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CRYPTOSPORIDIOSIS IN HIV/AIDS PATIENTS AND CHILDREN WITH DIARRHOEA IN ACCRA

By

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This thesis is submitted to the University of Ghana, Legon in partial fulfillment of the requirement for the award of Master of Philosophy degree in Microbiology.



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June, 2003.

DEDICATION

*I will want to dedicate this work to all who are concerned with the
plight of*

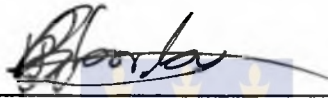
*HIV/AIDS patients, their families and those interested in the well
being of*

our children.



DECLARATION

With the exception of duly acknowledged references, I declare that this thesis had being a product of my toil and suffering and had not been presented for another degree.



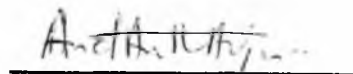
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TABLE OF CONTENT

Dedication	I
Declaration	II
Acknowledgements	III
List of Tables	X
List of Plates	XI
List of Maps	XII
List of figures	XIII
Abbreviation.....	XIV
Abstract	XVI
 CHAPTER ONE	
GENERAL INTRODUCTION	
1.1. INTRODUCTION.....	1
1.2. JUSTIFICATION AND SIGNIFICANCE	2
1.3. HYPOTHESIS	5
1.4. OBJECTIVES	5
1.5. ANALYSIS	6
 CHAPTER TWO	
LITERATURE REVIEW	
2.1 DIARRHOEAL DISEASE	7
2.2 CRYPTOSPORIDIOSIS AND ITS ETIOLOGY	7
2.2.1 The Parasite	7
2.2.2 Life Cycle	9

2.3	PATHOGENESIS, BIOLOGY, BIOCHEMISTRY, AND PATHOPHYSIOLOGY	11
2.3.1	Pathogenesis	11
2.3.2	Biology And Biochemistry	12
2.3.3	Pathophysiology	13
2.4	EPIDEMIOLOGY AND TRANSMISSION	14
2.4.1	Epidemiology Of Cryptosporidiosis	14
2.4.2	Immune Response To Cryptosporidiosis	15
2.4.3	Cryptosporidiosis In HIV/AIDS Patients	16
2.4.4	Cryptosporidiosis In Children	21
2.4.5	Water Outbreaks In The General Population	26
2.4.6	Transmission	27
2.5	DIAGNOSIS AND ASSESSMENT OF DIAGNOSTIC METHODS USED FOR CRYPTOSPORIDIOSIS	30
2.5.1	Diagnosis	30
2.5.2	Clinical Manifestations	34
2.6	CONTROL, PREVENTION AND TREATMENT	35
2.6.1	Treatment	35
2.6.2	Prevention	38
CHAPTER THREE		
MATERIALS AND METHODS		
3.1	STUDY DESIGN AND AREA	41
3.2	TARGET POPULATION AND SAMPLE SIZE	

CALCULATIONS	41
3.2.1 Criteria For Excluding a sample.....	43
3.3 QUESTIONNAIRE	43
3.4 SAMPLE COLLECTION (STOOL/ BLOOD)	44
3.5 METHODS OF EXAMINATION.....	44
3.5.1 Microscopy.....	45
(A) Wet Preparations.....	45
(B) Concentration Method.....	45
(C) Permanent Staining Techniques.....	45
3.5.2 Antigen-Antibody Techniques.....	46
(A) Elisa (Serological Techniques)	46
3.5.2.1 The procedure.....	47
CHAPTER FOUR	
RESULTS AND ANALYSIS	
4.1 AGE DISTRIBUTION OF PATIENTS.....	49
4.2 SEX DISTRIBUTION OF SUBJECTS.....	51
4.3 SYMPTOMS PRESENTED BY SUBJECTS WITH DIARRHOEA...	51
4.4 FREQUENCY OF PASSAGE OF STOOL AND <i>C.PARVUM</i> INFECTION.....	52
4.5 AGE RELATED <i>C.PARVUM</i> INFECTION IN CHILDREN.....	54
4.6 DURATION OF DIARRHOEA IN SUBJECTS AND <i>C.PARVUM</i> POSITIVITY.....	56
4.7 LABORATORY RESULTS (MACROSCOPY)	56
4.8 PATHOGENS ISOLATED FROM STOOL.....	57

4.9	RESULTS BASED ON ZN STAIN AND ELISA.....	59
4.10	<i>CRYPTOSPORIDIUM</i> INFECTION BY DISTRICTS IN ACCRA.....	62
4.11	SOURCE OF DRINKING WATER.....	63
4.12	PRESENCE OF DOMESTIC ANIMALS.....	63
4.13	BREAST FEEDING AND <i>CRYPTOSPORIDIUM</i> INFECTION.....	65
CHAPTER FIVE		
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS		
5.1	DISCUSSION.....	66
5.1.1	INTRODUCTION.....	66
5.1.2	HIV/AIDS AND NON-HIV/AIDS PATIENTS WITH DIARRHOEA.....	66
5.1.3	CHILDREN WITH DIARRHOEA AND WITHOUT DIARRHOEA.....	67
5.1.4	HIV/AIDS VERSUS CHILDREN.....	67
5.1.5	<i>CRYPTOSPORIDIUM</i> AND AGE AMONG HIV/AIDS PATIENTS.....	68
5.1.6	AGE AND <i>CRYPTO</i> .IN CHILDREN.....	68
5.1.7	SYMPTOMS PRESENTED AND <i>C.PARVUM</i>	70
5.1.8	FREQUENCY (NUMBER OF STOOLS PER DAY) AND <i>CRYPTO</i> . POSITIVITY.....	71
5.1.9	DURATION OF DIRRHOE.....	71
5.1.10	STOOLCONSISTENCY AND <i>CRYPTO</i> . POSITIVITY.....	72
5.1.11	PRESENCE OF DOMESTIC ANIMALS.....	73
5.1.12	OTHER PATHOGEN DETECTED.....	73
5.1.13	GEOGRAPHICAL AREA IN GREATER ACCRA	

REGION AND DISTRIBUTION.....	75
5.1.14 BREAST-FEEDING.....	76
5.1.15 SOURCE OF DRINKING WATER AND <i>C. PARVUM</i>	77
5.2 CONCLUSION.....	79
5.3 RECOMMENDATIONS.....	80
References	81
Appendices.....	99

LIST OF TABLES

Table 4.1	Age Distribution of Patients (HIV/AIDS)	49
Table 4.2	Age range of children with diarrhoea.....	50
Table 4.3	Sex distribution of subjects.....	51
Table 4.4	Symptoms presented by subjects with diarrhoea and <i>C. parvum</i> positivity.....	52
Table 4.5	Frequency (number of stools per day and <i>C. parvum</i> positivity.....	53
Table 4.6	<i>C. parvum</i> positivity and age.....	55
Table 4.7	Duration of diarrhoea in subjects and <i>C. parvum</i> positivity.....	56
Table 4.8	Stool consistency of subjects and controls.....	57
Table 4.9	Prevalence of <i>C. parvum</i> infection.....	58
Table 4.10	Results based on ZN Stain and ELISA.....	62
Table 4.11	<i>C. parvum</i> infection by districts.....	62
Table 4.12	Source of drinking water.....	63
Table 4.13	Presence of domestic animals in the home.....	64
Table 4.14	Breast-feeding and <i>C. parvum</i> infection.....	65

LIST OF PLATES

Plate 1 Green vegetable could be contaminated.....	29
Plate 2 Irrigated with a contaminated water from such a gutter.....	29
Plate 3 Stained picture of a positive sample observed under light microscope (Measuring bar is 10uM)	60
Plate 4 Set up of the Eliza Technique.....	61

LIST OF MAPS

Map 1A	Map of Ghana.....	42
Map 1B	The 5 Districts of the Greater Accra Region.....	42

LIST OF FIGURES

Figure 1	<i>C. parvum</i> Life cycle.....	11
Figure 4.1	Frequency of passage of stools and <i>C. parvum</i> positivity.....	54
Figure 4.2	Age related prevalence of <i>C. parvum</i> infection in children with diarrhoea.....	55
Figure 4.3	Pathogens isolated from stools.....	59

LIST OF ABBREVIATIONS

Crypto	-	<i>Cryptosporidium</i> species oocyst
CAMP	-	Cyclic Adenosine Monophosphate
CGMP	-	Cyclic Guanidine Monophosphate
CD	-	Cluster of differentiation
DNA		Deoxyribonucleic acid
RNA	-	Ribonucleic acid
rRNA		Ribosomal Ribonucleic acid
LDH	-	Lactose dehydrogenase
TNF-α	-	Tumour necrosis factor - alpha
IFN-γ	-	Interferon -gamma
IL	-	Interleukin
PMNs	-	Polymorphonuclear neutrophils
PCR	-	Polymerase Chain Reaction
MAB	-	Monoclonal Antibody
ELISA	-	Enzyme -Linked Immunosorbent Assay
HIV /AIDS	-	Human Immunodeficiency Virus /Acquired Immune Deficiency Syndrome
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
<i>et al</i>		and others
ml	-	Millilitre
etc	-	et cetera

%	-	Percentage
NAD	-	No Abnormality Detected
\leq	-	Less than or equal to
$<$		Less than
$>$		Greater than
\geq		Greater than or equal to
Yr		Year
K ^u Bu	-	Korle-Bu Teaching Hospital
UGMS		University of Ghana Medical School
M		Males
F		Females
HAART	-	Highly Active Antiretroviral Therapy

ABSTRACT

Although *cryptosporidium spp.* infections in acquired immunodeficiency syndrome patients (AIDS) and children with diarrhoea have been reported in several African countries, there is scanty information regarding *cryptosporidial* diarrhoea in Ghana. This study investigated occurrence of *C. parvum* in HIV/AIDS patients and children up to five years with diarrhoea in the Greater Accra Region. It was a cross sectional study, with Fevers Unit, Child Health Department in the Korle-Bu Teaching Hospital and Korle-Bu Polyclinic as the settings. A total of 278 samples were taken. Sixty-two (62) of these were clinically diagnosed HIV/AIDS patients with diarrhoea controlled by 96 HIV-seronegative patients also with diarrhoea. Among the children up to 5years, 63 had diarrhoea while 57 were without diarrhoea. *Cryptosporidium* oocysts were stained using the Kiyong's modified Ziehl Neelsen staining technique, examined microscopically and confirmed by the ELISA Technique. Analysis of stool specimen from clinically diagnosed HIV/AIDS (n=62) and HIV-seronegative patients (n=96) patients revealed *C. parvum* in 16 (25.8%) of HIV/AIDS and 0 (0%) of the HIV-seronegative patients, respectively. For the children under five, analysis of the 63 patients with diarrhoea and 57 without diarrhoea showed a prevalence of 15.9% and 3.5% respectively. There was no concomitant association between *C. parvum* and any other parasite found. In conclusion, this study demonstrates a higher prevalence of *Cryptosporidium sp.* in HIV/AIDS and children less than five years with diarrhoea in Accra. The prevalence of *Cryptosporidium* was found to be more prevalent in HIV/AIDS patients (25.8%) more than the control group who were HIV-seronegative but with diarrhoea ($\chi^2=21.69$, p value =0.0000032). With the children under five years it was found that prevalence was more in those children with diarrhoea (15.8%) than those without diarrhoea ($\chi^2=4.20$, p value =0.00405). However when the prevalence found in children with diarrhoea is compared to that in HIV/AIDS with diarrhoea, it was realized that statistically, there was no significant difference in prevalence ($\chi^2=1.23$, p value =0.2677). Among the children the most vulnerable group was those under 2 years.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 INTRODUCTION

Cryptosporidiosis is a diarrhoeal disease distributed worldwide in urban and rural population in both the developed and developing countries (Fayer *et al.*, 1997). Infection in humans and livestock seems to occur primarily with one species, *Cryptosporidium parvum*. In mammals, *C. parvum* mainly infests the brush-border of enterocytes of the small intestine resulting in villous atrophy, mild submucosal inflammation, reduction of brush-border enzymes and characteristic watery diarrhoea. Preventing and treating diarrhoea is a number one priority in reducing morbidity and mortality in children in developing countries (Bern *et al.*, 1992).

In healthy (immunocompetent) individuals, diarrhoeal illness is self-limiting and usually last less than one month. However, in immunocompromised individuals, such as HIV/AIDS patients, diarrhoeal diseases can persist for months [or through the entire life] and become life threatening (Casemore, 1990). Several studies have demonstrated *C. parvum*-related diarrhoea illness to be the most common cause of diarrhoea in AIDS patients, and recently the Centre of Diseases Control, USA, included chronic intestinal cyptosporidiosis as one of the AIDS-defining opportunistic infections (Kuby, 1997).

Cryptosporidial gastroenteritis account for up to 16% of AIDS diarrhoeal cases in the USA and about 50% in developing countries (Bartlett *et al.*, 1992; Lopez, 1988). In

Ghana, the prevalence of HIV/AIDS is on the increase thereby, prompting the need for cryptosporidiosis awareness among physicians, scientist and in the general public.

1.2 JUSTIFICATION AND SIGNIFICANCE

Diarrhoeal disease is a significant health problem in developed countries and a major problem in developing countries. Considerable efforts have been devoted to establishing the magnitude of the problem of diarrhoeal morbidity at a global level and identifying potential interventions for disease control (Bern *et al.*, 1992; Feachem, 1986; Martines *et al.*, 1993). Despite modern diagnostic techniques and treatment, diarrhoeal diseases continue to be the second most common cause of death of children younger than 5 years in less developed countries (World Bank, 1993).

Cryptosporidium spp. are coccidian parasites, which infect a wide variety of host species including humans. One species, *Cryptosporidium parvum*, invades the intestinal epithelial cells of humans and other mammals causing gastrointestinal illness (Fayer *et al.*, 1997). Diarrhoeal disease caused by the coccidian parasite *C. parvum* has become a major health concern since the first reported case of human cryptosporidiosis in 1976 (Nime *et al.*, 1976).

In individuals with a normal immune system the disease, is self limiting, lasting for several days with diarrhoea, vomiting, stomach cramps and fever. Nevertheless, much of the interest taken in cryptosporidiosis is due to its opportunistic behaviour in immunocompromised individuals, particularly AIDS patients who suffer far more serious forms of the disease resulting in prolonged, life-threatening, cholera-like illness. The role of cryptosporidiosis in HIV/AIDS in Ghana is actually unknown.

Epidemiological data from urban and rural settings of developing countries suggest that more than 20% of children suffer bouts of diarrhoea with attendant dehydration, wasting, malabsorption and weight loss due to infection with *Cryptosporidium* (Fayer and Unger, 1986). The morbidity and mortality is high (Boggaerts *et al.*, 1984; Blagburn and Soave, 1997; Current *et al.*, 1983) and unfortunately, no commercial chemotherapeutic agents have proven to be consistently effective against *C. parvum* infection (Blagburn, 1997; Riggs, 1997). The routes of transmission of this parasite are person-to-person, water contamination in community outbreaks with a common source, and food contamination (Mackenzie *et al.*, 1994).

Outbreaks of *Cryptosporidium*-related diarrhoeal illness in Ayreshire, Scotland (Rose *et al.*, 1997), Oxfordshire-Swindon, England, (Rose *et al.*, 1997), Carrollton, Georgia, Jackson, Oregon, and Milwaukee, Wisconsin, U.S.A. have been attributed to consumption of contaminated surface water containing oocysts (Mackenzie *et al.*, 1994; Rose *et al.*, 1997; Leland *et al.*, 1993). The importance of cryptosporidiosis in developed countries such as United States of America, United Kingdom, Japan, and Canada, has therefore been established.

Coming closer to our African continent, a study among Ethiopian AIDS patients (*appendix 1*) reveals the significance and importance of *Cryptosporidium* infection in AIDS patients with diarrhoea. In that study parasite species were identified in AIDS patients with chronic diarrhoea called “cases”, HIV seronegative diarrhoeal patients called “control 1” and HIV seropositive individuals without diarrhoea called “control 11”. From *appendix 1* it could be established, that, among the parasites involved with HIV infection, *Cryptosporidium* was found in about 26% of the subjects while none of

controls had the infection. This is a strong prove that *Cryptosporidium* is associated with HIV/AIDS.

In Ghana *C. parvum* related diarrhoea had not been well documented and the parasite is not tested for, routinely in stool examinations. Meanwhile there is the need for greater understanding of modes of transmission and pathogenesis. Earlier studies on *Cryptosporidium* in Ghana (Adjei *et al.*, 1987) revealed the occurrence of the parasite in some patients with diarrhoea in the Okomfo Anokye Teaching Hospital, Kumasi.

Cryptosporidiosis related diarrhoea is likely to be present in Ghana for two reasons. Firstly, various animals that are potential sources of transmission (dogs, pigs, and cattle) share human habitations. Secondly, the common sources of drinking water include tap water, which may be contaminated because of inadequacy of suitable standard water treatment methods to remove the parasite. There is, therefore, an urgent need to determine the incidence of cryptosporidiosis in Ghana.

Reports from other investigators have suggested that certain enteropathogenic agents, such as *Cyclospora*, *Enterocytozoon*, *Salmonella*, *Shigella*, *E. coli*, *Entamoeba histolytica*, *Giardia lamblia* cysts, *Ascaris lumbricoides* and *Campylobacter* are associated with *Cryptosporidium* infection. The role(s) if any the agents mentioned above play in promoting children to clinical illness with *Cryptosporidium* is not well understood. The study will therefore, assess existing correlation between *Cryptosporidium* and any of the more commonly encountered enteropathogens in Ghanaian patients, especially those in Accra.

1.3 HYPOTHESIS

Preventing and treating diarrhoea is a number one priority in reducing morbidity and mortality in children in developing countries. This is true especially in diarrhoeal diseases caused by *Cryptosporidium* where no commercial chemotherapeutic agents have been proven to be consistently effective. In Ghana, the involvement of *Cryptosporidium* in diarrhoeal diseases in children and HIV/AIDS patients is not well established. Accordingly, the study would like to test the hypothesis that *Cryptosporidium* is an important cause of diarrhoea in children and HIV/AIDS patients in Accra.

1.4 OBJECTIVES

1.4.1 *General objective:*

The main objective of the study was to find out the importance of *Cryptosporidium* as a causative agent of diarrhoea in children, and HIV/AIDS patients.

Specific objectives:

The specific objectives of the research were:

1. To investigate the involvement of *Cryptosporidium parvum* in diarrhoeal diseases in children up to 5 years and HIV/AIDS patients.
2. To determine the prevalence of *Cryptosporidium parvum* in children up to 5 years and HIV/AIDS patients.

1.5 ANALYSIS

Data and laboratory results were entered into a database and analysed statistically using Epi Info to a) identify prevalence of *Cryptosporidium* in the HIV/AIDS patients, children up to 5 years and their controls, b) investigate any relationship between *Cryptosporidium parvum* infection and HIV/AIDS in patients in Accra, c) identify any link between domestic animals, source of water and *Cryptosporidium parvum* infection, and d) find out whether breast-feeding habits has any correlation with *Cryptosporidium parvum* infection.

The data were analysed as follows; data from laboratory were descriptively analysed. Data from the questionnaire were analysed with the use of frequencies, percentages, proportions and deviations. For each response variable, tests of significance (p-values) were used to identify significant association with explanatory variables. Only factors considered to potentially explain variations in each response were included as explanatory variation.

The explanatory variable included; a) immune status of patient (HIV/AIDS), b) the age in children, c) source of water, d) presence of domestic animals and e) breastfeeding habits.

CHAPTER TWO

LITERATURE REVIEW

2.1 DIARRHOEAL DISEASES

Diarrhoeal diseases are a major cause of morbidity in the tropical areas where it underscores the global impact of tropical diseases. It is a common cause of infant mortality and morbidity and is linked to poverty and poor socio-economic status of people as well as poor sanitation. Diarrhoea could be caused by different pathogens including *Escherichia coli*, *Vibrio cholera*, *Shigella*, *Salmonella* and *Campylobacter jejuni*, *Balantidium coli*, *Giardia lamblia* and *Cryptosporidium parvum* among others. *Cryptosporidium parvum* can cause acute inflammatory self-limiting diarrhoea.

2.2 CRYPTOSPORIDIOSIS AND ITS ETIOLOGY

2.2.1 The parasite

Cryptosporidium parvum is a parasite of the gastrointestinal tract that belongs to the Genus *Cryptosporidium*, Family *Cryptosporiidae*, Order *Eucoccidiida*, Class *Sporozoa*, and Phylum *Apicomplexa*. It is found worldwide in urban/rural populations' in developed/developing countries. *Cryptosporidium parvum* causes malabsorption and diarrhoea and is found in the intestinal tract of man and several species of animal especially cattle (Current, 1988). There are two life forms of the organism; the trophozoite and cyst forms. The trophozoite form is the vegetative form while cyst or oocyst is the main form involved in transmission of the parasite.

Cryptosporidium parvum is an important cause of diarrhoea worldwide. *C. parvum* causes a potentially life-threatening disease in people with AIDS and contributes significantly to morbidity among children in developing countries. In immunocompetent adults, *Cryptosporidium* could be associated with waterborne outbreaks of acute diarrhoeal illness. Recent studies with human volunteers have indicated that *Cryptosporidium* is highly infectious. Although the mechanism by which *Cryptosporidium* causes diarrhoea is still poorly understood, the parasite and the immune response to it probably combine to impair absorption and enhance secretion within the intestinal tract. Important genetic studies suggest that humans can be infected by at least two genetically distinct types of *Cryptosporidium*, which may vary in virulence. This may, in part, explain the clinical variability seen in patients with cryptosporidiosis.

Although *Cryptosporidium* was initially described in mice in 1907, it was not until 1976 that it was first reported in association with diarrhoeal disease in humans, one case in an otherwise healthy child and one in an immunosuppressed adult (Flanigan *et al.*, 1996; Marshall *et al.*, 1997; Morgan *et al.*, 1998). In otherwise healthy individuals, *Cryptosporidium* spp. typically cause watery or mucoid diarrhoea with abdominal pain that can last several days and occasionally several weeks. Spontaneous recovery is the rule, and there is no effective specific therapeutic agent.

The infection is spread in a number of ways: from person to person, from animals, via food, and by water. Cryptosporidiosis is now a common waterborne disease in the United Kingdom and has been associated with drinking water and swimming pool contact (Griffiths *et al.*, 1998). Although not quite the commonest cause of waterborne diarrhoeal outbreaks in the United States, cryptosporidiosis is responsible for one of the largest

waterborne outbreaks ever described (LeChevallier *et al.*, 1995). *Cryptosporidium* causes far more serious disease in immunosuppressed individuals.

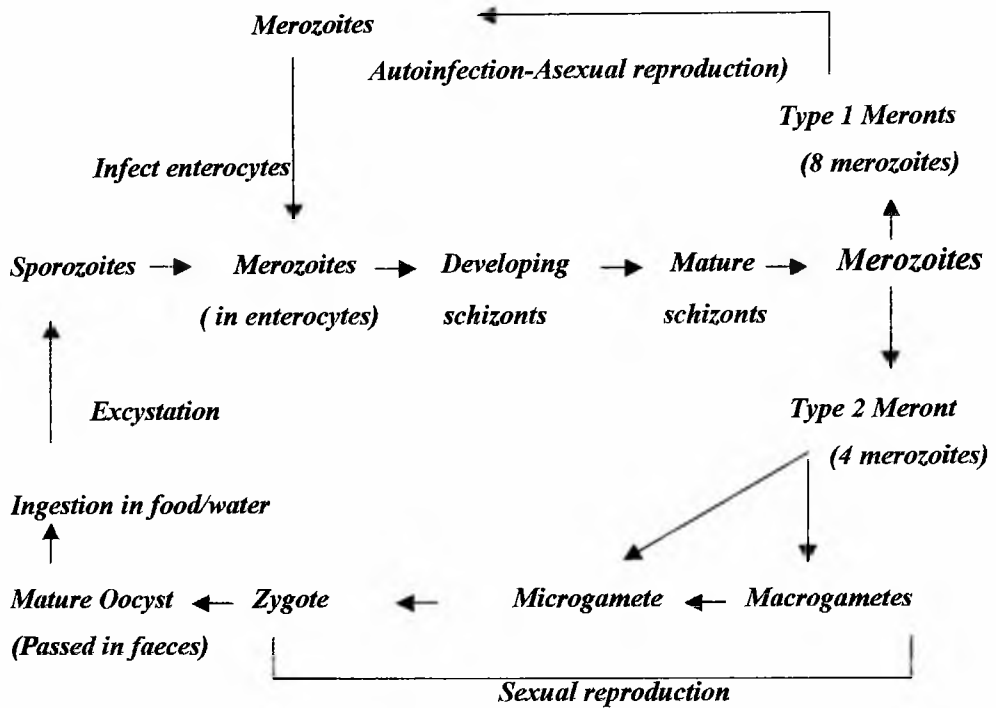
2.2.2 Life cycle

Cryptosporidium is an obligate intracellular parasite with a monoxenous life cycle, that is, the parasite is capable of completing its life cycle within a single host (Flanigan, 1992). *Cryptosporidium* exists in the environment as a hearty, 5- μ m-diameter oocyst, which contains four sporozoites. Humans and animals are infected by ingesting these oocysts through faecal-oral transmission. The oocysts travel through the gut lumen to the small intestine, where they rupture, releasing the four sporozoites. The sporozoites are motile, 5- by 1- μ m forms which adhere to and invade the absorptive epithelial cells that line the gastrointestinal tract and develops into schizonts at the luminal surface of the epithelium. The sporozoite, upon attachment develops into a trophozoite, and then a schizont that contains eight distinct merozoites. Merozoites from schizonts in turn re-initiate schizogony or enter into the sexual phase of the life cycle and develop into gametocytes.

This invasion process is likely to involve molecules discharged from parasite organelles (rhoptries, micronemes, and dense granules) found in the apical end of the sporozoite. Soon after attachment and discharge of these organellar contents, *Cryptosporidium* focally disrupts the microvilli, which cover the host cell and slides into the host cell, enveloping itself in the host cell membrane in the process. The parasite quickly establishes an intracellular niche, which is unique to *Cryptosporidium*, in which the parasite and the surrounding parasitophorous vacuole bulge into the gut lumen and are

separated from the host cell cytoplasm by a fascinating electron-dense structure. Here, the parasite replicates into eight merozoites, which then rupture out of the host cell, infect other host cells, and complete the asexual stage of the life cycle. At some point, merozoites differentiate into gamonts, which undergo sexual reproduction within the same host to ultimately regenerate oocysts, which are excreted in the faeces (Current *et al.*, 1985; Fayer *et al.*, 1986; Tzipori, 1998).

Fertilization of macrogametocytes by microgametocytes results in development of oocysts, which are released from the epithelial cells into the lumen of the gut, excreted via the faeces into the environment where they are infectious to the host. Asexual multiplication occurs by budding of first-stage meronts and progression to second-stage meronts of the development cycle. Multiple recycling of the organism in the asexual stage, production of auto infestive oocyst result in very heavy infection and massive shedding of oocysts in the faeces of the infected individual [Figure 1]. The developmental cycle of *C. parvum* is completed in three days [range, 1-8 days]

FIGURE 1 THE LIFE CYCLE OF *CRYPTOSPORIDIUM PARVUM*

2.3 PATHOGENESIS, BIOLOGY, BIOCHEMISTRY, AND PATHOPHYSIOLOGY

2.3.1 Pathogenesis

Cryptosporidium is an increasingly recognized agent of intestinal infection in both immunocompetent and immunocompromised humans, and in many other animals. The small intestine appears to be the most common site of infection. Histologic changes in

the intestine include blunted villi, crypt elongation, lamina propria cellular infiltration, mild submucosal inflammation and reduction of brush-border enzymes (Tziporis 1988; Lefkowitz, 1984). Enteropathy, characterized by D-xylose and B12 malabsorption, protein loss coupled with increased faecal clearance of alpha-1 antitrypsin has been reported in AIDS patients infected with *Cryptosporidium* (Modigliani *et al.*, 1985).

2.3.2 Biology and biochemistry

Like other protozoan parasites, *Cryptosporidium* appears incapable of de novo purine synthesis and relies on salvage pathways for hypoxanthine, guanine, and adenine. Studies with radiolabeled purine precursors (formate and glycine) indicate that these compounds are incorporated into host cells but not intracellular *C. parvum*. Enzymatic activity necessary for purine salvage (hypoxanthine, guanine, and xanthine phosphoribosyltransferase) was identified in *C. parvum* sporozoites and may localize to a single enzyme. Such an enzyme may serve as an antiparasitic drug target (Doyle *et al.*, 1998). Keithly *et al.*, (1997) have identified a polyamine biosynthesis pathway in *C. parvum*, which is found chiefly in plants and some bacteria but not mammalian cells.

The lead enzyme of this pathway, arginine decarboxylase, is sensitive to a specific, irreversible arginine decarboxylase inhibitor, which reduces the intracellular growth of *C. parvum*. Another potential drug target is the shikimate pathway, in which (in plants) chorismate is converted to *p*-aminobenzoic acid, folate, and other aromatic compounds (Roberts *et al.*, 1998). *Cryptosporidium* and other Apicomplexan parasites were found to be sensitive to glyphosate, an inhibitor of the shikimate pathway. This inhibition also provides circumstantial evidence for the existence of a plastid-like

organelle in *Cryptosporidium*, similar to that described for *Plasmodium* and *Toxoplasma* (Kohler *et al.*, 1997; McFadden *et al.*, 1996).

2.3.3 Pathophysiology

In general, diarrhoea develops when intestinal absorption is impaired or secretion is enhanced. Both of these processes are regulated by the intestinal epithelial cells, which are infected by *Cryptosporidium* (Clark *et al.*, 1996). Several investigators have identified impaired glucose-stimulated Na⁺ and H₂O absorption and/or increased Cl⁻ secretion in experimental models of cryptosporidiosis (Argenzio *et al.*, 1990, 1993; Moore *et al.*, 1995). In addition to these transport defects, abnormalities in the barrier properties of the intestinal epithelium, mediated in part by intercellular junctional complexes, contribute to *Cryptosporidium* diarrhoea. Two groups have found evidence of permeability defects and decreased resistance across *C. parvum*-infected intestinal cell lines (Adams *et al.*, 1994; Griffiths *et al.*, 1994). In addition, both groups found that *C. parvum* infection of these monolayers resulted in the release of cytoplasmic lactate dehydrogenase, consistent with cellular injury, which ultimately resulted in cell death.

Another group has suggested that *Cryptosporidium* induces apoptosis in biliary epithelial cells, but this mechanism of cell death has not been confirmed in vivo (Chen *et al.*, 1998). Malabsorption and abnormal intestinal permeability (decreased vitamin B₁₂ absorption, decreased D-xylose absorption, and abnormal lactulose/mannitol permeability test) have been confirmed in people with AIDS and cryptosporidiosis (Goodgame *et al.*, 1995; Stockman *et al.*, 1998). One mechanism for the induction of intestinal secretion by

Cryptosporidium may involve the stimulation of prostaglandin production by intestinal epithelial cells (Laurent *et al.*, 1998).

2.4 EPIDEMIOLOGY AND TRANSMISSION

2.4.1 Epidemiology of cryptosporidiosis

Cryptosporidium is found worldwide and infection has been reported in over 50 countries spanning 5 continents. The true prevalence of cryptosporidial infection is not known but surveys of selected populations have revealed infection rates ranging from 0.65% to 20% in developed countries and 4% to 40% in developing countries (Fayer *et al.*, 1997; Tziporis, 1988). Prevalence studies indicate that infection rates are higher in children less than 2 years of age and in periods when the weather is warm and humid (Zu, *et al.*, 1998; Tziporis, 1988).

In the U.S.A., *C. parvum* was detected in about 16% of patients with AIDS and diarrhoea; in Haiti and Africa, up to about 50% of the patients with AIDS had cryptosporidiosis (Bartlett *et al.* 1992; Lopez 1988). There is information that suggests that active or recent infection may be present in the general population. (Ungar, 1989; Casemore *et al.*, 1987). In immunocompetent individuals, diarrhoea is self-limiting whiles in immunocompromised individuals (HIV/AIDS) diarrhoea persist for months.

In a review on the epidemiology and clinical features of *cryptosporidium* infection in immunocompromised patients it was established that the most severe form of cryptosporidiosis is seen in individuals with defects in the T-cell response. The most commonly studied group is that of patients with AIDS. These patients suffer from more severe and prolonged gastrointestinal disease that can be fatal; in addition, body systems

other than the gastrointestinal tract may be affected. The widespread use of antiretroviral therapy does appear to be having a beneficial effect on recovery from cryptosporidiosis and on the frequency of infection in human immunodeficiency virus-positive patients.

Even though it is mostly not easy to define the phrase "immune-compromised persons", a guidelines from United Kingdom explained that it included patients whose T-cell function was compromised or who are immunosuppressed because of human immunodeficiency virus (HIV) infection, deficient CD40 ligand (known as hyper-immunoglobulin M syndrome), and children with severe immunodeficiency syndromes (Balatbat *et al.* 1996). This also includes the primary immunodeficiencies, which are uncommon or rare inherited defects in one or more aspects of the body's immune mechanisms. Cryptosporidiosis could also be expected in patients with malignant disease or following a solid-organ, bone marrow or renal transplants (Balatbat *et al.* 1996).

Immune response to cryptosporidiosis

The host immune response to *Cryptosporidium parvum* infection is still poorly understood (Macfarlane *et al.*, 1987; Pozio *et al.*, 1992). Many laboratory animals exhibit innate resistance to infection, and much experimental work has tried to determine the specific immune factors responsible for this resistance. The response that normally clears an established infection in a susceptible host probably involves similar mechanisms, but specific immune response mechanisms are probably also important. There is a general consensus that the mechanisms responsible for clearing *cryptosporidium* from the

gastrointestinal tract involve a role for gamma interferon, although the mechanism by which this cytokine imparts resistance is unclear.

It is also clear that CD4 T lymphocytes are necessary for the resolution of both acute and chronic cryptosporidiosis. 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, Interleukin-12 may play a role, possibly through its ability to induce gamma interferon production (Rossi *et al.*, 1998).

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 (Piper *et al.*, 1998). The role of CD8 T cells is unclear, but they appear to play a role in controlling infection in mice. Mast cells may play a role in innate resistance in mice. 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 (Pozio *et al.*, 1992).

Cryptosporidiosis in HIV/AIDS patients

Unlike immunocompetent adults, in whom cryptosporidiosis is usually self-limited, people with AIDS are susceptible to a devastating form of cryptosporidiosis manifested by chronic, voluminous diarrhoea (Gellin *et al.*, 1992; Petersen, 1992; Ramratnam *et al.*, 1997). The factors, which predispose these people to chronic

cryptosporidiosis rather than self-limited illness, appear to be immunologic (Pozio *et al.*, 1992). While a human immunodeficiency virus (HIV)-positive individual may acquire cryptosporidiosis at any point in the viral infection, the most severe, chronic form is limited to people with markedly impaired immune systems. Flanigan *et al.* (1992) have found that patients with CD4 cell counts of greater than 180 cells/mm³ cleared the infection spontaneously whereas 87% of patients with CD4 cell count less than 180 cells/mm³ had persistent disease.

Although AIDS-related cryptosporidiosis often manifests as a severe, persistent diarrhoeal illness, there is actually marked variability in the clinical presentation, including asymptomatic infection (Blanshard *et al.*, 1992; Goodgame *et al.*, 1993; Janoff *et al.*, 1990; McGowan *et al.*, 1993). Manabe *et al.* (1998) have identified four general clinical categories of AIDS-related cryptosporidiosis: a cholera-like illness requiring intravenous rehydration therapy (33%), a chronic diarrhoeal illness (36%), an intermittent diarrhoeal illness (15%), and a transient diarrhoeal illness (15%) (Manabe *et al.* 1998). These clinical manifestations are independent of the general immune status, since all patients in this study were markedly immunosuppressed. In addition to the obvious morbidity associated with AIDS-related cryptosporidiosis, mortality is also increased (Chaisson *et al.*, 1998; Hoxie *et al.*, 1997; Colford *et al.*, 1996).

Although the intestinal tract is the primary site of cryptosporidiosis, other involved organ systems have been described, including the lungs, middle ear, biliary tract, pancreas, and stomach (Rossi *et al.*, 1998; Dunand *et al.*, 1997; French *et al.*, 1995; Godwin, 1991). These sites most probably represent luminal extension of a primary infection of the intestine rather than a primary extraintestinal infection or a disseminated

infection. The biliary tract is the most common, clinically relevant site of extraintestinal infection. Vakil *et al.* (1996) found that HIV-positive patients exposed to a waterborne outbreak of cryptosporidiosis were at increased risk for biliary symptoms and death within 1 year if their CD4 cell counts were <50 cells/mm³.

Since the introduction of highly active antiretroviral therapy (HAART) to the treatment of HIV-infected individuals, the overall morbidity and mortality due to opportunistic infections has dramatically declined (Palella, 1998). Several studies have specifically documented a decreased prevalence of cryptosporidiosis subsequent to introduction of HAART (Kim *et al.*, 1998; Lemoing *et al.*, 1998; Miller, 1998). Also, it appears that reconstitution of immune system function with HAART may lead to the resolution of existing cryptosporidiosis in some patients.

A number of studies have investigated the prevalence and epidemiology of cryptosporidiosis in patients with HIV infection. The results of the studies investigating the prevalence of cryptosporidiosis in HIV-positive patients with diarrhoea have presented estimates that differ quite markedly from one another, ranging from 0 to 100% with a median of 32% (Agnew *et al.*, 1998; Carr *et al.*, 1998; Roberts *et al.*, 1998; Okhuysen *et al.*, 1997; Davis *et al.*, 1996; Flanigan *et al.*, 1996; Goodgame *et al.*, 1995; Fichtenbaum *et al.*, 1993).

It is not clear how much these differences may be explained by differences in study design, geographical location, population group, sensitivity of laboratory methods, or stage of disease. Nevertheless, it would appear that the prevalence of carriage *Cryptosporidium* spp. oocysts in the absence of diarrhoea is very low (Argenzio *et al.* 1990). There have been rather fewer studies that have attempted to define an overall

prevalence in HIV-positive individuals, irrespective of whether they have diarrhoea. In Europe, cryptosporidiosis seems to affect about 6.6% of HIV-positive individuals (Morgan *et al.*, 1998). An earlier U.S. study in Los Angeles found an overall infection rate of 3.8% (Peng *et al.*, 1997).

The risk of infection increases in more profoundly immunosuppressed persons, as measured by the CD4⁺ T-lymphocyte counts (Molbak *et al.*, 1997; Pallela *et al.*, 1998). Various social and behavioural factors also increase the risk of infection. For example, in a large multicenter European study the risk of cryptosporidiosis was significantly lower for intravenous drug users than for homosexual men (relative risk, 0.34; 95% confidence interval, 0.22 to 0.54) and for women than for men (relative risk, 0.43; 95% confidence interval, 0.21 to 0.87), suggesting that sexual behaviour may be an important risk factor (Morgan *et al.*, 1998).

The importance of sexual behaviour as a risk factor was also identified in a large U.S. study, where the prevalence of cryptosporidiosis was higher in persons whose suspected HIV exposure category was through sexual contact (3.9%) than among persons in other HIV exposure categories (2.6%, $P < 0.01$) and in immigrants from Mexico (5.2%) than in American-born patients (3.8%; $P < 0.01$). Blacks (2.7%) were less likely than whites (4.1%) and Latinos (4.2%) to be reported with cryptosporidiosis ($P < 0.001$) (Peng *et al.*, 1997). In another study, HIV-positive patients who owned dogs (but not other pets) also seem to be at increased risk of infection (odds ratio, 2.19; 95% confidence interval, 0.9 to 5.3; $P = 0.05$) (Godwin, 1991).

A group of scientists undertook a cross-sectional serological survey of HIV-positive individuals to look for anti-cryptosporidial antibodies (Centers for Disease

Prevention, 1998). They reported that an increased serological response to one or more antigens was related to a number of sexual practices such as having had sex within the past 2 years, having multiple partners during that time, having anal sex, and attending a spa or sauna.

Nosocomial outbreaks of cryptosporidiosis have also been described. For example, an outbreak in a hospital in Copenhagen affected some 18 HIV-positive patients (Neill *et al.*, 1996). The source of the outbreak was identified as ice from an ice machine in the ward, contaminated by an incontinent psychotic patient with cryptosporidiosis who was using his hands to pick out ice for cold drinks.

More recently, another group also conducted a retrospective cohort study of transmission between roommates in a hospital setting (Carraway *et al.*, 1996). They were able to identify 37 HIV-positive patients who had shared a hospital room with 21 *Cryptosporidium*-positive index patients and matched them to other HIV-positive individuals with a similar CD4 count but who had not shared a room with a *Cryptosporidium*-positive patient. None of the 37 exposed individuals became infected, and one of the unexposed controls became infected, suggesting that person-to-person transmission may not be common. However, extrapolation of this experience to other health care settings with different infection control practices may be difficult.

Molecular epidemiology of *Cryptosporidium* isolates has proved useful in determining sources of infection (MacPherson and MacQueen, 1993). There have been too few studies on the prevalence of different genotypes of *Cryptosporidium* in HIV-infected patients to date to draw firm conclusions. One American study characterized 13 strains from people with AIDS and found that 10 were *C. parvum* type 1 (the human type)

and 3 were *C. parvum* type 2 (the calf type) (Spano *et al.*, 1998). A subsequent American study found that 5 of 10 AIDS patients were infected by *C. parvum* genotype 1 and 1 was infected by *C. parvum* genotype 2 and the remaining isolates were other species (Moss *et al.*, 1998). A study of *Cryptosporidium* isolates from HIV-infected patients from Kenya, Switzerland, and the United States found that of 22 isolates examined, 6 were genotype 1, 8 were genotype 2, and 8 were non-*parvum* strains, including *C. meliagridis* (Mead *et al.*, 1990). The genotypes commonly found in humans are *C. parvum* genotypes 1 (the human strain) and 2 (the calf strain), although *C. meliagridis* and an as yet unnamed dog strain have been described (Morgan *et al.*, 1998; Spano *et al.*, 1998). Although *C. parvum* genotype 1 strains seem to predominate in AIDS patients in North America, it is unclear whether this represents increased person-to-person transmission.

2.4.4 Cryptosporidiosis in children

Cryptosporidium is a recognized cause of diarrhoea, particularly among children, in developing countries (Enriquez *et al.*, 1997; Molbak *et al.*, 1997; Neill *et al.*, 1996; Lima, 1992; Macfarlane and Horner-Bryce, 1987). Several studies have suggested that cryptosporidiosis is most common in children younger than 1 year and is associated with malnutrition. Because these studies were largely incidence studies, it was not clear if malnutrition predisposed children to cryptosporidiosis, if cryptosporidiosis led to malnutrition, or both. Checkley *et al.* (1997) observed a cohort of Peruvian children, aged 0 to 3 months on recruitment, for 2 years to address this question and found that the incidence of cryptosporidiosis in this cohort was high (45%); however, neither wasting nor low body weight was a significant risk factor for cryptosporidiosis.

Children with symptomatic cryptosporidiosis grew less during the first month of infection than did children without diarrhoea who were not infected. Interestingly, this study identified a large percentage of asymptomatic infections (63%). The effect of asymptomatic cryptosporidiosis was less severe, but these children also gained less weight than the controls did. Consequently, this study suggests that cryptosporidiosis leads to malnutrition in previously normal children. The factors, which determine whether a primary infection will be symptomatic or asymptomatic, are undefined.

In a study of Brazilian children, Agnew *et al.* (1998) identified a possible mechanism for malnutrition subsequent to cryptosporidiosis. In a case-control study of children monitored from birth, children younger than 1 year were found to experience excessive and protracted (nearly 2 years) episodes of diarrhoeal illness, which was not due to the initial episode or recurrent episodes of cryptosporidiosis. Only 14% of these children were initially coinfecting with a second pathogen (including *Salmonella* species, *Shigella flexneri*, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Giardia lamblia*). The mechanism of this subsequent diarrhoeal disease is unclear but may involve persistent malabsorption due to *Cryptosporidium*-induced intestinal injury or enhanced susceptibility to other enteric pathogens. Alternatively, immunologic abnormalities may be present in these children, analogous to the increased delayed mortality associated with high-titer measles vaccination in low socio-economic populations.

Cryptosporidiosis is more common and more severe in malnourished children. One early study from the West Indies reported on the investigation of 513 stool samples, of which 77 were from malnourished children (LeBlancq *et al.*, 1997). *Cryptosporidium* was detected in 25 (4.9%) of 513 samples and in 15 (19.5%) of the 77 samples from

malnourished children. All the malnourished children with cryptosporidiosis were admitted to hospital, where they presented with dehydration (87%), vomiting (93%), fever (100%), and diarrhoea (100%), which lasted an average of 15.3 days. Two of these children died. Of the 10 cases of cryptosporidiosis in well-nourished children, only 20% of the children presented with dehydration, 40% presented with vomiting, and 50% presented with fever. Diarrhoea was also less prolonged, and only four patients were admitted to hospital; all survived.

Many groups have also reported the higher prevalence of cryptosporidiosis in malnourished children. In a study from Israel, *Cryptosporidium* was detected in 30 (13.5%) of 221 children admitted to hospital with diarrhoea (Nichols *et al.*, 1991). In 77 outpatients with diarrhoea, *Cryptosporidium* was detected only in 4 (5.2%). Outpatients were better nourished than inpatients. Children with *Cryptosporidium*-positive stools were significantly more malnourished than children in whom *Cryptosporidium* was not detected. Moore *et al.*, (1995) reported a study of cryptosporidiosis in malnourished children.

The prevalence in the children at the Nutritional Recovery Center (the most severely malnourished individuals) was 8.5%, and the prevalence at an ambulatory undernourished center (less severely malnourished children) was only 1.9%. A case-control study of Peruvian children admitted to hospital with diarrhoea sought to determine the clinical differences between children with and without *cryptosporidium* (Novak and Sterling, 1991). There were 24 case-control pairs. The only significant difference was that children with cryptosporidiosis were significantly more likely to be malnourished. It was also noted that two severely malnourished children with

cryptosporidiosis died. In a study from India, *Cryptosporidium* was identified in 13 of 100 children with diarrhoea, of whom 6 (46%) were malnourished (Hoxie *et al.*, 1997). The same study found *Cryptosporidium* in 7 of 50 children with chronic diarrhoea, 6 (86%) of whom were malnourished.

In a study in Gabon, the carriage rate in children with malnutrition was 31.8%, almost twice that found in adequately nourished children (16.8%) ($P < 0.01$) (Feregriuno *et al.*, 1996). A study in Mexico also showed an increased risk of cryptosporidiosis in malnourished children, especially when those children were not being breast-fed (Jordan, 1996). In a study of 55 HIV-negative children with acute diarrhoea in Tanzania, 7 were positive for *Cryptosporidium* infection, and all 7 were malnourished (Chaisson *et al.*, 1998). However, these studies that show increased carriage of cryptosporidiosis in malnourished children do not distinguish between cause and effect. Given that cryptosporidiosis causes diarrhoeal disease, the infection may push children into the malnourished state rather than malnourishment being a risk factor for cryptosporidiosis.

This hypothesis is supported by a study of a cohort of 1,064 children from Guinea-Bissau (McGowan *et al.*, 1993). Children who developed cryptosporidiosis were no different in weight or height before the infection than were children who did not. However, boys and girls who developed cryptosporidiosis lost 392 and 294 g, respectively, on average, and this weight loss, relative to their peers, was not caught up during the period of the study. Other studies have also demonstrated the long-term impact on subsequent growth of individuals with symptomatic and asymptomatic cryptosporidiosis (Aguirre *et al.*, 1994; Chen *et al.*, 1993, 1998).

A point prevalence survey was done on 205 institutionalised orphans up to 61 months old in Bangkok, Thailand (Janoff *et al.*, 1990). *Cryptosporidium* was identified in 17 children (8%), *Giardia lamblia* was identified in 42 (20%), and 3 children (1%) harboured both parasites. Diarrhoea was present in 36% of children with *Cryptosporidium* alone, 10% of those with *G. lamblia* alone, and 20% of those with neither parasite. Chronic nutritional status (height/age) was similar in all groups, but acute nutritional status (weight/height) was lower in children with *Cryptosporidium* infection (Z score = -1.39 ± 0.13 [mean \pm standard error of the mean]) than in children with *G. lamblia* infection (Z score = -0.56 ± 0.26) or neither parasite (Z score = -0.78 ± 0.13 ; $P = 0.05$).

The study from Bangkok would suggest that cryptosporidiosis is a major factor in the causation of acute malnutrition rather than malnutrition being a risk factor for cryptosporidiosis. However, the results of all the studies do not fully explain the relationship between malnutrition and cryptosporidiosis may be more complex than it first appears. What is obvious is that *Cryptosporidium* infection has an effect on growth and this is likely to be more severe in children who are already malnourished. Such children also seem to be more at risk of death or prolonged illness.

Other epidemiological studies in children include the one on *Cryptosporidium* infection and diarrhoea in rural and urban areas of Jiangsu, People's Republic of China (Chen *et al.*, 1993). In that study, screening of infants and children under age 15 years for *Cryptosporidium* oocysts was carried out in the suburb of Xuzhou City and six rural areas of Jiangsu Province. The infection rate varied from 0.7 to 50.6%. The incidence was evidently higher in the group of children under age 4 years than it was in children from 4 to 15 years (P less than 0.01). Routine blood examination and immunoassay

performed on blood samples from some of the infected children indicated that more than half of them had anaemia and lower cellular immunity. Diarrhoea was the main symptom of cryptosporidiosis (Chen *et al.*, 1993).

In another study conducted to find out the prevalence of various parasites in elementary school children in northern Jordan, *Cryptosporidium* species was found in 40 specimens out of a total of 1000 specimens (4%). The symptoms reported most often were abdominal pain, cramps, malaise, nausea, and headache. The number of cases of infection was higher in villages, where contact with animals was evident and where contaminated drinking water could have been a major source of the infections.

2.4.5 Water outbreaks in the general population

Outbreaks of cryptosporidiosis due to drinking-water contamination, including a massive outbreak in Milwaukee, Wisconsin (Mackenzie *et al.*, 1995), have been increasingly recognized. Several recent reviews have dealt with this important public health concern (Fricker and Crabb, 1998; Marshall *et al.*, 1997; Smith and Rose, 1998). There are four major factors that contribute to these outbreaks: (i) the prevalence of *Cryptosporidium* in source water is high; (ii) *Cryptosporidium* oocysts are refractory to chlorine treatment of drinking water; (iii) coarse filtration methods normally performed on surface drinking waters do not efficiently remove *Cryptosporidium* oocysts, due to their small diameter; and (iv) the infectious dose of *Cryptosporidium* for humans is extremely low (LeChevallier and Norton, 1995).

Although water treatment deficiencies have been identified in some outbreaks, at least one outbreak has occurred in association with a modern treatment facility where

treatment was well documented and unremarkable (Goldstein *et al.* 1996). In the United States, routine testing of drinking water is mandatory for all surface water utilities serving populations of >100,000 persons; however, the current methods for *Cryptosporidium* detection may underestimate parasite numbers (Fricker and Crabb, 1998). In several situations, outbreaks were caused by contamination of recreational water in swimming pools and sprinklers (Centers for Disease Control and Prevention, 1998).

In addition to drinking water, food contamination has been implicated in several outbreaks of cryptosporidiosis (Centers for Disease Control and prevention 1995, 1998; Millard *et al.*, 1994). Presumably these outbreaks were due to faecal contamination of food by infected animals or food workers. Such incidents may lead to increased regulation of potentially infected food products imported into the United States. In the future, sensitive PCR-based assays may facilitate the detection and genotyping of *Cryptosporidium* in environmental sources.

2.4.6 Transmission

The main route by which *Cryptosporidium* oocysts are transmitted is faecal-oral. Transmission therefore ranges from (1) Water and Food borne (involving raw foods such as fresh oranges, raw milk and vegetables) (2) Zoonotic (Tree, 1997; Dawson, 1995) (3) Person to person via faecal / oral route (non-zoonotic) as in household contacts, day care centers and hospitals (Dawson 1995, Casemore *et al.*, 1987). Generally, transmission had proved to be very successful because the oocysts are environmentally robust, resistant to chlorination, wide host range, large number of oocysts excreted, low infectious dose. Agricultural methods such as the application of animal and human excreta to pasture and

cropland as fertilizer also influence the transmission of *Cryptosporidium* (Casemore *et al.*, 1985).

Contaminated water is reported to be responsible for infection among travellers and in communities, as *Cryptosporidium* oocysts are exceptionally resistant to most disinfectants and antiseptics, including chlorine, at concentrations significantly in excess of those routinely used in water treatment (Casemore *et al.*, 1987; Hayes *et al.*, 1988). Recent outbreaks of *cryptosporidiosis* due to water contamination in Milwaukee, Minnesota and New York have generated serious concerns among public health agencies and water management officials regarding the safety of drinking water reservoirs in both developed and developing countries (Mackenzie, 1994).

PLATE 1

**GREEN VEGETABLE COULD BE CONTAMINATED
WHEN IRRIGATED WITH CONTAMINATED WATER**



PLATE 2

**GUTTER FROM WHICH CONTAMINATED WATER
WAS COLLECTED TO IRRIGATE VEGETABLES**



2.5 DIAGNOSIS AND ASSESSMENT OF DIAGNOSTIC METHODS USED FOR CRYPTOSPORIDIOSIS

2.5.1 Diagnosis

The oocysts can be found throughout the gastrointestinal tract and some times can be found in the lungs (Hojlyng *et al.*, 1988). Microscopic examination of the morphological features of *Cryptosporidium* oocysts is very essential. This is done by the identification and/or detection of the oocysts in faeces or along the intestinal epithelial surface in biopsied tissue. Modified Ziehl-Nelson stain reveals the oocyst as red round unicellular cells about 4-6µm in diameter. There could also be serological detection of antigens in the stool. Immunofluorescence utilizing monoclonal antibodies have recently been developed for detection of oocyst in faeces, and are very useful for research particularly environmental studies that require rapid screening of specimens (Arrowood, 1989).

Polymerase Chain Reaction (PCR) could also be used for species differentiation. Strain variation (whether type I or II) have important medical implications. For example, it could explain the variability in the outcome of cryptosporidial infections in HIV positive patients and other patients with severe immunosuppression (Casemore *et al.*, 1987). Diagnosis of infection with this parasite has relied on identification of acid-fast oocysts in stool; however, it is known that new immunoassays or PCR-based assays may increase the sensitivity of detection.

In a study to evaluate three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in faecal specimens it was realized that, there is an increasing demand for diagnostic testing for *Cryptosporidium parvum*, with a priority

being placed on obtaining diagnostic results in an efficient and timely manner. Several commercial companies have developed rapid diagnostic tests that are simple to perform and can be completed in less time than traditional methods for detecting *Cryptosporidium*. (Johnston *et al.*, 2003) Comparing one of these rapid tests to the ImmunoCard STAT! (Meridian Bioscience, Inc.) Lateral-flow immunoassay, with the MERIFLUOR direct fluorescent-antibody (DFA) test, the ProSpecT microplate assay for *Cryptosporidium*, and modified Kinyoun's acid-fast stained smears for the detection of *Cryptosporidium* using 246 specimens. The MERIFLUOR DFA (Meridian Bioscience, Inc.) test detected the largest number of cases (37 *Cryptosporidium*) infections and was used to calculate the sensitivity and specificity of the other tests.

For detection of *Cryptosporidium*, the sensitivities of the ImmunoCard STAT!, the ProSpecT *Cryptosporidium* microplate assay (Alexon-Trend, Inc.), and modified Kinyoun's acid-fast stained smears were 68, 70, and 78%, respectively. Test specificities were equal to or greater than 99%. Specimens with very small numbers of organisms were not detected by the ImmunoCard STAT!, the ProSpecT microplate assay or modified Kinyoun's acid-fast stained smears.(Johnston *et al.*, 2003).A lot more efforts had been put into evaluation of diagnostic techniques

In a study conducted to detect Specific *Cryptosporidium parvum* Antigens by Enzyme Immunoassay of Serum Immunoglobulin G Antibodies. Human infection with *Cryptosporidium parvum* was found to usually elicit characteristic immunoglobulin G (IgG), IgA, and IgM antibody responses against two sporozoite surface antigens with apparent molecular masses of approximately 27 and 17 kDa. Two new enzyme-linked

immunosorbent assays (ELISAs) for the detection and quantitation of serum IgG antibodies against both antigens have been developed.

In an evaluation of a commercially produced enzyme-linked immunosorbent assay (ELISA; LMD Laboratories, Inc.) the advantages were clearly revealed. The ELISA is a fast, easy-to-read, and accurate method for the detection of *Cryptosporidium* spp. in stool specimens.

Epidemiologic and laboratory data suggest that some methods may fail to detect *Cryptosporidium* oocysts in stool specimens of infected patients. To improve the efficacy of stool concentration procedures, different steps of the Formalin-ethyl acetate (FEA) stool concentration technique were modified and these modifications were evaluated by examining stool samples seeded with known numbers of *Cryptosporidium* oocysts. Because these modifications failed to improve oocyst detection, a new stool concentration technique was developed, that includes FEA sedimentation followed by layering and flotation over hypertonic sodium chloride solution to separate parasites from stool debris was developed. Compared with the standard FEA procedure, this technique improved *Cryptosporidium* oocyst detection (Weber *et al.*, 1992).

Schmidt *et al.* (2001) carried out a study on the virologic and immunologic changes in both peripheral blood and the intestinal mucosa after highly active antiretroviral therapy (HAART) during cryptosporidiosis infection in a patient. They extracted nucleic acid from rectal biopsy specimens and blood samples and measured HIV RNA by reverse transcription PCR. Lymphocytes were isolated from rectal biopsy specimen after mechanical desegregation, and circulating and mucosal CD4 T cells determined by flow cytometry. They found that HAART led to clinical recovery and

eradication of cryptosporidiosis. In both blood and mucosa, HIV RNA decreased below the limit of detection and CD4 T cell increased especially the mucosal CD4 T cells. The findings therefore show a rapid repopulation of the intestinal mucosa with CD4 T cells after initiation of HAART that can effectively restore mucosal immunity, leading to eradication of opportunistic pathogens *C. parvum*

When *Cryptosporidium* isolates from human immunodeficiency virus-infected patients from Kenya, Switzerland, and the United States were examined at three genetic loci namely the 18S ribosomal DNA, HSP-70, and acetyl coenzyme A synthetase genes, four distinct *Cryptosporidium* genotypes were identified: (i) the *Cryptosporidium parvum* "human" genotype, (ii) the *C. parvum* "cattle" genotype, (iii) *Cryptosporidium felis*, and (iv) *Cryptosporidium meleagridis*. This was the first report of *C. meleagridis* in a human host. These results and those of others indicate that immunocompromised individuals are susceptible to a wide range of *Cryptosporidium* species and genotypes. (Una Morgan *et al.*, 2000)

An important advantage of the PCR test, is the ability to directly differentiate between different *Cryptosporidium* genotypes, and could be explored in determining the source of cryptosporidial outbreaks. Sensitivity, specificity, ability to genotype, ease of use, and adaptability to batch testing make PCR a useful tool for future diagnosis and studies on the molecular epidemiology of *Cryptosporidium* infections (Morgan *et al.*, 1998).

2.5.2 Clinical Manifestations

Since cryptosporidiosis is a diarrhoea disease, it exhibits similar symptoms. The most common presentation in both immunocompetent and immunocompromised subjects is the onset of gastroenteritis characterized by profuse watery offensive stool that may contain mucous but rarely blood and leucocytes. Associated symptoms include abdominal pain, vomiting, nausea, headache, low-grade fever (39°C), myalgia, malaise, anorexia and weight loss, even in immunocompetent subjects (Fayar *et al.*, 1997).

In immunocompetent individuals, the diarrhoea is self-limiting and usually last less than one month. However in immunocompromised patients the diarrhoeal disease is much more severe (Fayar *et al.*, 1997). In AIDS patients, the severity of the diarrhoeal is known to be related to CD4-cell count (Cegielski *et al.*, 1999). The stool frequency can be up to ten times a day and the volume up to 10 litres, and the body weight can drop by about 10%. The patients develop severe malabsorption and most patients with AIDS never clear the infection (Extambale *et al.*, 1989). About 20 – 30% of chronically infected patients develop raised alkaline phosphatase and γ -glutamyltransferase and γ -symptoms of biliary tract infection (Extambale *et al.*, 1989). The patients then develop severe malabsorption

One particularly problematic complication of gastric involvement is antral narrowing and gastric outlet obstruction (Chappell *et al.*, 1996; Garcia and Shimizu, 1997; Hays *et al.*, 1985; Millard *et al.*, 1994). Such gastric outlet obstruction can lead to nausea and vomiting and eventually may cause a severe reduction in nutrient intake.

Another unusual complication of cryptosporidiosis in AIDS patients is pneumatosis cystoides intestinalis (Current and Garcia, 1991; Nina *et al.*, 1992;

Okhuysen *et al.*, 1997). Pneumatosis cystoides intestinalis is characterized by the presence of thin-walled, gas-containing cysts in the intestinal wall. Sometimes these cysts can rupture, resulting in a pneumoretroperitoneum and pneumomediastinum.

2.6 CONTROL, PREVENTION AND TREATMENT

2.6.1 Treatment

There is no known effective therapy for cryptosporidial opportunistic infection of patients with AIDS. The absence of consistently effective anti-cryptosporidial chemotherapeutic agents, the lack of an effective vaccine, and the absence of an in vitro method for parasite cultivation exacerbate the consequences of the disease (Blagburn, 1997).

Persistent *Cryptosporidium* infection with diarrhoea in mammals and AIDS patients provides indirect evidence that T cells (possibly T helper cells) are essential for recovery of infection and the development of protective antibody (Aguirre, 1994; Adjei *et al.*, 1999). Immunologic intervention may be a reasonable strategy for the control of cryptosporidiosis since the duration and severity of infection are so linked with host immune competence. However, hyperimmune bovine colostrums when given to *Cryptosporidium*-infected humans had been noticed to fail to offer protection.

Of the antimicrobial agents available, spiramycin, paromomycin, azithromycin, neomycin and zidovudine offer some relief of symptoms during infection in the early stages of AIDS (Blagburn, 1997). In the absence of an effective treatment for cryptosporidiosis, supportive therapy is the only alternative intervention available. Both

immunocompromised and healthy individuals with cryptosporidiosis often require oral or parenteral rehydration therapy.

One of the most biologically intriguing, and clinically frustrating, features of cryptosporidiosis is its resistance to antimicrobial drugs. Unlike many of its relatives (*Toxoplasma gondii*, *Eimeria* and *Plasmodium*), there is no curative therapy for cryptosporidiosis, despite in vitro and in vivo testing of hundreds of compounds. One possible explanation for this is that *Cryptosporidium* establishes a compartment within the host cell, which is morphologically different from the setting used by the related parasites. This unique parasitophorous vacuole may somehow shelter the parasite from antimicrobial drugs (Griffiths *et al.*, 1998).

Because the clinical course of cryptosporidiosis depends largely on the immune status of the host, treatment options vary accordingly (Griffiths *et al.*, 1998). In immunocompetent adults and children, no specific therapy is indicated, since the disease is self-limiting; however, as in any diarrhoeal illness, hydration must be carefully monitored. In individuals with persistent diarrhoea, an underlying immunodeficiency (HIV infection, congenital immunodeficiency, etc.) should be considered. In developing countries, children with cryptosporidiosis often have associated (or subsequent) malnutrition, which should be addressed.

In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below $200/\text{mm}^3$, cryptosporidiosis can be life-threatening and must be treated aggressively. Initially, the nutritional, hydration, and electrolyte status of the patient should be assessed and corrected with intravenous hydration, if necessary. Antimotility agents, such as opiates and somatostatin analogues, may also be used. In people with

AIDS, the ideal treatment involves partial restoration of immune function with HAART. Several case reports have demonstrated the resolution of cryptosporidial diarrhea coincident with a rise in CD4 cell count upon combination antiretroviral therapy (Carr *et al.*, 1998; Foudraine *et al.*, 1998). While laboratories should be poised to respond to cases of cryptosporidiosis among individuals failing to respond to HAART, we have seen few such cases; most of the cases of AIDS-related cryptosporidiosis occur in patients who have never received HAART.

If HAART therapy is not possible, several antibiotics that have some efficacy against *Cryptosporidium* (paromomycin, nitazoxanide, azithromycin) have been reported and should be considered. Of these, paromomycin has been the most widely used and has consistently displayed at least partial activity in experimental systems and clinical trials (Fichtenbaum *et al.*, 1993; Flanigan *et al.*, 1996; Tzipori, 1998). A combination of paromomycin and azithromycin has also been proposed for the treatment of cryptosporidiosis (Smith *et al.*, 1998). Other experimental therapies, like bovine hyperimmune colostrum, may also be considered (Crabb, 1998). In patients with severe disease, infection with a copathogen such as cytomegalovirus should also be considered and treated.

Nitazoxanide (NTZ) is the latest drug to be widely tested against human cryptosporidiosis. NTZ is a nitrothiazole benzamide with broad antimicrobial activity. An open-label study of NTZ in 15 Mexican AIDS patients with cryptosporidiosis found parasite clearance in nearly 100% of patients, triggering larger studies in the United States (Feregriuno *et al.*, 1996). Another small, uncontrolled African study of NTZ also suggested that it has some efficacy (Doumbo *et al.*, 1997). Unfortunately, one larger

clinical trial and in vivo animal studies have been less encouraging (Davis *et al.*, 1996; Tzipori, 1998). Controlled clinical trials (ACTG 336) to determine the efficacy of NTZ in treating cryptosporidiosis are under way.

The best approach to prevention of cryptosporidiosis in HIV-infected individuals is the maintenance of immune system function by using HAART, since chronic cryptosporidiosis occurs only in severely immunosuppressed individuals. Also, it has been suggested that antibiotic regimens containing clarithromycin, aimed at preventing mycobacterial infections in severely immunosuppressed individuals, may inadvertently have a protective effect against cryptosporidiosis (Holmberg *et al.*, 1998; Jordan, 1996). Avoidance of tap water has been touted in the AIDS community, but no clinical trial has confirmed the efficacy of this approach. Despite this, numerous commercial filters are available to remove oocysts from drinking water. Bottled water has also been advocated to prevent cryptosporidiosis, but few regulations are in place to guarantee these products.

2.6.2 Prevention

Prevention of cryptosporidiosis is achieved mainly by avoiding contamination of objects, food and water by infected animal or human faeces. Important modes of prevention include; washing of the hands with soap especially before eating or preparing food, after gardening, and also after touching children in diapers; used clothing, bedding, toilets or bed pans. Even when gloves are worn, the hands should still be washed well afterwards.

Transmission may occur readily in hospitals, and many health care workers may become infected from a single patient even though *Cryptosporidium* is not spread by

contact with blood. Another potential source of infection is contaminated fresh vegetables and fruits that must be washed well if eaten uncooked (Casemore *et al.*, 1995). Peeling washed fresh vegetables and fruits before eating may further reduce transmission. Unpasteurised milk or dairy products should not be taken or collected in cooking utensils. The risk of getting *Cryptosporidium* is greatest from pets that are less than 6 months old, animals that have diarrhoea and stray animals. Puppies or kittens less than 6 months old must be tested for *Cryptosporidium* before domestication. Drinking water directly from lakes, rivers, streams or springs should be avoided as much as possible. Also, care should be taken about drinking tap water or iced tap water. Filtered water is safer, but water may be boiled to ensure sterility.

Transmission of cryptosporidiosis may occur when swimming in lakes, rivers, pools or jacuzzis and when using hot tubs. In this respect, one should avoid swallowing water. *Cryptosporidium* is not killed by the amount of chlorine normally used in swimming pools and water parks and can also remain alive in fresh and salt water for several days. Where necessary, swimming pools can be disinfected by using high concentrations of chlorine (3 mg/L of water for 53 hours or 8 mg/L for 20 hours) for long periods.

Cryptosporidium may be on the skin of infected people in the anal and genital areas, including the thighs and buttocks. Precautions should therefore be taken with any sex partner by avoiding kissing or licking the anus (rimming) or the genital part, even if partners wash well before that practice. Hands should always be washed well after touching the anal or rectal areas.

The link between *cryptosporidium* infection and drinking water has led authorities in both the United Kingdom and United States to issue advice to immunocompromised people to boil their drinking water under certain circumstances (Chaisson *et al.*, 1998; Dunand *et al.*, 1997). The United Kingdom guidelines published in 1998 stated "all water, from whatever source, that might be consumed by immune-compromised persons should be brought to the boil and allowed to cool before use.

CHAPTER THREE

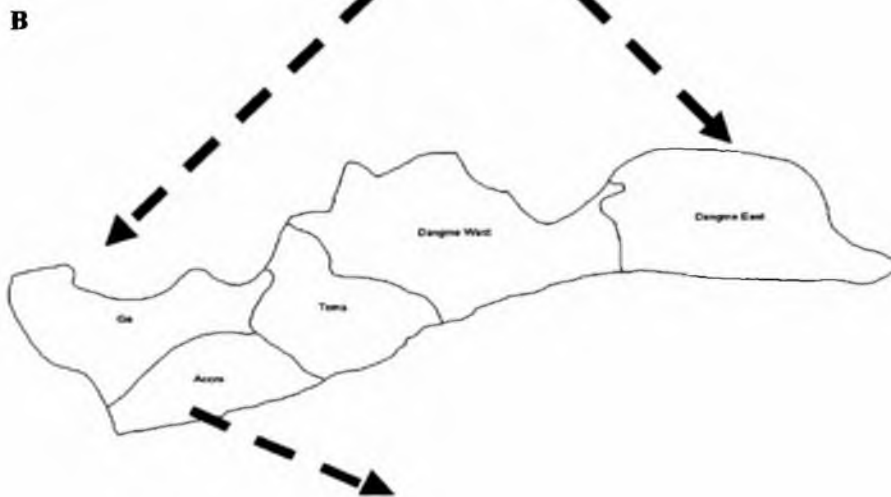
MATERIALS AND METHODS

3.1 STUDY DESIGN AND AREA

The study was a comparative and cross-sectional investigation to compare the occurrence and severity of diarrhoea caused by *Cryptosporidium* in HIV/AIDS and children. The study was conducted at the Korle Bu Teaching Hospital (KBTH). KBTH is Ghana's premier teaching hospital located in the south Ablekuma District of Accra. The population of Accra is heterogeneous and people who use this hospital comprises of residents and non residents, affluent and middle class people most of whom are well educated and observe good sanitary practices, live in neat residential and newly founded communities. Other users consist of people living and coming from semi-slum areas and shantytowns where over crowding and poor sanitary and environmental conditions exist.

3.2 TARGET POPULATION AND SAMPLE SIZE CALCULATIONS

Based on the assumption that infection rate is about 30% (for children/HIV/AIDS patients), a sample size of 300 was calculated for a confidence interval of 95% and a power of 90% using standard methods. Therefore a total of 300 diarrhoeal stool samples from children and HIV/AIDS patients were collected over a 1-year period in Accra.

MAP A**MAP B****Korle Bu Teaching Hospital****MAP 1.**

MAP A is the map of Ghana showing the greater Accra region while MAP B is the map of the Greater Accra region showing the five epidemiological districts and where the Korle Bu Teaching Hospital is located.

Samples were collected from each of the two main target groups at different departments of the Korle-Bu Teaching Hospital, Accra:

1. Children aged 0 to 5 years with acute diarrhoea attending the Paediatric Clinic, and
2. HIV/AIDS patients at the Fevers Unit.

To consider an individual as having diarrhoea, he /she should be passing loose or watery stool, at least 2 or 3 stools per day.

3.2.1 Criteria For Excluding a sample

1. Samples that were licking or not well labelled were rejected.
2. Samples that were not diarrhoeic but were intended for diarrheic ones and vice versa were also rejected.

Written informed consent from all adults and parents or guardians of children were obtained before eligibility in this study was granted.

3.3 QUESTIONNAIRE

Study subjects and the parents or guardians of the children were interviewed by a physician or community health nurse using a designed structured interviewed-administered questionnaire to collect information including; age, sex, socio-economic status, breast-feeding habits, dietary and weaning information, water source and treatment, and presence of domestic animals. Additional information was taken on the date of onset of disease, number of stools per day, duration of diarrhoea, clinical course,

complications, and anti-biotic treatment. A copy of the questionnaire used is shown in appendix 2.

3.4 SAMPLE COLLECTION (STOOL/ BLOOD)

Within each of the groups, stool samples were collected and preserved in sterile containers. Each specimen was divided into two portions and treated differently; one half in 10% formalin and the other placed in Cary-Blair transport medium to preserve bacteria. Specimens were transported to the laboratory at the Microbiology Department, University of Ghana Medical School where they were processed for the isolation of *Cryosporidium* oocysts using the modified Ziehl-Neelsen technique. Samples were also analysed for oocysts antigen using enzyme-linked immunoassay (ELISA). Five millilitres (5ml) of venous blood was collected and the serum separated and tested for antibodies against HIV-1 and HIV-2 by means of standard enzyme-linked immunoassay (ELISA).

3.5 METHODS OF EXAMINATION

For a thorough investigation the methods employed will include 1. Microscopy, involving wet preparations, concentration methods, and permanent staining techniques. 2. ELISA (serological techniques) were used. Bacteriological analysis was done but viral analyses were not.

3.5.1 Microscopy

3.5.1.1 (A) Wet Preparations

Wet microscopic preparations were used mainly to identify other organisms associated with *Cryptosporidium*. In the process, a small amount of each sample was mixed with normal saline and observed under the light microscope after a cover slip had been placed on it. Examination was made by systematic searching, as the slide was orderly moved on the stage. Observations were recorded immediately.

3.5.1.2 (B) Concentration Method

The formalin-ethyl acetate method among others concentrates the infecting organisms for identification irrespective of how few they are in the sample. Using the formalin-ethyl acetate method, four millilitres (4ml) of formalin – fixed stool suspension, 6ml of 10% formalin and 3ml of ethyl acetate were placed into a 15ml clinical glass centrifuge tube. These were mixed by shaking vigorously for 30sec and centrifuged at 500xg for 5min. The centrifugation separated the specimen into four layers (from top to bottom): ethyl acetate, plug of debris, formalin and sediment. The top three layers were decanted after loosening the faecal plug and the sediment examined by the staining technique.

3.5.1.3 (C) Permanent Staining Techniques

The Kinyoun's modified Ziehl-Neelsen staining was employed and the principle is that; Acid fast organisms hold fast to carbol fuchsin stain against washing with acid alcohol. The background then takes the counter stain while the organism still stands out

stained with the cold carbol fuchsin. The identification of *Cryptosporidium* oocysts using modified Ziehl-Neelsen staining technique was done as follows; 10 microlitres of sediment obtained by the formalin-ethyl acetate concentration method was placed on a slide using micropipette and then allowed to dry. The air-dried smears were then fixed in methanol for 3min. The slide was immersed in cold carbol fuchsin and stained for 15min followed by thorough rinsing in water. It was then decolourised in 1% HCl (v/v) in methanol for 10 seconds and then rinsed in tap water. The slide was counterstained with 0.4% malachite green for 30 seconds followed by thorough rinsing in tap water. Prepared slides were air-dried and scanned microscopically at x400 magnification. The presence of oocysts, which stained red and appeared as spheres on a pale green background, was confirmed under oil immersion objective lens x100 magnification.

3.5.2 ANTIGEN-ANTIBODY TECHNIQUES

3.5.2.1 (A) ELIZA (Serological Techniques)

The *Cryptosporidium* test (TechLab, Blaksburg, VA) uses monoclonal and polyclonal antibodies to a cell-surface antigen of the organism. The microtiter wells in the kit contain immobilized monoclonal antibody and the detecting antibody consisting of polyclonal antibody, both of which are specific for the cell surface antigen. In the assay, an aliquot of a diluted faecal specimen is transferred to a microtiter well. If the *Cryptosporidium* antigen is present, it binds to the immobilized monoclonal antibody. When the detecting antibody is added, it binds to the antigen/antibody complex. The bound diagnostic antibody is detected by using an anti-rabbit IgG-peroxidase conjugate. Any unbound materials are removed during the washing step following the addition of

substrate. A colour is detected due to the enzyme-antibody-antigen complexes that form in the presence of *Cryptosporidium* antigen.

3.5.2.1.1 The procedure for the ELIZA

Two wells were set for positive and negative controls* and a third one for each patient sample. Additional control wells were therefore not needed to run a greater number of samples. All wells were labelled. The positive control dropper bottle was shaken for five seconds. Two drops of the mixed positive control were added to the positive control well and 0.1ml of the 1X Wash solution* to the negative control well. Then 0.1ml of the prepared specimen was added to the test well and all the wells incubated at room temperature for one hour. The contents of the assay wells were then discarded. This was repeated three times. Each well was then washed using the wash solution in squirt bottle with fine tipped nozzle, directing the wash solution to the bottom of the well with force.

The wells were filled up, and the solution flipped out of the wells into a discard pan. The inverted plate was banged on dry paper towel and the washing step repeated 4X using dry paper towel each time. If any particulate matter is in the wells, continue washing until all the matter is removed. After washing, any residual liquid in the wells was completely removed by striking the plate onto a dry paper towel until no liquid was found. The paper towel and specimen containers were properly disposed off. A drop of detecting antibody was added to each well and incubated for 20 minutes at room temperature. The washing procedure was repeated. A drop of conjugate* was added to each well and incubated for 10 minutes at room temperature. The washing procedure was

again repeated. A drop of substrate A* was added to each well. Followed by 1 drop of substrate B.

The wells were gently tapped to mix contents and incubated at room temperature for 5 minutes. The wells were tapped 2 or 3 times during the incubation period to mix the contents. A drop of stop solution* was added to each well. The addition of the stop solution converted the blue colour to a yellow colour in the case of a positive sample. Since the ELISA reader was unavailable, the test was read visually in good light against a white background and this within ten minutes after the stop solution had been added.

*** For the compositions of the solutions, reagents and materials used please refer to “ELISA materials” under Appendix 2. (E.g. stop solution at number 5 is a 7ml of 1M sulphuric acid)**

CHAPTER FOUR

RESULTS AND ANALYSIS

4.1 AGE DISTRIBUTION OF PATIENTS

The ages of subjects both HIV/AIDS and the HIV sero-negative patients with diarrhoea ranged from less than 10 years to well over 80 years. Among the 62 HIV/AIDS group, 4.8% were in the 1-10 age group and 6.4% were in the 51 – 60 age group. More than 80% of the samples fell between the age ranges of 21 – 50 years (Table 4.1).

TABLE 4.1 AGE DISTRIBUTION OF PATIENTS (HIV/AIDS)

Age range	No in HIV	%	No in control	%
1-10	3	4.8	8	8.5
11-20	4	6.4	15	15.9
21-30	15	24.2	26	27.6
31-40	24	38.7	15	8.5
41-50	12	19.4	9	7.4
51-60	4	6.4	8	8.5
61-70	-		9	9.5
71-80	-		5	5.3
81-90	-		1	1.1
Total	62	100	96	100

The age distribution of the children's group is shown in Table 4.2. The ages ranged from 1 month to 62 months. Children between the ages of 7-12 months (1year) were the most predominant (42.9%) followed by 17.5% recorded in the age group 1-6 months (0.5yrs).

Apart from age range of 51-56 months (4.5yrs) where there were no subjects, there seem to be a gradual decrease in number from one year (1yr) onward. It is worth noting that as much as 80% of the subjects were between the ages of 0.5 to 2 years.

**TABLE 4.2 AGE DISTRIBUTION OF CHILDREN WITH
DIARRHOEA**

AGE: MONTHS (YEARS)	FREQUENCY	PERCENTAGE
1 - 6 (0.5yrs)	11	17.5%
7 -12 (1yr)	27	42.9%
13-18 (1.5yrs)	7	11.1%
19-24 (2yrs)	6	9.5%
25-32 (2.5yrs)	3	4.8%
33-38 (3yrs)	2	3.2%
39-44 (3.5yrs)	4	6.3%
45-50 (4yrs)	3	4.8%
51-56 (4.5yrs)	0	0
57-62 (5yrs)	0	0
TOTAL	63	100%

4.2 SEX DISTRIBUTION OF SUBJECTS

The sex distribution of the various subjects is shown in Table 4.3. Out of the 278 subjects enrolled in the study, 124 (44.6%) were males and 154 (55.4%) were females. Females were more in the HIV/AIDS group as well as its control group than in the children's group and their controls

TABLE 4.3 SEX DISTRIBUTION OF SUBJECTS

SAMPLE	MALES	FEMALE	TOTAL
HIV/AIDS	27(43.5%)	35(56.5%)	62
CONTROL (HIV/AIDS)*	42 (43.7%)	54(56.3%)	96
CHILDREN	32(50.8%)	31(49.2%)	63
CONTROL (CHILDREN)**	23(40.6%)	34(59.6%)	57
TOTAL	124	154	278

* HIV sero-negative patients with diarrhoea

** Children without diarrhoea

4.3 SYMPTOMS PRESENTED BY SUBJECTS WITH DIARRHOEA

Generally the subject who had diarrhoea presented five main symptoms. These were; abdominal pain, nausea, vomiting, fever and bloody stools. The two most common symptoms were abdominal pain and fever. Abdominal pain was the most common symptom (19/62) among the subjects who were HIV/AIDS patients while fever was found to be the most common symptom (27/63) among the children (Table 4.4).

Interestingly in both groups *Cryptosporidium parvum* infection seemed to be related to these symptoms.

TABLE 4.4 SYMPTOMS PRESENTED BY SUBJECTS WITH DIARRHOEA AND *C. PARVUM* POSITIVITY

SYMPTOMS	HIV/AIDS = 62 / CRYPTO. POSITIVE	CHILDREN =63 / CRYPTO. POSITIVE	TOTALS
ABDOMINAL PAIN	19 / 6	10 / 3	29 / 9
NAUSEA	7 / 3	5 / 0	14 / 3
VOMITING	9 / 3	15 / 1	24 / 4
FEVER	11 / 4	27 / 6	36 / 10
BLOOD IN STOOL	16 / 0	13 / 0	29 / 0

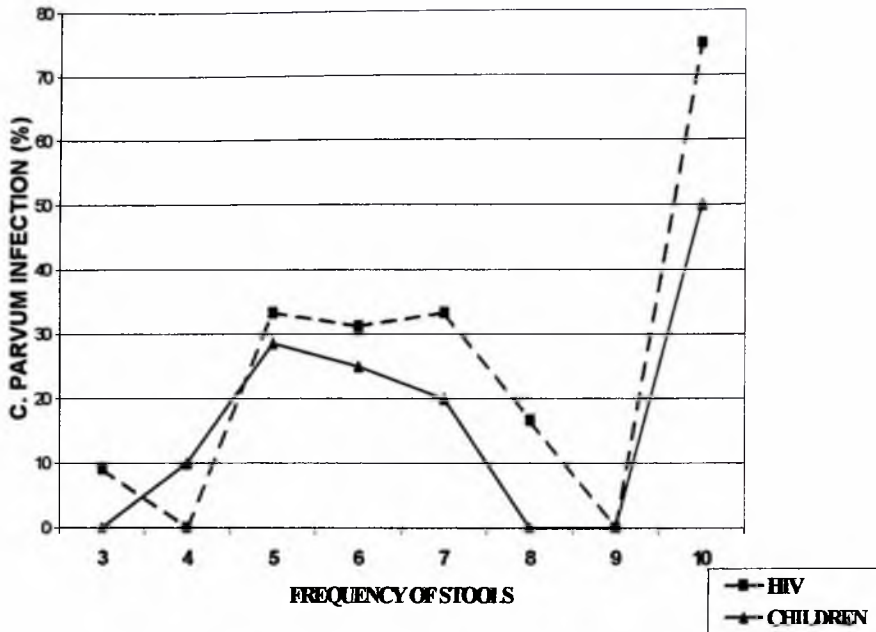
4.4 FREQUENCY OF PASSAGE OF STOOL AND *C. PARVUM* INFECTION

In Table 4.5 below, all the patients had the stool passage frequencies ranging from 3 times a day to as high as 10 times in a day. Noteworthy of the frequencies were those ranging between six and eight times (6, 7, and 8 times) daily for HIV/AIDS patients. Within these three frequencies, twelve out of the total of 16 positives (13/16) were from this group. Also for the HIV/AIDS group, three out of the four (70%) who passed stool ten times in a day were positive for *Cryptosporidium* infection. Among the children, the frequencies of stool passage ranged between five and six times daily. Generally the pattern of *Cryptosporidium* infection in the two groups is the same as shown in Figure 4.1.

**TABLE 4.5 FREQUENCY (NUMBER OF STOOLS PER DAY)
AND *C. PARVUM* POSITIVITY**

NUMBER OF TIMES OF PASSAGE OF STOOLS	HIV/AIDS / CRYPTO. POSITIVE	CHILDREN / CRYPTO. POSITIVE
3	11 / 1	9 / 0
4	4 / 0	10 / 1
5	6 / 2	14 / 4
6	16 / 5	12 / 3
7	9 / 3	5 / 1
8	12 / 2	7 / 0
9	0 / 0	4 / 0
10	4 / 3	2 / 1

FIGURE 4.1

FREQUENCY OF PASSAGE OF STOOLS AND
C. PARVUM POSITIVITY

4.5 AGE RELATED *C. PARVUM* INFECTION IN CHILDREN

The distribution of the positive cases as related to age in the children who had diarrhoea is shown in Table 4.6. Children from one year and below were found to have a high infection rate. 40% of the positive isolates were from the age 7-12 months. 80% of the infection was found among children below 2 years while the remaining 20% infection was associated with children between 2 and 5 years as shown in Figure 4.2 below.

TABLE 4.6 C. PARVUM POSITIVITY AND AGE

AGE:MONTHS (YEARS)	FREQUENCY	PERCENTAGE
1 - 6 (0.5yrs)	3	30%
7 -12 (1yr)	4	40%
13-18 (1.5yrs)	1	10%
19-24 (2yrs)	0	-
25-32 (2.5yrs)	1	10%
33-38 (3yrs)	0	-
39-44 (3.5yrs)	1	10%
45-50 (4yrs)	0	-
51-56 (4.5yrs)	0	-
57-62 (5yrs)	0	-
TOTAL	10	100%

FIGURE 4.2**AGE RELATED PREVALENCE OF C. PARVUM
INFECTION IN CHILDREN WITH DIARRHOEA**

□ Below 2 years
 ▨ Between 2-5 years

4.6 DURATION OF DIARRHOEA IN SUBJECTS AND *C. PARVUM* POSITIVITY

Table 4.7 shows a varied diarrhoeal duration ranging from below 4 weeks through a period of more than 12 weeks. From the HIV/AIDS group, the highest number, 22 out of 62 patients, (35.5 %) complained of having the diarrhoea for over 12 weeks. The same group recorded the highest prevalence (56.3 %) of *Cryptosporidium parvum* infection. Those who had had diarrhoea for about 4 weeks followed this. On the contrary most of the children were noted to have had diarrhoea for duration of 4 weeks and the highest prevalence of the infection was also recorded in this same group.



TABLE 4.7 DURATION OF DIARRHOEA IN SUBJECTS AND *C. PARVUM* POSITIVITY

DURATION	HIV/AIDS / CRYPTO. POSITIVE	CHILDREN / CRYPTO. POSITIVE
< 4 WEEKS	12 / 2	54 / 8
4 WEEKS	14 / 4	5 / 1
8 WEEKS	5 / 0	3 / 1
12 WEEKS	9 / 1	0 / 0
>12 WEEKS	22 / 9	1 / 0

4.7 LABORATORY RESULTS (MACROSCOPY)

Stool consistency ranged between watery, semi formed and loose. Most of the samples were watery or loose, only one sample was found to be micoid and non of the samples contained blood. From Table 4.8 it was observed that 41 out of the 62 stool samples examined from HIV/AIDS patients were watery (75%). 12 out of the 16

positives screened from the HIV/AIDS patients were from watery stools while the remaining 4 were obtained from loose stools. Macroscopic analysis of the control samples revealed that 56 of the samples were loose and the rest of the samples were either watery, semi formed or mucoid in fairly equal proportions. None of the samples from the control group tested positive for *Cryptosporidium parvum* infection.

TABLE 4.8 STOOL CONSISTENCY OF PATIENTS AND CONTROLS

STOOL CONSISTENCY	NUMBER OF HIV/AIDS/ CRYPTO. POSITIVE	NUMBER OF CONTROLS / CRYPTO. POSITIVE
SEMI-FORMED	2/0	15/0
LOOSE	18/4	56/0
WATERY	41/12	15/0
MUCOID	1/0	10/0
TOTAL	62/16	96/0

4.8 PATHOGENS ISOLATED FROM STOOL

Out of a total of 278 samples from the four different groups of subjects screened in the study, *Cryptosporidium* oocysts were detected in 28 samples (Table 4.9). Sixteen (16) of the positive samples were from HIV/AIDS patients with diarrhoea giving a percentage prevalence of 25.8 % from a total of 62 samples examined from that group. No *Cryptosporidium* oocysts were isolated from the HIV sero-negative persons with diarrhoea who were also screened. From the same data (Table 4.9), it was noted that among the children aged five (5) years and below who had diarrhoea, ten (10) out of the

63 samples examined were positive for *Cryptosporidium* infection (15.9%) while those without diarrhoea recorded a percentage of 3.5 out of a total of 57 samples.

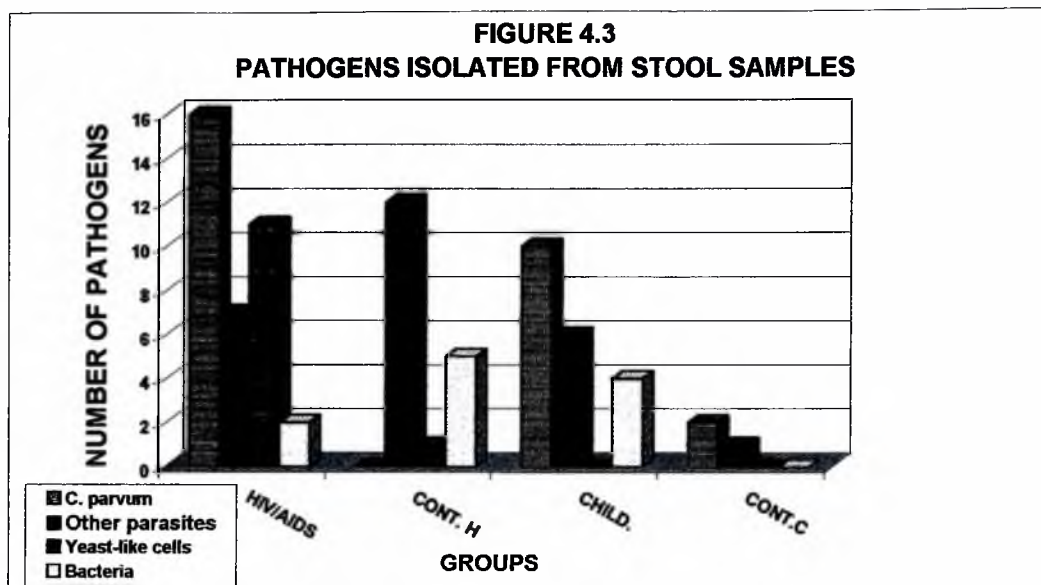
TABLE 4.9 PREVALENCE OF *C. PARVUM* INFECTION

SAMPLE GROUP	NUMBER OF SAMPLES	NUMBER OF POSITIVES
HIV/AIDS WITH DIARRHOEA	62	16 (25.8%)
NON HIV/AIDS WITHOUT DIARRHOEA	96	0 (0%)
CHILDREN WITH DIARRHOEA	63	10 (15.9%)
CHILDREN WITHOUT DIARRHOEA	57	2 (3.5%)
TOTAL	278	28

Figure 4.3 shows other enteropathogens isolated through the study. As mentioned earlier in the methodology section, there was no provision made for viral isolation aside of HIV/AIDS detection. Other parasites isolated ranged from 7, 12, 6, and 1 for HIV/AIDS patients their controls, children with diarrhoea and children without diarrhoea respectively, with the non-HIV/AIDS subjects with diarrhoea recording the highest. A number of yeast-like cells were also identified from the samples as shown in the figure.

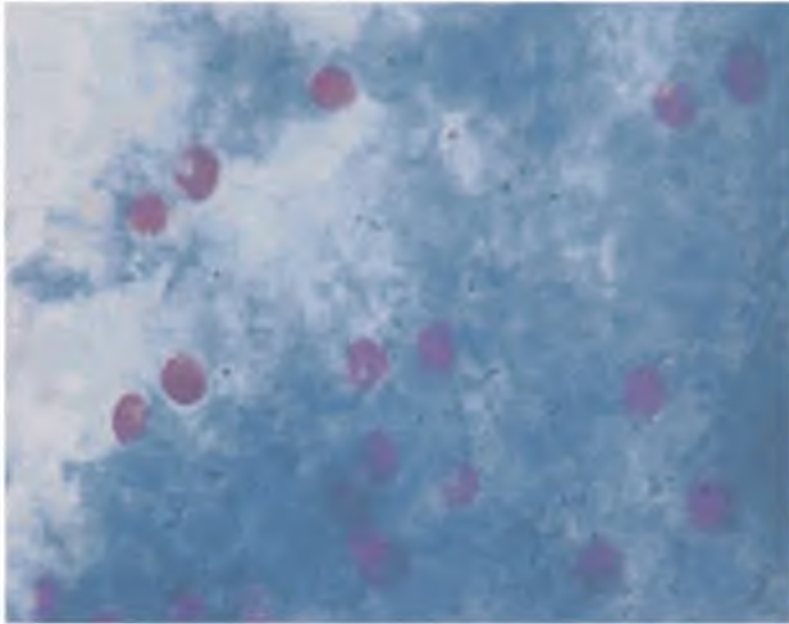
The HIV/AIDS group showed a strikingly high number of yeast-like cells (11) as compared with the 1 and 0 recorded for the children and control groups. The bacteria

isolated was generally low in number (2, 5, 4 and 0) through the four groups i.e. HIV/AIDS, non-HIV/AIDS, children with and children without diarrhoea respectively.

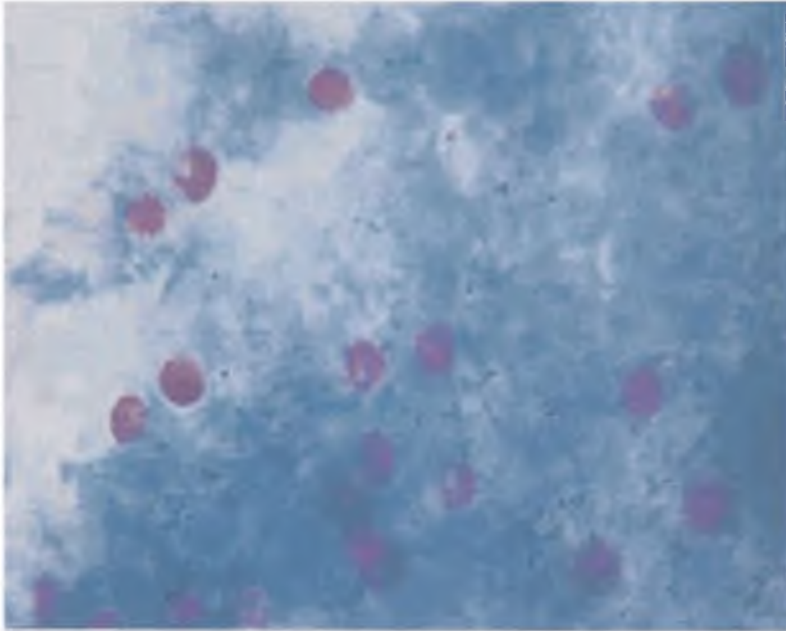


4.9 RESULTS BASED ON ZN STAIN AND ELISA

The results obtained by the ZN stain and ELISA were compared. From Table 4.10 it could be noted that, there is virtually no difference in sensitivity in detection of oocyst using any of the two methods since the results were similar. It is however important to note that the ELISA kit was able to detect one more positive case, which was not detected by microscopy. An example of the positive ZN stained slide is shown in Plate 3 and the ELISA kit is displayed in Plate 4 below.

PLATE 3**STAINED SLIDE OF *CRYPTOSPORIDIUM PARVUM***

The plate shows the ZN stained slide. The red looking cells are the Cryptosporidium oocysts against the greenish background of the counter stain (malachite green). Size ranges between 4-6µm.

PLATE 3**STAINED SLIDE OF *CRYPTOSPORIDIUM PARVUM***

The plate shows the ZN stained slide. The red looking cells are the *Cryptosporidium* oocysts against the greenish background of the counter stain (malachite green). Size ranges between 4-6µm.

Plate 4 SET UP FOR ELISA TECHNIQUE



- Monocolonal anti body coated wells (First from left)
- Detecting agent (Second from left)
- Conjugate (anti-rabbit IgG-peroxidase)-Third from left
- Substrate (colour detection)-Third from right
- Positive and negative controls-(First and second from right)
- Yellow coloured well showing positive and colourless showing negative

TABLE 4.10 RESULTS BASED ON ZN STAIN & ELISA

DIAGNOSTIC METHODS	POSITIVE	NEGATIVE	TOTAL
ZN STAIN	25	195	220
ELISA	26	195	221

4.10 CRYPTOSPORIDIUM INFECTION BY DISTRICTS IN ACCRA.

The distribution as seen in Table 4.11 shows that as much as 73% of the cases were from Accra 43% from Ga and the other 9% from the other three districts (Tema, Dangbe West, Dangbe East) respectively. Out of the positive case, it was noticed that most (17%) were from the Accra. 7 were from the Ga district and 2 from the other districts. Meanwhile out of the total samples taken 23.3% turned out positive among the patients from the Accra district, 16.2% from the Ga district and 22.2% from the others.

TABLE 4.11 C. PARVUM INFECTION BY DISTRICTS

EPIDEMIOLOGICAL DISTRICT	HIV/AIDS	CHILDREN	TOTAL/CRYPTO. POSITIVE
ACCRA DIST.	37	36	73 17 (23.3%)
GA DIST.	21	22	43 7 (16.3%)
OTHERS*	4	5	9 2 (22.2%)
TOTAL	62	63	125 26

* Tema, Dangbe West, Dangbe East

4.11 SOURCE OF DRINKING WATER

Table 4.12 indicates the types and sources of drinking water of the patients. Those that took water from wells and rivers were quite few. About 8.9% of the HIV/AIDS patients and 1.5% of the children consented to the use of well water. Only one HIV/AIDS patient who responded positive for *Cryptosporidium* infection claimed to use river water. Most (93%) of the HIV/AIDS patients use water from the tap or treated bottled water. 95.7% of the children also used tap or bottled water.

TABLE 4.12 SOURCE OF DRINKING WATER

SOURCE	HIV/AIDS / CRYPTO. + VE	CHILDREN / CRYPTO. + VE	TOTAL
PIPE	56	58	114
	15 (26.9%)	10 (17.2%)	25
WELL	5	1	6
	1 (20%)		1
RIVER	1		1
N/A	-	4*	4
TOTAL	62 16 (25.8%)	63 10(15.9%)	125 26

* Indicates children (4) who were exclusively breastfed and under 6 months.

4.12 PRESENCE OF DOMESTIC ANIMALS

The interview revealed that most Ghanaians studied cohabit with domestic animals. This was made clear when as much as 88.7% of the HIV/ADS patients and 76.2% from the mothers of the children readily answered yes to the question that inquired about the presence of domestic animals. The other 11.3% and 23.8% in HIV/AIDS

patients and children respectively, either answered no or were not sure of what to say at that time.

From Table 4.13, out of the 55 patients who had animals in their homes 15 (27.3%) of them were infected with *Cryptosporidium*. Out of the 7 who were not having domestic animals, 1(14.3%) was also positive. Those with the animals among the HIV/AIDS had more occurring than those without (Table 4.13). This suggests that some of the domestic animals could be reservoirs of *Cryptosporidium*.

From Table 4.13, a slightly different observation was made among the children. Eight 8 (16.7%) out of the 48 mothers who responded that they had animals at home had the infection. This was against the 2 positive out of the 15(13.3%) who had domestic animals.

TABLE 4.13 PRESENCE OF DOMESTIC ANIMALS IN THE HOME

PRESENCE OF ANIMALS*	HIV/AIDS / CRYPTO. POSITIVE	CHILDREN / CRYPTO. POSITIVE
YES	57 15 (27.3%)	48 8 (16.7%)
NO	5 1 (14.3%)	15 2 (13.3%)
TOTAL	62 16 (25.8%)	63 10 (15.9%)

*Types of animals include: dogs, cats, fowl and goats.

4.13 BREAST FEEDING AND *CRYPTOSPORIDIUM* INFECTION

A relationship between breast-feeding and prevalence of *Cryptosporidium* infection was also observed among the children. Out of the 63 children enrolled in the study, 42 (66.7%) were exclusively breast-fed while the remaining 21 (33.3%) were not. *Cryptosporidium* infection was found to be high in the children who were exclusively breast-fed as shown in Table 4.14. In all 33.3% of the children who were breast-fed were found to be infected with *Cryptosporidium*.

TABLE 4.14 BREAST FEEDING AND *C. PARVUM* INFECTION

BREAST FEEDING	NUMBER (%)	CRYPTO.POSITIVE (%)
EXCLUSIVE	42 (66.7%)	6 (14.3%)
NON-EXCLUSIVE	21 (33.3%)	4 (19%)
TOTAL	63 (100%)	10 (33.3%)

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. DISCUSSION

5.1.1 INTRODUCTION

Cryptosporidium parvum had been known to be one of the causes of opportunistic infections in HIV/AIDS and children (Kuby, 1997). As mentioned in the hypothesis it was expected that there would be a strong positive correlation between *Cryptosporidium* sp infection and diarrhoea in HIV/AIDS patients and children especially those under five years old with diarrhoea.

5.1.2 HIV/AIDS AND NON-HIV/AIDS PATIENTS WITH DIARRHOEA

A comparison of the results from the HIV/AIDS patients with that of the controls who were HIV/AIDS sero-negative with diarrhea, showed a clear association between *C. parvum* infections with HIV/AIDS or immunocompromised conditions. This was deduced from the observation that 25.8% of the HIV/AIDS patients with diarrhoea were *C. parvum* positive, while none (0%) of the non-HIV/AIDS patients with diarrhoea had the infection. This observation supports the findings of Flanigan *et al* who used low CD4 count to describe the seriousness of *Cryptosporidium* infection in immuno-compromised patients (Flanigan, 1992).

5.1.3 CHILDREN WITH DIARRHOEA AND WITHOUT DIARRHOEA

A higher prevalence of *C. parvum* infection was observed in the children with diarrhoea (15.7%) compared to those without diarrhoea (3.7%). This suggests a relationship between *Cryptosporidium* infections and diarrhoea in children. According to an investigation carried out by Frayer *et al*, (1997) invasion of the intestinal epithelial cells by *C. parvum* causes gastrointestinal disorders. This gives ample evidence that patients with *C. parvum* infection are more likely to present with diarrhoea. In few cases however there may be carriers who do not show any clinical symptoms, as was the case of the 3.5% of the children without diarrhoea. According to Goodgame *et al*, (1993) even though *Cryptosporidiosis* could be severe, there is marked variability in clinical presentation, thus it is possible to have symptomatic infections without diarrhoea.

The results obtained from the various study subjects, showed a 15.7% prevalence rate in the group of children with diarrhoea while there was no occurrence in the HIV/AIDS sero-negative subjects who were mostly adults with diarrhoea. It could be deduced from our results that there is a higher association between *C. parvum* and children with diarrhoea than adults with diarrhoea. This could be explained by the fact that these children are still in the process of building a stronger immune system compared to the adults.

5.1.4 HIV/AIDS VERSUS CHILDREN

Due to the observation that HIV/AIDS condition is associated with *Cryptosporidium* infections it could be expected that the HIV/AIDS group will have a distinctively and significantly higher prevalence than children.

Comparing the results from the children and adults it was revealed that 25.8% of the HIV/AIDS patients were *Cryptosporidium* positive, while only 15% were positive in the children's group. Even though numerically the prevalence rates look different, the difference was not statistically significant ($\chi^2 = 1.23$ p value = 0.2677). This observation indicates that as far as *Cryptosporidium* infection is concerned, children are as vulnerable as HIV/AIDS patients.

5.1.5 CRYPTOSPORIDIUM AND AGE AMONG HIV /AIDS PATIENTS

It is interesting to note that 25.8% of the HIV/AIDS patients who had diarrhoea and found to be infected with *Cryptosporidium* had ages ranging from 4 years to as old as 82 years. With reference to other studies on the prevalence of *Cryptosporidium* recently, our study actually reemphasizes similar observations that *Cryptosporidium* is associated with HIV/AIDS patients (Kuby, 1997; Casemore, 1990). These studies however did not critically look at the correlation between age and prevalence. It was observed that more than 80% of the *Cryptosporidium* positive cases were from the ages of 21-50 suggesting that *Cryptosporidium* infection just like AIDS itself, is associated with the reproductive ages (sexually active) among the HIV/AIDS group.

5.1.6 AGE AND C. PARVUM INFECTION IN CHILDREN

Generally age range of children who are prone to fatal presentation of diarrhoea had been known to be the under fives. All the subjects (children) enrolled in our study were less than five years. Comparing this age to the adults with diarrhoea seen in Table 4.1, it became apparent that although the two groups were all having diarrhoea, as much

as about 15.9% of the children had *Cryptosporidium* infection while none of the adults with diarrhoea was infected. This shows that children under five years of age are more vulnerable compared to the adults.

Whiles about 15.9% of the children with diarrhoea were positive only about 3.2% of the control group (children without diarrhoea) were found to be infected. Since *Cryptosporidium parvum* is known to be a diarrhoea-causing organism, (Frayer *et al*, 1997) their relative presence in the children with diarrhoea and absence in those without diarrhoea suggests that investigations into the aetiologic causes of diarrhoea among children include *Cryptosporidium parvum*. It is noteworthy that the data revealed that children with diarrhoea were mostly in the age of 0.5 – 2years. Incidentally this is the age range within which parents wean their children off breast milk and introduce them to solid food. It is likely that at this stage the children take in water and food, which may be contaminated with infective oocysts.

Another important observation is that about 90% of the *Cryptosporidium* positive cases were found to be among the age group less than or equal to 2 years. This shows that among children, those in these age groups are more vulnerable. This finding is in line with the result of the study conducted by Checkley *et al*, (1997). Checkley *et al*, (1997) found that children under 2 years had higher prevalence rate in *Cryptosporidium* infection and Nwabuisi in Nigeria also found that 86% of the total *Cryptosporidium* positives observed were between the ages of 0-2yrs.

5.1.7 SYMPTOMS PRESENTED AND *C. PARVUM*

As mentioned earlier the symptoms of *Cryptosporidium* ranges from fever, stomach or abdominal pains, headache, nausea, among others, which may also be exhibited in other diarrhoeal diseases. From our data (Table 4.4), abdominal pain and fever seem to be associated with *cryptosporidiosis*. It tells us therefore that abdominal pains and fever cannot be overlooked if one is suspecting cryptosporidiosis aside of the other diarrhoea diseases HIV/AIDS patients or children under five, complaining of abdominal pains and presenting with fever need to be followed up and investigated for the possibility of *Cryptosporidium* infection.

However the slight difference in number in the response given by the HIV/AIDS and the children, concerning the two symptoms could be attributed to the difference in age. The fact that abdominal pain was more mentioned among the HIV/AIDS patients than the children is most likely due to the fact that most children cannot fully describe what they feel when sick. On the other hand, the fact that the fever came out so clearly among the children might have been due to reports from their mothers. Certainly, the mothers could even feel the changes in temperature of their children before bringing them to the clinicians.

In contrast, there was no association between blood in stool and prevalence of *Cryptosporidium* infection HIV/AIDS patients and children. These findings are in line with what Tzipori observed in his survey, which similarly showed no association between the two conditions (Tzipori 1998).

5.1.8 FREQUENCY (NUMBER OF STOOLS PER DAY) AND *CRYPTOSPORIDIUM* POSITIVITY

It is clear that a normal (non-diarrhoeic) person will pass stool not more than three times a day. WHO's definition for diarrhoea states that, if stool is passed more than two times in a day, it could be considered a diarrhoeal case. All our patients fell into this classification, the stool frequency ranged from as low as 3 times to as high as 10 times in just a day (Table 4.5).

The number of stool passed that had the highest frequency (mode) was six times in the HIV/AIDS patients and five times in the children. This means that most of the cases had their diarrheic frequencies ranging between 5 and 6 times daily especially the *Cryptosporidium* related diarrhoea. This clearly shows the severity of *Cryptosporidium* related diarrhoea.

Even though there is no upward or downward trend in the frequencies, the slight difference in the frequencies associated with the HIV/AIDS patients and the children may be explained by the difference in abilities to stand dehydration. The HIV/AIDS patients who were mostly adults are able to cope with the rate of dehydration better and as a result survived while the children especially those under two years who happened to be the majority (80%) of the group sampled got to the severe stages.

5.1.9 DURATION OF DIARRHOEA

It has been established from other studies that diarrhoea in *Cryptosporidiosis* in immuno competent patients is self-limiting. In addition it is less severe and not that fatal. However cryptosporidial diarrhoea could last as long as 12 weeks as seen in the results of this study. It was realized by Walter *et al*, (1988) and Molbak *et al*, (1997) that most of

the patients infected with *Cryptosporidium* have prolonged diarrhoea ranging from 5 days to about 6 weeks.

The observations from this study are in line with Walter's findings and records even more than 12 weeks of diarrhoea. This study also certainly emphasized the fact that duration of diarrhoea in *Cryptosporidium* infection could really be prolonged. The difference in the duration in children and HIV/AIDS patients could be explained in that most parents in Accra are very much concerned about the health of their children and so report relatively early when they notice signs of diarrhoea. This is different from HIV/AIDS diagnosed patients, who already feel there is no hope and do not pay much attention to their health needs.

5.1.10 STOOL CONSISTENCY AND *CRYPTOSPORIDIUM PARVUM* POSITIVITY

Macroscopically, diarrhoea stool should be able to take the shape of the container. Most of the samples used for the study however were between semi formed, loose and the watery descriptions. For both cases, mucoid samples were few. However, it happened that there was a slight difference between the descriptions that had the highest number. While in the HIV/AIDS group, most had loose and watery stools, most of the samples from the controls had semi formed or loose stools. Our already discussed results which showed that about 25.8% of the HIV/AIDS patients had the *Cryptosporidium* infection and the infections lead to severe dehydration explains why the stools were mainly watery.

5.1.11 PRESENCE OF DOMESTIC ANIMALS

From the results more than 80% of the study population have domestic animals in their homes (Table 4.13). This is not surprising since in the introduction, it was mentioned that one of the reasons why *Cryptosporidium* infection is expected in Accra for that matter Ghana is because most people cohabit with one domestic animal or the other. The four most frequently mentioned animals were dogs, cats, fowls and goats with dogs being the most. As mentioned earlier, most Ghanaians keep dogs for security reasons. Some also keep them as pets while other use them for hunting purposes. Some also keep goats as livestock for additional income and food. There are similar reasons why people keep the other domestic animals like the goat. Yet they could be reservoir hosts for *Cryptosporidium*.

There was no significant difference between the prevalence of *C. parvum* infection among those who have animals at home and those who do not (p value=0.108). This is likely because the settlements of the region from which the samples were taken are not well organized. The difference seen in children could most probably be because most of the HIV/AIDS patients being adults keep themselves away from animals and also practice good personal hygiene.

5.1.12 OTHER PATHOGENS DETECTED

The prime objective of the investigation was the isolation of *Cryptosporidium parvum*. The focus of the screening of samples was not to look for other gastrointestinal pathogens but once found in the samples they were also recorded. Generally there was a

clear isolation of the *Cryptosporidium* oocyst and the detection of the surface antigen without interference.

For the *Cryptosporidium* infections, antibiotics administration did not have any influence on it being detected or not. So it was expected that whether the patient had taken any antibiotics or not, these parasites could still be isolated and they could be picked especially because of the use of the ELISA technique.

Among the other pathogens looked for were other parasites; bacteria and fungi. Investigations into diarrhoea causing viruses were not done. Generally, very few other pathogens were found most probably because the focus was on *Cryptosporidium* isolation. It could be noted from the above results that in both HIV/AIDS patients and children a few other parasites like *Strongyloides stercoralis* and *Ascaris lumbricoides*, *Entamoeba* sp., among others were also encountered. Among those isolates was *Entamoeba* sp which is also known to cause diarrhoea. However, no significant correlation was found between these two pathogens.

One striking and remarkable thing about Figure 4.3 is the clear distinction between the association between the prevalence of yeast like cells and HIV/AIDS. While the HIV/AIDS recorded as much as eleven (11) of yeast like cells there was virtually no yeast like cells found in the children's group. This observation is possibly due to the compromised immune system.

This suggests that yeast like cells are more linked with HIV/AIDS than with other persons. Even though numerically, it looks like the *Cryptosporidium* was more in the HIV/AIDS patients (25.8%) than the children (15.7%). Statistical analysis showed no significant difference ($\chi^2 = 1.23$, p value = 0.277).

The bacteria investigations could be influenced by antibiotic treatment. In the children for example majority (70%) had been given antibiotics and few were not sure whether they had taken antibiotics or not. Based on this, one could easily attribute the low number of such bacterial isolates such as *Salmonella sp*, *Shigella sp*, *E. coli* to the general use of antibiotics.

5.1.13 GEOGRAPHICAL AREA IN GREATER ACCRA REGION AND DISTRIBUTION

Accra the capital of Ghana was chosen for this study for some few reasons, one of which is convenience and for the results to serve as a pilot for a major project nation wide. This pilot study became important realizing that not much work had been done on *Cryptosporidium* in Ghana. Whiles the arguments made earlier about the reasons why *Cryptosporidium* is expected in Ghana applies to the whole nation, it stands to reason that conditions may vary within or between districts and regions.

As seen in the result the patients involved in the study came from the Greater Accra Region. This involves 5 districts namely Accra, Ga, Tema, Dangme West and Dangme East. Even though the Greater Accra Region contains the capital city problems associated with low socio-economic and sanitary conditions are prevalent. Besides the rearing of domestic animals for various reasons is also common in the region.

After analyses of where the patients were located in the region, they were geographically divided into three (3) main parts, namely; Accra, Ga and others. The others comprised Tema, Dangme West and Dangme East districts. This was adopted after noticing that the other districts excluding Accra and Ga, had less patients both at the

Fever's unit (HIV/AIDS clinic) and the children's block at the KBTH. This is most likely because of the distance from these sites to the Korle-Bu Teaching Hospital where the study was centered or conducted.

Sodemann *et al.*, (1999) reported that there is an association between the geographical area, environmental hygiene and the probability of *Cryptosporidium* infection. In this study however, our observations did not tally with the findings of Sodemann and his group. This might be due to lack of significant variation in the environmental conditions prevailing in the areas where the samples were collected. This indicates that technically all the five (5) districts in the Greater Accra Region could be classified under one geographical area with very similar hygienic conditions. There is the need for a bigger study over a wider geographical area, which may confirm Sodemann *et al.*, (1999) findings.

5.1.14 BREAST-FEEDING

Since 95% of the children in the study were exclusively breast-fed for the first six months, it shows that most parents in the Greater Accra Region had adopted the exclusive breast-feeding habit. In his survey in Liberia, Hojlng and colleagues (1998) mentioned that bottle-feeding and non-exclusive breast-feeding, expose children to cryptosporidiosis (Hojlng *et al.*, 1998). From this results however there was no significant difference between cryptosporidiosis in the two groups ($\chi^2 = 0.17$, p value = 0.467). This means that exclusive breast-feeding alone does not guarantee resistance to cryptosporidiosis. The explanation could take two lines of thought; (1) the general and (2) the psychological implication of the growth and developmental stage of children.

Concerning the general implications, firstly, there is the possibility that information given by the mothers with regards to exclusive breast-feeding might not be accurate. Secondly, there might also have been a possible default in the exclusive breastfeeding. The mothers unintentionally may have given the children other food apart from the breast milk. Thirdly the nipple of the breasts of mothers or their hands might have been contaminated.

From the psychological point of view, there is an interesting feature in human growth and development. In Freud's theory of infantile development, it was explained that children between 0-1 have the tendency of putting things into their mouths. This is the stage referred to as the 'oral' stage and is immediately followed by the 'anal' stage (1-2 years) where according to Staudomire (1997), children derive pleasure from playing with the anal regions. The behavioural patterns seen during these 'anal' and 'oral' stages (0-2 years) may explain why the children in that age group could easily get the infection.

5.1.15 SOURCE OF DRINKING WATER AND *C. PARVUM*

Millard *et al*, (1994) implicated contaminated drinking water in several out breaks of cryptosporidiosis. According to that study, types or sources of drinking water could have a bearing on the probability of having *Cryptosporidium* infection.

The results of our study reveal that about 93% of the HIV/AIDS patients used tap water or bottled water. This is also true among the children. As high as 95.7% of the children were given tap water or bottled water. This excluded four (Table 4.12) who were not given water because they were being exclusively breast-fed and were under 6 months. This suggests that most people have seemingly portable drinking water. How this does

not prevent the vulnerable HIV/AIDS and children groups from having the infection is rather uncertain. As mentioned earlier this is not surprising because our major source of water is treated by chlorination, which does not kill oocysts of *Cryptosporidium parvum*.

5.2 CONCLUSIONS

From our results and discussions, the following conclusions could be drawn.

- The prevalence of *C. parvum* in HIV/AIDS patients and children with diarrhoea in the Greater Accra Region were 25.8% and 15.9% respectively
- *C. parvum* is an aetiological agent of diarrhoea in both HIV/AIDS patients and children under 5 years.
- *C. parvum* infections are more prevalent in HIV/AIDS (25.8%) than in non-HIV/AIDS patients (10%).
- Also more prevalent in children with diarrhoea (15.9%) than those without. (3.6%).
- Statistically, there is no significant difference between prevalence of HIV/AIDS patients and children under 5 years (25.8% / 15.9%)
- Breast feeding, presence of animals and source of drinking water did not have any significant association to whether one will have disease or not in Accra.

5.3 RECOMMENDATION

- a. The link between *Cryptosporidium* infection and drinking water has led authorities in both the United Kingdom and United States to issue advice to immunocompromised people to boil their drinking water under certain circumstances. This recommendation could also be adopted
- b. Since most Ghanaians have some pets in their homes and these could be reservoir hosts of *cryptosporidium*, HIV/AIDS patients and children especially under 2 years may do well to keep themselves away from pets as much as possible.
- c. A study of bacteria infections in HIV/AIDS patients where culture is going to be the method to be used need to take in to consideration the use of antibiotics, since it can easily give false negatives. Or an immunological study if any could be done alongside to confirm culture results.
- d. Where less trained staff are those available at laboratories or for a project, it will be encouraged that an ELISA kit is used where available to confirm cases since they are more sensitive and easy to use than other reports.

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APPENDIX**Appendix 1**

Cases	Control 1	Control 11	
Parasite species	(n=147)	(n=56)	(n=43)
<i>Cryptosporidium spp</i>	38(25.9%)**	0	0
<i>Isospora belli</i>	2(1.4%)	0	1(2.4%)
<i>S. stercoralis</i>	5(3.4%)	1(1.8%)	0
<i>G. lamblia</i>	6(4.1%)	2(3.6%)	2(4.6%)
<i>A. lumbricoides</i>	17(11.850)	12(21.4%)	7(16.3%)
<i>T. trichura</i>	11(7.5%)	7(12.5%)	6(14.%)
<i>E. histolytica</i>	12(8.2%)	3(5.4%)	3(7.0%)
<i>Taenia spp</i>	4(3.4%)	2(3.6%)	1(2.4%)
<i>Blastocystis spp</i>	1(0.7%)	0	1(2.4%)
<i>S .mansoni</i>	0	1(1.8%)	1(2.4%)
<i>Hook worn spp</i>	0	2(3.6%)	2(4.6%)

Appendix 2

MATERIAL FOR METHODS USED

MICROSCOPY

WET PREPARATIONS

Materials needed include 1. Saline, 2 Slides and cover slips, 3. Light microscope

(B) CONCENTRATION METHOD

Materials needed include 1 Glass centrifuge tubes, 2 Guase, 3 Formalin, 4 Ethyl acetate

(C) PERMANENT STAINING TECHNIQUES

Materials needed include 1. malachite green, 2. cold carbol fuchsin, 1% acid alcohol, 4 methanol

(D) ELISA (SEROLOGICAL TECHNIQUES)

Materials needed include

1. Substrate A: 7.0ml (solution containing tetramethylbenzidine substrate).
2. Substrate B: 7.0ml (buffer solution containing peroxide), 3. Conjugate: 7.0 (anti-rabbit IgG-peroxidase in a buffered protein solution containing 0.02% Thimerosal),
- 4 .Detecting antibody: 7.0ml (rabbit polyclonal anti body to a cell-surface antigen of cryptosporidium in a protein buffer solution containing 0.02% thimerosal),
5. Stop solution: 7.0ml (1M sulfuric acid) ,Caution; avoid contact with skin. Flush with water immediately if contact occurs.
6. Positive control: 5.0ml (heat-inactivated bovine fecal material containing cryptosporidium antigen in a protein buffered solution with 0.02% thimerosal),
7. Wash buffer concentrate: 50 ml (20x concentrate containing phosphate-buffered saline, detergent and 0.2% thimerosal).
8. Microassay plate: 12 strips

each consisting of 8 well coated with monoclonal antibody of cryptosporidium cell-surface antigen (stored with desiccant). 9. 100 graduated disposable pipettes, 10. 2 plastic adhesive sheets, 11. 1 resealable bag, 12. Squirt bottle for wash reagent, 13. Vortex mixer, 14. Timer, 15. 950 ml distilled water for diluting wash reagent, 16. 1 liter bottle for diluted wash reagent, 17. ELISA reader capable of reading at 450nm, 18. Discard container and absorbent paper, 19. Test tubes, 20. Applicator sticks.

Appendix 3**CRYPTOSPORIDIOSIS RESEARCH QUESTIONNAIRE**

1.0 **INSTITUTION** 1. Korle-Bu Polyclinic 2. Fevers Unit 3. Children's
Block

2.0 BASIC DATA

2.1 Date.....(DD/MM/YY)

2.2 Study ID (Sample No.)
.....

2.3 Hospital Folder No.....

2.4 Name

2.5 Date of Birth(DD/MM/YY)

2.6 Age Months (Child)
.....

2.7 Years (Adult)
.....

2.8 Sex 1. Male 2. Female

2.9 Address (Location Area)
.....

3.0 SYMPTOMS

3.1 Stool Consistency 1. Solid 2. Loose 3. Watery

If solid skip 3.2.

3.2 Diarrhoea Duration (days)
.....

3.3 Number of stools per day

.....
 3.4 Blood present 1. Yes 2. No

3.5 Abdominal pain 1. Yes 2. No

4.0 **SOCIO-ECONOMIC FACTORS**

4.1 Mother's Education 1. None 2. Basic 3. Secondary 4. Voc/Tech 5. Tertiary

4.2 Mother's Work 1. None 2. unskilled 3. Skilled Labour 4. Professional
5. Trader

4.2 Father's Work 1. None 2. unskilled 3. Skilled Labour 4. Professional
5. Trader

4.4 Main Source of Drinking Water ... 1. Pipe in House 2. Buy Pipe Water 3. Well
4. River 5. Buy Sachet Water 6. Rain Water 7. Tanked Pipe Water

5.0 **NUTRITION**

5.1 Breast feeding (Children < 2 years old) 1. Exclusive to 6 months 2. Not Exclusive
3. Not Breastfed

5.2 Source of food (main source) 1. Cooked at home 2. Street food

5.3 Main type of food given before illness 1. Breast 2. Weaning 3. Family diet
4. Bottled 5. Street food

6.0 **SIGNS**

6.1 Degree of dehydration 1. None 2. Some 3. Severe

6.2 Nutritional Status 1. Malnourished 2. Borderline 3. Well nourished

6.3 Weight (Kg).....

6.4 Weight Percentile

(%).....

6.5 Height

(cm).....

6.6 HIV Status 1. Positive 2. Negative 3. Unknown

7.0 TREATMENT

- 7.1 Antibiotics 1. Given 2. Not given
- 7.2 Anti-Diarrhoeals 1. Given 2. Not given

8.0 PRESENCE OF ANIMALS

- 8.1 Domestic Animals 1. Yes 2. No
- 8.2 Specify Animals (If yes for 8.1) 1. Dog
- 8.3 Specify Animals (If yes for 8.1) 2. Cat
- 8.4 Specify Animals (If yes for 8.1) 3. Goat
- 8.5 Specify Animals (If yes for 8.1) 4. Sheep
- 8.6 Specify Animals (If yes for 8.1) 5. Fowls
- 8.7 Specify Animals (If yes for 8.1) 6. Other

Please indicate (If Other for 8.7)

.....

9.0 OUTCOME

Outcome 1. Referred 2. Discharged 3. Died

Administered By

.....

Date.....

Appendix 4

Consent form for the Survey of Cryptosporidiosis in HIV/AIDS patients and children/adults with diarrhoea in Ghana

Information.(to be read or translated to parents/guardians in their own mother tongue)

Dear Parents or Guardians:

We kindly ask your permission to enter or let your child enter into a study, which we will proceed to describe.

We would like to start by stressing that this study is strictly voluntary. Should you decide not to participate, it will have no consequences for the treatment. Should you, at any point during the study, decide that you do not wish to participate any further, you are free to terminate your participation or that of your child, effective immediately. Any such decision will be respected without any discussion.

The study in a few words.

The aim of this study is to examine what causes diarrhoea in children and adults and in particular those with HIV/AIDS. We are particularly interested in finding out the role, if any of the micro-organism, called *Cryptosporidium* in diarrhoea in Ghanaians. It is important because if we know the sources of the organisms, then we can suggest ways of preventing people from getting it.

We will do this by examining a small amount of stool samples from the individual. In addition we will collect a small amount of venous blood to test for antibodies. The blood

will be collected by a clinician or qualified individual. The amount of blood to be taken will not exceed 5ml (approx. $\frac{1}{2}$ spoonful) and will not affect your health or that of your child. The samples obtained for this study are obtained as part of samples required for standard or routine examinations. Therefore, the study does not impose any additional stress on you or your child.

Sterile techniques and disposable single-use equipment will be used at all times.

All information gathered will be treated in strict confidentiality.

If you have any questions, please feel free to ask the physician in charge.

Yours sincerely

Dr. Lorna Renner

Department of Child Health

Korle-Bu Teaching Hospital

Dr. Onike Rodrigues

Department of Child Health

Korle-Bu Teaching Hospital

I hereby grant permission for my child to enter into this study:

Signature/thumbprint of Parent or Guardian