PREVALENCE OF *CRYPTOSPORIDIUM* OOCYSTS ON VEGETABLES COLLECTED FROM SELECTED MARKETS IN ABUJA, NIGERIA.

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MAY, 2014.

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, IN PARTIAL FULFILMENT FOR AWARD OF DEGREE OF MASTER OF SCIENCE IN VETERINARY PARASITTOLOGY AND ENTOMOLOGY.

DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY,
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MAY, 2014.

DECLARATION

I hereby declare that the work in this thesis titled "Prevalence of *Cryptosporidium*Oocysts on Vegetables collected from selected Markets in Abuja, Nigeria" was performed by me in the Department of VeterinaryParasitology and Entomology, Ahmadu Bello University Zaria, under the supervision of Prof. Idris A. Lawal , Dr. A.J Natalaand Dr. S.N.A Saidu. The information derived from literature has been duly acknowledged and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Chinyere Susan Emeka **……..** **…….**
Signature Date

CERTIFICATION

This thesis titled "Prevalence of *Cryptosporidium*Oocysts on Vegetables Collected from Selected Markets in Abuja, Nigeria" byChinyere Susan Emeka, meets the regulations governing the award of the Degree of Master of Science in Veterinary Parasitology and Entomology of Ahmadu Bello University, and isapproved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to God Almighty for His grace and guidance and to my beloved parents for their love and support.

ACKNOWLEDGMENT

First and foremost, I would like to express my sincere gratitude to God for seeing me through this research work. My sincere gratitude goes to my supervisors Prof.Idris .A. Lawal, Dr. A.J. Natala and Dr. S.N.A Saidu for their continuous support, patience, motivation, enthusiasm and immense knowledge. Their guidance helped me throughout my research and in the course of writing this thesis. I could not have had better supervisors and mentors for this study than them. Besides, I would like to thank members of the Helminthology laboratory, department of Veterinary Parasitology and Entomology, Ahmadu Bello University Zaria, for their assistance and support. I truly appreciate my friends for their valuable help and I just want to say thank you. To all of you, I convey my best wishes, deepest respect and appreciation.

ABSTRACT

Fresh vegetables are important part of a healthy diet for people and can be agents of transmission of intestinal parasites. Some of these intestinal parasites like *Cryptosporidium* spp can be zoonotic with important consequences to the public health wellbeing of many of the consumers.It is for this reason that this study was conducted to determine the prevalence and risk factors for this parasite in three markets (Kubwa, Karu and Wuse) in the Federal Capital Territory (FCT), Abuja. In all four hundred and fifty vegetable samples made up of tomatoes, lettuce spinach, cabbage and carrot were collected from these markets, Nigeria. These samples were examined for the presence of *Cryptosporidium*oocysts using sucrose flotation medium and modified Ziehl Nelson staining technique. Nineteen (4.22%) out of the 450 samples examined, were positive for *Cryptosporidium*oocysts. Lettuce had the highest (5.56%) contamination rate, followed by spinach and carrots with 4.4% each, while tomato and cabbage had 3.3% contamination rate each. Among the markets, Wuse and Karu markets had the highest occurrence of contaminated samples with 4.67% each, while Kubwa had 3.3% contamination rate. There was no significant difference between the occurrence of *Cryptosporidium* oocysts and types of vegetables examined ($x^2 = 0.7693$, $p = 0.9425$), and the markets in which the vegetables were sold ($x^2 = 0.4396$, $p = 0.8027$).Vegetable samples had the highest contamination with *Cryptosporidium*oocysts in December (6.19%) followed by those in September (5.3%). Vegetable samples in August and November had a contamination rate of 2.68% and 2.65% respectively. There was no significant difference ($x^2=2.790$; $p=0.4251$) between the monthly prevalence and the occurrence of *Cryptosporidium* oocysts Twenty eight other parasites like mite egg 11(2.44), *coccidian* oocysts 10(2.22), *E. histolytica* 1(0.22), nematode larva 6(1.33) were also found on the vegetables studied. The use of well (35.6%), bore hole (26.7%) and tap water (37.8%) in rinsing or washing the vegetables could have significantly increased the chances for contamination of these vegetables. This study has

shown that vegetables sold within Abuja markets in Nigeria are contaminated with *Cryptosporidium* oocysts and this is of public health importance.

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LIST OF ABBREVIATIONS

Abbreviation or symbol	Term
AIDS	Acquired Immune Deficiency Syndrome
AMP	Adenosine monophosphate
CDC	Centre of Disease Control and Prevention
CF	Cystic fibrosis
CSA	*Cryptosporidium* Specific Antigen
C. spp	*Cryptosporidium species*
C-src	Proto-oncogene protein pp60
DFA	Direct immunofluorescent antibody test
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-Linked Immunosorbant Assay
FDA	Food and drug administration
FAST-ELISA	Falcon assay screening test enzyme-linked immunosorbent assay
FRET	Fluorescent resonance energy transfer
GMP	Guanosine monophosphate
GMRDC	Govan Mbeki Research Development Centre
QPCR	Quantitative PCR
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
HRP	Horseradish peroxidise
IFA	Immunofluorescent Antibody
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-8	Interleukin-8
mAbs	Monoclonal antibodies
MZN	Modified ZiehlNeelsen
NTZ	Nitrozoxanid
NF-KB	Nuclear factor-kappa B
PCR	Polymerase Chain Reaction
RDT	Recombinant DNA technology
RNA	Ribonucleic acid

ABSTRACT

Fresh vegetables are important part of a healthy diet for people and can be agents of transmission of intestinal parasites. Some of these intestinal parasites like *Cryptosporidium* spp can be zoonotic with important consequences to the public health wellbeing of many of the consumers.It is for this reason that this study was conducted to determine the prevalence and risk factors for this parasite in three markets (Kubwa, Karu and Wuse) in the Federal Capital Territory (FCT), Abuja. In all four hundred and fifty vegetable samples made up of tomatoes, lettuce spinach, cabbage and carrot were collected from these markets, Nigeria. These samples were examined for the presence of *Cryptosporidium*oocysts using sucrose flotation medium and modified Ziehl Nelson staining technique. Nineteen (4.22%) out of the 450 samples examined, were positive for *Cryptosporidium*oocysts. Lettuce had the highest (5.56%) contamination rate, followed by spinach and carrots with 4.4% each, while tomato and cabbage had 3.3% contamination rate each. Among the markets, Wuse and Karu markets had the highest occurrence of contaminated samples with 4.67% each, while Kubwa had 3.3% contamination rate. There was no significant difference between the occurrence of *Cryptosporidium* oocysts and types of vegetables examined ($x^2 = 0.7693$, $p = 0.9425$), and the markets in which the vegetables were sold ($x^2 = 0.4396$, $p = 0.8027$).Vegetable samples had the highest contamination with *Cryptosporidium*oocysts in December (6.19%) followed by those in September (5.3%). Vegetable samples in August and November had a contamination rate of 2.68% and 2.65% respectively. There was no significant difference ($x^2=2.790$; $p=0.4251$) between the monthly prevalence and the occurrence of *Cryptosporidium* oocysts Twenty eight other parasites like mite egg 11(2.44), *coccidian* oocysts 10(2.22), *E. histolytica* 1(0.22), nematode larva 6(1.33) were also found on the vegetables studied. The use of well (35.6%), bore hole (26.7%) and tap water (37.8%) in rinsing or washing the vegetables could have significantly increased the chances for contamination of these vegetables. This study has

shown that vegetables sold within Abuja markets in Nigeria are contaminated with *Cryptosporidium* oocysts and this is of public health importance.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Cryptosporidium, a coccidian protozoan parasite which has been described in sixteen different species of domestic and wild mammals, birds and reptiles (CDC, 2009), is an important causative agent of humans and animal gastrointestinal infection globally (Fayer, 2004) . The parasite is responsible for acute gastrointestinal and less frequently respiratory infections in humans. It is self-limiting in immunocompetent people but prolonged and potentially life threatening for the immunocompromised populations (Fayer, 2004). The incubation period is usually about one week, with clinical signs of watery diarrhoea which may be accompanied by abdominal pain, vomiting and fever (Fayer, 2004).

Studies have revealed that there are two main species commonly detected, *C. hominis* and *C. parvum* infecting humans of which *C. parvum* is more ubiquitous (Xiao and Ryan, 2008). The most susceptible species of animals to *Cryptosporidium* infection is cattle, the parasite is transmitted to susceptible animals through the ingestion of oocysts that are fully sporulated and infective when passed in faeces (Moon and Bermick, 1981). Oocysts are discharged in water by various animal hosts (Tariuwa *et al.*, 2007). The oocysts are resistant to most chemical disinfectants, but are susceptible to drying and ultra violet portion of the sunlight(USFDA/CFSAN, 2005).

In Nigeria, human cryptosporidiosis has been earlier reported from Northern (Kwaga *et al.,* 1988), Central (Banwat *et al.,* 2003*;* Udeh *et al.,* 2008), Eastern (Okafor and Okunji, 1994 and 1996) and Western (Reinthaler *et al*., 1987; Nwabuisi, 2001)parts of the country.The disease is a major public health problem in both developing and developed countries (Heylyng *et al.,* 1986). In developing countries, the disease probably exerts most of its impact

on young children resulting in stunted growth (Guerrant and Moore, 1999). In developed countries, water borne outbreaks of cryptosporidiosis have a significant economic impact(Checkley *et al.,* 1998; Molbak *et al.,* 1997).

Raw vegetables are common vehicles for the transmission of the parasites (Ozlem and Sener, 2005*).* The problem is becoming increasingly of concern because of the expanding number of susceptible people(Ozlem and Sener, 2005*).* Fresh vegetables are regarded as important part of a healthy diet. In many countries such leafy plants are eaten raw or lightly cooked to preserve taste and this practice may also favour the likelihood of food-borne parasitic infections (Ozlem and Sener, 2005*).* Food normally becomes a potential source of human infection by contamination, during production, collection, transportation and during processing (Kang *et al.,* 1998*).* The sources of zoonotic contamination are usually faeces, faecally contaminated soil or water(Damen *et al.,* 2007).In recent years, there has been an increase in the number of reported cases of food borne illness linked to consuming fresh vegetables (Ozlem and Sener, 2005*).* The consumption of raw vegetables plays a major epidemiological role in the transmission of parasitic food borne intestinal parasites widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene (Kang *et al.,* 1998*).* The deplorable hygienic condition of the environment as a result of indiscriminate defecation by humans can contaminate water sources (McEvoy *et al.,* 2003). Farm produce become contaminated when vegetables are washed with water containing *Cryptosporidium* oocysts (Glaberman *et al.,* 2002).

Contamination of food or water by cattle manure has been identified as one of the causes of several food borne and water borne outbreaks of cryptosporidiosis in man (Glaberman *et al.,* 2002). Reports of isolation of *Cryptosporidium spp* oocysts from vegetables include those in cilantro and lettuce in Costa Rica, in lettuce, cilantro and parley in Peru and lettuce and mung

bean sprouts in NorwayRica (Mongeand Arias, 1996, Ortega *et al.,* 1997, Robertson and Gjerde, 2001). In the United States of America alone, the number of reported cases of cryptosporidiosis in thousands per million persons reported through food net was 10.9 in 2003, 13.2 in 2004 and 29.5 in 2005.

The increasing public health importance of *Cryptosporidium* species has made the infection to be in the limelight through scientific investigations worldwide. Several reports on the epidemiology and outbreaks of cryptosporidiosis in humans from other parts of Africa and elsewhere abound in the literature (Endeshaw *et al.,* 2004;Jones *et al.,* 2006; Chalmers *et al.,* 2009;Ponka *et al.,* 2009).

This study therefore focuses on detecting the prevalence of *Cryptosporidium* oocysts on vegetables sold for human consumption in selected markets in Abuja with a view to determine to what extent humans are exposed to the infection.

1.2 Statementof Research Problem

Cryptosporidiosis is zoonotic and is transmitted by faeco-oral route. This infection is now well recognised to cause disease in humans, particularly those who are in some way immunosuppressed or immunodefficient. Cryptosporidiosis has been implicated as one of the more important opportunistic infections in patients with AIDS (Fayer, 2004). Calves and perhaps other animals serve as potential sources of human infection (Garcia, 2001). Cryptosporidiosis is not always confined to gastrointestinal tracts, in some cases respiratory cryptosporidiosis; cholecystitis, hepatitis and pancreatitis have been observed (Arora and Arora, 2005).

Cryptosporidiosis is a major public health problem in both developing and developed countries of the world (Heylyng *et al,* 1986). Children are among the high risk group for the

infection. Those less than five years of age are most susceptible (Huang *et al.*, 2004) and it may have long- term negative effects on their growth and cognitive development (Turwine *et al.*, 2003). Studies indicate that the prevalence varies geographically with increased prevalence in developing countries and temporally with higher rates reported during the rainy season in many tropical countries (Heylyng *et al*, 1986). The causative agent is *Cryptosporidium* which is described as single- celled obligate intracellular protozoa (CDC, 2009). Various epidemiological studies show that the prevalence of intestinal parasites was high, especially in developing countries, although in many of these the environmental risk factors have not been clearly elucidated (Nyarango *et al*., 2008).

The poor hygienic condition of the environment in Nigeria due to indiscriminate defecation by both humans and animals contaminate water sources. Vegetables washed with water from these sources could become contaminated with *Cryptosporidium* oocysts. The consumption of such raw vegetables in salads and other foods preparation with poor hygiene in production and marketing of these products in Nigeria exposes the consumer to *Cryptosporidium* infection. The use of animal dung as fertilizer in the production of vegetables due to scarcity of inorganic fertilizer also increases the chances of *Cryptosporidium* oocysts in vegetables sold in Nigerian markets.

Thus, the increasing consumption of leafy vegetables for a balanced diet coupled with increasing incidence of the disease necessitates the need for further studies of vegetables as a link to food borne outbreaks and cryptosporidiosis in Nigeria.

1.3 Justification

There is lack of reliable and documented information on vegetables as a possible source of transmission of *Cryptosporidium* infection in Abuja, although work has being done on meat and soil samples. Abuja is the capital City of Nigeria and mostly of working class

populations, who consume vegetables as a source of diet and do not have time for private cooking. The consumption of raw vegetables in salads and other food preparations against the background of poor hygiene in production and marketing of these products in Nigeria exposes the consumer to enormous risks of *Cryptosporidium* infection.

This research will therefore provide information on the prevalence and extent of contamination of vegetables sold in Abuja markets with *Cryptosporidium spp* oocysts. This will turn create awareness to the general public on the risks associated with consuming raw vegetables or lightly cooked vegetables.

1.4 Aim and Objectives

1.4.1 Aim;

The aim of the study was to determine the prevalence of *Cryptosporidium* oocysts on selected vegetables sold in three markets in Abuja.

1.4.2 Objectives of the study

The objectives of the study were to:

i. examine selected vegetables sold in Abuja markets for the presence of *Cryptosporidium* oocysts.
ii. determine the predisposing factors for possible cryptosporidiosis in humans in Abuja.

1.5 Research Questions.

i. Are there *Cryptosporidiumspp* oocysts on vegetables sold in Abuja markets in Nigeria?
ii. What are the predisposing factors involved in the marketing of vegetables in Abuja?
iii. Is there any relationship between factors such as type, nature, source of manure, and source of vegetables?

CHAPTER TWO

LITERATURE REVIEW

2.1 Cryptosporidium Species

Cryptosporidium species is an enteric protozoan pathogen that cause diarrhoea illness called cryptosporidiosis (Fayer *et al.,* 2000). The parasite invades and resides in the epithelial cells, most commonly in the small intestines of multiple mammalian species. There are fourteen named species of *Cryptosporidium* of which *Cryptosporidium hominis* (previously known as *Cryptosporidium parvum* human genotype or genotype I), is the species that almost exclusively infects humans. *Cryptosporidium parvum* (previously known as the *Cryptosporidium parvum* bovine genotype or genotype II) can infect not only humans but also ruminants and perhaps a few other animals (Sestak *et al*., 2002). Other species that infect human but are less common include *Cryptosporidium andersoni* (cattle), *Cryptosporidium baileyi* (chicken and some other birds), *Cryptosporidium canis* (dogs), *Cryptosporidium felis* (cats), *Cryptosporidium galli* (birds), *Cryptosporidium meleagridis* (birds and humans), *Cryptosporidium molnari* (fish), *Cryptosporidium muris* (rodents and some other mammals), *Cryptosporidium wrairi* (guinea pigs), *Cryptosporidium saurophilum* (lizards and snakes), and *Cryptosporidium serpentis,* snakes and lizards (Xiao *et al*., 2004). Recent literature has also described *Cryptosporidium parvum* as the most common species isolated from human stools from diarrhoea infected and non-infected individuals (Abdel-Messih *et al*., 2005).

There is genetic heterogeneity among *Cryptosporidium species* isolated from humans, thus research surveys suggest that *Cryptosporidium species* undergoes genetic changes following human infection and/or that there may be distinct pathways of *C. species* transmission from bovine to human (Carryway *et al*., 2001).

2.2 Classification

Cryptosporidium species are classified as eukaryotes in the Phylum Apicomplexa (possessing an apical complex of secretory organelles), Class Sporozoasida (reproduce by asexual and sexual cycles, with oocysts formation), Subclass Coccidiasina (life cycle involving merogony, gametogony and sporogony), Order Eucoccidiida (schizogony occurs), Suborder Eimeriina (independent micro and macrogamy development), Family cryptosporiidae (four naked sporozoites within oocysts) (Tzipori and Widmer, 2000).

Due to the relative uniform appearance, different genotypes have shown that some are very host specific while others have a broad host range and these have been utilized to classify *Cryptosporidium spp* into 14 valid species that are currently recognised on the basis of oocyst morphology and site of infection and they include *Cryptosporidium parvum, C. hominis, C. muris, C. meleagridis, C. baileyi, C. serpentis, C. nasorum, C. felis, C.andersoni, C. canis, C. galli, C. molnari, C. wrairi, C.saurophilum* (Hoepelman and O'Donoghue, 2004). Among these species, only *Cryptosporidium parvum and Cryptosporidium hominis* are responsible for most of the human cases of infection with some geographical differences (Xiao and Fayer, 2008). The others are known to have less infection in humans.

Polymerase chain reaction analysis of the 18S RNA based on *Cryptosporidium felis* and *Cryptosporidium canis* has been implicated as an unusual potential causative agent in immunocompetent individuals after morphometric evaluation of oocysts isolated from human faeces in England (Pedraza-Diaz *et al*., 2000). Additionally, new *Cryptosporidium spp* genotypes such as *Cryptosporidium cervine* have been identified in human stools (Ong *et al*., 2002). It appears likely that other species may cause occasional zoonotic infections in humans in the future through which the sporozoites exit during excystation.

2.2.1 Taxonomy

The genus *Cryptosporidium spp* is classified taxonomically within the same group as other coccidian parasites such as *Toxoplasma, Eimeria and Isospora spp*. A key difference between *Cryptosporidium spp* and other coccidian parasites is that the organism progresses through a complex life cycle characterised by the host‘s expulsion of double-walled, sporulated infective sporozoites oocysts.The current taxonomic position of the genus *Cryptosporidium* is described by Fayer *et al.,* (2000) as follows:

Phylum: Apicomplexa

Class: Conoidasida

Subclass: Coccidiasina

Order: Eucoccidiorida

Suborder: Eimeriorina

Family: Cryptosporididae

2.3 Morphology of the Oocyst

The small size of *Cryptosporidium* oocysts for the various species are difficult to distinguish from each other during routine faecal examination based solely on morphology by light microscopy (Fall *et al.*, 2003). Oocysts are spherical in appearance, and have many distinct phases. They contain four parallel sporozoites surrounded by a tough protective smooth oocyst wall, measuring 3 – 6µm in diameter (Fayer *et al*., 2000).In the wall, a faint suture can be seen through which the sporozoites exit during excystation (Morgan- Ryan *et al.*, 2002). Depending on the host species, recovered oocysts can vary in size (3 to 8 µm in width by 3 to 9 µm in length) and they consist of many small granules and a membrane-bound globule with a residuum that is extremely resilient to various unconducive environmental conditions (Fayer *et al*., 2004).

2.3.1 Life cycle

Cryptosporidium species are reported to have a monoxenous life cycle where all stages of development (asexual and sexual) occur within one host to form oocysts (O'Donoghue, 2002). Each oocyst contains four infective stages termed sporozoites which are fully sporulated when excreted by the host into the environment. The naked sporozoites chromosomes consisting of 10.1-10.4 million base pairs of DNA with very few introns are released along with parasitized epithelial cells of the *Cryptosporidium* by the infected host through faeces and possibly other routes such as respiratory secretions (Bankier *et al*., 2003). Ingested oocysts excyst in the ileum, releasing sporozoites that are capable of penetrating individual epithelial cells in this region (O'Donoghue, 2002).

Cryptosporidium is capable of completing all stages of its development (asexual and sexual) within a single host as shown in Appendix III (Chen *et al.*, 2002). Unlike other coccidian species, the organism does not require a period of maturation (sporulation) outside the host to become infective. The thick- walled oocysts are fully sporulated and infectious when excreted in the faeces of an infected host (O'Donoghue, 2002). The life cycle of *Cryptosporidium species* progresses through five phases, where it begins with the ingestion of the oocysts by the new host. When these oocyts are ingested, the suture in the oocyst wall opens (excystation), triggered by the body temperature and the interaction with stomach acid, bile salt and pancreatic enzymes. Four infective motile sporozoites are released from the cyst to produce thin-walled and thick-walled oocysts that invade the small intestine of the host and infect the intestinal epithelia cells (enterocytes), mainly in the jejunum and ileum. The freed sporozoites attach to epithelial cells and become enveloped within parasitophorous vacuoles, developing attachment organelles, a stage referred as trophozoites. Trophozoites reside beneath the cell membrane of the epithelial cells but outside the cytoplasm, where they

transform into several life stages in an asexual merogony, releasing 8 and 4 merozoites respectively.

The four merozoites released from the second merogony give rise to sexual developmental stages, through gametogony to produce microgamonts (male) and macrogamonts (female). The release of microgametes, and their fusion with macrogametes give rise to a zygote which undergoes further two asexual divisions to form the environmentally robust resistant oocysts containing 4 sporozoites (Smith *et al*., 2005). The majority of oocysts that are produced at this stage are usually thick-walled and are excreted with faecal materials from the host. Thin-walled oocysts can excyst within the same host and start a new life cycle, known as autoinfection (O'Donoghue *et al.*, 2002). This may lead to a heavily infected epithelium of the small intestine, resulting in malabsorptive or secretory diarrhoea characterized by copious volume of fluid faeces that may contain blood, mucus, and /or bile (O'Donoghue *et al.*, 2002). The infectious stage of this disease may persist for several days or months depending on the parasite host.

2.4 Epidemiology in Humans

The prevalence of this infection varies widely from country to country and from one region to another. The disease is more in developing countries than in developed countries, whereby infection is influenced by ethnic background, gender, socioeconomic conditions, different locations and age group (Xiao *et al*., 2004). Research surveys have been conducted to gain some idea about the prevalence of the parasite in industrialised nations. An average of about 2-3 and 5% of patients hospitalized for diarrhoea in United Kingdom and United States of America were infected with chronic cryptosporidiosis respectively and 40% of those in some third world countries (Xiao *et al*., 2004). The reported analysis of 29 storm water samples in

the United States of America (USA) revealed the presence of 14 *Cryptosporidium* genotype oocysts each year for every square meter of surface area (Xiao *et al*., 2004).

A longitudinal study of *Cryptosporidium* infections in children in North Eastern Brazil documented a cryptosporidiosis prevalence of 94% (Lee *et al*., 2005). The most common symptoms were persistent diarrhoea (16.5%) and acute diarrhoea (8.4%) (Xiao *et al*., 2004). Analysis of stool specimens from the immunocompetent, adult population with gastrointestinal symptoms revealed a prevalence of infection close to 2% in Germany (Steeb *et al*., 1987). Remarkably, children aged 1 to14 years, were found with prevalence close to 2% (Krause *et al*., 1995). As expected, the prevalence of cryptosporidiosis was higher (4-7.7%) among Human Immunodeficiency Virus (HIV) patients (Heise *et al.,* 1988).

Cryptosporidiosis in the Netherlands was found to be responsible for 2.2 % of gastroenteritis cases (Mank, 1997). The prevalence was found to be highest in patients with acute diarrhoea complaints (lasting < 7 days) being 5.4%. In patients with persistent diarrhoea complaints, the prevalence of *Cryptosporidium spp*. was found to be 3.3% (Mank, 1997).

Furthermore, the distribution of infection appears to be age-related, with the highest prevalence of 7.0% found in patients between 0 and 4 years of age whereas the lowest prevalence (1.1%) was found in patients between 45 and 65 years of age. However, in the region of Aragon, *Cryptosporidium* oocysts were identified in 87 (1.93%) patients. (Clavel *et al*., 1996). A prospective study in Acquired Immune Deficiency Syndrome (AIDS) patients with chronic diarrhoea in the Madrid area of Spain, showed an overall prevalence of intestinal cryptosporidiosis of 15.6% (Lopez-Velez *et al*., 1995). The prevalence was found to be higher in homosexual patients (33.3%) than in intravenous drug abusers (10.6%) (Lopez-Velez *et al*., 1995). Extra-intestinal infection was present in 30% of the patients with known intestinal cryptosporidiosis (Lopez-Velez *et al*., 1995). Eight of the 13 (61.5%) patients with

extra- intestinal cryptosporidiosis had *Cryptosporidium* in the bile and 7 (16.28%) of them had it in the sputum. Interestingly, of the seven patients with *Cryptosporidium* in the sputum, four had respiratory symptoms and an abnormal chest radiograph (Lopez-Velez *et al*., 1995). *Cryptosporidium* was the only organism detected in induced sputum and bronchoalveolar lavage specimens. Another study performed in the Zaragoza area of Spain, found a prevalence of 3% to 8% in immunocompromised HIV-positive adults (Moles *et al.*, 1998).

2.4.1 Cryptosporidiosis in Nigeria

Several works have been done on cryptosporidiosis in Nigeria. Some of the documented studies include the reports of 32.6%, 12.0%, and 7.6% prevalence of oocysts amongst pigs, goats, and cattle respectively in Ile-Ife by Ayeni *et al* (1985). Furthermore, a prevalence of 38.5% was also recorded in calves in Jos by Pam and Onwuliri (2009).They noted that infection was more common in female calves (38.7%) than in males (38.3%). A prevalence of 21% was reported in humans in Zaria (Kwaga *et al.*, 1988) and he noted that infection was higher among females (27%) than males (17%) and among adult (29%) than children (8%).

In North-Central Nigeria, Nwabuisi (2001) reported a prevalence rate of 15.1% among children aged 0 to 14 years with diarrhoea while Banwat *et al.* (2003) reported a prevalence rate of 4.8% among malnourished children aged between 0 and 5 years. In Osun State a prevalence rate of 52.7% was reported among HIV patients indicating that cryptosporidiosis may be an opportunistic parasitic disease contributing to the incidence of diarrhoea among HIV infected patients in Nigeria. A study conducted in Ilorin amongst patients with diarrhoea reported a prevalence of 14% (Nwabuisi, 2001). Egberongbe *et al*. (2010) reported a prevalence of 21.4% among children of Ijebu and Remo areas of Ogun state Nigeria. The infection was more common among children using nearby bush as toilet and those using stream or pond as a source of water.

2.5 Transmission of Infection

Evidence from several investigations suggests that transmission of *Cryptosporidium* is epidemiologically linked with the oocyst, where it is transmitted from an infected host to a susceptible one by the faecal-oral route. The infectious stage of the parasite is the oocyst which is excreted in great numbers along with faeces of infected hosts. The infective dose is not accurately known, but is believed to be very low (Egger *et al*., 2000). A mathematical model based on data from the Milwaukee outbreak suggested that some individuals developed cryptosporidiosis following the ingestion of only one oocyst (DuPont *et al*., 1995,Mac Kenzie *et al*., 1994;).

During the exogenous stage of the organism, the oocyst is difficult to remove from drinking water supplies because of its resistance to chlorine disinfection and inefficiency in filtering (Xiao *et al*., 2004).

2.5.1 Routes of transmission

Collectively, the various species of *Cryptosporidium* have a broad host range affecting gastro-intestinal epithelial cells. The durability of oocysts, along with their diminutive size, lend themselves to the incurable transmittable diseases via municipal drinking water, as they can survive many disinfectants, thus having the potential to cause community- wide outbreaks (Robertson and Gjerde, 2007). Outbreaks illustrate different routes of the transmitted diseases namely person-to-person contact with infected patients (family members, health care workers, users of communal swimming pools and travellers), human-animal contact (zoonosis) (veterinarians, farmers), or via indirect transmission through the environment (particularly by water) (Chen *et al*., 2002). The guidelines of world health organisation (WHO) for drinking water, classifies *Cryptosporidium* as a pathogen of significant public health importance (WHO, 2006).

2.5.1.1 Human-human transmission

The mechanisms of human transmitted infections are usually caused by direct person to person faecal-oral transfer, as illustrated by health care centres. Transmissions have been reported in day care centres, veterinarians, within households, in urban communities and in public institutions where there is poor hygiene or inadequate sanitary conditions leading to a high prevalence of cryptosporidiosis in developing countries (Robertson *et al*., 2000). Another risk factor that is generally found in these studies include sexual practices that imply oro-anal contact with diarrhoeic faeces or HIV/AID patients, which has lead to high yield exposure to *Cryptosporidium* world-wide (Alireza *et al*., 2009).

2.5.1.2Animal to human transmission

Over the past 20 years cattle have been identified as the main reservoir hosts that carry the parasite which was then transmitted to humans (Sulaiman *et al*., 1998). The role of zoonotic infections in human cryptosporidiosis in developing countries appears much more important than in the industrialized world since 60 – 90% of human infections in developing countries results from *Cryptosporidium parvum* (WHO, 2006). Mixed *Cryptosporidium* zoonotic transmission among *Cryptosporidium parvum* and *Cryptosporidium hominis* have improved our knowledge of understanding about the prevalence of zoonotic infections in humans as the leading cause of persistent diarrhoea in developing countries, and this has led to a major threat to the South Africa water supply. However, a review study on *Cryptosporidium spp* suggests that only the cattle genotype of *Cryptosporidium* is capable of zoonotic transmission whereas the roles of companion animals are considered less important (Xiao *et al.*, 2004).

There are also molecular epidemiological studies documenting transmission of *Cryptosporidium* between humans and livestock by direct contact through animal handling (Casemore *et al*., 1997). The high prevalence of infection in cattle and sheep and the high

number of oocysts shed by infected animals make cattle and sheep important sources of environmental contamination with *Cryptosporidium* oocysts that are able to infect humans. Waterfowl and insects are also known as transport vehicles of infection, where they pick up oocysts from contact with human or cattle faeces and may deposit them in water or on food (Graczyk *et al*., 2000; Szostakowska *et al*., 2004). However, the significance of waterfowl in contamination of watersheds with oocysts that may infect humans is unknown.

2.5.1.3 Transmission through water

Previous studies indicate that *Cryptosporidium* is the most frequently recognized cause of recreational water associated outbreaks of gastroenteritis, particularly in treated (disinfected) venues. The risk of waterborne transmission of *Cryptosporidium* is a serious global issue in drinking water safety. Oocysts from this organism are extremely robust, prevalent in source water supplies and capable of surviving in the environment for extended periods of time (Haas and Rose, 1994). The importance of waterborne *Cryptosporidium* transmission is evaluated based on the number of waterborne cases reported and on the various economic, regulatory, recreational, environmental and social aspects of waterborne disease control (Haas and Rose, 1994).

Outbreaks where many people are affected usually occur when surface water becomes contaminated through the discharge of untreated and treated sewage and run-off of manure (Juranek, 1995). Surveys have indicated that *Cryptosporidium* is found in all types of water sources, and their presence is more common in surface water than in groundwater (Glaberman *et al*., 2002). However, the highly environmentally resistant cyst of *Cryptosporidium spp* allows the pathogen to survive during water filtrations and chemical treatment such as chlorination. These levels may increase rapidly through contamination from agricultural and human effluents. In fact, the incidence of human cryptosporidiosis tends to

increase during the spring and summer seasons in South Africa (O'Donoghue, 2002). Currently, no curative therapy exists for the complete removal of *Cryptosporidium* from an infected host. Therefore, the presence of this parasite in drinking water represents a serious threat to public health (Glaberman *et al*., 2002). The relative importance of both human and agricultural pollution on surface water reserves has yet to be determined, but both sources have great potential for waterborne contamination, as high numbers of *Cryptosporidium* have been isolated from agricultural and human effluent in many geographical locations (Smith & Rose, 1998).

2.5.1.4 Transmission through food

The consumption of certain foods such as raw milk and meat, farm-made apple cider, fermented milk, salads and raw vegetables has resulted in cases of human cryptosporidiosis (Casemore *et al*., 1997). Food borne transmission, although not a major route of infection is possible if food becomes faecally contaminated. Moreover, infections have been associated with inappropriate food handling and/or using contaminated water to wash food (Quiroz *et al*., 2000). Interestingly, eating of properly washed raw vegetables has been found to be a protecting factor against *Cryptosporidium* in a case-control study in the United Kingdom (Hunter, 2003). This could serve as a route and build-up of protective immunity.

2.6 Factors Influencing Water Borne Transmission

Several factors facilitate waterborne transmission of *Crytosporidium*. These are considered below;

2.6.1 Resistance to chemical disinfection

Disinfection with chlorine has always been an important barrier for waterborne pathogens. However, the high resistance of *Cryptosporidium* oocysts against chlorine disinfection renders this process ineffective for oocysts inactivation in drinking water treatment (Ransome

et al., 1993). Chlorine dioxide is slightly more effective, but still requires a high measure of disinfectant dose (Ransome *et al*., 1993). However, exposure of *Cryptosporidium* oocysts to multiple disinfectants has been shown to be more effective than was to be expected from both disinfectants alone and synergism between environmental stress and filtration has also been observed to be effective (Liyanage *et al*., 1997). The multiple stresses that oocyst encounter in the environment and during treatment might limit the infectivity of oocysts. A previous study suggests that Ultra-Violet (UV) systems have a limited effect on *Cryptosporidium* viability, probably due to the use of *in vitro* viability assays that over-estimate infectivity (Clancy *et al*., 1998). More successive studies have shown that oocysts are sensitive to low or medium wavelength pressure Ultra-Violet (UV), but this is not proven yet (Craik *et al*., 2001).

2.6.2 Persistence in the environment

Oocysts can survive for months in surface water and estuarine waters for over 12 weeks at 20^0C but less in seawater (Robertson *et al*., 1992). However, under natural conditions, the survival rate of *Cryptosporidium* oocysts in water is very high. In other studies, survival was monitored with *in vitro* assays such as excystation or dye exclusion and it was confirmed that the longevity of oocysts in fresh water was high (King *et al*., 2005). A longer period of survival (120 days) of oocysts in soil has also been reported. Oocysts in soil do not survive well when artificially frozen/thawed in the soil or under field conditions in Norwegian soil (Kato *et al*., 2002).

2.6.3 Oocyst wall

The disulfide bond-rich oocyst wall provides a protective barrier for infective sporozoites (Mitschlere *et al.*, 1994). Unlike other coccidians, the oocyst wall of *Cryptosporidium* is bilayered, consisting of a distinct inner and outer layer. This structure perhaps represents a

unique feature of *Cryptosporidium spp* oocysts. However, the outer layer of the oocyst wall is comprised of acidic glycoproteins and is at least partially removed by treatment with sodium hypochlorite, an *in vitro* excystation solution (Reduker *et al*., 1985). Previous studies revealed that the rigidity and elasticity of the oocyst wall is a consequence of the central glycolipid/lipoprotein layer, and the thick inner filamentous layer, which is believed to be composed of glycoproteins (Bonnin *et al*., 1991).

Interestingly, researchers have examined the oocyst wall structure of *Cryptosporidium* and its susceptibility to different treatments. A filamentous array on the inner surface was seen by transmission electron microscopy following thin sectioning and negative staining of isolated oocyst walls. It was seen that this filamentous array can be depleted by digestion with proteinase K and trypsin, but pepsin was less effective. Ultrasonication of untreated oocyst walls produced almost no lysis. Treatment with proteinase K was the most effective in releasing the internal fibrillar layer from the oocyst wall, as compared to treatments with trypsin and pepsin. Chloroform treatment and phenol extraction did not disrupt the oocyst wall (Harris and Petry, 1999).

2.6.4 Small size

Compared to other protozoan parasites, the oocysts of *Cryptosporidium* are very small and are more difficult to remove during soil passage, in bank filtration and in rapid or slow sand filtration in drinking water treatment, thus making the organism a particular challenge to effective water treatment methods. Although, the state in which oocysts in water suspension are attached to particles is important for water treatment through boiling. Little information is available on oocyst attachment to sand particles in batch experiments (Dai and Boll, 2003).

2.6.5 High infectivity

The infectivity of oocysts has been shown to be very high, although uncertainty exists concerning the dose required to induce *Cryptosporidium* infection (Chappell *et al*., 1999). Studies suggest that very small inoculum are capable of inducing infection (Chappell *et al*., 1999). A comparative study completed a *Cryptosporidium parvum* human feeding investigation among healthy volunteers which determined that the dose at which 20 percent of the subjects were infected was 30 oocysts while the median infective dose of *Cryptosporidium parvum* in healthy adult volunteers was 132 oocysts (DuPont *et al*., 1995). Based on a mathematical model on data from previous outbreak, some individuals might develop cryptosporidiosis after ingestion of only one oocyst (Haas and Rose, 1999).

Similar studies employing different *Cryptosporidium spp* isolates recorded doses causing infection in fifty percent of the population from below 100 to 1000 oocysts (Okhuysen *et al*., 1999). Interestingly, an infectious dose did not significantly affect the severity of symptoms, length of the incubation, or number of oocysts shed (Okhuysen *et al*., 1999). The risk of infection following ingestion of one oocyst has been estimated at 0.028 (Okhuysen *et al*., 1999). Significant virulence differences exist between strains of *Cryptosporidium*, but this may not accurately describe the infectivity of other species. However, during acute infection, oocysts can be found in high numbers in the faeces of the host, although this is facilitated by auto-infection of the host.

2.6.6 Period of maturation

Unlike other coccidian parasites and helminths, *Cryptosporidium* oocysts do not require a period of maturation of the oocysts after shedding in faeces. They are immediately able to infect a new host when excreted by another (Okhuysen *et al*., 1999).

2.6.7 Environmental factors

Cryptosporidium oocysts can persist and survive in the environment for several days or months (Pokorny *et al*., 2002). In general, aged oocysts are more susceptible to disruption by environmental changes and disinfectants. Temperature has been shown to have an effect on the ability of oocysts to survive in water, including seawater especially at temperatures ranging from 4^0C to 22^0C. Under ambient conditions, the duration of oocyst infectivity decreases as the temperature increases from 4^0C to 23^0C (Pokorny *et al*., 2002). Warmer temperatures can accelerate oocyst degradation, although oocysts are known to remain infective for up to 12 weeks when stored in water at 25^0C. At extreme temperatures, oocyst viability and infectivity are adversely affected. As the oocyst wall is composed of numerous proteins, their denaturation at elevated temperatures may disrupt oocyst wall integrity and expose the sporozoites to conditions detrimental to their survival (Harris and Petry, 1999).

2.7 Pathogenesis of *Cryptosporidium* Species

Cryptosporidium possesses numerous surface glycoproteins thought to play a role in the pathogenesis of the disease. The mechanism involved in the invasion of *Cryptosporidium* sporozoites into epithelial cells is not clear. So far, the few antigens shown to be important for the attachment and invasion of *Cryptosporidium* into the host cell are all mucin-like glycoproteins such as Gp60 and Gp900 (Xiao *et al*., 2002). The Gp900 localizes at the apical end of sporozoites and in micronemes of merozoites where it attaches intimately to the microvillous membrane and causes loss of microvilli and effacement, which results in malabsorption (Xiao *et al*., 2002). The organism immediately activates a second-signal pathway, such as the nuclear factor-kappa B (NF-κB) and Proto-oncogene protein pp60 (C-src) systems. Activation of (NF-κB) induces the production of cytokines and chemokines, such as interleukin-8 (IL-8), to trigger an inflammatory reaction and stimulates anti-apoptotic survival signals in directly infected cells (presumably facilitating the organism's ability to

survive and propagate). Activation of C-src is associated with host-cell cytoskeletal reorganization and perhaps dysfunction of tight junctions (Cevallos *et al*., 2002). Human immunodeficiency virus (HIV) type 1 infection can further amplify damage by the action of soluble factors, such as tat protein with varying degrees of villous atrophy by an unknown mechanism, resulting in epithelial damage.

Histological findings of severe villous atrophy, crypt hyperplasia and presence of several developmental stages of the organism on the mucosa have been found associated with an altered glucose-stimulated Na+, water absorption, and increase in Cl− secretion (Argenzio *et al*., 1990). However, glucose stimulated sodium absorption was inhibited, thereby increasing chlorine secretion, and epithelial cell damage. Therefore it is still thought that diarrhoea is a combination of secretory sodium/glucose malabsorption due to villous atrophy and epithelial damage.

Immunological and molecular methods have identified several possible virulence factors which may affect the adhesion of *Cryptosporidium* to the host intestinal epithelial layer and those factors like ATP transport to cause membrane disruption (Riggs *et al*., 1997). These events could ultimately lead to malfunctioning in the small intestine epithelium, induced by *Cryptosporidium parvum*-infected individuals. Villi often are blunt, shorter, and wider than normal and are sometimes fused to other villi, whereas cryptosporidium are elongated and hyperplasic. Changes in villous and crypt structures may be accompanied by an inflammatory infiltrate consisting of lymphoid cells, macrophages, and neutrophils in the underlying lamina propria (Xiao *et al*., 2001).

2.8 Clinical Features

The symptoms of cryptosporidiosis differ greatly between immunocompetent and immunocompromised hosts. In the immunocompetent patient, the disease is acute and self-

limiting, producing diarrhoea illness that typically occurs for one to two weeks (Smith and Corcoran, 2004). Children are much more susceptible to *Cryptosporidium* infections than other age groups, probably due to their immunological immaturity (Smith and Corcoran, 2004). However, infection takes advantage of the impaired or destroyed immune system, causing a persistent infection that seriously dehydrates the patient as up to 20 litres of excreta are passed out per day which ultimately results in a higher mortality rate (Keusch *et al*, 1995). The most common clinical signs of *Cryptosporidium* infection are profuse and watery diarrhoea, crampy abdominal pain, fever, nausea, vomiting and low grade fever (Smith and Corcoran, 2004). A plethora of such signs is often associated with a marked weight loss. Patients can have chronic diarrhoea that can last for more than two months, shedding oocysts in stool during the entire period. This may lead to severe dehydration, weight loss, extended hospitalizations, and mortality (Shin *et al*., 2001). Thus, the duration and severity of clinical signs reflect the immune status of the patients.

Due to the immunological deficiency, HIV/AIDS patients are at a high risk of contracting cryptosporidiosis. In such patients, infection produces persistent diarrhoea that worsens with time and eventually contributes to death. Infections are not always confined to the small intestine and parasites have been found in the oesophagus, stomach, appendix, colon and rectum (Smith and Corcoran, 2004).

2.9 Diagnosis

Laboratory techniques are usually applied for identification of *Cryptosporidium*. The need to identify the organism in diarrhoea patients is useful to avoid misuse of drugs, to reduce the spread of the disease in a community, as well as to follow the need for control and preventive measures. For many years, microscopy has been the only tool available for the detection

of*Cryptosporidium* parasites through inspection of blood smears, tissue specimens, faeces, lymph node aspirates, bone marrow, and even cerebrospinal fluid (Cruz *et al*., 2006).

Diagnosis of cryptosporidiosis is made microscopically by examination of stool samples for the presence of oocysts. Mature *Cryptosporidium* oocysts recovered from stool can be easily identified using differential staining methods such as safranin-methylene blue stain, modified Kinyoun‘s acid-fast method, Ziehl-Neelsen and DMSO-carbol fuchsin stain which stain oocysts red and counterstain the background to blue (Cruz *et al*., 2006). However, sample preparation for direct observation is time-consuming, labour intensive, and proper diagnosis depends on qualified laboratory technicians (Cruz *et al*., 2006). Because detection of *Cryptosporidium* is difficult, patients may be asked to submit several stool samples for identification. In endemic regions, where resources are limited, this proves to be difficult and misdiagnosis can significantly affect a patient. In reality, all major intestinal helminth infections are still solely dependent on microscopy for diagnosis. As for other parasite infections, many are confirmed by the use of microscopy in conjunction with other methods of diagnosis including serology-based assays and more recently molecular-based assays. Evaluation of *Cryptosporidium* infection by serology is much more sensitive than detection of oocysts in stool samples, especially in populations chronically exposed to *Cryptosporidium* through contaminated food or drinking water, and has been widely used for epidemiological studies (Moro and Schantz, 2009). Serology-based assays include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), and all its derived tests such as the Falcon assay screening test ELISA (FAST-ELISA) and the dot-ELISA. Other assays include the hemagglutination test, indirect or direct immunofluorescent antibody (IFA) or Direct immunofluorescent antibody (DFA) tests, complement fixation (CF) test, and immunoblotting and rapid diagnostic tests (Moro and Schantz, 2009). Although the ease of use and turn-around times for serologic assays are similar to microscopy, it becomes

important for individuals exhibiting low-parasitamia and/or who are asymptomatic (Moro and Schantz, 2009).

Finally, having these tests readily available allows for the monitoring of parasite clearance following therapy. However, cross-reaction leading to false-positives and misdiagnosis is a problem, especially in Africa regions where more than one parasite is endemic (Burbelo *et al*., 2008). Furthermore, antibody-detection tests cannot be used in parasitic infections that do not develop a significant antibody response. This has been observed in some individuals carrying *Echinococcus* cysts (Moro and Schantz, 2009). For all these reasons, there is still a need to improve on the current diagnosis approaches available. Since the advent of the polymerase chain reaction (PCR), parasitologists have turned to molecular-based approaches in the hope to better the existing diagnosis tools (Diez *et al*., 2007).

The limitations of microscopy and serology-based assays have influenced parasitologists towards the use of gene amplification methods made possible with the advent of polymerase chain reaction. This technique offers many advantages over other techniques for the clinical and environmental detection of *Cryptosporidium species* and is capable of differentiating the taxonomic membership between *Cryptosporidium* strains that infect humans. Cost-effective and amenable to quantitation, PCR allows for the analysis of multiple samples in a short time frame. Besides the traditional PCR, including nested and multiplexed PCR, the implementation of the quantitative PCR (q-PCR) for the detection of several parasitic infections have also emerged as possible new approaches for the diagnosis of parasitic diseases (Gasser, 2006).

Molecular-based approaches based on nucleic acids have been found to offer greater sensitivity and specificity over the existing diagnostic tests (Zarlenga and Higgins, 2001). They permit the detection of infections from very low parasitized samples including those

from asymptomatic patient‘s samples (Zarlenga and Higgins, 2001). Moreover, multiplexed PCR allows for the detection of multiple sequences in the same reaction tube proving useful in the diagnosis of several parasitic infections simultaneously (Gasser, 2006). The q-PCR system, unlike the conventional PCR, allows for the quantification of the original template‘s concentration through the use of various fluorescent chemistries, such as Sybergreen, Taqman probes, fluorescence resonance energy transfer (FRET), and Scorpion primers (Muldrew, 2009). This eliminates the need to visualize the amplicons by gel electrophoresis thereby greatly reducing the risk of contamination and the introduction of false-positives (Muldrew, 2009). Running the multiplex assay not only reduce the cost per test but also allow for a rapid turnaround time, in a single reaction tube even in very low parasitized samples and it allows for the high-throughput analysis of different sequences in one single-closed tube reaction. It is a clear advantage over microscopy which is labour intensive and time-consuming with slow turnaround times especially during high-throughput settings, these examples demonstrate that efficient and early diagnosis can directly impact patients care and that PCR-based approaches have the potential to help in making the right choice for treatment.

Although DNA-based methods have shown excellent sensitivity and specificity, the introduction of these methods in daily laboratory practice is still uncommon, especially in rural endemic regions. In addition, as observed with many serology-based assays, PCR-based methods also suffer by the lack of standardization (Murray and Cappello, 2005). Deoxyribonucleic acid extraction, choice of primer sets, and use of various amplification protocols are all factors that may cause diversification in results. Adding an automated DNA extraction step would certainly improve PCR assays for use in the diagnosis of parasitic diseases. However, the presence of inhibitors, sample contamination and precipitation of

oocyst nucleic acids represent challenges facing the current PCR techniques. A shortcoming of PCR is that it does not directly assess viability (Toze, 1999).

2.10 Treatment

Currently, no drugis known to be effective in treating the recurrence of cryptosporidiosis infection, 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 related parasites. This unique parasitophorous vacuole may somehow shelter the parasite from effects of drugs (Griffiths *et al*., 1998). Regardless of the significance of *Cryptosporidium* infection in childhood health, there has been a lack of serious effort to invest in the development of affordable and effective therapeutics specifically targeting *Cryptosporidium* species. Existing therapeutics for other apicomplexan diseases are ineffective against *Cryptosporidium* infection, perhaps because of the unique intracellular location of *Cryptosporidium* and the poorly understood host-parasite interface. In addition, difficulties in laboratory ideal cell culture methods, have limited drug screening. Clinical course of cryptosporidiosis depends largely on the immune status of the host, treatment with options vary accordingly (Griffiths *et al*., 1998). Healthy persons with normal immune systems usually get better on their own. The recommended treatment is to drink plenty of fluids and to get extra rest. In immunocompetent adults and children, no specific therapy is indicated, since the disease is self-limiting. Individuals with persistent diarrhoea, an underlying immunodeficiency (HIV, congenital immunodeficiency, among others) might need special treatment from a health-care provider to replace fluids lost during the illness.

However, some encouraging results following the use of supportive therapy remains the preferred option for the management of symptoms arising from the disease. Several drugs

have been tested in the laboratory. An acknowledged report suggests that paromomycin is an antimicrobial drug that can alleviate cryptosporidiosis symptoms with a reduction of oocyst excretion (Steiner *et al*., 1997). Paromomycin is typically administered at a dose of 500 mg four times daily for 4–8 weeks, with a recommended maintenance dose of 500 mg twice daily following the initial treatment period (Hoepelman, 1996). However, clinical evaluations of these drugs have been disappointing, mainly because they failed to clear the parasite from patients with HIV/AIDS. To date, nitazoxanide (NTZ) has been shown to be the most promise against *Cryptosporidium.* Nitazoxanide, also known as Alinia, is an antiprotozoal drug believed to interfere with the pyruvate ferredoxin oxidoreductase-mediated electron transfer reaction that is essential for anaerobic energy metabolism in the parasite (Herbert and Hoffman, 2002). Presently, nitazoxanide is the only drug approved by the USA Food and Drug Administration (FDA) for the treatment of diarrhoea caused by *Cryptosporidium* in children. The drug was found to be effective in AIDS-related cryptosporidiosis during a double-blind placebo-controlled trial of 66 HIV patients in Mexico (Rossignol *et al*., 1998). When administered at a dose of 500 or 1000 mg twice daily for 14 days, nitazoxanide resulted to a cure rate of 63% and 67% of patients, respectively.

2.11 Prevention

As cryptosporidiosis is initiated by ingestion of infective oocysts, therefore control measures are aimed at reducing or preventing oocyst transmission. In locations such as hospitals, laboratories and day-care centres, contact with potential sources of infection should be minimized. This entails sound personal hygiene, proper handling and disposal of bio-hazardous wastes, and boiling of water prior to consumption. Individuals with weakened immune system are advised to always use a home micro-straining water filter, capable of removing particles less than 1 micrometre in size. Avoidance of sexual practices that can result in hand or mouth exposure to stool, along with direct exposure to cattle and other farm

animals is important. If exposure cannot be avoided, washing hands well immediately after contact is recommended (Roberson and Bruno, 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Abuja, the Federal Capital of Nigeria. It is also the seat of the Federal Capital Territory authority (FCT). The Territory was formed in 1976 from parts of former Nasarawa, Niger and Kogi States with the express purpose of supporting Abuja city encircling it within a womb of nature.

The territory is located just north of the confluence of the River Niger and Benue River. Abuja is bordered by Niger state to the North-west, Kaduna state to the Northeast, Nasarawa state to the east and Southeast and Kogi state to the Southwest. Abuja is geographically located in the centre of the country, it lies between latitude 8.25'N and 9.20'N, and longitudes 6.45' and 7.39'E. Based on the Nigerian National census of 2006, the FCT has a population of about 1,405,201 people and a landmass of approximately 7,315km^2, situated within the northern Guinea savannah zone with moderate climatic conditions. The FCT is made up of six Area Councils, comprising Abuja municipal, Abaji, Bwari, Gwagwalada, Kuje, and Kwali Area Councils (Figure 3.1).

Abuja climate features a tropical wet and dry climate. The FCT experiences three weather conditions annually. They include a warm, humid-rainy season and a blistering dry season. The annual total rain fall is in the range of 1100mm to 1600mm.

The samples were collected from three markets in Abuja that included Kubwa, Karu and Wuse.

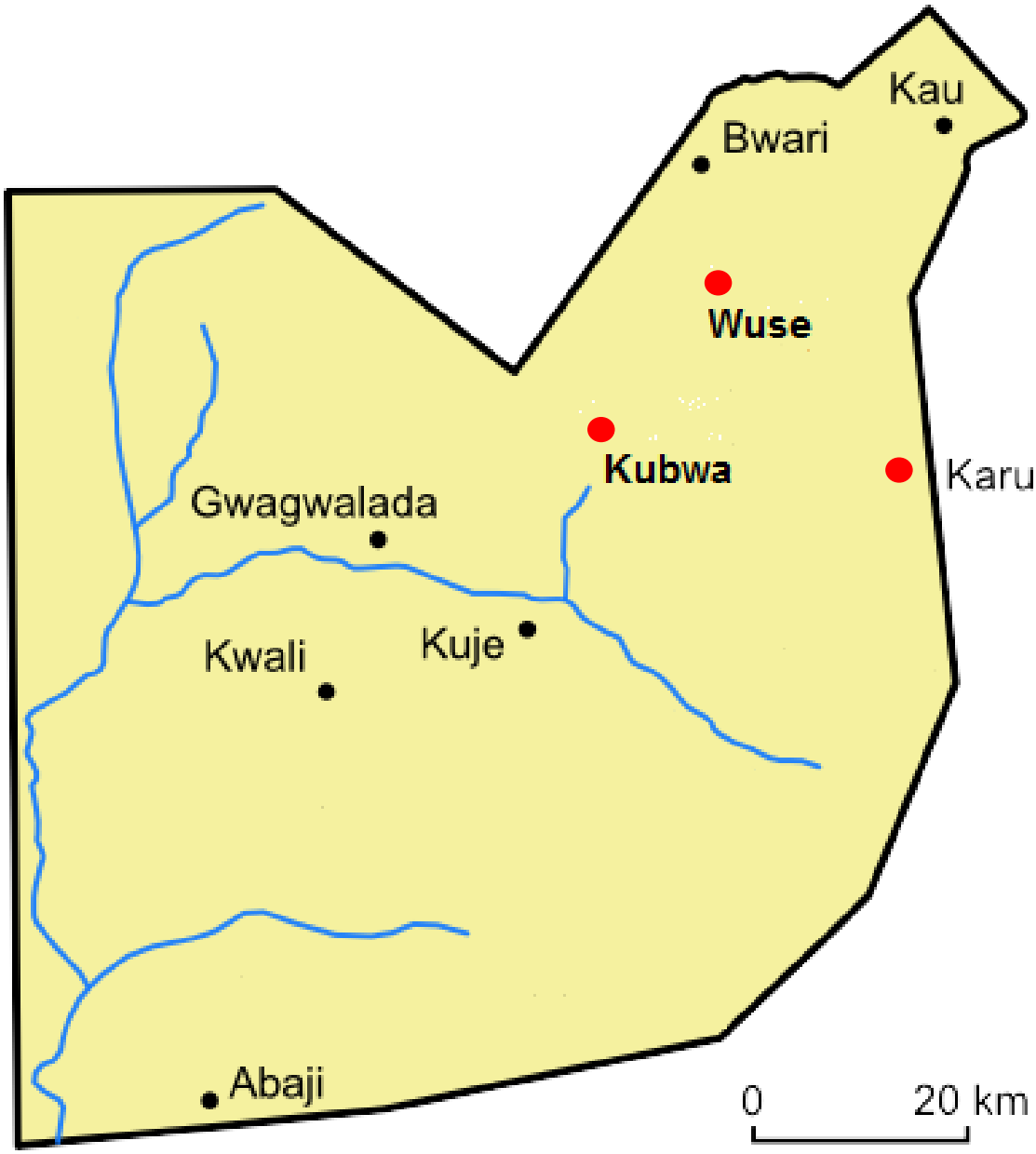


Figure 3.1 Map of Federal Capital Territory, Nigeria showing the sampling sites.

3.2 Collection of Vegetable Samples

The samples were collected at randomfrom Kubwa, Karu and Wuse markets in Abuja.. One hundred and fifty vegetable samples were collected from each market (thirty for each vegetable). Samples were collected during the morning hours into clean polythene bags and were transported to the heminthology laboratory in the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria.Samples were then processed for the detection of *Cryptosporidium* oocysts.

Table 3.1 Common name, botanical name and nature of vegetables collected.

Common name	Botanical name	Nature of vegetable
Tomato	*Lycopersicum esculentum*	Fruit
Lettuce	*Lactuca sativa*	Leafy
Spinach	*Spinacea oleracea*	Leafy
Cabbage	*Brassica oleracea var capitata*	Leafy
Carrot	*Daucus carota var sativa*	Root

3.3 Laboratory Isolation of the Oocysts

Each sample was treated according to the protocol described by Ortega *et al*. (1997) as follows. One hundred grams of each sample collected was placed into plastic containers and washed with 250ml physiological saline solution (0.95% NaCl). The wash was left in the plastic container for 24 hours to sediment. The supernatant was discarded and the residue was transferred into a centrifuge tube and spun for 5 minutes at 365 Rcf (relative centrifuge force). The supernatant was discarded and the residue was agitated gently in sucrose flotation medium of 1.21 specific gravity. Each test tube was then topped to the brim with the flotation medium to form a meniscus. A cover slip was placed on the test tube for 3 minutes and this was removed and placed on a clean glass slide and viewed under the microscope (x40) for the presence of the parasitic oocysts. Oocysts-positive slides were allowed to air-dry and stained using modified Ziehl –Neelsen technique (WHO, 1999) by fixing the air- dried

slides in methanol for 2-3 minutes. The slides were flooded with cold carbol fuschin for 5-10 minutes and then with 1% hydrochloric-acid ethanol until colour ceased to flow out and rinsed in tap water. It was then counter- stained with 0.25% methylene blue for 30 seconds and then rinsed in tap water again and air-dried.

3.4 Identification of *Cryptosporidium* Oocysts.

Each slides were examined using a light microscope at ×40 and ×100 magnification. The cryptosporidial oocysts appeared as bright pink spherules. Oocysts were confirmed under a higher power magnification (× 100).

3.5 Assessment of Predisposing Factors

The risk factors such as source of vegetables, manure, water, and transportation, duration of harvest and sale etc. were assessed with the aid of questionnaires (appendix ix).

3.6 Data Analysis

Data was analyzed using SPSS version 17 (Inc. Chicago., USA). Chi-square analysis was used to calculate the significant difference between the prevalence of *Cryptosporidium* on vegetables in different markets. $P \leq 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1: Prevalence of *Cryptosporidium* Oocysts Based on Vegetable Types Sampled.

Nineteen (4.22%) of the 450 vegetables sampled during the study were positive for *Cryptosporidium* oocysts. All the 5 types of vegetable examined were positive for *Cryptosporidium* oocysts. However, lettuce had the highest prevalence (5.56%) followed by spinach and carrots (4.44%) each, while tomatoes and cabbage had a prevalence of 3.3% each. There was no significant difference between occurrence of *Cryptosporidium* oocysts and type of vegetables examined (Table 4.1). The identified *Cryptosporidium* oocysts in the various vegetables sampled are presented in plates I to IV.

4.2 Prevalence of *Cryptosporidium* Oocysts in Vegetables Sold in Abuja Markets

Table 4.2 shows the occurrence of*Cryptosporidium* oocysts recovered from vegetables sold in different markets in Abuja. Seven (4.67%) of the vegetables examined from Wuse and Karu markets were positive for *Cryptosporidium* oocysts respectively, and 5 (3.33%) were observed for Kubwa market. There was no significant difference between occurrence of *Cryptosporidium* oocysts and the markets in which the vegetables were sold.

4.3 Prevalence of *Cryptosporidium* Oocysts based on Months Sampled

Table 4.3 shows monthly variation (August, September, November, and December) of *Cryptosporidium* oocysts on vegetables examined from the different markets. Vegetable samples had the highest contamination with *Cryptosporidium* oocysts in December (6.19%) followed by those in September (5.3%). Vegetable samples in August and November had a contamination rate of 2.68% and 2.65% respectively. There was no significant difference between the monthly prevalence and the occurrence of *Cryptosporidium* oocysts .

Table 4.1: Prevalence of Cryptosporidium oocyts based on the type of vegetables sampled in Abuja, Nigeria.

Vegetables	**Total No. Sampled**	**Vegetable With *Crypt.* (%)**
Cabbage	90	3 (3.33)
Carrot	90	4 (4.44)
Lettuce	90	5 (5.56)
Spinach	90	4 (4.44)
Tomatoes	90	3 (3.33)
Total	**450**	**19 (4.22)**

$X^{2 =}$ 0.7693 P =0.9425 df =4

Table 4.2 Prevalence of *Cryptosporidium* oocysts based on markets sampled in Abuja, Nigeria.

Markets	**Total Sampled**	**No Of Vegetables With *Crypt*. (%)**
Karu	150	7 (4.67)
Kubwa	150	5 (3.33)
Wuse	150	7 (4.67)
Total	**450**	**19 (4.22)**
$X^2 = 0.4396$	P = 0.8027.	df =2

Table 4.3: Prevalence of *Cryptosporidium* oocysts based on monthly samplingin Abuja, Nigeria.

Months	**Total No. Sampled**	**No. With *Crypt*. (%)**
August	112	3 (2.68)
September	112	6 (5.36)
November	113	3 (2.65)
December	113	7 (6.17)
Total	**450**	**19 (4.22)**
$X^{2 =}$ 2.790	P = 0.4251	df =3

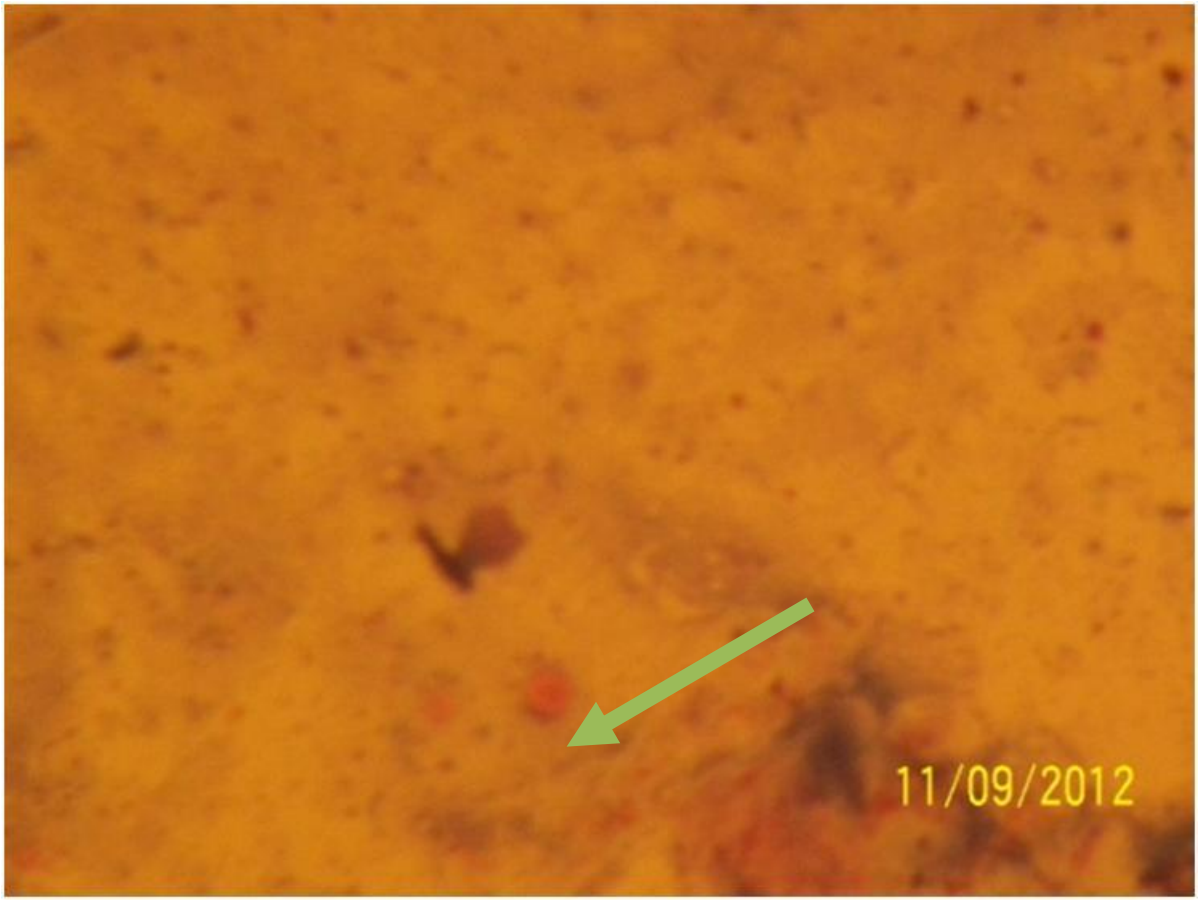


Plate I: Photomicrograph of *Cryptosporidium* oocysts (arrow) (x400 Magnification) isolated from lettuce



Plate II: Photomicrograph of *Cryptosporidium* oocysts (arrow) (x400 Magnification) isolated from spinach

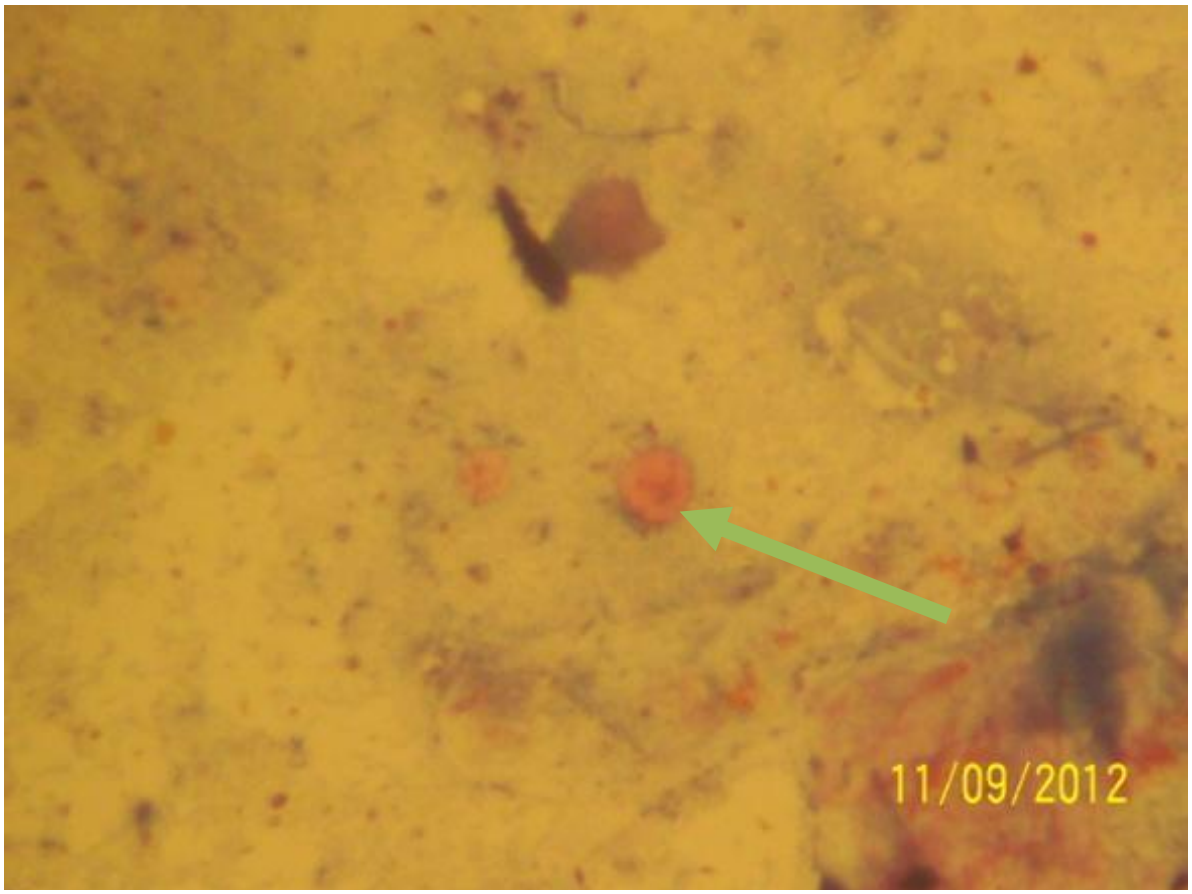


Plate III: photomicrograph of *Cryptosporidium* oocysts (arrow) (x400 Magnification) isolated from tomatoes



Plate IV: Photomicrograph of *Cryptosporidium* oocysts (arrow) (x400 Magnification) isolated from cabbage

4.4 Other Parasite Oocysts and Eggs Detected from theVegetable Samples

Other parasites oocysts and eggs that were also detected from this study and which could also cause diseases, if ingested are presented in Table 4.4.These parasites include mite eggs 11 (2.44%), coccidian oocysts 10 (2.22%), Nematode larva 6(1.33%) and *Entamoeba histolytica* cysts 1 (0.22%). These parasites were detected in tomatoes, cabbage, carrot and spinach respectively. Plate V to VIII present the various parasites detected.

4.5 Sources of Vegetables, Water, Transportation andManure.

A total of 45 questionnaires were served to vegetable sellers and used to assess the sources of vegetables sold in the market, water used in washing or rinsing the vegetables in the markets, transportation and source of manure. Of these, 26 (57.8%), 13 (28.9), and 6(13.3%) respondents were from markets, commercial farms and personal farms respectively (Fig 4.1 and Appendix VI). Furthermore, the water used for washing the vegetables in the markets was from tap water 17 (37.8%), borehole 12 (26.7%) or well 16 (35.6%). (Fig 4.1 and Appendix VII).

The vegetable farms were fertilized using cow dung and poultry manure with 9(69.3%) using cow dung while 4 (30.7%) used poultry manure (Table 4.5). The vegetables were transported to the markets using vehicles 22(48.9%), motorcycles 17 (37.8%) and wheel barrow 6(13.3%) (Table 4.6).

The period between harvest and sending the vegetables to the markets was captured for vegetables from personal farms (Table 4.7).The vegetables were either taken to the markets a day after harvest by 11(84.6%) farmers or two days after harvest by 2(15.4%) of the farmers.

Table 4.4: Prevalence of other Parasites Oocysts, Cysts and Egg Found on the Vegetables in Abuja, Nigeria.

Parasites	**No. found (%)**
Mite egg	11(2.44)
Nematode larva	6 (1.33)
Coccidia oocysts	10 (2.22)
E.histolytica cysts	1 (0.22)
Total	**28 (6.21)**

Table 4.5 Source of manure for personal Vegetable farmers in Abuja, Nigeria.

Source of manure	Frequency	Percentage (%)
Cow dung	9	69.3
Poultry	4	30.7
Others	0	0
Total	**13**	**100**

Table 4.6 Means of transporting Vegetables to the market in Abuja, Nigeria.

Source of transportation	Frequency	Percentage (%)
Motor cycle	17	37.77
Vehicle	22	48.88
Wheel barrow	6	13.33
Total	**45**	**100**

Table 4.7 Duration between harvest and sale of vegetables in Abuja, Nigeria.

Duration (day)	Frequency	Percentage (%)
One	11	84.6
Two	2	15.4
Others	0	0
Total	**13**	**100**

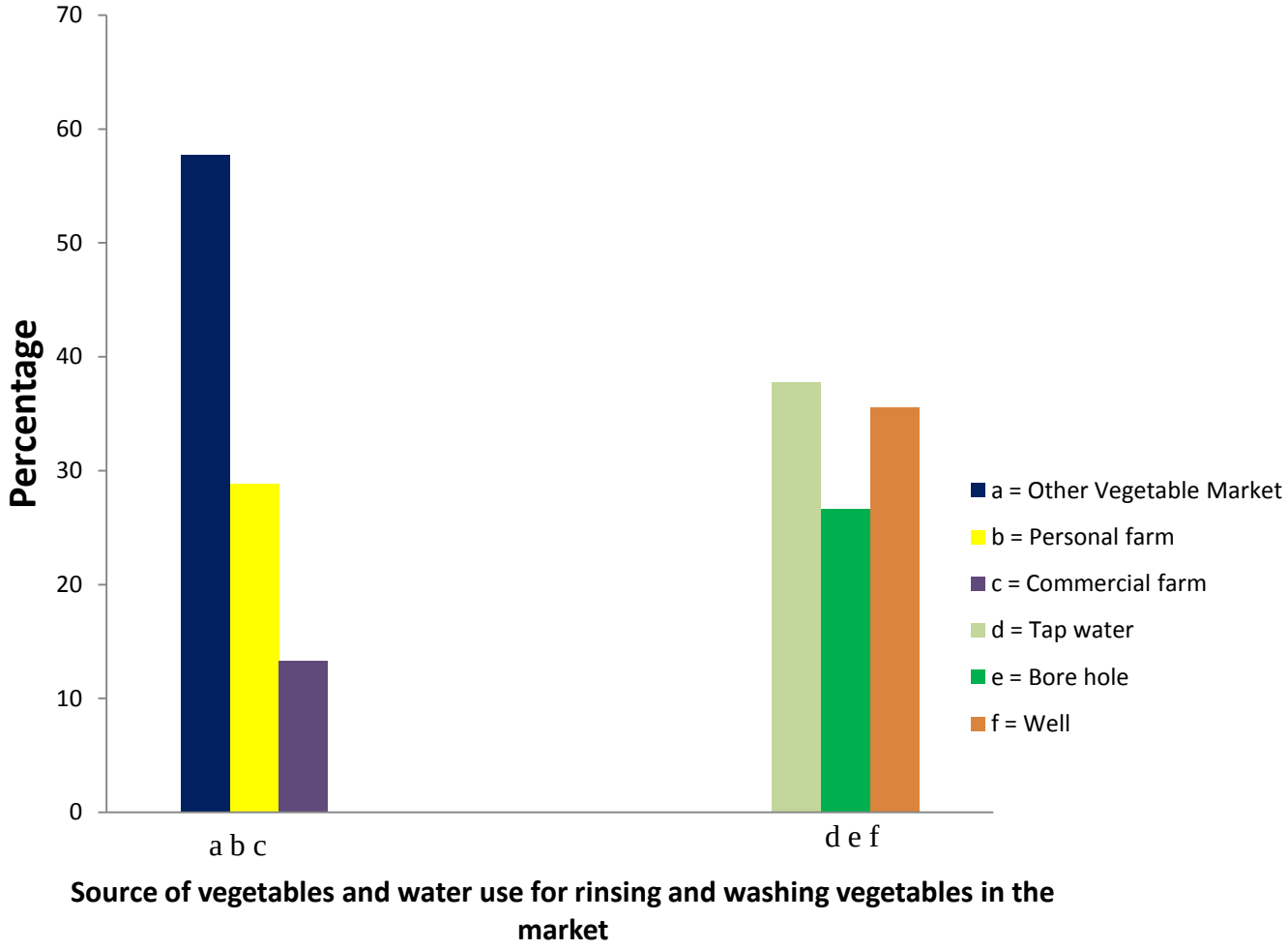


Figure 4.1: Sources of vegetables and water used for washing vegetables in the markets.

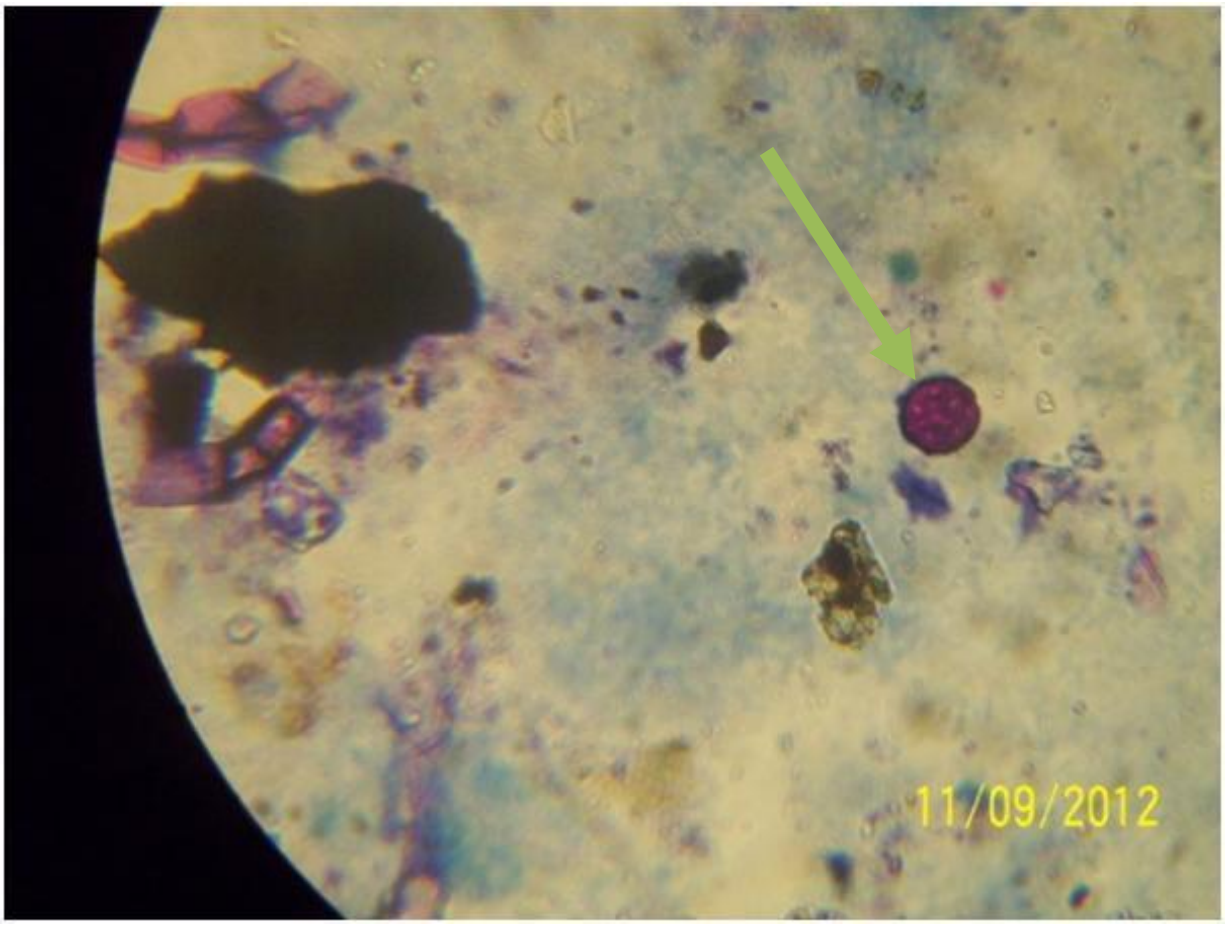


Plate V: photomicrograph of *E. histolytica* (arrow) (x400 Magnification) isolated from spinach



Plate VI: photomicrograph of mite egg (arrow) (x400 Magnification) isolated from tomatoes.

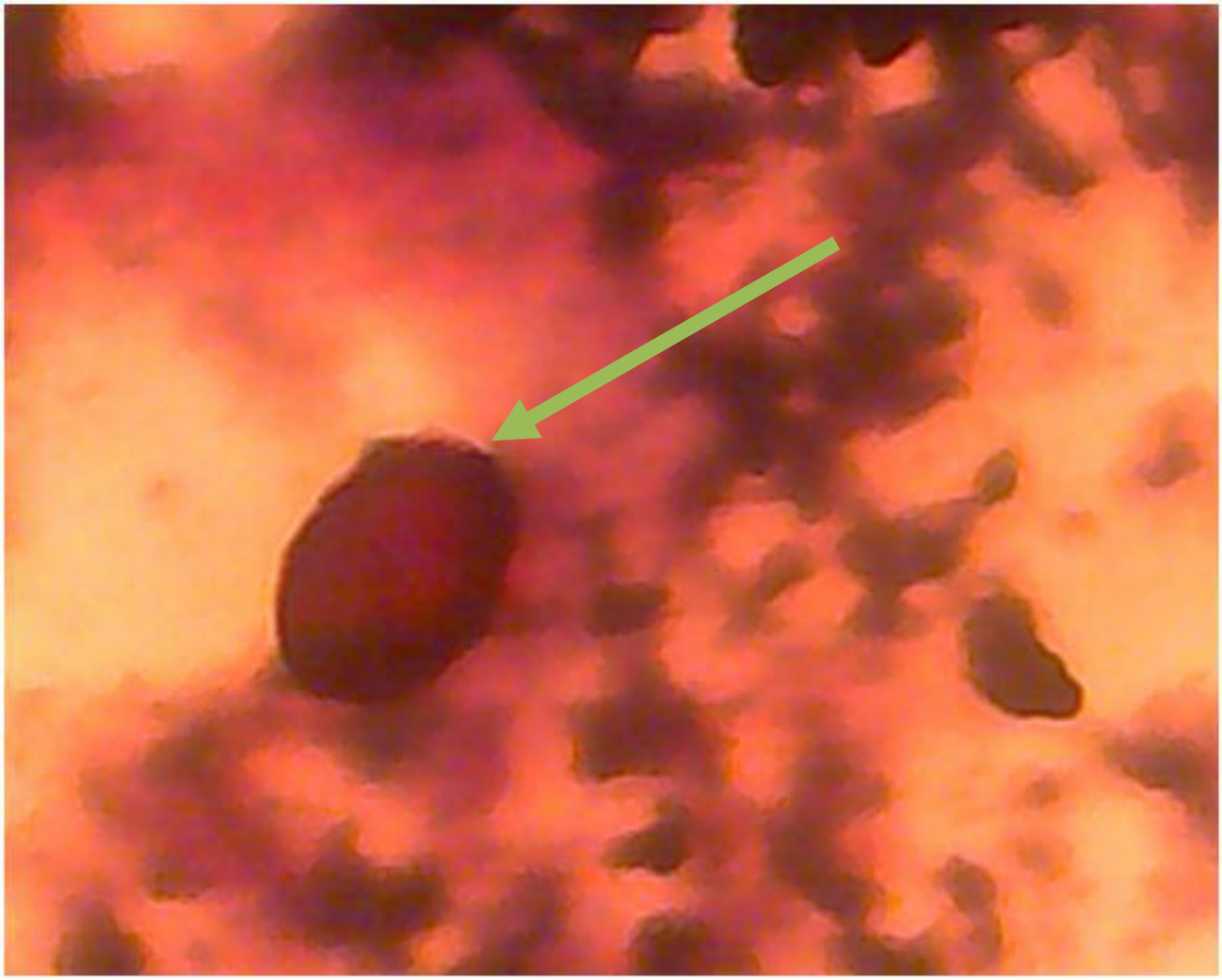


Plate VII: Phomicrograph of Eimeria oocysts (arrow) (x400 Magnification) isolated from cabbage

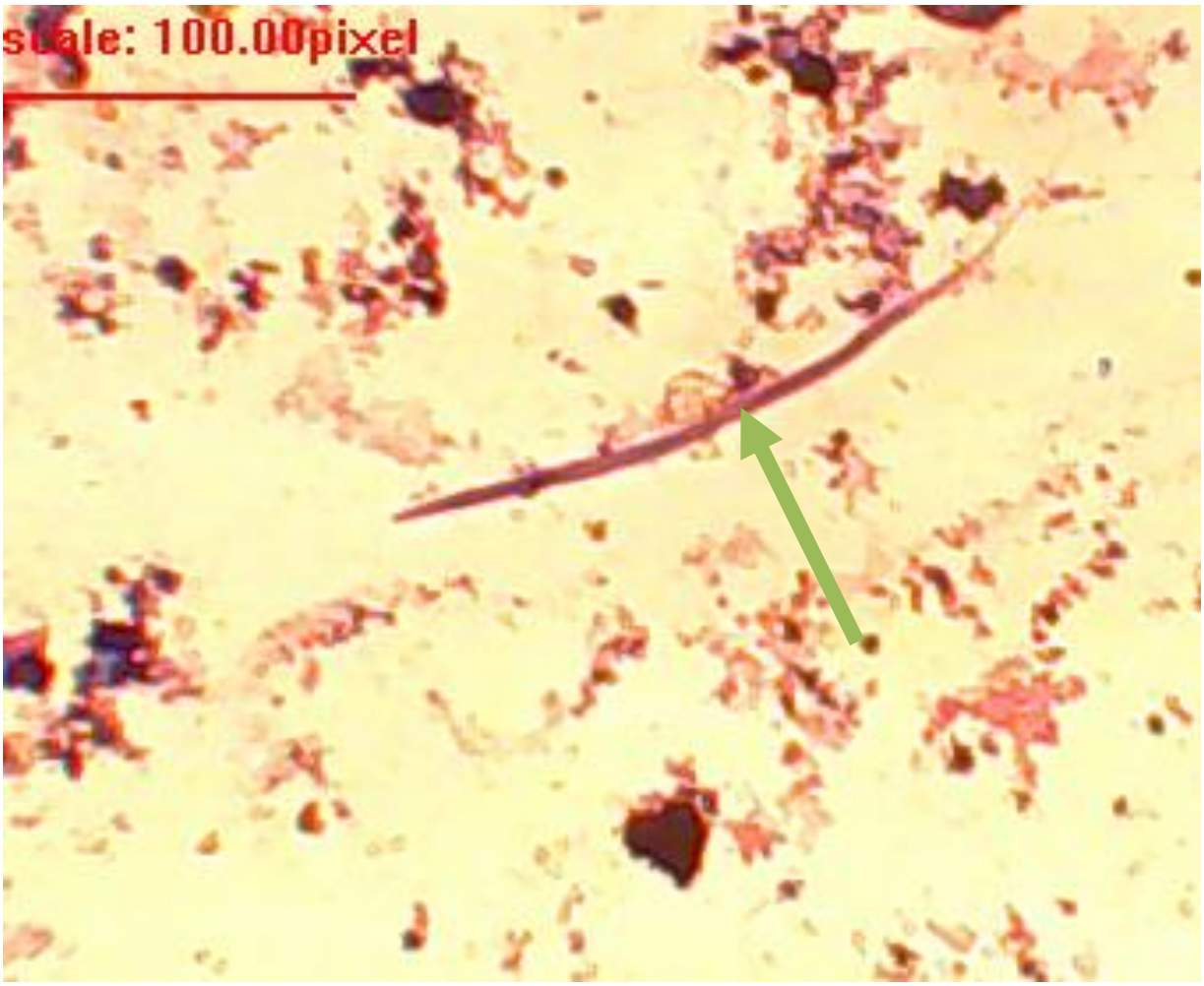


Plate VIII: Photomicrograph of nematode larva (x400Magnification) isolated (arrow) from spinach

CHAPTER FIVE

DISCUSSION

From the study, all the vegetables were shown to harbour the organism at more or less same prevalence level. This suggests a risk potential for those who are in the habit of eating them especially raw. The fact that salad eating is commonly practised by public servants and low income earners, especially traders it means that these group of individuals may be at greater risk of the infection than others. The risk is even higher for those that patronize local restaurants who are not in the habit of washing such vegetables properly before serving them as salad in food as reported by Ozlem and Sener, 2005. The occurrence level of 4.22% as recorded in this study is lower than the 14.5% reported by Ortega *et al*. (1997) on the vegetable markets of the semi- urban slums of Peru. The difference in the contamination rates may be due to the poor hygiene practices and the method of handling the vegetables. Again the study of Ortega was in a semi-urban slum markets.

The high occurrence of *Cryptosporidium* oocysts in lettuce may be due to its broad leaves which may enhance the entering of a higher number of oocysts from contaminated sources (Robertson and Gyerde, 2001). Contamination in carrot may be that as a root crop it is continuously exposed to soil contaminants (Robertson and Gyerde, 2001). Again the contamination in spinach may be from the manure used in fertilising the soil (Glaberman *et al*., 2002) while the overlapping nature of the leaves of cabbage may sieve contaminated water thereby attracting more of the parasite (Robertson and Gyerde, 2001).

Cryptosporidium oocysts were identified in vegetable samples from all the three markets studied. This has indicated a wide spread potential for the parasites in these markets. It is an indication that the organism may be wide spread in the area under study. Monge and Arias, 1996, in Costa Rica and Ortega *et al,* 1997 in Peru reported that the organism has common

occurrence on vegetables. The fact that all the markets recorded positive samples might indicate that the farmers farming practices could be similar.

From the study, the organism was isolated from the vegetable samples during all the months of the study, though with higher occurrence in the month of September and December. The high incidence in these two months tends to indicate a higher exposure potential, especially for children at boarding schools and during Christmas festivals. Children are among the high risk group for *Cryptosporidium* infection and those less than five years of age are more susceptible (Guerrant *et al* ., 2004). The high incidence of the parasites in December could be due to the fact that it coincided with the dry season and such vegetable farms can only get their water through irrigation and not rain fall. This further incriminates irrigation water since such water is from effluents from the sewage from the communities.

The finding of other parasites apart from *Cryptosporidium* oocysts has further exposed the risk of the consumption of these parasites in addition to *Cryptosporidium* spp. These vegetables could lead to individuals coming down with helminth infection should such vegetable be highly contaminated with these nematode eggs or larvae. Studies in Nigeria have shown the prevalence of helminths infection to be high (Ejezie, 1981). Kang *et al.*, (1998) reported intestinal parasites to be widely prevalent in developing countries, due to poor sanitation and inadequate personal hygiene. The eggs of these helminths could have been from soil as a result of contamination from human and animal wastes. Soil is a basic problem in the transmission of parasitic infection as larvae are developed in the soil until they become infective (Gharavi et al., 2002).

The *Cryptosporidium* oocysts identified in the vegetables from the study could have come from the water or manure used in the vegetable farms. The water from the wells could have been contaminated as it is common for people to defaecate in the bush and with rain water

flooding around it could easily run into such wells and where such stool was contaminated could result in contaminating the water from the wells with the *Cryptosporidium* oocysts. The fact that Dai and Boll, (2003) reported that these parasites are small they could find their way into tap and well water and therefore be sources of contamination of such vegetables. Surveys have indicated that *Cryptosporidium* oocysts are found in all types of water sources and their presence is more common in surface water than in ground water (Glaberman *et al*., 2002).

More importantly, the cow dung and poultry manure could also be incriminated to harbour the organism since Glaberman *et al,* (2002) reported identifying the organism in cattle and poultry manure. To prevent Cryptosporidium infection vegetables individuals should adopt strict personal hygiene and avoid indiscriminate defaecation. Also consumers of raw vegetables should thoroughly wash vegetables before consumption.

There is therefore the need to conduct more detailed study on the exposure rate of the various operators of the vegetable production chain with a view to finding a better solution for controlling the exposure to the parasites.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study has shown that vegetables sold within Abuja markets, Nigeria were contaminated with *Cryptosporidium* oocysts. The study also showed that the more populated and more patronized Wuse and Karu markets were more contaminated with *Cryptosporidium* oocysts than Kubwa market. The study established that vegetables obtained in the months of December and September have a higher rate of contamination with *Cryptosporidium* oocysts than in the months of August and October. The finding of contamination of vegetables with oocysts from Abuja markets, Nigeria is of great public health importance.

6.2 Recommendations

i. *Cryptosporidium* oocysts that contaminate vegetables should be identified to species level by using molecular techniques to determine the specific type contaminating vegetables.

ii. There is the need to conduct further studies to determine the level of contamination of well, tap and bore hole water with *Cryptosporidium* sp with a view to advising vegetable farmers more appropriately on how to protect their vegetable farms.

iii. Vegetable farmers, sellers and the general public should be educated on the effects of the use of contaminated water on vegetable farms while consumers should thoroughly wash vegetables before consumption.

iv. Government should educate the general public on the hazards of indiscriminate defaecation and enjoin them to adopt strict personal hygiene.

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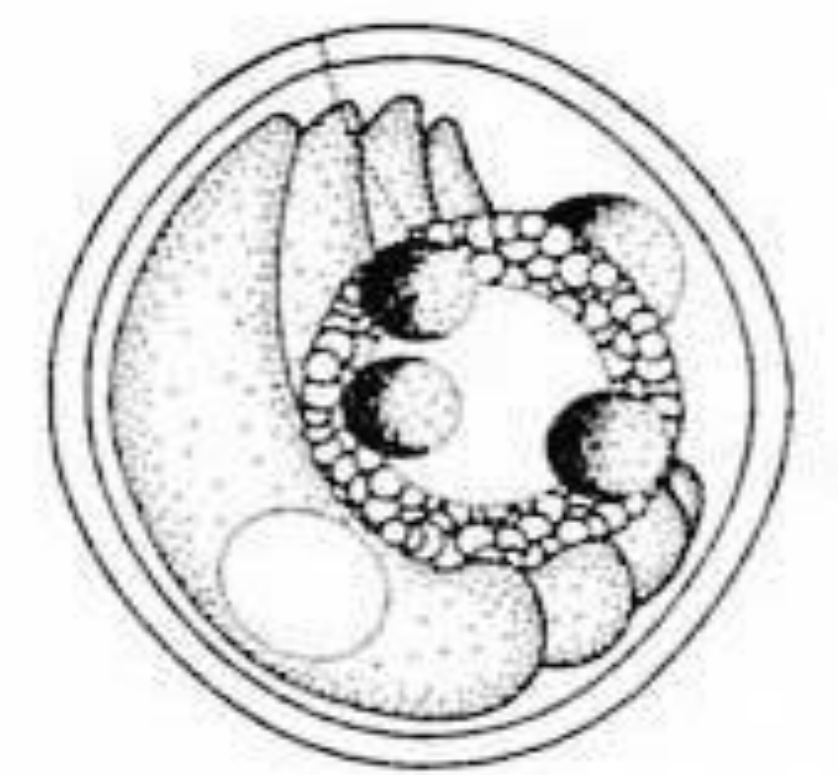
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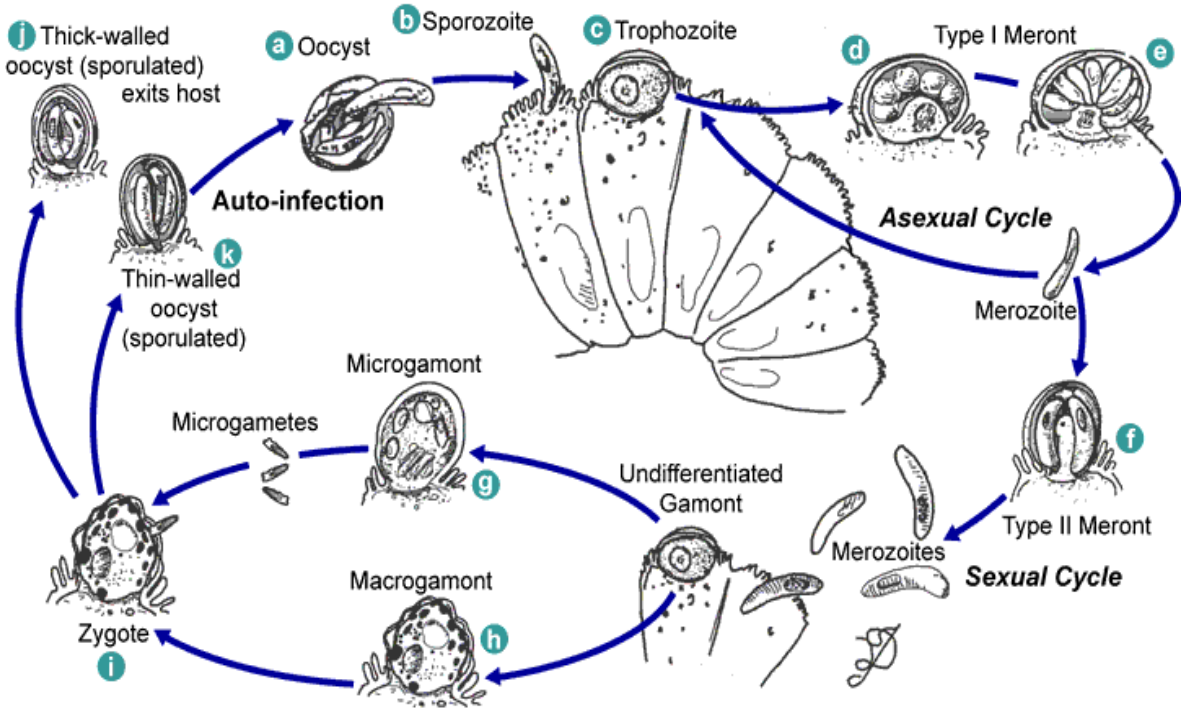
APPENDIX I

Appendix I Recognized *Cryptosporidium species*, their predominant host specificities and primary site of infection (Egyed *et al.*, 2003).

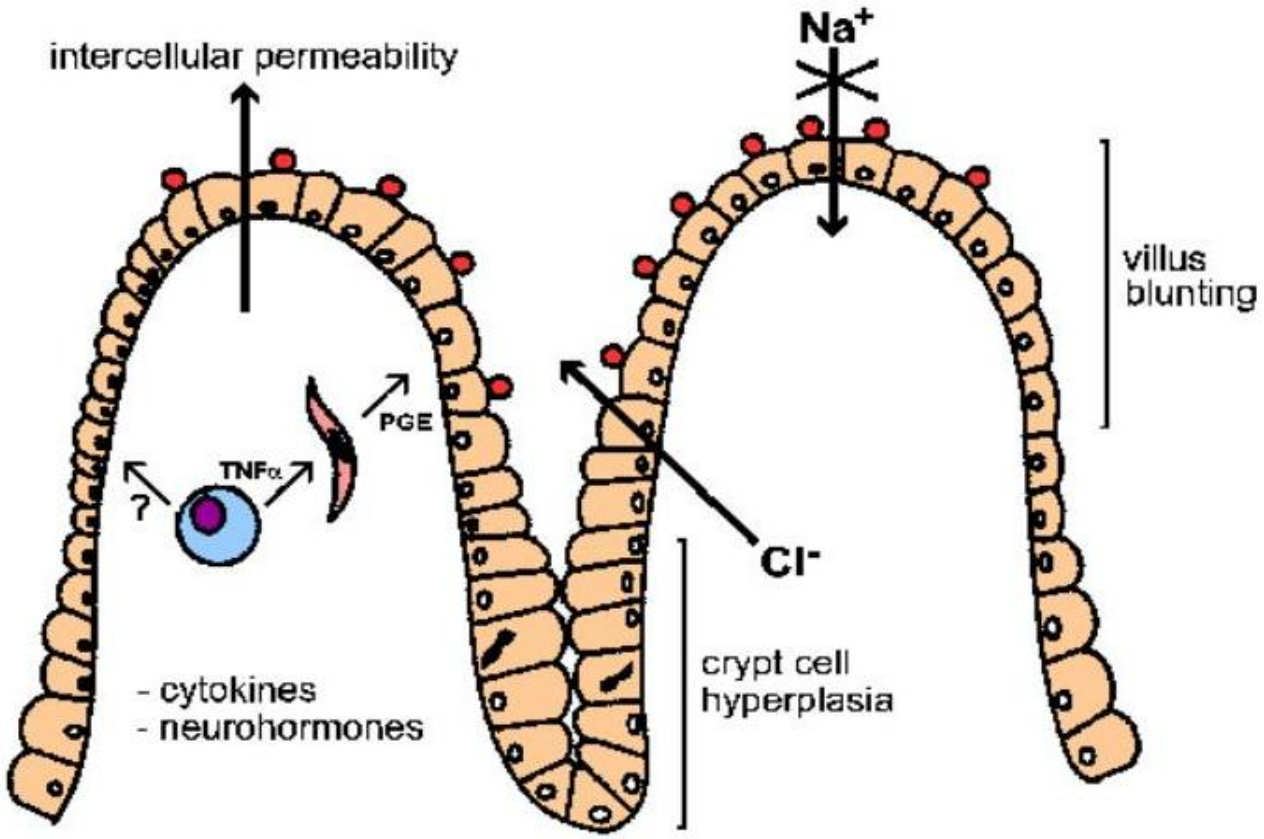
Species	Predominant Host	Primary site of Infection	Waterborne	References
C. hominis	Humans	Small intestine	Yes	Morgan-Ryan *et al.*, 2002
C. parvum	Mammals	Small intestine	No	Tyzzer, 1907
C. baileyi	Chickens	Brusa of fabricius, cloaca	No	Ditrich *et al*., 2001
C. meleagridis	Turkeys, Humans	Small intestine	No	McDougald, 1998
C. felis	Cats	Small intestine	No	Pedraza-Diaz *et al*., 2001
C. canis	Dogs	Small intestine	No	Pedraza-Diaz *et al*., 2001
C. suis	Pig	Small intestine	No	Xiao *et al.,* 2002
C. muris	Rodents, Ruminants	Stomach	No	Palmer *et al.*, 1990
C. andersoni	Cattle, camel.	Abomasums	No	Pieniazak *et al.*, 1999
C. wrairi	Guinea pigs	Small intestine	No	Widmer *et al.,* 2000
C. galli	Birds	Stomach	No	Ryan *et al.,* 2003
C. serpentis	Snakes	Small intestine, stomach	No	Ryan *et al.*, 2003
C. saurophilum	Lizard	Stomach, small intestine	No	Ryan *et al.*, 2003
C. molnari	Sea bass, sea bream	Small intestine, stomach	No	Ryan *et al.*, 2003



APPENDIX II: *Cryptosporidium* oocysts (Upton and Current, 2002).


j Thick-walled oocyst (sporulated) exits host
a Oocyst
b Sporozoite
c Trophozoite
d Type I Meront
e
Auto-infection
Asexual Cycle
k Thin-walled oocyst (sporulated)
Merozoite
Microgamont
Microgametes
f
g
Undifferentiated Gamont
Type II Meront
Merozoites
Sexual Cycle
Macrogamont
h
Zygote
i

APPENDIX III: lifecycle of Cryptosporidium oocysts (Chen et al., 2002).



APPENDIX IV: Schematic representation of Cryptosporidium pathogenesis. (Clark and Sears, 1996)

APPENDIX V

Common Name, Botanical Name and Nature of Vegetable

Common name	**Botanical name**	**Nature of vegetable**
Tomato	*Lycopersicum esculentum*	Fruit
Lettuce	*Lactuca sativa*	Leafy
Spinach	*Spinacea oleracea*	Leafy
Cabbage	Brassica oleracea var capitata	Leafy
Carrot	*Daucus carota var sativa*	Root

APPENDIX VI

Sources of Vegetables

Source of Vegetable	**Frequency**	**Percentage (%)**
Personal farm	13	28.88
Commercial farm	6	13.33
Other vegetable markets	26	57.77
Total	**45**	**100**

APPENDIX VII

Source of water for washing and rinsing vegetables in the market before sale

Source of water frequency	**Frequency**	**Percentage (%)**
Tap	17	37.77
Borehole	12	26.66
Well	16	35.55
Total	**45**	**100**

APPENDIX VIII

QUESTIONNAIRES

1. What is your source of vegetables?
   a. Personal
   b. Commercial farm
   c. Other vegetable markets

2. If personal, what is your source of manure?
   a. Animal dung
   b. Poultry dung
   c. Others.

3. What is the duration of harvest and sale for personal farmers
   a. One day
   b. Two days
   c. Others

4. What is your source of transportation to the markets?
   a. Motorcycles
   b. Vehicles
   c. Wheel barrow

5. Source of water for washing or sprinkling vegetables in the markets
   a. Tap
   b. Borehole
   c. well