it can seen while by ELISA method it is the antigen that are been detected thus making it more sensitive than microscopy. ELISA methods are expensive and so rarely used for diagnosis. Elgun and Koltas (2011) in a study carried out in Turkey showed a higher prevalence by ELISA (24.03%) than Microscopy (5.19%) and El-settawy and Fathy (2012) in a study carried out in Egypt also showed a higher prevalence by ELISA (20.9%) than microscopy (18.6%). Studies to compare microscopy method and newer ELISA methods have not been carried out in Zaria.

1.2 Justification

Cryptosporidiosis being an opportunistic infection is becoming more prevalent in HIV seropositive patients and its prevalence is usually under estimated because conventional diagnostic techniques do not efficiently detect parasite. Also detection of *Cryptosporidium* by microscopy is not routinely carried out in the laboratory. Therefore there have been underreporting of the prevalence of *Cryptosporidium* infection in Nigeria and not many studies

have addressed this problem. From the foregoing, there is therefore the need to document the prevalence of *Cryptosporidium* infection among HIV seropositive patients.

Microscopy is the most frequently used method of diagnosis of *Cryptosporidium* infection in Nigeria, therefore there is need compare these two methods to see which best detect the parasite in the study population hence providing information on the most sensitive method for detecting the parasite. There is also the need to provide data that will be useful for clinical diagnosis and treatment of the disease and finally information on the factors associated with the spread of the disease with the aim of aiding the disease control and prevention. No such study has been conducted in study area therefore there is the need to carry out this study.

1.3 Aim

The aim of this research work was to determine the prevalence of *Cryptosporidium* infection among HIV seropositive patients attending three ARV (Antiretroviral) Clinics in Kaduna metropolis, Nigeria.

1.4 Specific Objectives

The objectives of this study were:

1. To determine the prevalence of *Cryptosporidium* infection in HIV seropositive patient with or without diarrhoea, using the modified Ziehl-Neelsen staining method (Kinyoun's method).
2. To compare the sensitivity of Kinyoun's method as a conventional staining method to newer ELISA method.
3. To determine the risk factors associated with *Cryptosporidium* infection among study population using questionnaires.

4. To correlate *Cryptosporidium* infection with CD_4^+ T-cell count of HIV patients

1.5 Research Hypothesis

H_o: *Cryptosporidium* infection is not associated with HIV seropositive patient within Kaduna metropolis, Kaduna state, Nigeria.

H_a: *Cryptosporidium* infection is associated with HIV seropositive patient within Kaduna metropolis, Kaduna state, Nigeria.

1.6 Research Questions

1. What is the prevalence of *Cryptosporidium* infection among HIV seropositive patient within Kaduna metropolis?
2. What is the sensitivity of the Kinyoun's method as compare to the ELISA method?
3. What is the prevalence of *Cryptosporidium* infection according to sociodermographic factors among the study population?
4. What are the risk factors associated with *Cryptosporidium* infection in the study population?
5. What is the correlation between the infection and CD_4^+ T-cell count?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 Taxonomy of Cryptosporidium

Cryptosporidium is a genus of protozoan parasites within the phylum *Apicomplexa*, and its taxonomy continues to evolve (Jonathan and Yoder, 2007). However, the current taxonomic position of the genus *Cryptosporidium* is described as follows (Fayer *et al*., 2001):

Phylum: Apicomplexa

Class: Conoidasida

Sub-class: Coccidiasina

Order: Eucoccidiorida

Sub-order: Eimeriorina

Family: *Cryptosporidiidae*

Currently there are 20 described species of which *Cryptosporidium hominis, C. parvum, C. meleagridis, C. felis, C. canis, C. suis, C. muris* and *C. andersoni* and 6 unnamed species (*C. cervine*, monkey, skunk, rabbits, horse and chipmunk genotype) infect immunocompetent and immunocompromised humans (Jirku *et al*., 2008; Chalmer *et al*., 2009; Smith and Nichols, 2009). *Cryptosporidium hominis* and *Cryptosporidium parvum* are the primary species of *Cryptosporidium* that infect humans. *Cryptosporidium hominis* has an anthroponotic transmission cycle, while *C. parvum* is zoonotic, infecting cattle and other ruminants, in addition to humans (Feltus *et al.*, 2006).

Cryptosporidiosis is a gastrointestinal illness caused by *Cryptosporidium* (Chen *et al.,* 2002). *Cryptosporidium spp*. is a major cause of diarrhoea in developing countries mainly affecting children and HIV-infected individuals with low CD4 counts. The infection is self-

limiting in immunocompetent hosts, but can be severe and persistent in the immunocompromised and malnourished (Ajjampur *et al.,* 2008). Similarly, diarrhoea is one of the most common AIDS-related illness causing a significant morbidity and mortality in HIV- infected patient (Suddiqui *et al*., 2007).

2.1.1 History of Cryptosporidium

Cryptosporidium was first described by E.E. Tyzzer in 1907. He identified the parasite in the gastric glands of a mouse and described it as in formed flask shaped either spheriodal or ellipsoidal. All forms during the period of growth posses a relatively thin limiting membrane, an organ of attachment and each contains one or more globule of fat which during segmentation are found in the residual body. Although during this time the steps involved in maturation and fertilization were not yet identified, Tyzzer noticed that each oocyst contains 4 sporozoites which are about 10 microns in length, slender, fusiform without demonstrable structure. At this time, the parasite was named *Cryptosporidium muris* and classified as possible in the family *Asporocystidae* . It is a zoonotic pathogen first noted to cause disease of turkeys in 1955 (Slavin, 1955) and of calves in 1971 (Panciera and Thomassen, 1971). *Cryptosporidium* infects the respiratory or gastrointestinal tracts of a large number of hosts including fish, birds, reptiles, and mammals (Fayer and Ungar, 1986). It was not until 1976, however, that the first human cases were identified. One of the afflicted humans was a 3-year-old child who developed severe enterocolitis that resolved on its own. The other was a 39-year-old immunosuppressed man. His condition improved after he discontinued one of his medications, cyclophosphamide (John and Petri Jr, 2006). The Centers for Disease Control (CDC) in her Morbidity and Mortality Weekly report (1982) documented the importance of *C. parvum* as a major human pathogen in 1982, reporting outbreaks of cryptosporidial diarrhoea in 21 patients with advanced HIV disease and in

immunologically normal hosts who had been exposed to *Cryptosporidium* from infected calves. In 2004, cryptosporidiosis was added to the WHO's 'Neglected Diseases Initiative' which includes diseases that occur mainly in developing countries and were linked to poverty and lack of access to services (Savioli *et al*., 2006).

Cryptosporidium was first recognized as water borne pathogen during an outbreak in Brain Station, Texas, where more than two thousand individuals were infected with Cryptosporidiosis (D'Antonio *et al*., 1995; Graczyk *et al*., 1998). Since that time, out breaks affecting over a million individuals have been documented throughout North America and Europe, with the single largest epidemic occurring in Milwaukee, Wisconsin, in 1993 (Mackenzie *et al*., 1994). Most deaths related to these outbreaks occurred in AIDS patients (Hoxie *et al*., 1997). Recently most water–borne outbreaks of cryptosporidiosis in the USA have occurred due to contamination of recreational water sources such as swimming pools and water parks (Yoder *et al*., 2010).

2.1.2 Transmission

Transmission is via the faecal oral route, the infective dose depending on the strain could be as low as 10 oocyst (Okhuysen *et al*., 1999). Chlorination and treatment of water supplies are not always sufficient to eliminate *Cryptosporidium* (Winn Jr. *et al.,* 2006). Thus, the oocysts may reside in swimming pools and chlorine-treated or filtered drinking water (Chen *et al.,* 2002). A person can contact the disease via direct contact with infected animals or by eating food contaminated by faeces (Winn Jr. *et al*., 2006). There are often outbreaks in hospitals and day-care centers, showing that *Cryptosporidium* can be transmitted from one human to another (Ryan and Ray, 2004). Yet another way to become infected as reported by CDC in 2009 is through exposure to faeces during sexual activities that may involve contact with faeces.

2.1.3 Life Cycle

The life cycle of *Cryptosporidium* consists of an asexual stage and a sexual stage. After being ingested, the oocysts excyst in the small intestine, they release sporozoites that eventually get attached to the microvilli of the epithelial cells of the small intestine. From there they transform into trophozoites that reproduce asexually by multiple fission, a process known as schizogony. The trophozoites develop into Type 1 meronts that contain 8 daughter cells (Ryan and Ray, 2004). These daughter cells are Type 1 merozoites, which get released by the meronts. Some of these merozoites can cause autoinfection by attaching to epithelial cells; others become Type II meronts (Chen *et al*., 2003) which contain 4 Type II merozoites (Ryan and Ray, 2004). These merozoites, once released attach to the epithelial cells, from there they develop to either macrogamonts or microgamonts (Chen *et al*., 2003), these are the female and male sexual forms, respectively (Ryan and Ray, 2004). Zygotes are formed by microgametes from the microgamont penetrating the macrogamonts developing into oocysts of two types (Chen *et al*., 2003). About 20% of oocysts have thin walls and so can reinfect the host by rupturing and releasing sporozoites that start the process over again (Ryan and Ray, 2004). Others develop into thick-walled mature and infective oocyst and are excreted into the environment (Chen *et al.,* 2003; Ryan and Ray, 2004). The life cycle of *Cryptosporidium parvum* is presented in figure 2.1.

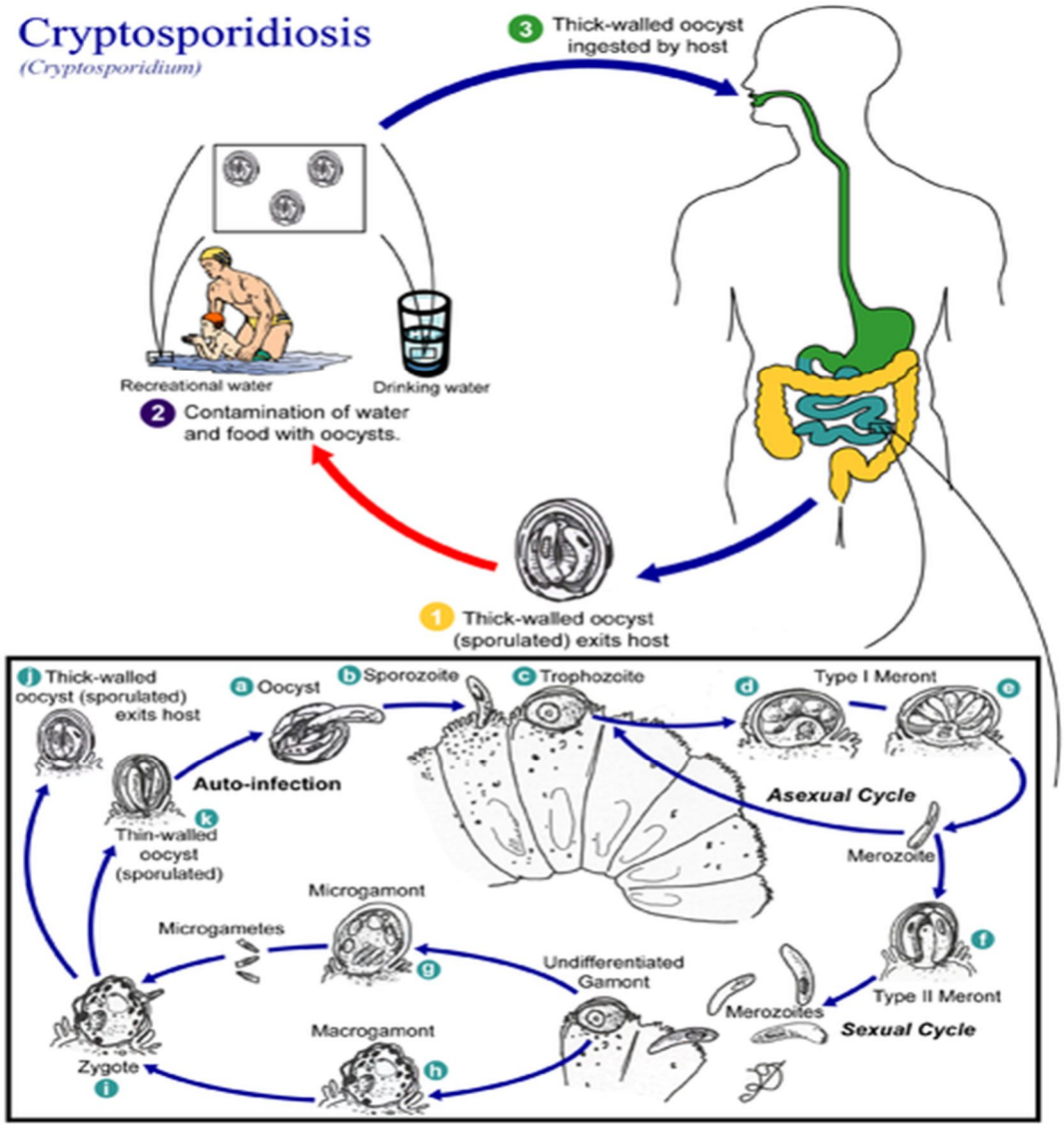


Figure 2.1: Life cycle of *Cryptosporidium parvum*.

Source (CDC, 2009).

2.1.4 Epidemiology

Cryptosporidiosis is found worldwide. In developing countries, 8-19% of diarrhoeal diseases can be attributed to *Cryptosporidium* (Gatei *et al*., 2006), with 10% of the population in developing countries excreting its oocysts. In developed countries, the rate is lower (1-3%) (Chen *et al*., 2002). Infection by *C. parvum* has been reported in six continents and identified in patients aged 3 days to 95 years old (Flanigan and Soave, 1993). Persons most likely to be infected with *Cryptosporidium* include: infants and younger children in day-care centres, those whose drinking water is untreated, those involved in farming practices such as lambing, calving, and muck-spreading, those engaging in sexual practices that brings a person into oral contact with faeces of an infected individual, patients in a nosocomial setting with other infected patients or health-care employees, veterinarians who come in contact with farm animals and travelers to areas with untreated water living in densely populated urban areas and owners of infected household pets (rare) (Keusch, *et al*., 1995; Casemore, *et al*., 1994; Goodgame, 1996; Juranek, 1995; Flanigan and Soave, 1993).

The high resistance of *Cryptosporidium* oocysts to disinfectants such as chlorine bleach enables them to survive for long periods and still remain infective (Carpenter *et al.,* 1999). Furthermore the oocyst are much too small (4-6um) to be removed by the conventional sand filters used in the purification of drinking water (Willey *et al.,* 2011). Infection with small oocyst usually occurs directly by faecal-oral transmission, water or food-borne (Joachim, 2004). The infectious dose is low; feeding studies have demonstrated that the ingestion of as few as 10-30 oocysts can cause infection in healthy persons (Du pont *et al.,* 1999; Okhuysen *et al.,* 1999*)*. Infected persons have been reported to shed 10^8-10^9 oocysts in a single bowel movement and to

excrete oocysts for up to 50 days after cessation of diarrhoea (Chappell *et al.,* 1996; Jokipii and Jokipii*,* 1986).

High prevalence of cryptosporidiosis have been reported in HIV/AIDS patients (Ghimire *et al*., 2004; Adesiji *et al*., 2007; Zaidah *et al*., 2008) though the prevalence varies depending on location, age of study population, stage of disease and laboratory methods used (Zaidah *et al*., 2008). In a similar vein, the few report in Nigeria show varying prevalence depending on the region of the country and age of patients (Adesiji *et al*., 2007). In persons with AIDS, the rate of cryptosporidiosis is higher in developing countries, ranging from 12-48% of persons with AIDS who have diarrhoea (White, 2005; Wumba, 2010).

2.1.5 Immunity

The host immune response to *Cryptosporidium parvum* infection is still poorly understood. Experimental-infection studies with mice and calves show that immunity is dependent on the number of CD4 T cells increasing within the intestinal intraepithelial lymphocytes, and generating gamma interferon and Interleukin-12 may play a role, possibly through its ability to induce gamma interferon production (Tsaihong *et al*., 1990). Antigen-driven interleukin-12 production in macrophages requires interaction between CD40 on antigen-presenting cells and CD40 ligand on $CD4^+$ T lymphocytes. No difference was found between cryptosporidiosis in normal and B-cell-depleted neonatal mice, suggesting that antibody production may play a less important role in recovery from infection (Sreedharan *et al*., 1996). The role of CD8 T cells is unclear, but they appear to play a role in controlling infection in mice (Taghi-Kilani *et al*., 1990). Mast cells may play a role in innate resistance in mice (Taghi-Kilani *et al*., 1990). Interleukin-2, tumor necrosis factor alpha, and $\gamma\delta^+$ T cells are probably not involved in innate resistance to *C. parvum*, and the role of natural killer (NK) cells remains unclear

(Taghi-Kilani *et al*., 1990). The potential importance of gamma interferon has recently been highlighted in several studies. Theodos *et al*., (1997) developed a nonhealing mouse model of infection with a targeted disruption of the gamma interferon (Tanvuksel *et al*., 1995). These mice developed an overwhelming infection of the entire gastrointestinal tract and usually died within 2 to 3 weeks; in contrast, healing mice were free of infection by day 30. Mead and You, (1998) also demonstrated significant differences in survival between mouse strains with and without the ability to produce gamma interferon (Mc Gowan *et al*., 1993). In a more recent study in mice with severe combined immunodeficiency (SCID mice), it was shown that SCID mice remained infected with *Cryptosporidium* spp. for a long time without symtoms but that those with an inability to produce gamma interferon died rapidly (Hashmey *et al*., 1997). They suggested that gamma interferon might be important in immunity against secondary infections, and other evidence suggests that it plays a role in innate immunity.

In a study of human volunteers, jejunal biopsy specimens were examined for gamma interferon mRNA (Venturu *et al*., 1997). Gamma interferon m-RNA was not detected prior to challenge with *Cryptosporidium* spp. but was detected in 13 samples from 26 people after deliberate infection. After infection, the gamma interferon mRNA was more commonly found in volunteers with pre-existing antibody and in those who did not shed oocysts in the faeces indicating that gamma interferon probably plays a more important role in the secondary rather than primary infections.

2.1.6 Diagnosis

There are many diagnostic tests for *Cryptosporidium*. They include microscopy, staining, and detection of antigens. Microscopy can help identify oocysts in faecal matter (Chen *et al*., 2002). To increase the chance of finding the oocysts, the diagnostician should inspect at least 3

stool samples (Gatei *et al*., 2006). There are several techniques to concentrate either the stool sample or the oocysts. The modified formalin-ethyl acetate (FEA) concentration method concentrates the stool (Meamar *et al*., 2006). Both the modified zinc sulfate centrifugal flotation technique and the Sheather's sugar flotation procedure can concentrate the oocysts by causing them to float (Gatei *et al*., 2006).

Other staining techniques include acid-fast staining (Chen *et al*., 2003) which will stain the oocysts red (Winn Jr *et al* 2006). One type of acid-fast stain is the Kinyoun technique (modified Ziehl-Neelsen stain). Giemsa staining can also be performed (Ryan and Ray, 2004). Biopsy of the small intestine can be stained with hematoxylin and eosin (H & E), which will show oocysts attached to the epithelial cells (Winn Jr *et al*., 2006).

Detecting antigens is yet another way to diagnose the disease. This can be done with direct fluorescent antibody techniques (Chen *et al*., 2002). It can also be achieved through indirect immunofluorescence assay (Murray *et al*., 2005). For greatest sensitivity and specificity, immunofluorescence microscopy is the method of choice followed by Enzyme immunoassays (Morgan-Ryan *et al.,* 2002).

Polymerase chain reaction (PCR) has been developed for the diagnosis *Cryptosporidium* with high sensitivity and can be used for species characterization (Chen *et al.,* 2002). If the patient is thought to have biliary cryptosporidiosis, then an appropriate diagnostic technique is ultrasonography. If that returns normal results, the next step would be to perform endoscopic retrograde cholangiopancreatography (Chen *et al*., 2002). Figure 2.2 shows pictures of *Cryptosporium* oocyst in modified Ziehl-Neelsen stain.

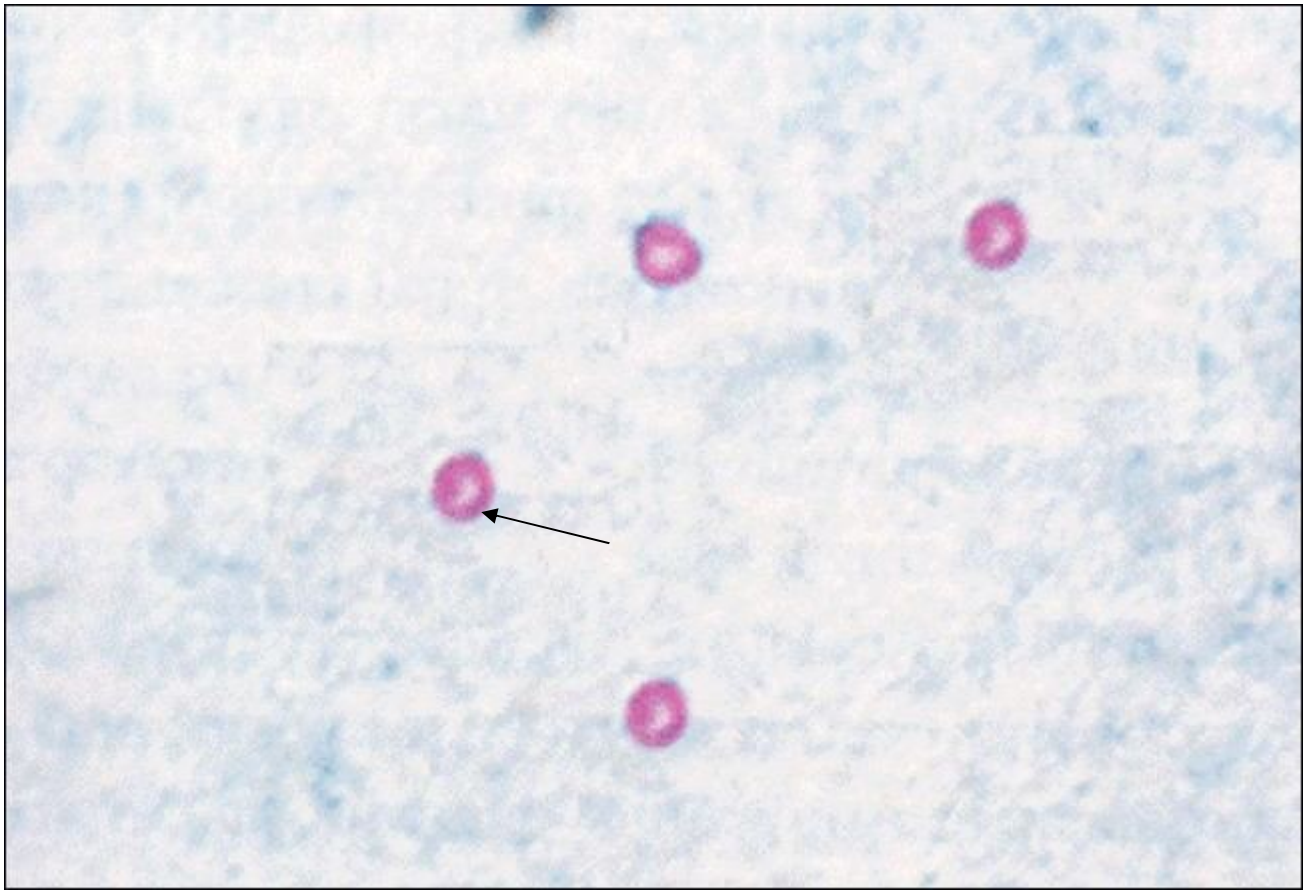


Figure 2.2: Arrow shows *Cryptosporidium* ooysts in modified Ziehl-Neelsen-stained faecal smear (x 450)

Source (Arora and Arora, 2009)

2.1.7 Treatment

The use of highly active antiretroviral (HAART) in persons with AIDS has reduced the prevalence of infection with *Cryptosporidium parvum* and the length and severity of its clinical course. This effect has in most cases been attributed to the recovery of the host immunity (Mele *et al*., 2003). However the emergence of drug- resistant HIV variant and failure or discontinuation of HAART has been associated with re-emergence of *Cryptosporidium* infection in these patients (Maggi *et al*., 2000; Nannini and Okhuysen, 2002). Even patients with advanced AIDS who are on HAART have recently been reported to have *Cryptosporidium* infection (Werneck-Silva and Prado, 2009).

In immunocompetent people cryptosporidiosis often resolves on its own and treatment is not necessary. The main therapy consists of compensating for fluid lost from diarrhoea (Murray *et al*., 2005). Pregnant women and young children are the most prone to become dehydrated. Nitazoxanide is one of the FDA approved for use in immunocompetent people to combat diarrhoea. Spiramycin can help shorten the length of time oocysts are passed as well as the duration of diarrhoea in children (Chen *et al*., 2002). The drug can also help treat diarrhoea in patients who are in the early stages of AIDS (Murray *et al*., 2005).

In one AIDS patient from Iran, who had pulmonary cryptosporidiosis in addition to intestinal cryptosporidiosis, azithromycin and paromomycin helped to clear the infection (Meamar *et al*., 2006). Parenteral octreotide acetate can help decrease the number of stools passed (Ryan and Ray, 2004). Generally, though, for AIDS patients, the best treatment is to use HAART to strengthen the immune system (Chen *et al*., 2002). A treatment for patients with biliary cryptosporidiosis who have cholangitis in addition to papillary stenosis is endoscopic sphincterotomy (Chen *et al*., 2002).

Currently research is being conducted in molecular-based immunotherapy. For example, synthetic isoflavone derivates have been shown to fight off *Cryptosporidium parvum in vitro* and in a gerbil. Derivates of nitazoxanide, which are synthetic nitro- or non nitro- thiazolide compounds, have also shown promising results in vitro (Gargal , 2008).

2.1.8 Clinical Manifestation

In apparently healthy persons, clinical illness is characterized by watery diarrhoea, which can be accompanied by abdominal cramps, loss of appetite, low-grade fever, nausea, vomiting and weight loss, however asymptomatic infection occur frequently (Hellard *et al*., 2000). Persons with compromised immune system can suffer life threatening chronic diarrhoea, especially when their CD_4^+ lymphocyte count fall below 200 cell/µl (Cama *et al.,* 2006). There are four clinical presentations of cryptosporidiosis for patients with AIDS, 4% have no symptoms, 29% have a transient infection, 60% have chronic diarrhoea and 8% have a severe cholera-like infection. With transient infections, diarrhoea ends within 2 months and *Cryptosporidium* is no longer found in faeces. Chronic diarrhoea last for 2 or more months, the most severe form results in the patients excreting at least 2 litres of watery diarrhoea per day (Chen *et al.,* 2002), and losing of up to 25 litres of body fluid per day (Ryan and Ray, 2004). Patients with AIDS can pass up to 10 stools per day, they experience severe malabsorption and have 10% weight loss and many of them never completely eliminate *Cryptosporidium* from their body (Chen *et al.,* 2002).

When *Cryptosporidium* spread beyond the intestine, as it can predominate in patients with AIDS, it can reach the lungs, middle ear, pancreas and stomach. Thus one symptom is pain in the right upper quadrant (Chen *et al.,* 2003). The parasite can invade biliary tract, causing biliary cryptosporidiosis. This can result to cholecystitis and cholengitis (Ryan and Ray, 2004).

Half of AIDS patients who contract cryptosporidiosis die in less than 6 months (Ryan and Ray, 2004).

2.1.9 Prevention and Control

The best way to prevent getting and spreading cryptosporidiosis is to practice good hygiene and sanitation (Murray *et al*., 2005). Prevention is through regular washing of hands especially after going to the toilet, and before eating. People should avoid contact with animal faeces (Chen *et al*., 2003). They should also avoid contaminated food and water. Additionally, people should refrain from engaging in sexual activities that can expose them to faeces (Murray *et al*., 2005).

Standard water filtration may not be enough to eliminate *Cryptosporidium*, According to CDC report of 2009, filtration has a high effectiveness in removing Cryptosporidium when using an absolute less than or equal to 1 micron filter (NSF Standard 53 or 58 rated "cyst reduction / removal" filter) and boiling for at least one minute (three minutes above 6,500 feet (2,000 m) of altitude) will decontaminate it. Water Research Foundation-funded research discovered UV's efficacy in inactivating *Cryptosporidium* (Rochelle *et al*., 2004). Bottled drinking water is less likely to contain *Cryptosporidium*, especially if the water is from an underground source. Immunocompromised people should take care to protect themselves from water in lakes and streams (Chen *et al*., 2003). They should also stay away from animal stools and wash their hands after touching animals. Fresh fruits and vegetables should be thoroughly washed and/or peeled before eating (Gracia, 2001). Hand washing, use of gloves and improved personal hygiene minimise the risk of acquiring the infection in the hospital (Arora and Arora, 2009).

2.2.0 Human Immunodeficiency Virus/Acquired Immune deficiency Syndrome

Human immunodeficiency virus is a lentivirus and a member of the *Retroviridae* family that causes AIDS (Weiss,1993; Douek *et al*., 2009), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Most untreated people infected with HIV-1 eventually develop AIDS (Migueles and Connor, 2010). These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system (Lawn, 2004). Infection with HIV progresses to AIDS at a variable rate influenced by viral, host, and environmental factors. Most infection will progress to AIDS within 10 years of HIV infection, some will progress much sooner, and some will take much longer (Buchlinder *et al*., 1994).

2.2.1 History of HIV/AIDS

Genetic research indicates that HIV originated in West-Central Africa during the late nineteenth or early twentieth century (Gao *et al*., 1999; Worobey *et al*., 2008). Acquired Immunodeficiency Syndrome was first recognized by the Centers for Disease Control and Prevention (CDC) in 1981 and its cause, HIV, identified in the early 1980s (Gallo, 2006). The first two cases of HIV and AIDS in Nigeria were identified in 1985 and were reported at an international AIDS conference in 1986. Although two types of HIV have been characterized (HIV-1 and HIV-2). HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. Human immunodeficiency virus-1 is more virulent, more infective (Gilbert *et al.,* 2003) and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West African sub region (Reeves and Doms, 2003).

2.2.2 Transmission

Infection with HIV occurs by contact with blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unprotected sex, contaminated needles, breast feeding, and transmission from an infected mother to her baby at birth. Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world.

Heterosexual transmission remains the dominant mode of transmission and accounts for about 85% of all HIV-1 infections. Southern Africa remains the epicentre of the pandemic and continues to have high rates of new HIV-1 infections (Hayes, 2006). Sexual transmission can occur when infected sexual secretions of one partner come into contact with the genital, oral, or rectal mucous membranes of another. In high-income countries, the risk of female-to-male transmission is 0.04% per act and male-to-female transmission is 0.08% per act. For various reasons, these rates are 4 to 10 times higher in low-income countries (Boily *et al*., 2009). According to UNAIDS (2006) report on the global AIDS epidemics, outside of sub-Saharan Africa, a third of all HIV-1 infections are acquired through injecting drug use, most (an estimated 8·8 million) of which are in Eastern Europe and Central and Southeast Asia. A defining feature of the pandemic in the current decade is the increasing burden of HIV-1 infections in women (Quinn and Overbaugh, 2005), which has additional implications for mother-to-child transmission. The transmission of the virus from the mother to the child can occur in utero (during pregnancy), intrapartum (at childbirth), or via breast feeding. In the absence of treatment, the transmission rate between conception and time of birth increases to about 25% (Coovadia, 2004). However, where combination antiretroviral drug treatment and

Caesarian section are available, this risk can be reduced to as low as one percent (Coovadia, 2004). An estimate of 430,000 children were infected worldwide in 2008 (19% of all new infections), primarily by this route, and that a further 65,000 infections were averted through the provision of antiretroviral prophylaxis to HIV-positive women (UNAIDS, 2009) hence in 2010 an estimate of 370,000 children were infected (UNAIDS, 2010).

The risk of HIV transfusion through infected blood products exceeds that of any other risk exposure. Ninety percent of recipients transfused with HIV antibody-positive blood were found to be HIV infected at follow-up (Donegan, 1994)

2.2.3 Pathogenesis

Human immunodeficiency virus infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells (Cunningham *et al*., 2010). The typical course of untreated HIV infection spans for about a decade. Stages include the primary infection, dissemination of virus to lymphoid organs, clinical latency, elevated HIV expression, clinical disease and death. The duration between primary infection and progression to clinical disease averages about ten years. In untreated cases, death usually occurs within two years after the onset of clinical symptoms (Brooks *et al*., 2010). In the absence of HAART, progression from HIV infection to AIDS has been observed to occur at median of between nine to ten years and the median survival time after developing AIDS is only 9.2 months (Morgan *et al*., 2002). Ultimately, HIV causes AIDS by depleting $CD4^+$ T helper lymphocytes. This weakens the immune system and allows opportunistic infections. T lymphocytes are essential to the immune response and without them, the body cannot fight infections or kill cancerous cells. The mechanism of $CD4^+$ T cell depletion differs in the acute and chronic phases (Hel *et al.,* 2006) Following primary infection, there is a 4 to 11 day period between mucosal infection and

initial vireamia, the vireamia is detected for about 8-12 weeks and is widely disseminated throughout the body and the lymphoid organs become seeded. An acute mononucleosis like syndrome develops in many patients (50-75%) 3-6 weeks after primary infection (Brooks et al., 2010). During the acute phase, HIV-induced cell lysis and killing of infected cells by cytotoxic T cells accounts for $CD4^+$ T cell depletion, although apoptosis may also be a factor. Although the symptoms of immune deficiency characteristic of AIDS do not appear for years after a person is infected, the bulk of $CD4^+$ T cell loss occurs during the first weeks of infection, especially in the intestinal mucosa, which harbors the majority of the lymphocytes found in the body (Mehandru *et al*., 2004). The reason for the preferential loss of mucosal $CD4^+$ T cells is that a majority of mucosal $CD4^+$ T cells express the CCR5 coreceptor, whereas a small fraction of $CD4^+$ T cells in the bloodstream do so (Brenchley *et al*., 2004). Human immune deficiency virus seeks out and destroys CCR5 expressing $CD4^+$ cells during acute infection. An immune response to HIV occurs 1week to 3months after infection, plasma viremia drops, and levels of CD4 cells rebound, however the immune response is unable to clear the infection completely and HIV-infected cells persist in the lymph nodes (Brooks *et al*., 2010).

During the chronic phase, which can last for months to years, virus replication continues and the number of $CD4^+$ T cells in the blood begins to significantly decrease. Because this T-helper are critically important in the generation of acquired immunity, individuals at the stage develop a variety of symptoms including fever, weight loss, malaise, fatigue, anorexia, abdominal pain, diarrhoea, headaches and lymphadenopathy (Willey *et al*., 2011). Continuous HIV replication results in a state of generalized immune activation persisting throughout the chronic phase (Appay and Sauce, 2008). Immune activation, which is reflected by the increased activation state of immune cells and release of proinflammatory cytokines, results from the

activity of several HIV gene products and the immune response to ongoing HIV replication. Another cause is the breakdown of the immune surveillance system of the mucosal barrier caused by the depletion of mucosal $CD4^+$ T cells during the acute phase of disease (Brenchly *et al*., 2006). This results in the systemic exposure of the immune system to microbial components of the gut's normal flora, which in a healthy person is kept in check by the mucosal immune system. The activation and proliferation of T cells that results from immune activation provides fresh targets for HIV infection. However, direct killing by HIV alone cannot account for the observed depletion of $CD4^+$ T cells since only 0.01–0.10% of $CD4^+$ T cells in the blood are infected.A major cause of $CD4^+$ T cell loss appears to result from their heightened susceptibility to apoptosis when the immune system remains activated. Although new T cells are continuously produced by the thymus to replace the ones lost, the regenerative capacity of the thymus is slowly destroyed by direct infection of its thymocytes by HIV. Eventually, the minimal number of $CD4^+$ T cells necessary to maintain a sufficient immune response is lost, leading to AIDS

2.2.4 Epidemiology

The disease is a major health problem in many parts of the world, and is considered a pandemic, a disease outbreak that is not only present over a large area but is actively spreading (Kallings, 2006). According to UNAIDS (2009) report, some 60 million people have been infected worldwide since the start of the pandemic, with some 25 million deaths, and 14 million orphaned children in southern Africa alone. The UNAIDS reported that, at the end of 2010, 34 million people were globally living with HIV, 2.7 million were newly infected and 1.8 million died of AIDS related illness. The region most heavily affected remains Sub- Saharan Africa with South Africa having more people living with it (an estimate of 5.6 million). According to the 2010 seroprevalence sentinel survey, the prevalence rate of HIV in Nigeria is

4.1% with Kaduna state having 5.1 %. Women were more susceptible to HIV-1 infection due to hormonal changes, vaginal microbial ecology and physiology, and a higher prevalence of sexually transmitted diseases (Sagar *et al*., 2004; Lavrey *et al*., 2004).

According to WHO/UNAIDS/UNICEF (2011), the global incidence of HIV infection has stabilized and on the decline in many countries with generalized epidermics. The number of people receiving antirectroviral therapy continues to increase with 6.65 million people getting treatment at the end of 2010. New surveillance data confirm that the epidemic disproportionately affect sex workers, men who have sex with men, transgender people, people who inject drugs, prisoners and migrants in both concentrated and generalized epidemics. These people are not included in natural AIDS plan even though they face formidable legal and other structural barrier to accessing HIV services.

2.2.5 Immunity

Human immune deficiency Virus infected persons develop both humoral and cell mediated responses against HIV related antigens. Antibodies to a number of viral antigens develop soon after infection (Brooks *et al*., 2010). Many forms of HIV commonly use CCR5 and/or CXCR4 as a co-receptor to enter its target cells. A few individuals carry a mutation known as CCR5-Δ32 in the CCR5 gene, protecting them against these strains of HIV. Several chemokine receptors can function as viral coreceptors, but CCR5 is likely the most physiologically important coreceptor during natural infection. The normal ligands for this receptor, RANTES, MIP-1β, and MIP-1α, are able to suppress HIV-1 infection *in vitro*. In individuals infected with HIV, CCR5-using viruses are the predominant species isolated during the early stages of viral infection (Anderson and Akkina, 2007) suggesting that these viruses may have a selective advantage during transmission or the acute phase of disease.

2.2.6 Treatment

There is currently no cure for HIV infection, though the search is on and many drugs are at various stages of testing. Antiviral drugs currently approved for use according to Willley *et al* (2011) are:

1. **Nucleoside reverse transcriptase inhibitors (NRTIs)** Examples include Zidovudine (AZT or Retrovir), didanosine (Videx), zalcitabine (ddc orHIVID), tenofovir (Viread), emtricitabine (Emtriva or Coviracil), stavudine (Zerit), and lamivudine (Epivir or3TC).
2. **The nonnucleoside reverse transcriptase inhibitors (NNRTIs)** include delavirdine (Rescriptor), efavirenz (Sustiva), and nevirapine (Viramune).
3. **The protease inhibitors (PIs)** Examples include indinavir (Norvir), nelfinavir (Viracept), lopinavir (Kaletra), Fosamprenavir (Lexiva), atazanavir (Reyataz), and saquinavir (Invirase).
4. **The fusion inhibitors (FIs)** Example is enfuvirtide (Fuzeon).

Therapy with HAART became available in 1996, it often can suppress viral replication to below limit of detection in plasma, decrease viral loads in lymphoid tissue, allow the recovery of immune response to opportunistic pathogens, and prolong patient survival (Brooks *et al*., 2010).

2.2.7 Diagnosis

The diagnosis of HIV-1 infection is based on the detection of specific antibodies, antigens, or both, and many kits have been developed and are available commercially. Serological tests are generally used for screening. A major advance has been the availability of rapid HIV-1 antibody tests. These assays are easy to do and provide results rapidly (Green wald *et al*., 2006), enabling specimen collection and proper diagnosis at the same visit. Rapid tests are important tools for surveillance, screening, and diagnosis, and can be reliably done on plasma, serum, whole blood,

or saliva by health-care providers with little laboratory expertise. The two limitations of these serological tests are detection of infection during primary infection when antibodies are absent, and in infants younger than 18 months who might bear maternal HIV-1 antibodies. In these instances direct virus detection is the only option (e.g., quantification of viral RNA [standard] or p24 antigen in heat denatured serum [less expensive]).

For staging purposes, measurement of $CD4^+$ cells and viraemia is required. Plasma viral load is widely used to monitor therapeutic success of antiretroviral treatment. Several commercially available tests provide sensitive quantification of plasma HIV-1 RNA copies. The newer versions of the Amplicor and Quantiplex (Roche, Indianapolis, IN, USA, and Bayer Diagnostics, Walpole, MA, USA, respectively) assays have overcome initial suboptimum performance for non-B subtypes (Berger *et al*., 2005). While the viral load determines the rate of destruction of the immune system, the number of $CD4^+$ cells reveals the degree of immunodeficiency and is, therefore, used to assess the stage of infection. The $CD4^+$ cell counts together with clinical manifestations (e.g., occurrence of opportunistic infections) are key criteria for HIV-1 disease classification.

Flow cytometry analysis is the standard method for $CD4^+$ cells quantification. Standard methods for quantifying viral load and $CD4^+$ cell counts need advanced laboratory infrastructures, and assays require a specimen to be tested within a short time of collection. These requirements pose challenges for resource-constrained settings. The use of dried blood spot specimen has resolved some of the difficulties associated with transportation of samples needed for virological assessments (Uttaymakul *et al*., 2005). Similarly microcapilliary flow-based systems, $CD4^+$ chips, or total white counts (panleucocyte gating) provide alternatives for

establishment of the level of immunodeficiency in resource-limited settings (Rodriguez *et al*., 2005; Ceffa *et al*., 2005).

2.2.8 Symptoms

Infection with HIV generally occurs by introduction of bodily fluids from an infected person into the body of an uninfected person. A period of rapid viral replication ensues, leading to an abundance of virus in the peripheral blood. During primary infection, the level of HIV may reach several million virus particles per milliliter of blood (Piatak *et al*., 1993). This response is accompanied by a marked drop in the numbers of circulating $CD4^+$ T cells. Thus acute viremia is associated in virtually all patients with the activation of $CD8^+$ T cells, which kill HIV-infected cells, and subsequently with antibody production, or seroconversion. The $CD8^+$ T cell response is thought to be important in controlling virus levels, which peak and then decline, as the $CD4^+$ T cell counts rebound. A good $CD8^+$ T cell response has been linked to slower disease progression and a better prognosis, though it does not eliminate the virus (Pantaleo *et al*., 1997). During this period (usually 2–4 weeks post-exposure) many individuals develop an influenza or mononucleosis-like illness called acute HIV infection, the most common symptoms of which may include fever, lymphadenopathy, pharyngitis, rash, myalgia, malaise, mouth and esophageal sores, and may also include, but less commonly, headache, nausea and vomiting, enlarged liver/spleen, weight loss, thrush, and neurological symptoms.

Infected individuals may experience all, some, or none of these symptoms. The duration of symptoms varies, averaging 28 days and usually lasting at least a week (Kahn and Walker, 1998). Because of the nonspecific nature of these symptoms, they are often not recognized as signs of HIV infection. Even if patients go to their doctors or a hospital, they will often be misdiagnosed as having one of the more common infectious diseases with the same symptoms.

As a consequence, these primary symptoms are not used to diagnose HIV infection, as they do not develop in all cases and because many are caused by other more common diseases. However, recognizing the syndrome can be important because the patient is much more infectious during this period (Daar *et al*., 2001).

2.2.8.1 Chronic Infection

A strong immune defence reduces the number of viral particles in the blood stream, marking the start of secondary or chronic HIV infection. The secondary stage of HIV infection can vary between two weeks and 20 years. At this phase of infection, HIV is active within lymph nodes, which typically become persistently swollen, in response to large amounts of virus that become trapped in the follicular dendritic cells (FDC) network (Burton *et al*., 2002). The surrounding tissues that are rich in $CD4^+$ T cells may also become infected, and viral particles accumulate both in infected cells and as free virus. Individuals who are in this phase are still infectious; $CD4^+$ $CD45RO^+$ T cells carry most of the proviral load (Clapham and Mcknight, 2001).

2.2.8.2 Acquired Immune Deficiency Syndrome

The symptoms of AIDS are primarily the result of conditions that do not normally develop in individuals with healthy immune systems. Most of these conditions are opportunistic infections caused by bacteria, viruses, fungi and parasites that are normally controlled by the elements of the immune system that HIV damages (Kallings *et al*., 2003). These infections affect nearly every organ system. People with AIDS also have an increased risk of developing various cancers such as Kaposi's sarcoma, cervical cancer and cancers of the immune system known as lymphomas. Additionally, people with AIDS often have systemic symptoms of infection like

fevers, sweats (particularly at night), swollen glands, chills, weakness, and weight loss (Guss, 1994a; Guss, 1994b). Opportunistic infection usually do not occur in HIV-infected patients until CD4 T cell count have dropped from normal level of about 1000 cells/µl to less than 200 cells/µl.

The most common opportunistic infection in untreated AIDS patient reported by Brooks *et al.* (2010) include:-

- **Protozoa**: *Toxoplasma gondii, Isospora belli, Cryptosporidium species*.
- **Fungi**: *Candida albicans, Cryptococcus neoformans, Coccidiodes immitis, Histoplasma capsulatum, Pneumocystis jiroveci.*
- **Bacteria**: *Mycobacterium avium-intracellulare, M tuberculosis, Listeria monocytogenes, Nocardia asteroids, Salmonella species, Streptococcus species.*
- **Viruses**: *Cytomegalovirus, Herpes simplex virus, Varicella-zoster virus, Adenovirus, polyomavirus, JC virus, Hepatitis B virus, Hepatitis C virus*

Diarrhoea is one of the most common presenting complaints in HIV-infected individuals. The infectious etiological agents include both opportunistic agents that consistently cause severe, chronic or frequent gastrointestinal disease and non-opportunistic agents that usually cause acute, treatable diarrhoeal illness (Saha *et al*., 2011). Chronic diarrhoea, defined as persistence of diarrhoea beyond four weeks (Saha *et al*., 2011) is a common symptom in HIV-infected patients in the tropics. Due to the delayed diagnosis of HIV in these individuals, the patients usually take over-the-counter drugs or local medications for symptomatic relief and the underlying disease is left untreated. The World Health Organisation defines diarrhoea, wasting syndrome along with a positive HIV serology test to be an AIDS-defining illness (Alemu *et al*., 2011).

2.2.9 Prevention

Prevention and control of AIDS is achieved primarily through education (Willey *et al*., 2011). The health education for the general public has been summarized as follows (Brooks *et al*., 2010):

- Any sexual intercourse (outside of mutually monogamous HIV antibody-negative relationships) should be protected by a condom.
- Do not share unsterile needles or syringes.
- All women who have been potentially exposed should seek HIV antibody testing before becoming pregnant and, if the test is positive, should consider avoiding pregnancy.
- All HIV-infected mothers should avoid breast feeding to reduce transmission of the virus to their children if safe alternative feeding options are available.

Population Action International in 2008 reported that even before the term 'ABC' was conceived, materials provided by WHO, the Global Program on AIDS (later succeeded by UNAIDS) and governments and organisations around the world, contained information on how abstinence, fidelity and condom use could prevent the sexual transmission of HIV. According to UNAID's 2004 Global Report on the AIDS Epidemic, 'ABC' stands for:

- Abstinence (not engaging in sex, or delaying first sex)
- Being safer, by being faithful to one's partner or reducing the number of sexual partners
- Correct and consistent use of condoms.

The following are broader strategies for preventing HIV among children as reported by WHO/UNAIDS

- Preventing HIV infection among prospective parents by making HIV testing and other prevention interventions available in services related to sexual health such as antenatal and postpartum care and focusing on preventing HIV in women of a child-bearing age.
- Avoiding unwanted pregnancies among HIV positive women by providing appropriate counselling and support to women living with HIV to enable them to make informed decisions about their reproductive lives. Ensuring that contraception is available to women who want it.
- Preventing the transmission of HIV from HIV positive mothers to their infants during pregnancy, labour, delivery and breastfeeding.
- Integration of HIV care, treatment and support for women found to be positive and their families.

2.3.0 CD4 T- Cells

The cluster of differentiation 4 (CD4) is a glycoprotein expressed on the surface of T helper cells, regulatory T cells, monocytes, macrophages, and dendritic cells. The CD4 is used by HIV-1 to gain entry into host T-cells. This is achieved by binding of the viral envelope protein known as gp120 to CD4 (Kwong *et al*., 1998). The cardinal feature of HIV is the depletion of T helper-inducer lymphocytes-the result of HIV replication in this population of lymphocytes as well as of the death of uninfected T cells by indirect mechanism (Brooks *et al*., 2010). The CD4 count is a strong predictor of HIV progression to AIDS and death and is by consensus considered the best laboratory marker for deciding when to initiate antiretroviral treatment (Giorgi, 1993; Sterling *et al*., 2001). Flow Cytometers are used in cell counting and sorting application (Nurez, 2001). Normal blood values are 500-1200x10^6/L (Bofill *et al*., 1992).

Highly active antiretroviral therapy decreases the viral load and gives $CD4^+$ time to regenerate to be able to fight infections. Figure 2.3 shows a computer generated image of a human $CD4^+$ white blood cell infected by HIV particles. In this computer generated image, the large object is a human CD4+ white blood cell, and the spots on its surface are the spiky blue objects in the foreground represent HIV particles.

Cryptosporidiosis is normally self-limited in HIV-infected individuals with $CD4^+$ T-cell counts over 180 cells/µl and usually chronic in HIV-infected individuals with lower counts, and it may be fulminant in some patients with $CD4^+$ T-cell counts below 50 cells/µl (Blanchard *et al*., 1992; Flanigan *et al*., 1992; Manabe *et al*., 1998). However, asymptomatic oocyst shedding has been noted in apparently healthy individuals and also in HIV-positive individuals (Esteban *et al*., 1998; Yu *et al* 2004; Houpt *et al* 2005).

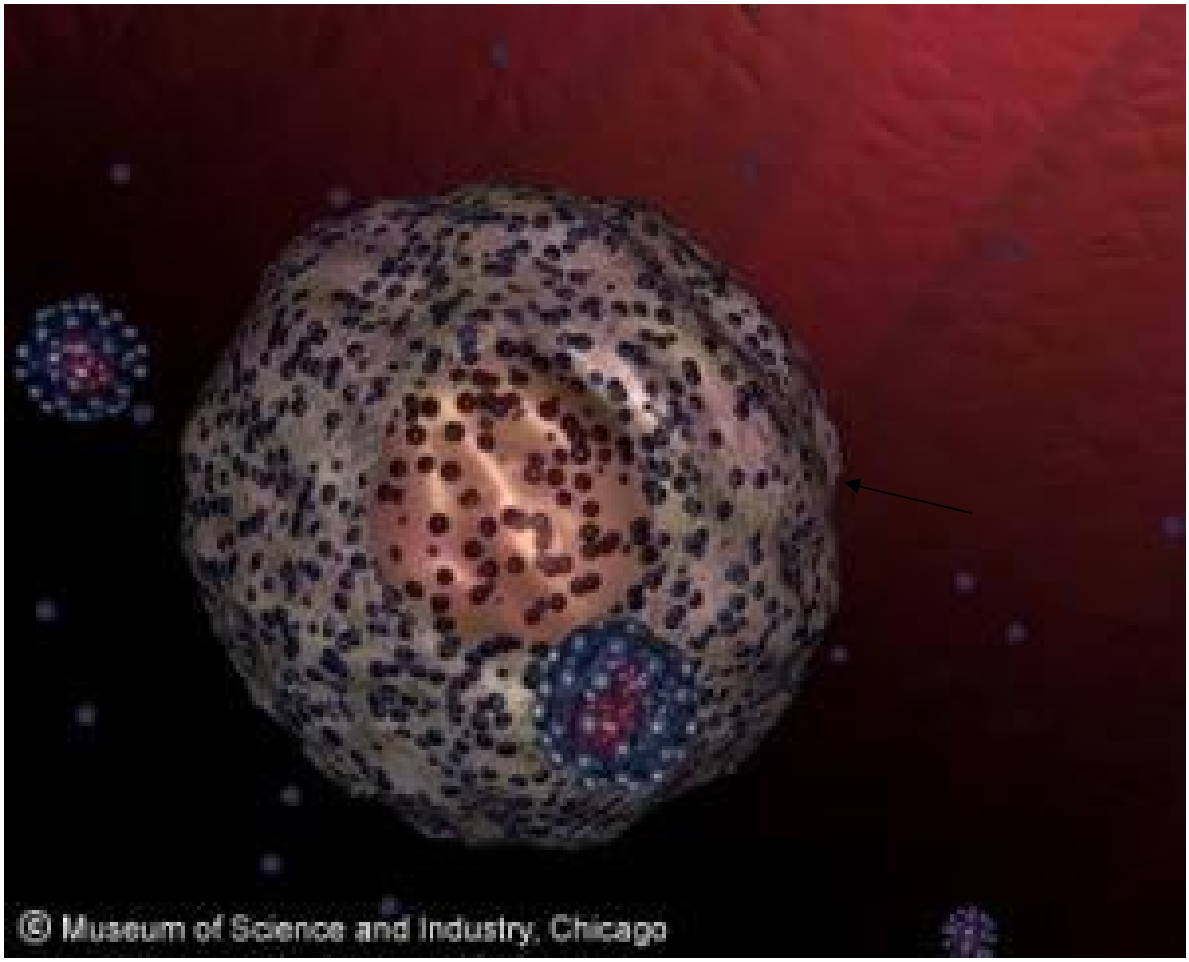


Figure 2.3: Arrow shows a computer generated image of a human $CD4^+$ white blood cell infected by HIV Particles.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Kaduna metropolis, Kaduna state Nigeria between October 2011 and January 2012. According to C-GIDD report of 2008, Kaduna metropolis covers an area of 3,080 km^2 and derives its name from the Hausa word 'Kada' meaning Crocodile and 'Kaduna' is the Hausa plural word for crocodiles many of which abounded in River Kaduna. It is a major hub for the surrounding agricultural areas with its rail and road junction. Kaduna is an industrial centre of Northern Nigeria, housing manufacturing industries for products such as textile, machinery, steel, aluminium, petroleum products and bearings. The population of Kaduna metropolis is about 760,084 according to the 2006 census. It has a characteristic southern guinea savannah climatic condition; where the two rainfall dry and wet seasons match the vegetation type typical of the zone.

3.2 Study Population

The study was carried out among HIV seropositive patients attending Antiretroviral Clinics in three health care facilities within Kaduna metropolis. These facilities were Barau Dikko Specialist Hospital located in Kaduna North, Gwamna Awan General Hospital located in Kaduna South and Yusuf Dantsoho General Hospitals located Kaduna West. The study population included male and female, of ages 16 to 65 years old, from all works of life attending the Antiretroviral Clinics.

3.3 Control Population

Blood and stool samples were collected from 30 (10% of the study population) non-HIV patients with or without diarrhoea, who served as control group for the study.

3.4 Inclusion Criteria

The inclusion criteria were all consenting HIV positive patients attending ARV clinics within the study period and non HIV patients who gave consent.

3.5 Exclusion Criteria

The exclusion criteria were all non consenting non- HIV patients and non consenting HIV patients attending the facilities during the study period.

3.6 Ethical Approval and consent

Approval to carry out the research was given by the hospital ethical committee of Kaduna State Ministry of Health (Appendix I). The consent of individuals recruited for the study was obtained and they were educated on the purpose and benefits of the study (Appendix II).

3.7 Sample Size

The sample size was determined using the following equation as described by Naing *et al.* (2006) as follows:-

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where n = sample size for both stool and blood.

z = statistics for a level of 95% confidence interval = 1.96

p = prevalence rate of *Cryptosporidium* infection among HIV seropositive patient = 22.2% (Akinbo *et al*., 2010)

d = precision (allowable error) = 5% = 0.05

thus $n = \frac{(1.96^2) \times 0.222 \times (1-0.222)}{(0.05)^2}$

$$n = \frac{0.6636}{0.0025}$$

$= 265.44 \approx 265$

The calculated sample size was 265, which was the minimum sample to be used for the study.

3.8 Study Design

Samples were collected consecutively from 300 consenting HIV seropositive patients who came to the clinic for $CD4^+$ cell count during the study period. All consenting patients were picked for the study regardless of whether the patients were on HAART or not. One hundred samples were collected randomly from each of the three Hospitals. Control samples were randomly collected consecutively from 30 consenting individuals who came for voluntary counseling and testing and were tested negative. Ten controls were collected from each of the three Hospitals.

3.9 Data Collection using Questionnaire

Prior to sample collection a predesigned structured questionnaire was used to collect socio-demographic and clinical data of each of the study participants. Information concerning potential risk factors associated with cryptosporidiosis was also obtained using the questionnaires (Appendix III).

3.10 Sample Collection

Five millilitres (5 ml) of blood was collected by a trained phlelabotomist aseptically from each participant into a vacutainer tube containing EDTA and placed on a roller mixer, this was used for $CD4^+$ cell enumeration. Stool specimens were also collected by the participants into a wide-mouthed stool specimen container and used for detecting Cryptosporidium by microscopy and ELISA. One hundred blood samples and one hundred stool samples were collected from each Hospital. Blood Samples for $CD4^+$ cell count and stool for microscopy were processed

immediately after collection while stool sample for ELISA were stored at -20℃ and transported to the Microbiology Department, Faculty of Science Ahmadu Bello University Zaria for analysis.

3.11 Sample Analysis

3.11.1 Formol Ether Concentration and Modified Ziehl-Neelsen (ZN) Method Analysis of Stool Samples by Microscopy

The method described by Cheesebrough 2005 was used. About one gram of stool was emulsified in about 4ml of 10% formol water in a tube. 3ml of 10% V/V formol water was added. The tube was capped and sample mixed properly by shaking. The emulsified stool was sieved into a beaker. The supernatant was transfered into a centrifuge tube and 3ml of diethyl ether was added. The tube was stoppered and mixed for 1 minute. A tissue was wrapped around the top of the tube and used to loosen the stopper. It was then centrifuged immediately at 1000rpm for 1 minute. A pasteur pipette was used to carefully remove the entire column of fluid below the faecal debris and ether, this was transferred to another centrifuge tube.

Formol water was added to make the volume up to 10ml, this was then centrifuge at 3000rpm for 5. The supernatant was removed and the bottom of the tube tapped to resuspend and mix the sediment. A smear from the sediments was prepared on a slide. The smear was air dried and fixed with methanol for 3 minutes.

The smear was stained with unheated carbol fuchsin for 15 minutes and then washed off with water. The smear was decolourised with 1% acid alcohol for 15 seconds and washed off with water. The smear was counterstained with 0.5% malachite green for 30 seconds and washed off with water. The slide was placed on a draining rack for the smear to dry. Stained smear were observed with an Olympus microscope for oocysts, using x10 objective to detect the oocyst and the x100 objective to identify them. The diameter of the oocyst (4-6um) was measured using a

microscope equipped with an ocular micrometer and calibrated against a stage micrometer, which has uniformly spaced lines of known distance etched on it.

3.11.2 ELISA Method for Detection of *Cryptosporidium*

All stool samples were tested for the presence of *Cryptosporidium* antigen by a commercially available ELISA kit *Cryptosporidium* Faecal kit (Diagnostic Automation, Inc. USA). The assay was carried out according to manufacturer's instructions.

3.11.2.1 Principle of Test

Cryptosporidium antigens if present in the stool supernatant are captured by antibodies attached to the wells during the first incubation. The second incubation adds an additional anti-*Cryptosporidium* antibody that "sandwiches" the antigen. The third incubation adds an anti-second antibody conjugated to peroxidase. After washing four times to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the color to yellow.

3.11.2.2 Test Procedure

Sample dilutions were prepared in labeled tubes. An applicator stick was used to pick about a pea size of faecal sample; this was added to 0.3 ml of dilution buffer and was thoroughly mixed. Watery specimens were mixed and 0.1 ml of the sample was added to 0.3 ml dilution buffer. Wells were labeled and a micropipette was used to dispense 100 µl of negative control to well 1 and 100 µl of positive control to well 2. A micropipette was used to dispense 50 µl of dilution buffer to each sample well. Fifty (50) microlitre of sample was added to each sample well with dilution buffer. The plate was then incubated for 60 minutes at room temperature (25^{o}C) after which the wells were washed 4 times manually. The plates were dried on a clean

absorbent tissue to remove excess wash buffer. Two drops of enzyme conjugate were then added to each well and incubated for 30 minutes at room temperature after which the wells were washed. The plates were then tapped gently on a clean absorbent paper to remove excess wash buffer. Two drops of chromogen was added to each well and incubated for 10 minutes at room temperature after which 2 drops of stop solution was added to each well and mixed by gently tapping the side of the strip holder with the index finger for approximately 15 seconds. Reactions were read within 5 minutes after adding stop solution using an ELISA plate reader (GF-M3000 B. Bran Scientific and Instrument Company England) at 450nm.

3.11.2.3 Interpretation of Result

Absorbance values of 0.15 Optical Density(OD) units and above indicates that the sample contains *Cryptosporidium* antigen (positive) while Absorbance values less than 0.15 OD units indicates the sample does not contain detectable levels of *Cryptosporidium* antigen (negative).

3.11.3 $CD4^+$ T Cell Count

Blood samples were analyzed on a Cyflow partec device (071040020 Partec GmbH Germany) which counted the $CD4^+$ cells.

3.11.3.1 Principle of Test

Leukocytes in the subpopulations are determined because of their CD-markers and for this specific antibodies are used which recognize a specific epitope on the cell of interest. Because of the link between the antibody and the specific fluorescence light when exited by a 532nm laser. The fluorescence is detected in the FL 2 channel of the Cyflow counter and assigned to a specific cell.

3.11.3.2 Test Procedure

Blood sample were placed on the roller mixer for at least 15 minutes for proper mixing. 20µl of CD_4^+ easy count-CD_4^+ monoclonal antibody was added to a partec test tube. To this was added 20µl of blood sample and incubated for 15minutes in the dark at room temperature (25-30^{o}c) mixing at intervals. After which, 800µl of CD_4^+ easy count-No lyse buffer was added to the tube and shaken gently.

3.12 Sensitivity and Specificity

Sensitivity and Specificity of microscopy was calculated using ELISA as a gold standard.

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive + False Negative}}$$

$$\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative + False Positive}}$$

3.13 Data Analysis

The data obtained was analysed using statistical software SPSS, version 17.00. Results obtained were converted to percentages and presented in tables and figures. Chi-square test was use to test for significant association and p-value <0.05 was considered significant.

CHAPTER FOUR

4.0 RESULT

Stool samples from 300 HIV seropositive patients attending three hospitals in Kaduna Metropolis were screened for *Cryptosporidium* infection using ELISA and microscopy methods. *Cryptosporidium* was detected in the stool of 177 (59.0%) patients by ELISA and in 45 (15.0%) by microscopy. There was a significant difference in the prevalence of *Cryptosporidium* obtained by ELISA (χ^2 =38.7764, df=2, p=0.000) and by microscopy (χ^2 = 8.941, df=2, p=0.012) among the selected Hospitals. The highest prevalence was recorded in Yusuf Dantsoho Hospital for ELISA (72.0%, 72/100) and (22.0%, 22/100) for microscopy while the lowest was in Gwamna Awan Hospital (34.0%, 34/100) for ELISA and (7.0%, 7/100) for microscopy (Table 4.1). A typical oocyst of *Cryptosporidium* seen under the microscope is shown in Figure 4.1. A Venn diagram of the number of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis by ELISA and Microscopy is shown in Figure 4.2.

Table 4.1: Prevalence of *Cryptosporidium* infection among HIV seropositive patients attending some selected Hospitals within Kaduna metropolis, Nigeria.

Hospital	*ELISA		**Microscopy		Total
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Barau Dikko	71 (71.0)	29 (29.0)	16 (16.0)	84 (84.0)	100
Yusuf Dantsoho	72 (72.0)	28 (28.0)	22 (22.0)	78 (78.0)	100
Gwamna Awan	34 (34.0)	66 (66.0)	7 (7.0)	93 (93.0)	100
Total	177 (59.0)	123 (41.0)	45 (15.0)	255 (85.0)	300

*(χ^2 =38.7764, df=2, p=0.000) **(χ^2 = 8.941, df=2, p=0.012)



Figure 4.1: Arrow shows *Cryptosporidium* oocyst in the stool of patient as seen under the microscope.

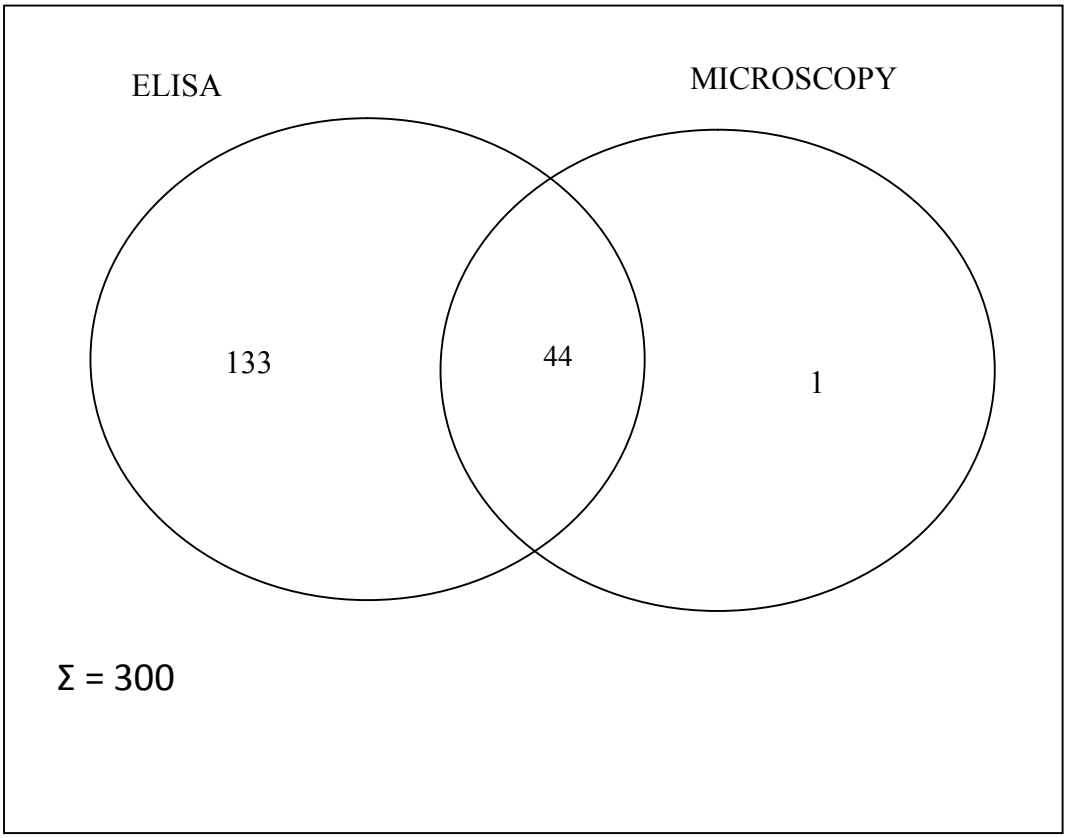


Figure 4.2: Venn diagram of the number of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis by ELISA and Microscopy.

The results obtained and the sociodermographic data from questionnaires were analysed and the results are presented in Table 4.2. There was a marked difference in the distribution of *Cryptosporidium* among the patients by age group. Individual in the age group 16-25 years had the highest prevalence (25.7%: 9/35) of *Cryptosporidium* and 36-45 years had the lowest prevalence (10.4: 10/96). However there was no significant difference (χ^2 = 4.975, df=4, p=0.290) between the prevalence obtained for the parasite and the various age groups.

Of the 45 patients who were positive for *Cryptosporidium* infection 18 (19.8%) were male and 27 (12.9%) were female. Rate of infection in male was higher than that of female, however there was no significant difference (χ^2 = 2.341, df=1, p=0.126) in the observed prevalence between male and female.

In relation to marital status, infection was highest (17.0%: 33/194) among married patients and lowest (0%: 0/8) among the divorced. However there was no significant difference (χ^2 =3.891, df=3, p=0.274) in their prevalence.

Regarding educational status, there was no significant difference in the prevalence of *Cryptosporidium* (χ^2 = 5.233, df=3, p=0.156). However the highest prevalence (26.1%: 6/23) was detected in those with no formal education while the lowest prevalence (11.1%:15/135) was detected in those with secondary education.

The result was further analysed according to occupation of patients and there was no statistical significant association (χ^2 = 3.170, df=4, p=0.530) between *Cryptosporidium* infection and type of occupations. However the highest prevalence (25.0%: 6/24) was seen in those with other type of occupations, while the lowest (6.7%: 1/15) was seen in patients who are retired.

Table 4.2: Distribution of *Cryptosporidium* infection by demographic factors among HIV seropositive patients within Kaduna metropolis, Nigeria.

Demographic factors	Total Number	No positive by *ELISA (%)	No positive **Microscopy (%)	P value
Age (years)				
16-25	35	26 (74.3)	9 (25.7)	
26-35	117	71 (60.7)	17 (14.5)	*p=0.255
36-45	96	51 (53.1)	10 (10.4)	**p=0.290
46-55	40	23 (57.5)	7 (17.5)	
56-65	12	6 (50.0)	2 (16.7)	
Gender				
Male	91	56 (61.5)	18 (19.8)	*p=0.555
Female	209	121 (57.9)	27 (12.9)	**p=0.126
Marital status				
Single	49	30 (61.2)	8 (16.3)	
Married	194	119 (61.3)	33 (17.0)	*p=0.417
Divorced	8	4 (50.0)	0 (0)	**p=0.274
Widowed	49	24 (49.0)	4 (8.2)	
Educational status				
Primary	101	59 (58.4)	19 (18.8)	
Secondary	135	80 (59.3)	15 (11.1)	*p=0.986
Tertiary	41	25 (61.0)	5 (12.2)	**p=0.156
None	23	13 (56.5)	6 (26.1)	
Occupation				
Civil servant	59	38 (64.4)	8 (13.6)	
Self employed	118	67 (56.8)	16 (13.6)	*p=0.581
Unemployed	84	47 (56.8)	14 (16.7)	**p=0.530
Retired	15	15 (53.3)	1 (6.7)	
Others	24	17 (70.8)	6 (25)	

The result was analysed according to the possible risk factors that might be associated with *Cryptosporidium*, and the result is presented in Table 4.3. There was significant association (χ^2 = 9.614, df=4, p=0.047) between *Cryptosporidium* infection and water source. *Cryptosporidium* infection was higher (50%: 1/2) in those who drank unboiled river water. The parasite was not detected in the stool of patients who drank water from other sources such as packaged and bottled water.

Prevalence of *Cryptosporidium* was higher (16.0%: 42/262) in patients who do not boil their drinking water than in those who boil their drinking water (7.9%: 3/38). However, there was no statistical significant difference in the prevalence obtained (χ^2 = 1.723, df=1, p=0.189).

Patients who defaecated in opened layouts had higher prevalence of *Cryptosporidium* infection (50.0%: 3/6) while those who used water cistern had lower prevalence (10.6%: 19/180). There was significant association (χ^2 = 10.948, df=2, p=0.004) between *Cryptosporidium* infection and type of toilets used.

Infection was more prevalent (22.7%: 10/44) among animal owners than non animal owners (13.7%: 35/256) although there was no significant association (χ^2 = 2.415, df=1, p=0.120) in the prevalence.

There was no significant association (χ^2 = 0.27, df=1, p=0.869) between *Cryptosporidium* infection and swimming in polls. Prevalence was however higher (16.7%: 2/12) in swimmers than in non swimmers (14.9%: 43/288)

There was no significant association (χ^2 = .083, df=1 p=0.773) between the infection and overseas travel, however the parasite was detected with a higher prevalence in those that have never travelled (15.1%: 43/284) than those who had travelled overseas (12.5%: 2/16).

Table 4.3: Distribution of *Cryptosporidium* infection in relation to risk factors among HIV seropositive patients within Kaduna metropolis, Nigeria

Possible Risk factor	Total Number	No positive *ELISA (%)	No positive **Microscopy (%)	P value
Source of water				
Tap	202	119 (58.90)	25 (12.4)	
Well	93	56 (60.2)	19 (20.4)	*p=0.217
River	2	2 (100)	1 (50.0)	**p=0.047
Others	3	0 (0)	0 (0)	
Boiling of water				
Yes	38	20 (52.6)	3 (7.9)	*p=0.393
No	262	157 (59.9)	42 (16.0)	**p=0.189
Type of toilet				
Water cistern	180	97 (53.9)	19 (10.6)	*p=0.061
Pit latrine	114	75 (65.8)	23 (20.2)	**p=0.004
Open layout	6	5 (83.3)	3 (50.0)	
Animal contact				
Yes	44	27 (61.4)	10 (22.7)	*p=0.730
No	256	150 (58.6)	35 (13.7)	**p=0.120
Swimming				
Yes	12	5 (41.7)	2 (16.7)	*p=0.213
No	288	172 (59.7)	43 (14.9)	**p=0.869
Overseas travel				
Yes	16	9 (56.3)	2 (12.5)	*p=0.818
No	284	168 (59.2)	43 (15.1)	**p=0.773

The results and data from the questionnaire concerning the symptoms of *Cryptosporidium* infection were analysed and the results are presented in Table 4.4. The results, showed significant association (χ^2 = 104.669, df=1, p=0.000) between *Cryptosporidium* infection and diarrhoea. Similarly, there was a significant association (χ^2 = 117.073, df=4, p=0.000) between the duration of diarrhoea and *Cryptosporidium* infection. The prevalence was more (75.0%: 15/20) in those with diarrhoea that had lasted between 3-5 days however *Cryptosporidium* was not detected (0.0%) in those with diarrhoea that had lasted for 2-4 weeks.

Other symptoms associated with *Cryptosporidium* were fever, anorexia, nausea malaise and abdominal cramps. There was a highly significant association (p=0.000) between *Cryptosporidium* infection and these symptoms. The highest prevalence (60.0%: 3/5) was detected in those with anorexia and the lowest (7.7%: 1/13) in those with malaise, however there was no significant association.

Table 4.4: Distribution of *Cryptosporidium* infection in relation to clinical symptoms among HIV seropositive patients within Kaduna metropolis, Nigeria

Symptom	Total	No positive *ELISA (%)	No positive **Microscopy (%)	P value
Diarrhoea				
Yes	59	45 (76.3)	34 (57.6)	*P=0.002
No	241	132 (54.8)	11 (4.6)	**p=0.000
Duration of diarrhoea				
No diarrhoea	241	132 (54.8)	11 (4.6)	
1-2 days	31	24 (77.4)	15 (48.4)	*p=0.05
3-5 days	20	17 (85.0)	15 (75.0)	**p=0.000
1 week	6	4 (66.7)	4 (66.7)	
2-4 weeks	2	0 (0)	0 (0)	
Other symptoms				
NCR	187	107 (57.2)	15 (8.0)	
Fever	43	30 (69.8)	11 (25.6)	*p=0.459
Abdominal cramp	40	22 (55.0)	11 (27.5)	**p=0.000
Anorexia	5	4 (80.0)	3 (60.0)	
Nausea	12	8 (66.7)	4 (33.3)	
Malaise	13	6 (46.2)	1 (7.7)	

NCR=No *Cryptosporidium* related symptoms.

Two hundred and seven of the patients enrolled in the study were on highly active antirectroviral therapy. The result showed a significant association ($\chi 2 = 4.327$, df=1, p=0.038) between *Cryptosporidium* infection and patients on HAART. Patients on HAART had higher prevalence (17.9%: 37/207) than those not on HAART (8.6%: 8/93) (Table 4.5).

Table 4.5: Prevalence of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis by their HAART status

HAART	*ELISA		**Microscopy		Total
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
Yes	122 (58.9)	85 (41.1)	37 (17.9)	170 (77.70)	207
No	55 (59.1)	38 (40.9)	8 (8.6)	85 (91.4)	93
Total	177 (59.0)	123 (41.0)	45 (15.0)	255 (85.0)	300

* (χ^2 = 001, df=1, p=0.974) **(χ2 = 4.327, df=1, p=0.038)

The result were analysed according to duration of HAART drugs. The result shows that HIV patients who were on drugs for less than 24 months, had the highest prevalence to *Cryptosporidium* infection (24.6%: 29/118) and zero prevalence was observed in those on drugs for more than 97-120 months. There was significant association (χ^2 = 10.167, df=4, p=0.001) between the infection and the period of being on HAART (Figure 4.3).

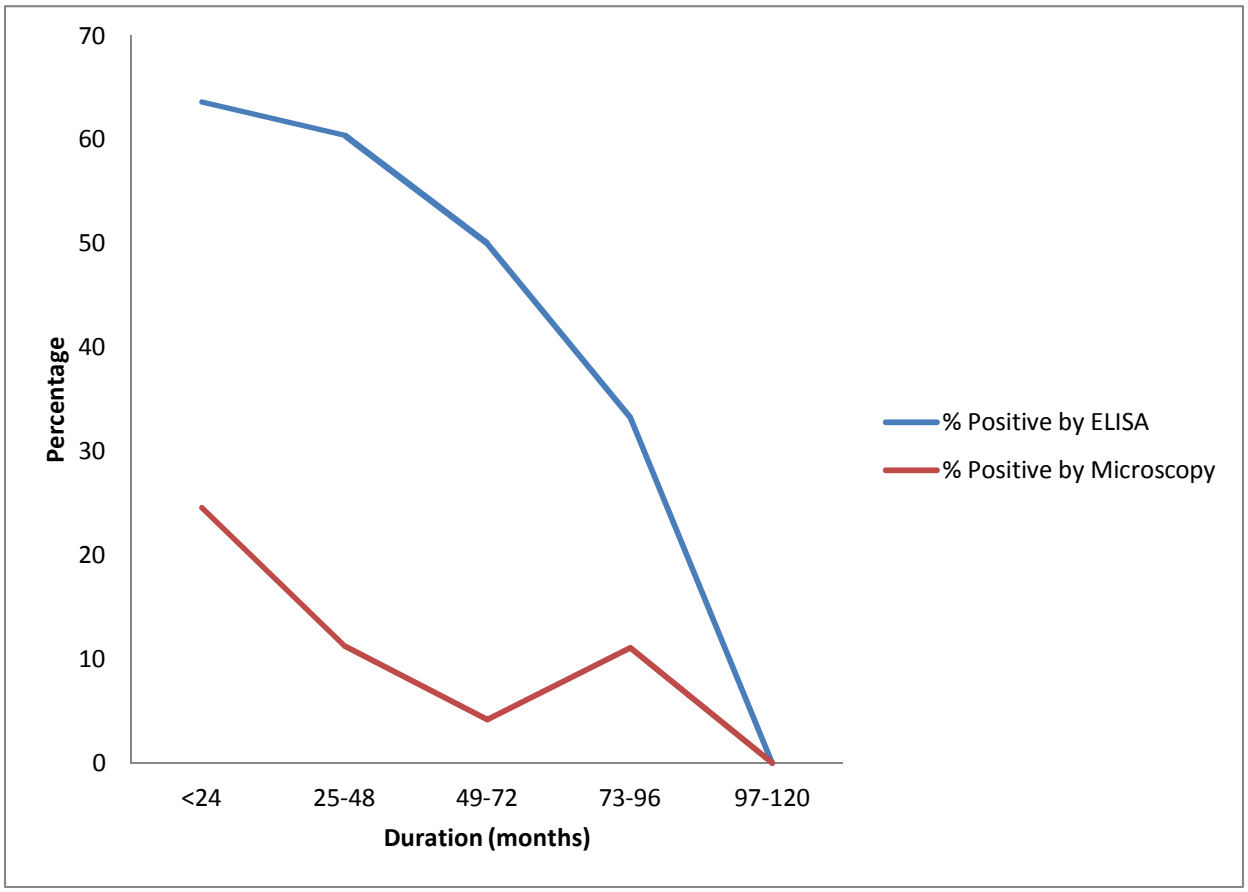


Figure 4.3: Prevalence of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis in relation to duration on HAART.

The result were analysed according to CD4+T-cell counts of HIV patients, there was a highly significant association (χ^2 = 24.888, df=9, p=0.000) between *Cryptosporidium* infection and $CD4^+$ count. Mean $CD4^+$ count was 409.86 while the median was 382. Patients with $CD4^+$ count between 101-200 had the highest prevalence of the infection by ELISA (75.9%: 22/29) and microscopy (51.7%: 15/29) while patients with $CD4^+$ between 901-1000 had the lowest prevalence by ELISA (0.6%) and those with $CD4^+$ >1000 were not infected with *Cryptosporidium*. (Table4.6). There was a decrease in infection as $CD4^+$ count increase by microscopy method (Figure 4.4).

Table 4.6: Prevalence of *Cryptosporidium* infection among HIV seropositive patients in relation to $CD4^+$ T cell count.

	*ELISA		**Microscopy		Total
CD^+4	Positive (%)	Negative (%)	positive (%)	Negative (%)	
<200	38 (67.9)	18 (32.1)	25 (44.6)	31 (55.4)	56 (18.67)
201-350	41 (61.1)	26 (38.9)	16 (23.9)	51 (76.1)	67 (22.33)
351-500	49 (53.8)	32 (39.5)	3 (3.3)	78 (96.2)	81 (27.00)
501-1000	45 (50.6)	44 (49.4)	1 (1.1)	88 (98.9)	89 (29.67)
>1000	4 (57.1)	3 (42.9)	0 (0.0)	7 (100)	7 (23.33)
Total	177 (59.0)	123 (41.0)	45 (15.0)	255 (85.0)	300 (100)

*(χ^2 = 9.754, df=4, p=0.002**)** **(χ^2 = 24.888, df=4, p=0.000)

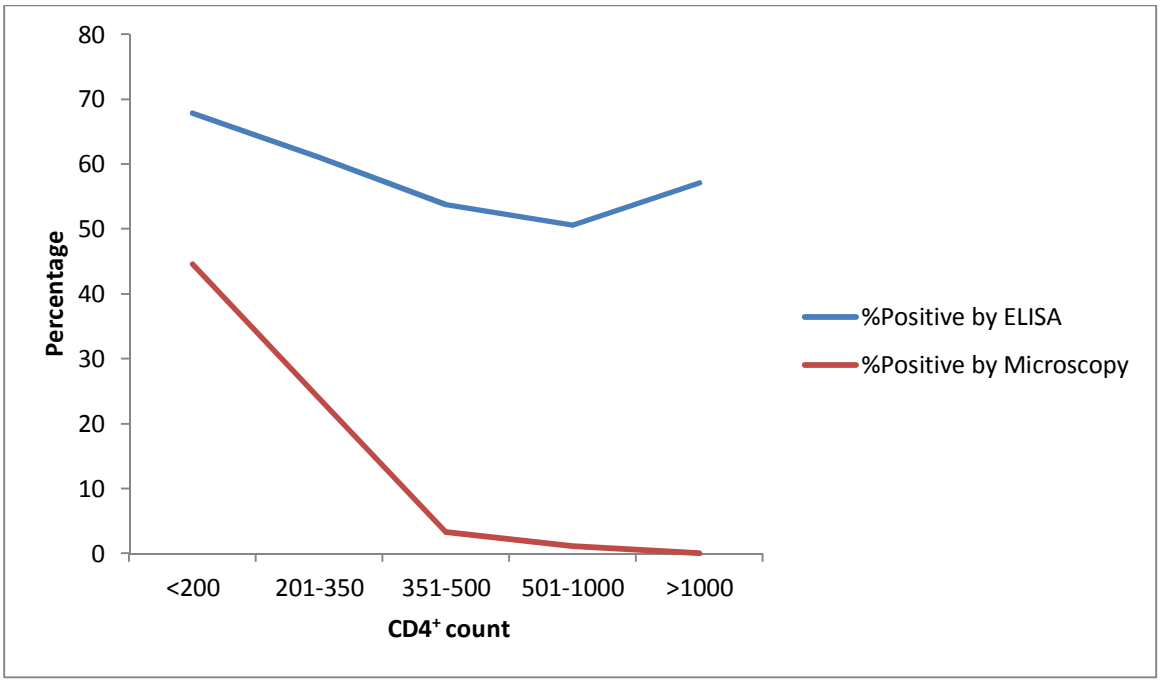


Figure 4.4: Prevalence of Cryptosporidium infection among HIV seropositive patients in relation to $CD4^+$ count.

The sensitivity of microscopy using ELISA as the Gold standard was determined. Out of the 45 patients that were positive by Microscopy, 44 (True positive) were also positive by ELISA while 1 (False positive) was negative by ELISA. Sensitivity of microscopy was 24.9% and specificity was 91.2% (Table 4.7).

Table 4.7: Determination of Sensitivity and Specificity of Microscopy using ELISA Test as a standard.

Microscopy	ELISA		
	Positive	Negative	Total
Positive	44	1	45
Negative	133	122	255
	177	123	300

Sensitivity = 24.9% Specificity = 91.2%

CHAPTER FIVE

5.0 DISCUSSION

A prevalence of 15% was obtained for *Cryptosporidium* in this study to determine the prevalence of *Cryptosporidium* infection among HIV seropositive patients in Kaduna state during the period of October 2011 to January 2012. The prevalence of *Cryptosporidium* obtained in this study is similar to the15% prevalence reported in a study carried out in Venezuela (Certad *et al.*2005). The prevalence is also similar to earlier findings in Nigeria by Akujobi and Ogunsola (2005) who reported a prevalence of 18.7% among HIV patients with diarrhoea in Lagos. In contrast, Nwokediuko *et al.* (2002) and Oyerinde *et al.* (1989) reported 0.0% prevalence of *Cryptosporidium* oocyst in similar studies carried out among HIV patients in Enugu and Lagos respectively. The reasons for these variations may be related to the fact that infections tend to vary from one locality to another and from one country to another depending on the level of associated risk factors.

The results obtained by using the two methods varied, a higher prevalence of *Cryptosporidium* was obtained by ELISA (59%) than by microscopy (15%) indicating that ELISA detected more *Cryptosporidium* infection than microscopy. The result obtained in this study is similar to the findings of Elgun and Koltas (2011) in Turkey, in which prevalence was higher by ELISA (24.03%) than microscopy (5.19%). This may be due to microscopy being specific but less sensitive for the diagnosis of *Cryptosporidium* in faeces while ELISA has a higher sensitivity. Specificity of microscopy is due to the fact that the organism has to be intact before it can be seen while for ELISA it is the antigen that is being detected. Bialek *et al.,* 2000, reported high sensitivity of ELISA as compared to microscopy.

Higher prevalence was seen in age group 16-25 years, however there was no significant difference. The result is similar to the findings of Ibrahim *et al*. (2007) in which a higher prevalence in age group 15-24 years was observed. This may be because incidence of HIV is highest between ages 15-35 years (Ungar, 2000). This is because people in this age bracket are the most sexually active.

In this study, prevalence of *Cryptosporidium* was not significantly associated with gender although prevalence was higher in male than female. This finding agrees with that of Akujobi and Ogunsola (2005), Ikeh *et al.* (2006) and Akinbo *et al.* (2010) which showed higher prevalence of *Cryptosporidium* in male than female. The result however contrasts the report of Ibrahim *et al.* (2007) in which prevalence of intestinal parasitosis was more in female than male. Ikechukwu *et al.* (2011) in a study on cryptosporidiosis in Imo state also reported that more female were infected than male. The finding in this study may be explained by the fact that more male are exposed to occupational hazards such as rearing of animals and since it is a zoonotic infection it can be transmited to man and also female are more conscious of their personal hygiene than male most especially hand washing after defeacating.

In relation to marital status, prevalence was not significantly associated ($p>0.05$) among the five groups. This is similar to the report of Ibrahim *et al.* (2007) which showed no significant association between intestinal parasitosis and marital status, this observation may be explained by the fact that all the groups are exposed to similar predisposing risk factors.

With regards to educational status, the prevalence observed were similar ($p>0.05$). This is contrary to the findings of Akinbo *et al*. (2010) which showed that level of education significantly affected prevalence of intestinal parasitic infection.

There was no association between the infection and the type occupation of patients. This is in agreement with the findings of Ibrahim *et al.* (2007) in which there was no prevalence of intestinal parasitosis in relation to the occupational status of patients, but this findings contrast that of Assefa *et al.* (2009) who reported that prevalence of infection was associated with occupational status. This may be explained by the fact that only urban dwellers were used in this study, rural dwellers whose main occupation is farming were not part of the study.

Patients whose source of drinking water was tap water had the lowest rate of infection, while those who drank from rivers had the highest. This agrees with the findings of Akinbo *et al.* (2010) and Egberongbe *et al.* (2010) and contrasts the report of Ikeh *et al.* (2006). A significant association existed between water source and *Cryptosporidium* infection. High rate of infection among those whose source of drinking water was river may be because cryptosporidiosis is a largely water borne infection and lot of activities like bathing, defecating, washing etc take place in the river thereby contaminating the water.

Patients who boiled water before drinking had lower prevalence of infection than those who did not. This does not agree with the findings of Hunter *et al.* (2004), who found no association with drinking of unboiled water. Reasons for this finding may be due to the fact that boiling kills the oocyst of *Cryptosporidium*.

In this study, there was significant association between the type of toilets used. Those who used open layout had the highest prevalence followed by those who used pit latrines. This finding is similar to that of Akinbo *et al.* (2010) and Egberongbe *et al.* (2010) who reported that defeating in nearby bushes resulted in significantly increase prevalence of infection. This may be because flies in this environment can be able to transmit the oocyst to food thereby infecting them when they consume such foods and since there is no water in this environment hand

washing is not regularly practiced therefore oocyst are transferred from the hands to food or mouth.

Patients who had farm animals had higher prevalence than those who did not, however there was no significant association between the infection and animal contact. This agrees with the findings of Hunter *et al.* (2004), who identified contact with cattle as a main risk factor of *Cryptosporidium parvum* infection. Morgan *et al*. (2000) also identified animal contact in patients infected with zoonotic isolates from Kenya, Switzerland and United states. The higher prevalence in this category is due to the fact that *Cryptosporidium* infection is a zoonotic disease and so can be contacted by contact with new born pets and animals with diarrhoea.

In this study prevalence was higher in patients who swim than in those who do not swim in pools. However there was no significant association between swimming and prevalence of the infection. This does not agree with the findings of Hunter *et al*. (2004) in which swimming was not identified as a factor for *Cryptosporidium* infection. This finding may be because chlorination is not enough to kill the oocyst therefore infection can be contacted in swimming pools.

For overseas travel, prevalence was higher in people who had never travelled overseas than those who had travelled. There was no significant association between prevalence of the infection and overseas travel. This finding is in agreement with that of Aragon *et al*. (2003) but contrasts the report of Hunter *et al*. (2004). This finding may be because the patients in this study did not travel to endemic countries.

Diarrhoea is a major symptom in cryptosporidiosis and findings in this study showed that cryptosporidiosis was significantly associated with diarrhoea. This is similar to reports of Banwat *et al*. (2003), Ajjampur *et al*. (2008), Gupta *et al*. (2008), and Egberongbe *et al* (2010)

who reported that there was high association between the infection and diarrhoea. Reason for this may be because in HIV infection diarrhoea is a major sign of progression to AIDS.

Regarding the duration of diarrhoea, patients with diarrhoea that lasted between 3-5 days had higher prevalence of *Cryptosporidium* by both methods. This may be because cryptosporidiosis causes a short self limited watery diarrhoea which can resolve as immune status recovers and most patients used in this study were on HAART which helps resolve immune status.

The present study found a higher prevalence of cryptosporidiosis among patients who had anorexia, while those with malaise had the lowest. There was a very high significant association between the infection and the clinical symptoms.

Patients on HAART had higher prevalence than those not on HAART. There was significant association between *Cryptosporidium* infection and being on HAART. In regards to the period of being on HAART, there was significant association between *Cryptosporidium* infection and the period of being on HAART. Prevalence was higher in patients who were on HAART for <24 months. There was decrease in prevalence with increase in period of being on HAART. No *Cryptosporidium* infection was detected in patients who have been on HAART for 97-120 months. This suggests that HAART reduces the prevalence of *Cryptosporidium* infection by boosting immunity.

There was a very high significant association between *Cryptosporidium* infection and $CD4^+$T-cell count. Prevalence was highest in patients with $CD4^+$T-cell count <200 cell/µl. This finding is similar to that of Assefa *et al*. (2009) and Gupta *et al*. (2008) which showed that the rate of parasitic infection decreases with increase in $CD4^+$T-cell count. This finding indicates

that there is low opportunity for this parasite to get established as the patients $CD4^{+}$T-cell count increases and confirms the fact that the organism is an opportunistic infection.

CHAPTER SIX

6.0 CONCLUSION

The study has shown a high prevalence of *Cryptosporidium* infection among HIV seropositive patients within the Kaduna metropolis and that ELISA technique is more sensitive than microscopy in detection of *Cryptosporidium* infection.

An overall prevalence of 15% and 59% were obtained by use of microscopy and ELISA respectively.

The source of water and type of toilet were the major risk factors associated with *Cryptosporidium* infection in this study.

The study showed that $CD4^+$ T-cell counts are inversely related with prevalence of *Cryptosporidium* amongst HIV positive patients, where HIV patients with high $CD4^+$ T-cell counts had low prevalence of *Cryptosporidium*.

The study also showed that HAART reduces the prevalence of *Cryptosporidium* infection.

6.1 Recommendation

Since there is high prevalence of *Cryptosporidium* infection among HIV seropositive patients within Kaduna metropolis, diagnosis for *Cryptosporidium* should be incorporated as a routine laboratory test in follow up management of HIV/AIDS patients as this will help reduce the infection.

Although microscopy method is the most common method for diagnosis, it lacks sensitivity is low therefore ELISA method should be used in diagnosis for *Cryptosporidium* as this will provide early and better diagnosis for early intervention .

All HIV patients should be educated on how to prevent water borne infections. They should be encouraged to boil water before drinking and should also be enlightened on how to use different types of sanitary facilities, and the use of open layout should be discouraged. They should also be educated on the need to maintain personal hygiene most especially hand washing after contact with animal and human faeces and avoid contamination of food.

All HIV patients $CD4^+$ T-cell count should be frequently checked to ensure that it is still high and if low patients should be placed on HAART to help boost their immunity as this will be a major defense against the infection hence reducing the prevalence.

More research should be carried out using different diagnostic methods so as to ascertain the actual state of the infection to help in planning intervention program hence meeting the UNAIDS target of zero AIDS- related deaths.

6.2 Limitations of Study

Microscopy method of diagnosis of *Cryptosporidium* is laborious, time consuming and requires high level of technical skill.

The *Cryptosporidium* oocyst can be missed by microscopy method if small quantity of it is excreted in stool hence small quantity per gram of stool or not at all in the gram that will be taken for diagnosis.

In the ELISA method, a negative result can occur from an antigen level lower than the detection limits of the assay.

Lack of funds to compare all the available methods of diagnosis of *Cryptosporidium* to see which the best is and also to carry out molecular studies to know the predominant species among HIV positive patients.

REFERENCES

Adesiji, Y.O, Lawal, R.O, Taiwo, S.S, Fayemiwo, S.A. and Adeyeba O.A. (2007).Cryptosporidiosis in HIV- infected patients with diarrheoa in Osun State South Western Nigeria. *European Journal of General Medicine*, 4: 119-122.

Ajjampur, S.S., Sankaran, P. and Kang, G. (2008). *Cryptosporidium* species in HIV-infected individual in India: an overview. *National Medical Journal of India*. 21(4): 178-184.

Akinbo, F.O., Okaka, C.E. and Omeregiel, R. (2010). Prevalence of intestinal parasitic infections among HIV patients in Benin City, Nigeria. *Libyan Journal of Medicine*, 5: 5506. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3066785/ Accessed July, 2011.

Akujobi, C.N. and Ogunsola, F.T. (2005).Prevalence of *Cryptosporidium* species in patient with Human Immunodeficiency Virus (HIV) / Acquired Immunodeficiency Syndrome (AIDS) presenting with diarrhoea in Lagos. *Nigeria Quarterly Journal of Hospital Medicine,* 15 (3): 119-122.

Alemu, A., Shiferaw, Y., Getut, G., Yalew, A. and Addis, Z. (2011). Opportunistic and other intestinal parasites among HIV/AIDS patients attending Gambi Higher Clinics in Bashir Dar City. North West Ethiopia. *Asian Pacific Journal of Tropical Medicine*, 4 (8): 661-665.

Aragon, T.J., Novothy, S., Enanoria, W., Vulgia,D.J., Khalakdina, and Katz, M.H. (2003). Endemic Cryptosporidiosis and exposure to municipal tap water in person with Acquired Immunodeficiency Syndrome (AIDS): Acase-control study. *BioMed Central Public Health.*3: 2. http://www.biomedcentral.com/1471-2458/3/2/ Accessed March 11, 2012.

Aminu, M. and Yakubu, S.E. (2008). Prevalence of asymptomatic intestinal coccidian parasite infections among non-diarrhoeic HIV positive children in Zaria, Nigeria. *South African Journal of Science*, 4: 9 – 10.

Anderson, J. and Akkina, R. (2007). Complete Knockdown of CCR5 by lentiviral vector-expressed siRNAs and protection of transgenic macrophages against HIV-1 infection. *Gene Therapy*. 14: 1287–1297.

Appay, V. and Sauce, D. (2008). Immune activation and inflammation in HIV-1 infection: causes and consequences. *Journal of Pathology*, 214 (2): 231–241.

Arora, D.R. and Arora, B. (2009). AIDS-associated parasitic diarrhoea. *Indian Journal of Medical Microbiology*, 27 (3): 185 – 190.

Assefa S., Erko B., Medhin G., Assefa Z. and Shimelis T. (2009). Intestinal parasitic infections in relation to HIV/AIDS status, diarrhoea and CD4 T-cell count. *BioMed Central Infectious Diseases* .9**:** 155. http://www.biomedcentral.com/1471-2334/9/155. Accessed May 2, 2012.

Banwat, E.B., Egah, D.Z., Onile, B.A., Angyo, R.A. and Audu, E.S. (2003). Prevalence of Cryptosporidium infection among undernourished children in Jos Central Nigeria. *Nigeria Postgraduate Medical Journal*, 10 (2): 84-97.

Berger, A., Scherzed, L., Sturmer, M., Preiser, W., Doerr, H.W. and Rabenau, H.F. (2005) Comparative evaluation of the Cobas Amplicor HIV-1 Monitor Ultrasensitive test, the new Cobas AmpliPrep/Cobas Amplicor HIV-1 Monitor Ultrasensitive test and the Versant HIV RNA 3.0 assays for quantitation of HIV-1 RNA in plasma samples. *Journal of Clinical Virology*, 33: 43–51.

Bialek, R., Binder, N., Dietz, K., Joachim, A., Knobloch, J. and Zelek, U.E. (2002). Comparism of Flourescence, Antigen and PCR assays to detect Cryptosporidium parvum in faecal specimens. *Dignostic Microbiology and Infectious Diseases*, 43: 283-288.

Blanchard, C., Jackson, A.M., Shanson, D.C., Francis, N. and Gazzar, B.G. (1992). Cryptosporidiosis in HIV Seropositive patients. *Quarterly Journal of Medicine*, 85: 813-823.

Bofill , M., Janossy, G., Lee, C.A., Mac Donald- Burns, D., Phillips, A.N., Sabin, C., Timms, A., Johnson, M.A. and Kernoff, P.B. (1992). Laboratory control values for CD4 and CD8 T lymphocytes . Implications for HIV diagnosis. *Clinical and Experimental Immunology*, 88: 242-252.

Boily, M.C., Baggaley, R.F., Wang, L., Masse, B., White, R.G., Hayes, R.J. and Alary, M. (2009). Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. *The Lancet Infectious Diseases*, 9 (2): 118–129.

Brenchley, J.M., Schacker, T.W., Ruff, L.E., Price, D.A., Taylor, J.H., Beilman, G.J., Nguyen, P.L., Khoruts, A., Larson, M., Haase, A.T. and Douek, D.C. (2004). $CD4^+$ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal stract. *Journal of Experimental Medcine*, 200 (6): 749–759.

Brenchley, J.M., Price, D.A., Schacker, T.W., Asher, T.E., Silvestri, G., Rao, S., Kazzaz, Z., Bornstein, E., Lambotte, O., Altmann, D., Blazar, B.R., Rodriguez, B., Teixeira-Johnson, L., Landay, A., Martin, J.N., Hecht, F.M., Picker, L.J., Lederman, M.M., Deeks, S.G. and Douek, D.C. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nature Medicine*, 12 (12): 1365–1371.

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A. and Mietzner, T.A. (2010). AIDS and Lentiviruses. *Jawetz, Melnick and Adelberg's Medical Microbiology*. 25th ed. New York:Mc Graw Hill, pp 609-623.

Buchlinder, S.P., Katz, M.H., Hessol, N.A., O'Malley, P.M. and Holmberg, S.D. (1994). "Long-term HIV-1 infection without immunologic progression. *AIDS* 8 (8): 1123-1128.

Burton ,G.F, Keele ,B.F, Estes, J.D, Thacker, T.C. and Ghartner S. (2002). "Follicular dendritic cell contributions to HIV pathogenesis". *Seminar in Immunology.* 14 (4): 275– 284.

Cama, V., Gilman, R.H., Vivar, A., Twicona, E., Ortega, Y., Bern, C. and Xiao, L. (2006). Mixed *Cryptosporidium* infections and HIV. *Emerging Infectious Diseases*, 12 (6): 1025 – 1028.

C-GIDD (Canback Global Income Distribution Database) 2008. Canback Dangel. http://www.cgidd.com. Accessed March 3, 2011.

Casemore, D.P., Garder, C.A. and O'Mahony, C. (1994). "Cryptosporidial infection, with special reference to nosocomial transmission of *Cryptosporidium parvum*: a review." *Folia Parasitologica*, 41 (1): 17-21.

Ceffa, S., Erba, F., Assane, M., Coelho E., Calgaro, M. and Brando, B. (2005). Panleucogating as an accurate and affordable flow cytometric protocol to analyse lymphocyte subsets among HIV-positive patients on HAART treatment in Mozambique. *Journal of Biological Regulators and Homeostatic Agents*, 19 (3-4): 169-175.

Centers for Disease Control and Prevention (2009). A Guide to Drinking Water Treatment and Sanitation for Backcountry & Travel Use. http://www.cdc.gov/healthywater/drinking/travel/backcountry_water_treatment.htm Accesed April 9, 2011

Centers for Disease Control and Prevention (1982). Epidemiologic Notes and Reports Cryptosporidiosis: Assesment of Chemotherapy of males with Acquired Immune Deficency Syndrome (AIDS). *Morbidity and Mortality Weekly Report,* 31: 589-592.

Centers for Disease Control and Prevention (2009), HIV/AIDS Surveillance Report 2007, 19. http://www.cdc.gov/hiv/topics/surveillance/resource/reportts. Accessed June 6, 2011.

Certad, G., Arenas-Pinto, A., Pocaterra, G., Castro, J., Bello, A. and Nunez, L. (2005). Cryptosporidiosis in HIV infected Venezuelan adult is strongly associated with acute or chronic diarrhoea. *The American Journal of Tropical Medicine and Hygiene,* 73 (1): 54-57.

Chalmers, R.M., Robinson, G., Elwin, K., Hadfield, S.J., Xiao, L., Ryan, U., Modha, D. and Mallaghan, C. (2009). *Cryptosporidium* spp rabbit genotype, a newly identified human pathogen. *Emerging Infectious Diseases*, 15: 829 – 830.

Chappell, C.L., Okhuysen, P.C., Sterling, C.R. and Du Pont, H.L. (1996). *Cryptosporidium parvum*: Intensity of infection and oocyst excretion pattern in healthy volunteers. *Journal of Infectious Diseases*, 173: 232-236.

Cheesbrough, M.(2005). *Parasitological test. District Laboratory practice in tropical countries.* Part 1. 2nd edn. Cambridge University Press, pp 178‑309.

Chen, W., Harp, I.A. and Harsen, A.G. (2003). *Cryptosporidium parvum* infection in gene targeted B cell-deficient mice. *Journal of Parasitology*, 89 (2): 391 – 393.

Chen, X. M., Keithy, J.S., Pava, C.V. and La Russo, N.F. (2002). Cryptosporidiosis. *New England Journal of Medicine.*346 (22): 1723-1731.

Chen, X.M., Splinter, P.L., O'Hara, S.P. and LaRusso, N.F. (2007). A cellular micro-RNA, let-7i, regulates Toll- like receptor 4 expression and contributes to cholangiocyte immune responses against *Cryptosporidium parvum* infection. *Journal of Biological Chemistry,* 282: 28929–28938.

Clapham P.R, McKnight A. (2001). HIV-1 receptors and cell tropism. *British Medical Bulletin,* 58 (4): 43–59.

Coovadia, H. (2004). "Antiretroviral agents: how best to protect infants from HIV and save their mothers from AIDS". *New England Journal of Medicine,* 351 (3): 289–292.

Cunningham, A., Donaghy, H., Harman, A., Kim, M. and Turville, S. (2010). "Manipulation of dendritic cell function by viruses". *Current Opinion in Microbiology,* 13 (4): 524–529.

Daar, E.S., Little, S., Pitt, J., Santangeto, J., Ho, P., Harawa, N., Kerndt, P., Glorgi, J.V, Bai, J., Gaut, P., Richman, D.D., Mandel, S. and Nichols, S. (2001). "Diagnosis of primary HIV-1 infection. Los Angeles County Primary HIV Infection Recruitment Network". *Annals of Internal Medicine*, 134 (1): 25–29.

D'Antonio, R.G., Winn, R.E., Taylor, J.P., Gustafson, T.L., Currant, W.L., Rhodes, M.M., Gary, G.W. and Zajac, R.A. (1985). A waterborne outbreak of Cryptosporidiosis in normal host. *Annals of Internal Medicine*, 103: 886-888.

Donegan, E., Lee, H., Operskalski, EA., Shaw, G.M., Kleinman, S.H., Busch, M.P., Stevens, C.E., Schiff, E.R., Nowicki, M.J., Hollingsworth, C.G. and Mosley, J.W. and the transfusion safety study group (1994). Transfusion transmission of retroviruses: Human T-lymphotropic virus types I and II compared with human immunodeficiency virus type 1.*Transfusion,* 34: 478-483.

Djuretic, T., Wall, P.G., Ryan, M.J., Evan, H.S., Adak, G.K. and Cowden, J.M. (1996). General outbreak of infectious intestinal diseases in England and Wales: 1992 to1994. *Communicable Disease Report Review,* 6: 57-63.

Douek, D.C., Roeder, M. and Koup, R.A. (2009). "Emerging concepts in the immunopathogenesis of AIDS. *Annual Review of Medicine*, 60: 471-84.

Du pont, H.L., Chappell, C.L., Sterling, C.R., Okhuysen, P.C., Rose, J.B. and Jakabowski, W. (1999). The infectivity of *Cryptosporidium parvum* isolates for healthy adults. *Journal of Infectious Diseases*, 173: 232-236.

Egberonge, H.O., Agbolade, O.M., Adesetan, T.O., Mabekoje, O.O. and Olugbode, A.M. (2010). Cryptosporidiosis among children in relation to toilet facilities and water sources in Ijebu and Remo area, South Western Nigeria. *Journal of Medicine and Medical Science,* 1 (10). 485-489.

Elgun, G. and Koltas, I.S. (2011). Investigation of *Cryptosporidium spp*. Antigen by ELISA method in stool specimens obtained from patients with diarrhoea. *Parasitology Research,* 108 (2): 395-397.

El-Settawy, M.A. and Fathy, G.M. (2012). Evaluation and comparism of PCR, coproantigen ELISA and microscopy for diagnosis of *Cryptosporidium* in human diarrhoeic specimens. *Journal of American Science,* 8(12):1378-1385.

Esteban, J. G., Aguirre, C., Flores, A., Strauss, W., Angles, R., and Mas-Coma, S. (1998). *Cryptosporidium* prevalences in healthy Aymara children from the northern Bolivian Altiplano. *American Journal of Tropical Medicine and Hyg*iene, 58**:**50-55.

Fayer, R., Speer, C.A. and Dubey, J.P. (2001). Basic biology of *Cryptosporidium.* In: *Cryptosporidium and Cryptosporidiosis*. 2nd ed.CRC press, Boca Raton, Florida, pp 288.

Fayer, R. and Ungar, B.L.P. (1986). *Cryptosporidium spp*. and Cryptosporidiosis. *Microbiological Review,* 458-483.

Federal Ministry of Health, Department of Public Health. National AIDS/STI Control Programme. Technical report 2010 National HIV seroprevalence sentinel survey.HIV Prevalence, pp.16.

Feltus, D.C., Giddings, C.W., Schneck, B.L., Monson, T., Warshauer, D. and McEvoy, J.M. (2006). Evidence supporting zoonotic transmission of Cryptosporidium spp. in Wisconsin. *Journal of Clinical Microbiology*, 44 (12): 4303-4308.

Flanigan, T.P. and Soave, R. (1993). Cryptosporidiosis. Progress in Clinical and Parasitological Research, 3: 1-20.

Flanigan, T., Whalen. C., Turner, J., Soave, R., Toerner, J., Havlir, D. and Kotler, D. (1992) *Cryptosporidium* infection and CD4 counts. *Annals of Internal Medicine,*116: 840–844.

Framm, S.R. and Soave, R. (1997). Agents of diarrhea. *Medical Clinics of North America*, 81: 427-447.

Gallo, R.C (2006). A reflection on HIV/AIDS research after 25 years. *Retrovirology,* 3: 7. http://www.retrovirology.com/content/3//72. Accesed September 30, 2011.

Garcia, L.S. (2001). Intestinal protozoa (coccidia and microsporidia) and algae. In: *Diagnostic Medical Parasitology*. 4th ed. ASM press, American Society for Microbiology, street NW, Washington DC. p. 60-105.

Gargala, G. (2008). Drug treatment and novel drug target against *Cryptosporidium. Parasite*, 15 (3): 275-281.

Gatei ,W., Wamae C.N., Mbae C., Waruru, A., Mulinge E., Waithera, T., Gatika, S.K., Kamwati, S.K., Revathi, G, and Hart, C.A. (2006). Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. *American Journal of Tropical Medicine and Hygiene,* 75 (1): 78–82.

Getaneh, A., Medhin, G. and Shimelis T. (2010). *Cryptosporidium and Strongiloides stercoralis* infection among people with and without HIV infection and efficiency of diagnostic method for Strongiloides in Yirgalen Hospital, Southern Ethiopia. *BioMedcentral*, Research Notes, 3: 90. http://www.biomedcentral.com/1756-0500/3/90. Accesed December 10, 2011.

Ghimire, P., Sapkota, D. and Manandhar S.P. (2004). Cryptosporidiosis: Oppportunistic infection in HIV/AIDS patients in Nepal. *The journal of Tropical Medicine and Parasitology*, 27: 7-10.

Gilbert, P.B., McKeague, I.W., Eisen, G., Mullins, C., Guéye-Ndiaye, A., Mboup, S. and Kanki, P.J (2003). Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. *Statistics in Medicine,* 22 (4): 573–593.

Giorgi, J.V. (1993). Characterization of T Lymphocytes subsets alteration by Flow Cytometry in HIV disease. Ann. *New York Academy of Science,* 677: 126-139.

Gao, F., Bailes, E., Robertson, D.L., Chen, Y., Rodenburg, C.M., Micheal, S.F., Cummins, L.B., Arthur, L.O., Peeters, M., Sharp, P.M. and Hahn, B.H. (1999). Origin of HIV-1 in the Chimpanzee Pantroglodytes troglodytes. *Nature,* 397 (6718): 436–444.

Goh, S., Recher, M., Caemore, D.P., Verlander, N.Q. and Chalmer, S. (2004). Sporadic Cryptosporidiosis, North Cumbria, England, 1996-2000. *Emerging Infectious Diseases*, 10 (6): 1007 – 1015.

Goodgame, R.W. (1996). Understanding intestinal spore-forming protozoa: *Cryptosporidia, Microsporidia, Isospora, and Cyclospora. Annals of Internal Med*icine, 124 (4): 429-41.

Graczyk, T.K., Fayer, R. and Cranfield, M.R. (1998). Zoonotic transmission of *Cryptosporidium parvum*: Implications for waterborne Cryptosporidiosis. *Parasitology Today*, 13: 9:348-351.

Greenwald, J.L., Burstein, G.R., Pincus, J., Branson, B. (2006). A rapid review of rapid HIV antibody tests. *Current Infectious Disease Report*, 8: 125–131.

Gupta, S., Narong, S., Nanavata, V. and Singh, S. (2008). Chronic diarrhoea in HIV: prevalence of coccidian parasites. *Indian Journal of Medical Microbiology*, 26 (2): 172 –175.

Guss D.A. (1994 a). The acquired immune deficiency syndrome: an overview for the emergency physician, Part 1. *Journal of Emerging Medicine*, 12 (3): 375–384.

Guss, D.A. (1994 b). The acquired immune deficiency syndrome: an overview for the emergency physician, Part 2. *Journal of Emerging Medicine,* 12 (4): 491–497.

Hashmey, R., Smith, N.H., Cron, S., Graviss, E.A., Chappell, C.L., and White Jr, A.C. (1997). Cryptosporidiosis in Houston, Texas. A report of 95 cases. Medicine, 76**:** 118–139.

Hayes, R. and Weiss H. (2006) Epidemiology. Understanding HIV epidemic trends in Africa. *Science*, 311: 620–21.

Hel, Z., McGhee, J.R. and Mestecky, J. (2006). HIV infection: first battle decides the war. *Trends in Immunology*, 27 (6): 274–281.

Hellard, M.E., Sinclair, M., Hogg, G.G., Fairley, C.K. (2000). Prevalence of enteric pathogens among community based asymptomatic individuals. *Gastroenterology and Hepatology*, 15: 290-293.

Houpt, E. R., Bushen, O.Y., Sam, N.E., Kohli, A., Asgharpour, A., Ng, C.T., Calfee, D.P., Guerrant, R.L., Maro, V., Ole-Nguyaine, S., and Shao, J.F. (2005). Short report: asymptomatic *Cryptosporidium hominis* infection among human immunodeficiency virus-infected patients in Tanzania. *American Journal of Tropical Medicine and Hygiene*, 73**:** 520-522.

Hoxie, N.J., Davis., J.P., Vergeront, J.M., Nashold, R.D. and Blair, K.A. (1997). Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. *American Journal of Public Health*, 87: 2032–2033.

Hunter, P.R., Hughes, S., Woodhouse, S., Syed, Q., Verlander, N.Q., Chalmer, R.M., Morgan, K., Nichols, G., Beeching, N. and Osborn, K. (2004). Sporadic Cryptosporidiosis case control study with genotyping. *Emerging Infectious Diseases*, 10 (7): 1241-1249.

Hunter, P.R. and Nichols, G.(2002). Epidemiology and Clinical Features of *Cryptosporidium* Infection in Immunocompromised Patients *Clinical Microbiology Rev*iew, 15: 145-154.

Hunter, P.R., Syed, Q. and Maumova, E.N. (2001). Possible undetected outbreaks of cryptosporidiosis in areas of the North West of England supplied by an unfiltered surface water source. *Communicable Disease and Public Health*, 4: 136-138.

Ibrahim, A.K., Ikeh, E.I., Malo, A.O., Okeke, E.N. and Damen, J.G. (2007). Intestinal parasitosis in human immunodeficiency virus (HIV) infected adults with chronic diarrhoea in Jos University Teaching Hospital, Jos, Nigeria. *The Internet Journal of Parasitic Diseases,* 2 (1): 1559-4629.

Ikechukwu, D., Benjamin, N. and Uchechukwu, C. (2011). Cryptosporidiosis in Imo state, Nigeria, *Journal of Rural and Tropical Public Health,* 10: 106-110.

Ikeh, E.I., Obadofin, M., Brindeiro, B., Baugher, C., Frost, F., Vanderjagt, D. And Glew, R. (2006). Intestinal parasitism in rural and urban area of North Central Nigeria: An update. *The Internet Journal of Microbiology*, 2: 1-12.

Johnson, S.P., Ballard, M.M., Beach, M.J., Causer, L. and Wilkins, P.P. (2003). Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organism in faecal specimens. *Journal of Clinical Microbiology*, 41: 623-626.

Jokipii, L. and Jokipii, A.M.M. (1986). Timing of symtoms and oocyst excretion in human Cryptosporidiosis. *New England Journal of Medicine*, 315: 1643-1647.

Joshi, M., Chowdhary, A.S., Dalal, P.J. and Maniar, J.K. (2002). Parasitic diarrhoea in patients with AIDS. *National Medical Journal of India,* 15: 72-74.

Jirku, M., Valigurova, A., Koudel, B., Krizet, J., Modey, D. and Slapetu, J. (2008). New species of *Cryptosporidium tyzzer*, 1907 (Apicomplexa) from amphian host, morphology, biology and physiology. *Folia Parasitologica*, 55: 81-94.

Juranek, D.D. (2001). Crytosporidiosis. Sources of infection and guidelines for prevention. *Center for Disease Control and Prevention*, 47; 273-294.

Kahn, J. O. and Walker, B. D. (1998). Acute Human Immunodeficiency Virus type 1 infection. *New England Journal of Medicine*, 331 (1): 33–39.

Kalling Holmes C.B., Losina, E., Walensky, R.P., Yazdanpanah, Y. and Freedberg K.A (2003). Review of human immunodeficiency virus type 1-related opportunistic infections in Sub- Saharan Africa. *Clinical Infectious Diseases*, 36 (5): 656–662.

Kaplan, J.E., Masur, H. and Holmes, K.K. (2002). Guideline for preventing opportunistic infections among HIV infected persons. *Morbidity, Mortality Weekly Report*, 51: 1-46.

Keusch, G.T., Hamer, D., Joe, A., Kelley, M., Griffiths, J., and Ward, H. (1995) "*Cryptosporidia*-- who is at risk?" *Schweiz Med Wochenschr*, 125 (18): 899-908.

Kosek, M., Akanlara, A., Lima, A.A.M. and Guerrant, R.L. (2001). Cryptosporidiosis: an update. *Lancet Infectious Diseases*, 1: 262 – 269.

Kwong, P.D., Wyatt R., Robinson J., Sweet R.W., Sodroski J., and Hendrickson W.A. (1998). Structure of an HIV gp120 envelope glycoprotein in complex with the $CD4^+$ receptor and a neutralizing antibody. *Nature,* 393: 648-659.

Lavreys, L., Baeten, J.M., Martin, H.L., *Overbaugh, J., Mandaliya, K., Ndinya-Achola, J., and Kreiss, J. K.* (March 2004). Hormonal contraception and risk of HIV-1 acquisition: results of a 10-year prospective study. *AIDS,* 18 (4): 695–697.

Lawn, S.D. (2004). AIDS in Africa: the impact of co infection on the pathogenesis of HIV-1 infection without immulogic progression. *AIDS,* 8 (8): 1123-1128.

Mac Kenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S, Blair, K.A., Peterson, D.E., Kazmierczak, J. and David, J. (1994) A massive outbreak in Milwaukee of *cryptosporidium* infection transmitted through the public water supply. New *England Journal of Medicine,* 331: 161–167.

Maggi, P., Larocca, A.M., Quarto, M., Serio, G., Brandonisio, O. and Angarano, G. (2000). Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1. *European Journal of Clinical Microbiology and Infectious Diseases*, 19: 213–217.

Manabe, Y.C., Clark, D.P., Moore, R.D., Lumadue, J.A., Dahlman, H.R., Belitsos, P.C., Chaisson, R.E. and Sears, C.L. (1998). Cryptosporidiosis in patients with AIDS: correlates of disease and survival. *Clinical Infectious Disease Journal,* 27:536-542.

Martin, K., Dana, K., Bohamil, S. and Oley, D. (2009). *Cryptosporidium* pig genotype II in immunocompetent man. *Journal of Infectious Diseases*, 15 (6): 982 – 983.

McGowan, I., Hawkins, A.S. and Weller, I.V. (1993). The natural history of cryptosporidial diarrhoea in HIV-infected patients. *AIDS,* 7**:**349–354.

Meamar, A., Rezaian M., Rezaie S., Mohraz, M., Kia, E.B.,, Houpt E.R., and Solaymani-Mohammadi, M. (2006) *Cryptosporidium parvum* bovine genotype oocysts in the respiratory samples of an AIDS patient: efficacy of treatment with a combination of azithromycin and paromomycin. *Parasitology Research*, 98: 593-595.

Mead, J. R., and You, X. (1998). Susceptibility differences to *Cryptosporidium parvum* infection in two strains of gamma interferon knockout mice. *Journal of Parasitology,* 84**:** 1045-1048.

Mehandru, S., Poles, M.A., Tenner-Racz ,K., Horowitz, A., Hurley, A., Hogan, C., Boden, D., Racz, P. and Markowitz, M. (2004). Primary HIV-1 infection is associated with preferential depletion of $CD4^+$ T lymphocytes from effector sites in the gastrointestinal tract. *Journal of Experirimental Medicine*, 200 (6): 761–770.

Mele, R., Gomez Morales, M.A., Tosini, F. and Pozio E. (2003). Indinavir reduces *Cryptosporidium parvum* infection in both in vitro and in vivo models. *International Journal of Parasitology*, 33 (7): 757-764.

Molley, S.F., Smith, H.V., Kirwin, P., Nicholas, R.A.B., Asaolu, S.O., Connely, L. and Hollen, C.V. (2010). Identification of a high diversity of *Cryptosporidium* species genotype and subgenotypes in paediatric population in Nigeria. *American Journal of Tropical Medicine and Hygiene*, 82 (4): 608-613.

Morgan, D., Mahe, C., Mayanja, B., Okongo, J.M., Lubega, R., Whitworth, J.A. (2002). HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries? *AIDS* 16 (4): 597–632.

Morgan-Ryan, U.M., Fall, A., Ward, L.A., Hijjawi, N., Sulaiman, I., Fayer, R., Thompson, R.C., Olson, M., Lal, A. & Xiao, L. (2002). *Cryptosporidium hominis* n. sp. (Apicomplexa: *Cryptosporidiidae*) from Homo sapiens. *Journal of Eukaryotic Microbiology*, 49: 433-440.

Murray, P. R., Rosenthal, K.S. and Pfaller, M.A. *Medical Microbiology*. 5th ed. Philadelphia: Elsevier Inc., 2005: 855-856.

Nannini, E.C. and Okhuysen P.C. (2002). HIV1 and the gut in the era of highly active antiretroviral therapy. *Current Gastroenterology Report*, 4: 392–398.

Nunez, R. (2001). Flow Cytometry: Principles and Instrumentation. *Current Issues in Molecular Biology*, 3 (2): 39-45.

Nwokediuko, 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. *Nigerian Postgraduate Medical Journal,* 9 (2): 70-73.

Okhuysen, P.C., Chappell, C.L., Crabb, J.H., Sterling, C.R, and DuPont., H.L. (1999). Virulence of three distinct *Cryptosporidium parvum i*solates for healthy adults. *Journal of Infectious Diseases*, 180: 1275–1281.

Oyerinde, J.P., Odugbemi, T., Benson, R.I., Alonge, A.A. and Roberts, J.I. (1989). Investigation of *Cryptosporidium* in relation to other intestinal parasites at the Lagos University Teaching Hospital, Lagos, *West African Journal of Medicine,* 8(4): 264-269.

Panciera, R.J, Thomassen, R.W, Garner, F.M. (1971). Cryptosporidial infection in a calf. *Veterinary Pathology*, 28: 187-188.

Pantaleo, G., Demarest, J.F., Schacker, T., Vaccarezza, M., Cohen, O.J., Daucher, M., Graziosi, C., Schnittman, S.S., Quinn, T.C, Shaw, G.M., Perrin, L., Tambussi, G., Lazzarin, A., Sekaly, R.P., Soudeyns, H., Corey, L. and Fauci, A.S. (1997). The qualitative nature of the primary immune response to HIV infection is a prognosticator of disease

progression independent of the initial level of plasma viremia. *Proceedings of National Academy of Sciences U S A.* 94 (1): 254–258.

Piatak, M., Jr, Saag, M. S., Yang, L. C., Clark, S. J., Kappes, J. C., Luk, K. C., Hahn, B. H., Shaw, G. M. and Lifson, J.D. (1993). High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* ,259 (5102): 1749–1754.

Population Action International (2008) Reclaiming the ABCs - The Creation and Evolution of the ABC Approach' http://www.avert.org/abc-hiv.htm.

Quinn, T.C. and Overbaugh, J. (2005). HIV/AIDS in women: An expanding epidermic. *Science*, 308 (5728): 1582-1583.

Reeves, J. D. and Doms, R. W (2002). Human Immunodeficiency Virus Type 2. *Journal of General Virology,* 83: 1253–1265.

Rochelle, P.A., Fallar, D., Marshall, M.M., Montelone, B.A., Upton, S.J. and Woods, K. (2004). Irreversible UV inactivation of *Cryptosporidium spp*. despite the presence of UV repair genes. *Journal of Eukaryotic Microbiology,* 51 (5): 553–562.

Rodriguez, W.R., Christodoulides, N., Floriano, P.N., Graham, S., Mohanty, M.D., Hsiang, M., Peter, T., Zavahir, S., Thior, I., Romanovicz, D., Bernard, B., Goodey, A.P., Walker, B.D. and Mc Deuitt, J.T. (2005) . A microchip CD4 counting method for HIV monitoring in resource-poor settings. *Public Library of Science Medicine*, 2: 182. http://www.plosmedicine.org/article/info:doi/10.1371/journal.pmed.0020182. Accessed October 20, 2011.

Ryan, K. J. and Ray C.G. (2004). *Sherris Medical Microbiology*: An Introduction to infectious Disease. 4th ed. New York: McGraw-Hill, 2004: 727-773.

Sagar, M., Lavreys, L., Baeten, J.M., Richardson, K. and Mandaliya, B.H. (2004). Identification of modifiable factors that affect the genetic diversity of the transmitted HIV-1 population. *AIDS,* 18 (4): 615–619.

Saha, K., Firdaus, R., Santra, P., Pal, J., Roy, A., Bhattacharya, M.K., Chakrabarti, S. and Sadhukhan, P.C. (2011). Recent pattern of Co-infection amongst HIV seropositive individual in tertiary care Hospital Kolkata. Virology Journal, 8 (1): 116. http://www.virologyj.com/8/1/116. Accessed May 12, 2012.

Savioli, L., Smith, H. and Thompson, A. (2006). *Giardia* and *Cryptosporidium* join the Neglected Diseases Initiative. *Trends in Parasitology*, 22: 203–208.

Sepkowitz, K.A. (2001). AIDS – the first 20 years. *England Journal of Medicine*, 344 (23): 1764–1772.

Slavin, D. (1955). *Cryptosporidium meleagridis* (sp.nov). *Journal of Comparative Pathology,* 65:262-266.

Smith, H.V. and Corcoran, G.D. (2004). New drugs and treatment for cryptosporidiosis. *Current Opinions on Infectious Diseases*, 17 (6): 557–564.

Smith, H.V. and Nicholas, R.A.B. (2010). *Cryptosporidium*: detection in water and food. *Experimental Parasitology*, 124(1): 61-79.

Sreedharan, A., Jayshree, R.S. and Sridhar, H. (1996). Cryptosporidiosis among cancer patients: an observation. *Journal of Diarrhoeal Disease Research*, 14: 211– 213.

Sterling, T.R., Chaisson, R.E. and Moore, R.D. (2001). HIV-1 RNA, CD4T-Lymphocytes, and clinical response to highly active antirectroviral therapy. *AIDS*, 15:2251-2257.

Suddiqui, U., Bini, E.J., Chandara, K., Leong, J., Ramsetly, S., Schilliro, D. and Poles, M. (2007). Prevalence and impact of diarrhea on health related quality of life in HIV-infected patients in the era of highly active antirectroviral therapy. *Journal of Clinical Gastroenterology*, 41 (5); 484-490.

Srisuphanunt, M., Suvedyathavorn, V., Suputtamonkol, Y., Arnantapunpong, S., Wiwanitkit, V., Satitvipawee, P. and Transupasawadikul, S. (2008). Potential risk factors for *Cryptosporidium* infection among HIV/AIDS patients in Thailand. *Journal of Public Health*, 16: 173-182.

Taghi-Kilani, R., L. Sekla, and K. T. Hayglass. 1990. The role of humoral immunity in *Cryptosporidium* spp. infection. Studies with B cell-depleted mice. *Journal of Immunology,* 145: 1571–1576.

Tanyuksel, M., H. Gun, and L. Doganci. (1995). Prevalence of *Cryptosporidium* spp. in patients with neoplasia and diarrhea. *Scandinavian Journal of Infectious Disease,* 27: 69–70.

Theodos, C.M., Sullivan, K.L., Griffiths, J.K. and Tzipori, S. (1997). Profiles of healing and nonhealing *Cryptosporidium parvum* infection in C57BL/6 mice with functional B and T lymphocytes: the extent of gamma interferon modulation determines the outcome of infection. *Infectious Immunology*, 65: 4761–4769.

Tsaihong, J. C., Guiguli P., M. Y. Liou M.Y., and Ma P. (1990). Cryptosporidiosis in AIDS and non-AIDS patients. *China Journal of Microbiology and Immunology*, 23: 155–161.

UNAIDS (2004) Report on the Global AIDS Epidemic http://data.unaids.org/Global-Reports/Bangkok-2004/unaidsbangkokpress/gar2004html/GAR2004_03_en.htm#P237_35114. Accessed May 22, 2012.

UNAIDS 2009 AIDS Epidemic Update. http://www.unaids. Org/en/dataanalysis/knowyourepidemic/epidemiologypublications/2009aidsepidemicupdate/. Accessed May 22, 2012.

UNAIDS-AIDS Epidemic Update: December 2006. Available from:http://www.data.unaids.org. Accessed May 23, 2012.

UNAIDS Fact Sheet 2009. Sub-Saharan Africa, 20091124. http://data.unaids.org/pub/FactSheet/2009/20091124_fs_ssa_en.pdf http://. Accessed May 23,2011.

UNAIDS Report on the global AIDS epidemic 2010. http://www.unaids.org/globalreport. Accessed May 23, 2012.

Ungar B.L. P (2000). *Cryptosporidium*. In: Mandell, G. L., Bennett, J.E. and Dohn, R. ed. Mandell , Douglas and Bennett's principle and practice of infectious diseases. 5th ed. Philadelphia, Pa: Churchill Livingstone. 2903-2915.

Uttayamakul, S., Likanonsakul, S., Sunthornkachit, R., Kuntiranout, K., Louisirirochanakuh. S., Chaovanunich, A., Thiamchai, V., Tanprasertsuk, S, and Suttheat, R. (2005). Usage of dried blood spots for molecular diagnosis and monitoring HIV-1 infection. *Journal of Virology Methods*, 128: 128–134.

Ventura, G., Cauda,R., Larocca L.M., Riccioni sM.E., Tumbarello.M, and Lucia M.B. (1997). Gastric cryptosporidiosis complicating HIV infection: case report and review of the literature. *European Journal of Gastroenterology and Hepatology*, 9: 307–310.

Weiss, R.A. (1993). How does HIV cause AIDS? *Science Journal*, 260 (5112): 1273 – 1279.

Werneck-Silva, A.L. and Prado, I.B. (2009). Gastroduodenal opportunistic infections and dyspepsia in HIV- infected patients in the era of highly active antiretroviral therapy. *Journal of Gastroenterology and Hepatology*, 24: 135–139.

White A.C Jr. (2005). Cryptosporidiosis (*Cryptosporidium hominis, Cryptosporidium parvum,* other species). In: Mandell GL, Bennett JE, Dolin R, ed. *Principles and Practice of Infectious Diseases*. 6th ed. Philadelphia, PA: Elsevier Churchill Livingstone. 3215-3228.

Willey, J.M, Sherwood, L.M and Woolverton, C.J. (2011). Human diseases caused by fungi and protists, Food-borne and water borne diseases and Human diseases, caused by viruses and prions, direct contact diseases In: Willey,J.M, Sherwood, L.M and Woolverton, C.J.(editors) *Prescott's Microbiology*. 8th Edition, New York. The McGraw Hill Companies. International Edition. pp 928-1008 and 897-931.

Winn Jr., Washington, Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Paul Schreckenberger, and Gail Woods. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2006: 1267-1270.

WHO Progress Report (2010). More developing countries show universal access to HIV/AIDS. Service is possible. 20th September, 2010. http://www.who.int/hiv/pub/2010progressreport/en/ Accessed May 30, 2012.

WHO/ UNAIDS/ UNICEF (2011). Global HIV/AIDS response: Epidemic update and health sector progress report. November 30. http://www.who.int/hiv/pub/progress_report2011/en/index.html. Accessed May 30, 2012.

Worobey, M., Gemmel, M., Teuwen, D.E., Haselkorn, T., Kunstman, K., Bunce, M., Muyembe, J., Kabongo, J. M., Kalengayi, R.M., Van Marck, E. and Gilbert, M.T. (2008). "Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960". *Nature* 455 (7213): 661-664.

Wumba, R., Longo-Mbenza, B., Mandina, M., Odio, W.T., Biliqui, S., Sala, J., Breton, J. and Thellier.M. (2010). Intestinal parasites infections in hospitalized AIDS patients in Kinshasa, Democratic Republic of Congo. *Parasite*. 17(4): 321-328.

Yoder, J.S., Harral, C, and Beach, M.J. Cryptosporidiosis surveillance: United States, 2006–2008. *Morbidity and Mortality Weekly Report Surveillance Summumaries,* 2010; 59: 1–14.

Yu, J. R., Lee, J.K., Seo, M., Kim, S.I., Sohn, W.M., Huh, S., Choi, H.Y. and Kim,T.S. (2004). Prevalence of cryptosporidiosis among the villagers and domestic animals in several rural areas of Korea. *Korean Journal Parasitology*, 42: 1-6.

Zaidah, A.K., Chen, Y.Y., Asma, H.S., Abdullahi, S., Nurhaslindawati, A.R, and Saleh, M. (2008). Detection of *Cryptosporidium parvum* in HIV infected patients in Malaysia using a molecular approach. *South east Asian Journal of Tropical Medicine and Public Health,* 39: 511-516.

APPENDIX I: Ethical Approval

MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 248084
(062) 248252

Indepedence Way,
P.M.B. 2014,
Kaduna.
Kaduna State, Nigeria

MOH/ADM/744/VOL.I/38 **20TH October, 2011**

Head of Department,
Microbiology,
A.B.U. Zaria.

APPROVAL TO CARRY OUT RESEARCH

Approval has been granted comfort Ndaks to carry out a research work on the topic prevalence of cryptosporidium infection among HIV seropositive patients within Kaduna metropolis.

You are requested to grant her the necessary assistance needed.

F.A. Kurah
Secretary, Ethical Committee.

APPENDIX II: Informed Consent

DEPARTMENT OF MICROBIOLOGY
SCHOOL OF POSTGRADUATE STUDIES
AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

INFORMED CONSENT FORM (ICF)

Serial No.: …….. ***Hospital No.: ……*** ***Age: ……..*** ***Phone No.: ……..……***

This Informed Consent Form is basically for males and females within the age group 16 – 65 years, who attend VCT center in Hospitals within Kaduna metropolis, Nigeria. We are inviting you to participate in this research work titled "Prevalence of Cryptosporidium infection and its correlation with $CD4^+$ Count among HIV seropositive patients within Kaduna metropolis''. It involves the collection of blood and stool samples of participants. The procedure involved will be fully explained to all participants. The results obtained thereby may be used in any way to improve the understanding and management of the disease in our community. All participants may be given copies of their test results. Individual data will be treated with strict confidentiality. Participation is strictly voluntary and refusal to participate in the research study will not in any way affect your right and benefit in this clinic/hospital.

CERTIFICATE OF CONSENT

I ……..of …….. do hereby consent to participate in this study. The full procedures of the test/study have been explained to me by the investigator. I understand that sample of my blood and stool will be taken for test. I therefore give this consent voluntarily without being subjected to any pressure.

Name of Participant ……..…

Signature/right thumb print of participant ……. Date …….

Statement by Witness:

I have witnessed the accurate reading of the consent form and detail explanation of the study to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness……

Signature of witness……..…. Date ……..

Statement by the Researcher/Person Taking Consent

I confirm that sufficient information, including about risks and benefits, to make an informed decision have been fully explained to the participant. The participant was given an opportunity to ask questions about the study, and all the questions asked by participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher …….

Signature ……... Date. ……

APPENDIX I II: Questionnaire

QUESTIONAIRE

SECTION A: BIODATA

1. Serial no.:_____
2. Residential address _____
3. Age: 16 – 25 [ ] 26 – 35 [ ] 36 – 45 [ ] 46 – 55 [ ] 56 – 65 [ ]
4. Sex: Male [ ] Female [ ]
5. Marital status: Single [ ] Married [ ] Divorced [ ] Separated [ ] Widowed [ ]
6. Educational status: Primary [ ] Secondary [ ] Tertiary [ ] Others [ ]
7. Occupation: Civil servant [ ] Self employed [ ] Unemployed [ ] Retired [ ] Others [ ]

SECTION B: MEDICAL INFORMATION

8. Source of water supply: Tap water [ ] Well [ ] River [ ] Streams [ ]
9. Do you boil water before drinking? Yes [ ] No [ ]
10. Types of toilet used. Water cistern [ ] Pit latrine [ ] Open layout [ ] Others [ ]
11. Any case of diarrhoea? Yes [ ] No [ ]
12. Duration of diarrhoea: 1 – 2 days [ ] 3 – 5 days [ ] 1 week [ ] 2 – 4 weeks [ ]
13. Do you have any contact with farm animals? Yes [ ] No [ ]
14. Do you experience any of these? Fever [ ] abdominal cramp [ ]
    Anorexia [ ] Nausea [ ] Malaise [ ]
15. Do you swim in the swimming pool? Yes [ ] No [ ]
16. Have you travelled out of the country before? Yes [ ] No [ ]
17. How often do you travel?
18. Dehydration Status Dehydrated [ ] Not dehydrated [ ]
19. Are you on anterectroviral drugs? Yes [ ] No [ ]
20. How long?

APPENDIX IV ELISA Report

Analysis Report

Plate No.:1
1st Item:Cryptosporidium
Measure:ABS Analysis:ABS
Sample:Single Sample Aavelength:450
Blank ABS:0.000

	1	2	3	4	5	6
Hole	S1	S2	S3	S4	S5	S6
OD	0.116	0.084	0.084	0.116	0.112	0.281
Quant	0.116	0.084	0.084	0.116	0.112	0.281
Quali						
Hole	S13	S14	S15	S16	S17	S18
OD	0.231	0.207	0.311	0.115	0.254	0.326
Quant	0.231	0.207	0.311	0.115	0.254	0.326
Quali						
Hole	S25	S26	S27	S28	S29	S30
OD	0.292	0.249	0.302	0.214	0.127	0.554
Quant	0.292	0.249	0.302	0.214	0.127	0.554
Quali						
Hole	S37	S38	S39	S40	S41	S42
OD	0.313	0.343	0.239	0.255	0.136	0.253
Quant	0.313	0.343	0.239	0.255	0.136	0.253
Quali						
Hole	S49	S50	S51	S52	S53	S54
OD	0.387	0.224	1.221	0.410	0.478	0.350
Quant	0.387	0.224	1.221	0.410	0.478	0.350
Quali						
Hole	S61	S62	S63	S64	S65	S66
OD	0.443	0.344	0.253	0.174	0.221	0.157
Quant	0.443	0.344	0.253	0.174	0.221	0.157
Quali						
Hole	S73	S74	S75	S76	S77	S78
OD	0.332	0.568	0.357	0.395	0.342	0.427
Quant	0.332	0.568	0.357	0.395	0.342	0.427
Quali						
Hole	S85	S86	S87	S88	S89	S90
OD	0.695	0.353	0.372	0.371	0.297	0.499
Quant	0.695	0.353	0.372	0.371	0.297	0.499
Quali						

	7	8	9	10	11	12
Hole	S7	S8	S9	S10	S11	S12
OD	0.241	0.279	0.190	0.322	0.314	0.325
Quant	0.241	0.279	0.190	0.322	0.314	0.325
Quali						
Hole	S19	S20	S21	S22	S23	S24
OD	0.297	0.245	0.340	0.227	0.271	0.208
Quant	0.297	0.245	0.340	0.227	0.271	0.208
Quali						
Hole	S31	S32	S33	S34	S35	S36
OD	0.236	0.167	0.176	0.338	0.335	0.399
Quant	0.236	0.167	0.176	0.338	0.335	0.399
Quali						
Hole	S43	S44	S45	S46	S47	S48
OD	0.164	0.243	0.269	0.318	0.378	0.362
Quant	0.164	0.243	0.269	0.318	0.378	0.362
Quali						
Hole	S55	S56	S57	S58	S59	S60
OD	0.325	0.154	0.303	0.364	0.235	0.348
Quant	0.325	0.154	0.303	0.364	0.235	0.348
Quali						
Hole	S67	S68	S69	S70	S71	S72
OD	0.242	0.267	0.243	0.414	0.360	0.402
Quant	0.242	0.267	0.243	0.414	0.360	0.402
Quali						
Hole	S79	S80	S81	S82	S83	S84
OD	0.368	0.351	0.372	0.344	0.393	0.430
Quant	0.368	0.351	0.372	0.344	0.393	0.430
Quali						
Hole	S91	S92	S93	S94	S95	S96
OD	0.429	0.387	0.667	0.363	0.459	0.394
Quant	0.429	0.387	0.667	0.363	0.459	0.394
Quali						

Test date:2012-02-15 Test time:23:15:54
Print date:2012-02-15 Print time:23:26:38
User: Verifier:

Analysis Report

Plate No.:1
1st Item:Ctyptosporidium
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.000

	1	2	3	4	5	6
Hole	S1	S2	S3	S4	S5	S6
OD	0.117	0.124	0.134	0.110	0.160	0.207
Quant	0.117	0.124	0.134	0.110	0.160	0.207
Quali						
Hole	S13	S14	S15	S16	S17	S18
OD	0.144	0.137	0.163	0.099	0.118	0.152
Quant	0.144	0.137	0.163	0.099	0.118	0.152
Quali						
Hole	S25	S26	S27	S28	S29	S30
OD	0.128	0.176	0.173	0.251	0.147	0.171
Quant	0.128	0.176	0.173	0.251	0.147	0.171
Quali						
Hole	S37	S38	S39	S40	S41	S42
OD	0.134	0.127	0.087	0.075	0.100	0.113
Quant	0.134	0.127	0.087	0.075	0.100	0.113
Quali						
Hole	S49	S50	S51	S52	S53	S54
OD	0.200	0.144	0.164	0.188	0.194	0.199
Quant	0.200	0.144	0.164	0.188	0.194	0.199
Quali						
Hole	S61	S62	S63	S64	S65	S66
OD	0.144	0.141	0.168	0.149	0.130	0.125
Quant	0.144	0.141	0.168	0.149	0.130	0.125
Quali						
Hole	S73	S74	S75	S76	S77	S78
OD	0.557	0.117	0.137	0.172	0.108	0.111
Quant	0.557	0.117	0.137	0.172	0.108	0.111
Quali						
Hole	S85	S86	S87	S88	S89	S90
OD	0.097	0.091	0.343	0.129	0.124	0.118
Quant	0.097	0.091	0.343	0.129	0.124	0.118
Quali						

	7	8	9	10	11	12
Hole	S7	S8	S9	S10	S11	S12
OD	0.198	0.169	0.184	0.168	0.233	0.242
Quant	0.198	0.169	0.184	0.168	0.233	0.242
Quali						
Hole	S19	S20	S21	S22	S23	S24
OD	0.141	0.174	0.159	0.148	0.145	0.175
Quant	0.141	0.174	0.159	0.148	0.145	0.175
Quali						
Hole	S31	S32	S33	S34	S35	S36
OD	0.180	0.196	0.198	0.170	0.191	0.200
Quant	0.180	0.196	0.198	0.170	0.191	0.200
Quali						
Hole	S43	S44	S45	S46	S47	S48
OD	0.105	0.132	0.136	0.148	0.140	0.169
Quant	0.105	0.132	0.136	0.148	0.140	0.169
Quali						
Hole	S55	S56	S57	S58	S59	S60
OD	0.203	0.236	0.186	0.153	0.135	0.206
Quant	0.203	0.236	0.186	0.153	0.135	0.206
Quali						
Hole	S67	S68	S69	S70	S71	S72
OD	0.132	0.108	0.162	0.247	0.108	0.201
Quant	0.132	0.108	0.162	0.247	0.108	0.201
Quali						
Hole	S79	S80	S81	S82	S83	S84
OD	0.139	0.151	0.241	0.260	0.293	0.266
Quant	0.139	0.151	0.241	0.260	0.293	0.266
Quali						
Hole	S91	S92	S93	S94	S95	S96
OD	0.178	0.150	0.109	0.163	0.123	0.207
Quant	0.178	0.150	0.109	0.163	0.123	0.207
Quali						

Test date:2012-02-15 Test time:23:17:18
Print date:2012-02-15 Print time:23:27:58

Plate No.:1
1st Item:Ctyptosporidium
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.000

	1	2	3	4	5	6
Hole	S1	S2	S3	S4	S5	S6
OD	0.091	0.093	0.097	0.115	0.105	0.106
Quant	0.091	0.093	0.097	0.115	0.105	0.106
Quali						
Hole	S13	S14	S15	S16	S17	S18
OD	0.101	0.211	0.109	0.078	0.082	0.071
Quant	0.101	0.211	0.109	0.078	0.082	0.071
Quali						
Hole	S25	S26	S27	S28	S29	S30
OD	0.104	0.124	0.142	0.140	0.115	0.127
Quant	0.104	0.124	0.142	0.140	0.115	0.127
Quali						
Hole	S37	S38	S39	S40	S41	S42
OD	0.120	0.111	0.103	0.102	0.093	0.100
Quant	0.120	0.111	0.103	0.102	0.093	0.100
Quali						
Hole	S49	S50	S51	S52	S53	S54
OD	0.115	0.132	0.133	0.167	0.130	0.124
Quant	0.115	0.132	0.133	0.167	0.130	0.124
Quali						
Hole	S61	S62	S63	S64	S65	S66
OD	0.102	0.088	0.085	0.103	0.108	0.124
Quant	0.102	0.088	0.085	0.103	0.108	0.124
Quali						
Hole	S73	S74	S75	S76	S77	S78
OD	0.104	0.124	0.115	0.127	0.137	0.133
Quant	0.104	0.124	0.115	0.127	0.137	0.133
Quali						
Hole	S85	S86	S87	S88	S89	S90
OD	0.099	0.091	0.138	0.098	0.108	0.095
Quant	0.099	0.091	0.138	0.098	0.108	0.095
Quali						

	7	8	9	10	11	12
Hole	S7	S8	S9	S10	S11	S12
OD	0.152	0.213	0.832	0.154	0.159	0.216
Quant	0.152	0.213	0.832	0.154	0.159	0.216
Quali						
Hole	S19	S20	S21	S22	S23	S24
OD	0.077	0.098	0.136	0.109	0.096	0.130
Quant	0.077	0.098	0.136	0.109	0.096	0.130
Quali						
Hole	S31	S32	S33	S34	S35	S36
OD	0.146	0.160	0.144	0.180	0.164	0.304
Quant	0.146	0.160	0.144	0.180	0.164	0.304
Quali						
Hole	S43	S44	S45	S46	S47	S48
OD	0.130	0.147	0.113	0.138	0.138	0.134
Quant	0.130	0.147	0.113	0.138	0.138	0.134
Quali						
Hole	S55	S56	S57	S58	S59	S60
OD	0.147	0.158	0.136	0.185	0.155	0.189
Quant	0.147	0.158	0.136	0.185	0.155	0.189
Quali						
Hole	S67	S68	S69	S70	S71	S72
OD	0.138	0.139	0.129	0.136	0.124	0.143
Quant	0.138	0.139	0.129	0.136	0.124	0.143
Quali						
Hole	S79	S80	S81	S82	S83	S84
OD	0.158	0.148	0.150	0.209	0.168	0.186
Quant	0.158	0.148	0.150	0.209	0.168	0.186
Quali						
Hole	S91	S92	S93	S94	S95	S96
OD	0.097	0.112	0.088	0.178	0.091	0.115
Quant	0.097	0.112	0.088	0.178	0.091	0.115
Quali						

Test date:2012-02-15 Test time:23:18:20
Print date:2012-02-15 Print time:23:29:03
User: Verifier:

Analysis Report

Plate No.:1
1st Item:Ctyptosporidium
Measure:ABS Analysis:ABS
Sample:Single Sample Wavelength:450
Blank ABS:0.000

	1	2	3	4	5	6
Hole	S1	S2	S3	S4	S5	S6
OD	0.641	0.383	0.194	0.287	0.395	0.434
Quant	0.641	0.383	0.194	0.287	0.395	0.434
Quali						

Hole	S13	S14	S15	S16	S17	S18
OD	0.324	0.336	0.573	0.232	0.168	0.383
Quant	0.324	0.336	0.573	0.232	0.168	0.383
Quali						

Hole	S25	S26	S27	S28	S29	S30
OD	0.413	0.400	0.371	1.192	0.479	0.379
Quant	0.413	0.400	0.371	1.192	0.479	0.379
Quali						

Hole	S37	S38	S39	S40	S41	S42
OD	0.132	0.200	0.169	0.120	0.179	0.148
Quant	0.132	0.200	0.169	0.120	0.179	0.148
Quali						

Hole	S49	S50	S51	S52	S53	S54
OD	0.067	0.055	0.062	0.069	0.064	0.058
Quant	0.067	0.055	0.062	0.069	0.064	0.058
Quali						

Hole	S61	S62	S63	S64	S65	S66
OD	0.055	0.074	0.056	0.069	0.063	0.058
Quant	0.055	0.074	0.056	0.069	0.063	0.058
Quali						

Hole	S73	S74	S75	S76	S77	S78
OD	0.068	0.097	0.061	0.055	0.083	0.067
Quant	0.068	0.097	0.061	0.055	0.083	0.067
Quali						

Hole	S85	S86	S87	S88	S89	S90
OD	0.079	0.050	0.063	0.067	0.054	0.050
Quant	0.079	0.050	0.063	0.067	0.054	0.050
Quali						

	7	8	9	10	11	12
Hole	S7	S8	S9	S10	S11	S12
OD	0.339	0.279	0.490	0.433	0.658	0.367
Quant	0.339	0.279	0.490	0.433	0.658	0.367
Quali						

Hole	S19	S20	S21	S22	S23	S24
OD	0.593	0.454	0.188	0.407	0.315	0.284
Quant	0.593	0.454	0.188	0.407	0.315	0.284
Quali						

Hole	S31	S32	S33	S34	S35	S36
OD	0.409	0.300	0.293	0.424	0.532	0.475
Quant	0.409	0.300	0.293	0.424	0.532	0.475
Quali						

Hole	S43	S44	S45	S46	S47	S48
OD	0.154	0.367	0.237	0.187	0.144	0.256
Quant	0.154	0.367	0.237	0.187	0.144	0.256
Quali						

Hole	S55	S56	S57	S58	S59	S60
OD	0.054	0.065	0.050	0.048	0.045	0.048
Quant	0.054	0.065	0.050	0.048	0.045	0.048
Quali						

Hole	S67	S68	S69	S70	S71	S72
OD	0.046	0.066	0.043	0.043	0.044	0.044
Quant	0.046	0.066	0.043	0.043	0.044	0.044
Quali						

Hole	S79	S80	S81	S82	S83	S84
OD	0.059	0.060	0.043	0.055	0.051	0.070
Quant	0.059	0.060	0.043	0.055	0.051	0.070
Quali						

Hole	S91	S92	S93	S94	S95	S96
OD	0.054	0.209	2.222	2.068	0.203	0.359
Quant	0.054	0.209	2.222	2.068	0.203	0.359
Quali						

Test date:2012-02-15 Test time:23:19:27