EVALUATION OF CRYPTOSPORIDIUM OOCYSTS ON SOME VEGETABLES SOLD IN SELECTED MARKETS WITHIN MINNA METROPOLIS, NIGER STATE, NIGERIA

BY

SALAWU, Murtala Eneji
(MTech/SLS/2018/8435)

DEPARTMENT OF MICROBIOLOGY

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA

SEPTEMBER, 2023

ABSTRACT

Cryptosporidium is one of the most significant parasites responsible for short-lived gastroenteritis in humans. This infection can cause a veritable risk to public health, economy and physical and cognitive development primarily among the minors in developing worlds. Fresh vegetables are important part of a healthy diet for human and can serve as means of transmission of parasites that inhabit the intestine. In this study, Modified Zeihl-Neelsen staining technique was used to assess the occurrence of *Cryptosporidium* oocyst on fresh vegetables sold in selected Markets within Minna metropolis, Niger State. A total of 600 samples were randomly selected from three different Markets (Kure, Gwari and Bosso) in Minna. A total of 200 samples were collected from each Market. Four different vegetables that were selected for the study are; Cabbage, Carrot, Lettuce and Tomato. For each vegetable type, 150 samples were collected. Five (0.83%) out of 600 samples assessed were positive for *Cryptosporidium* oocyst. Lettuce and Cabbage had 2 (1.33%) contamination rate each, Carrot had 1 (0.67%) and Tomato had no contamination. Vegetables from Gwari Market had the highest contamination of oocyst with 3 (1.5%) followed by Kure Market with 2 (1%) and Bosso Market without oocyst contamination. The month of August recorded the highest oocyst contamination of 3 (2.5%) followed by the months of June and July with 1 (0.83%) and no contamination rate was recorded in the months of November and December respectively. Chi square analysis ($P<0.05$) showed significance difference between occurrence of oocyst and vegetable types. Sedimentation method was used to recover other parasites of medical importance from the vegetable samples. One hundred and thirty-four (22.33%) samples were positive for other parasites. The highest was *Entamoeba histolytica* positive for 35 (5.83%) samples and the lowest was *Trichiuris trichiura* positive for 6 (1.00%) samples. The presence of these parasite on the vegetable samples is of major public health concern. Proper washing of fruits and vegetables with clean water before consumption is highly recommended.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

Abbreviation	Meaning
AIDS	Acquired Immunodeficiency Syndrome
WHO	World Health Organisation
FAO	Food and Agricultural Organisation
EIAs	Enzyme Immunoassays
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain reaction
ZN	Ziehl-Neelsen
HCl	Hydrochloric acid
DRC	Democratic Republic of Congo
HSP	Heat Shock Protein
SPSS	Statistical Package for Social Science
HIV	Human Immunodeficiency Virus
CDC	Centre for Disease Control
NNDSS	National Notifiable Disease Surveillance System
IGCC	International Giardia and Cryptosporidium conference
IMS	Immuno-magnetic Separation
EPA	Environmental Protection Agency
EDTA	Ethyl diamine tetra acetic acid
ISO	International Standard Organisation
GBD	Global burden of Disease

GEMS	Global enteric multicentre Study
CD4	Cluster of differentiation
IgM	Immunoglobulin M
ICCA	Immuno-chromatographic cartridge assays
RFLP	Restriction Fragment Length Polymorphism
CDPKs	Calcium dependent protein kinase
BKIs	Bumped Kinase inhibitors
CpCDPK6	Calcium dependent protein kinase 6
TCAMS	Tres cantos Antimalaria Set
IFM	Immunofluorescence Microscopy
EFSA	European food safety Authority
qPCR	quantitative polymerase chain reaction
DALYs	Disability-adjusted life years
GI	Gastrointestinal tract
SSrNA	Small subunit ribonucleic Acid
COWP	*Cryptosporidium* Oocyst wall protein
HSP70	Heat Shock protein 70
IYFV	International Year for Fruits and Vegetables
UN	United Nation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

The global causes of diarrhoea in children under two years are rotavirus and *Cryptosporidium* (Kotloff *et al.* 2019). Asides from being responsible for high mortality in children at young age, *Cryptosporidium* can also cause persistent infection, mental functions related problems, malnourishment, and retardation of growth (Platts-Mills *et al.*, 2015; Khalil *et al*., 2018). It also causes high economic losses in case of morbidity and mortality by affecting the gastrointestinal tract of sheep, goat, pig, cattle and human (Vanathy *et al.*, 2017). The incubation period is usually about one week, with clinical signs of profuse, offensive, watery diarrhoea, which may be accompanied by abdominal pain, vomiting and fever. In the immune compromised individuals, such as those with Acquired Immunodeficiency Syndrome (AIDS) and severe malnourished children, Cryptosporidiosis is a common and life threatening condition causing profuse and intractable diarrhoea leading to severe dehydration, malabsorption and wasting. The parasite has become prominent in public health impact due to the severity of the disease it causes especially in immunocompromised individuals couple with the none readily available safe drugs or vaccines for its treatment and prevention.

Cryptosporidium belongs to protozoan group of parasite under the phylum apicomplexan. Ernest Edward Tyzzer was the very first person to observe the parasite in 1907 (Tyzzer, 1907). At first, the parasite was thought to be incapable of causing harm, up to the 1970s, when it was reported to be aetiological agent of diarrhoea (Nime *et al.*, 1976). As of 2019, there were over 40 named *Cryptosporidium* species, as acknowledged by host specificity, morphology, and

molecular biology studies (Gharpure *et al.,* 2017; Chalmers *et al.,* 2019). *Cryptosporidium* species can cause infection in immunocompetent and immunosuppressed individuals.

Cryptosporidium species are faecal-oral protozoan parasites, and humans and animals can be major hosts of this parasite. Coccidian oocyst stages are highly resistant to environmental stress and chemical disinfection and this is attributed to a durable oocyst wall, a complex protective barrier consisting of a double layer of a protein-lipid carbohydrate matrix (Naseer, 2022). Transmission of *Cryptosporidium* may be by direct routes, person to person or animal to person, or by indirect environmental routes, through faecally contaminated food or water (Ahmed and Karanis, 2018; Ryan *et al*., 2018).

Consumption of fruits and vegetables may result in a decreased risk of chronic diseases, including heart disease, diabetes, and certain cancers. Moreover, including fruits and vegetables on a daily basis in our diet may be an important strategy for weight control. Fruits and vegetables are good sources of many important nutrients, including potassium, vitamin C, folate, fibre, and numerous phytochemicals. Food and Agriculture Organization and World Health Organization report recommends a minimum of 400 g of fruit and vegetables per day for the prevention of chronic diseases, such as heart disease, cancer, diabetes, and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (FAO/WHO, 2004).

The International Year of Fruits and vegetables (IYFV) was officially pronounced in 2021 by the United Nation (UN). This is to educate the general populace about the health benefits and nutritive values of fruits and vegetables

as integral part of our healthy diet. It also targets the reduction in wastages of these produce through policy making as they are subject to easy and fast deterioration (FAO, 2020). When talking about healthy food practice, one thing that is foremost on our menu are fruits and vegetables. They are of different colour, vitamin-mineral and fibre-rich. For proper functioning of human system, fruits and vegetables are key components optimal performance. There are wide range advantages of consuming fruits and vegetables in our diets. Fresh fruits and vegetables benefits not only the consumers, but also the food chain system. It has generally improved biodiversity, sustainable environment and the sustenance of both Farmers and employee operating in line with the value chain (FAO, 2020).

Some vegetables are eaten raw as salad to retain the natural taste and preserve heat labile nutrients (Gupta *et al.,* 2010). Ingestion of raw vegetables represented an important means of transmission of several infectious diseases because of their complex surface and porosity, which unfortunately facilitate pathogen attachment and survival (Orlandi *et al.,* 2002).

The consumption of raw vegetables plays a major epidemiological role in the transmission of parasitic food-borne diseases (Abougrain *et al.,* 2010). Intestinal parasites are widely prevalent in developing countries, probably due to poor sanitation and inadequate personal hygiene (Fallah *et al.,* 2012).

Consumption of raw vegetables may result in gastroenteritis by infectious enteric pathogens present on the crops. *Cryptosporidium* oocysts are among the over 150 enteric pathogens that may be transmitted by contaminated fruits and vegetables. Contamination of vegetables by

oocysts of *Cryptosporidium* can occur during production, transportation, storage, and commercialization; however, cultivation conditions involving the quality of irrigation water, fertilizer type, the presence of animals in the property, and direct contamination from farm workers are the main sources of contamination (Almeida *et al.,* 2013; Dixon *et al.,* 2013). Contamination of vegetables by *Cryptosporidium* oocysts can also originate from agricultural runoff from grazing land, slurry, and water reuse of inadequately treated effluents from wastewater treatment plants. Infective *Cryptosporidium* oocysts can remain viable in the environment (especially under low temperatures), serving as a reservoir of infection (Naseer, 2022). Numerous outbreaks of Cryptosporidiosis were reported mainly from industrialized countries, whereas most of the studies reporting a high prevalence of *Cryptosporidium* oocysts in fresh vegetables and fruits were recorded in less industrialized countries (El Sherbini *et al.,* 2016; Utaaker *et al.,* 2017).

1.2 Statement of the Research Problem

The burden of *Cryptosporidium*-associated diarrhoea is greatest in Sub-Saharan Africa, especially Nigeria and the Democratic Republic of the Congo (DRC) where about 48% of the under-5 associated deaths occur (Khalil *et al.,* 2018). *Cryptosporidium* has emerged as the most frequently recognized cause of recreational water–associated outbreaks of gastroenteritis, particularly in treated (disinfected) venues. The disease is likely underestimated, since the diarrhoea usually resolves without any treatment (Gerace *et al.*, 2019). Domestic animals such as cattle, goats and sheep are vital sources of zoonotic *Cryptosporidium* species (Walter *et al*., 2021).

Consumption of raw vegetables may result in gastroenteritis by infectious enteric pathogens present on the crops. *Cryptosporidium* oocysts are among the over 150 enteric pathogens that may be transmitted by contaminated fruits and vegetables. Contamination of vegetables by oocysts of *Cryptosporidium* can occur during production, transportation, storage, and commercialization (Naseer, 2022).

Cryptosporidium diarrhoea damages gut endothelial cells and microvilli as such absorption of macronutrients, and micronutrients are impaired (Robertson *et al.*, 2020). Furthermore, *Cryptosporidium*-related malnutrition results in secondary impairment of cell-mediated immunity, which is associated with increased susceptibility to other infectious diseases. Other long-term anomalousness resulting from *Cryptosporidium* infection include reduced cognitive development, poor school performance, and elevated risk of cardiovascular and metabolic diseases later in life (Sudfeld *et al.*, 2015), all likely to have a disproportionate effect on the global poor populations. Furthermore, with increasing importance of Cryptosporidiosis in immunocompromised individuals, there is need to ascertain the risk of its infection associated with consumption of fruits and vegetables in the study area.

1.3 Aim and Objectives of the Study

The aim of this study was to determine the incidence of *Cryptosporidium* Oocysts on some vegetables sold within selected Markets in Minna metropolis, Niger State, Nigeria.

The Objectives of the Study were to:

i. analyse the selected vegetables sold in Markets within Minna metropolis for the presence of *Cryptosporidium* oocysts.

ii. determine the kinds of vegetable most likely to be contaminated by *Cryptosporidium* oocysts.

iii. determine the predisposing factors for possible cryptosporidiosis in humans in Minna, Niger State.

iv. identify other parasites of medical importance on the vegetable samples.

1.4 Justification for the Research

Consumption of fresh fruits and vegetables is on the rise. They form major part of our diet due to their nutritional values. Vegetables may become contaminated by enteric pathogens (parasites, bacteria, and viruses) by irrigation with contaminated water, fertilization with fresh animal manure, or by infected food handlers (Naseer, 2022).

Despite the many negative health effects of these four parasites (*Cryptosporidium*, *Giardia*, *Cyclospora* and *Entamoeba*), not much has been done on the prevalence of these parasites in water and raw vegetables in Nigeria. In addition, limited studies have been conducted to determine the occurrence of the parasite in fresh produce which have been reported to be common routes for several parasites (Carrero *et al.*, 2020). Considering the problems with the parasite and the public health hazard status, seeking answers to the fundamental questions about the incidence and the common types of vegetable that are

contaminated is necessary for effective public health education (Dhal *et al.*, 2022).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Species of *Cryptosporidium*

Currently, there are more than 40 morphologically and molecularly different *Cryptosporidium* species (Adamu *et al.,* 2014; Ahmed and Karanis, 2020), which infect mammals (Bovidae, Primates, Carnivora, Hares, Equidae, Rabbits, Rhinocerotidae, and Tapiridae), amphibians, birds, and reptiles. Additionally, more than 157 mammalian species were listed as hosts for *Cryptosporidium* infection. However, *Cryptosporidium* species including *C. hominis, C. bovis, C. parvum, C. ryanae, C. andersoni, C. fayeri, C. canis, C. felis, C. macropodum, C. muris, C. suis,* and *C. wrairi* have been isolated from mammals. *C. meleagridis, C. baileyi,* and *C. galli* have been isolated from birds (Helmy *et al.,* 2017) while *C. varanii* and *C. serpentis* have been isolated from reptiles and *C. fragile* has been isolated from amphibians (Mamedova and Karanis, 2020). Additionally, *C. rubeyi* has been isolated from squirrels, *C. scophthalmi* from turbot (flat fish), *C. huwi* from fish, and *C. erinacei* from horses and hedgehogs (Zahedi *et al.,* 2016). Human cryptosporidiosis is caused by *C. hominis*, while *C. parvum* is considered the zoonotic species of human cryptosporidiosis. Both *C. hominis* and *C. parvum* are responsible for more than 90% of human cryptosporidiosis. Although there is host specificity of the *Cryptosporidium* species, other species such as *C. meleagridis, C. baileyi, C. andersoni, C. canis, C. felis, C. bovis, C. suis, C. fayeri, C. scrofarum, C. tyzzeri, C. erinacei,* and *C. muris* have been detected in animal hosts as well as in humans. The aforementioned species and *C. parvum* have been considered potentially

zoonotic species (Helmy *et al.,* 2013; Ryan *et al.,* 2014). Additionally, humans can also be infected with *C. viatorum*, *C. cuniculus*, *C. ubiquitum*, Chipmunk genotype I, *Cryptosporidium* horse, and *Cryptosporidium* mink genotype (CDC, 2020).

Currently, there are more than 60 reported genotypes of *Cryptosporidium* that differ in their molecular sequences (Ahmed and Karanis, 2020). *Cryptosporidium* subtypes are distinguished by the number of repeats in each strand. Short, repetitive sequences (R) appear directly after the trinucleotide repeats in some subtypes. In *C. parvum*, 11 subtype families (IIa- IIk) have been discovered with at least 78 subtypes. Furthermore, in *C. hominis*, six subtype families have been detected (Ia, Ib, Id, Ie, If, and Ig) with at least 78 subtypes (Valenzuela *et al.*, 2014). In *C. meleagridis*, seven subtype families have been identified (IIIa- IIIg), while six subtype families were identified in *C. fayeri* (IVa-IVf), and two in *C. cuniculus* (Va, Vb), Horse genotype (VIa, VIb), and *C. tyzzeri* (IXa, IXb), whereas one subtype was identified in *C. erinacei* (XIIIa), Mink genotype (Xa), Ferret genotype (VIIIa), and *C. wrairi* (VIIa) (Ryan *et al.*, 2014). Several highly preserved genes, including (1) small subunit rRNA (18S rRNA), (2) *Cryptosporidium* oocyst wall protein (COWP), (3) heat shock protein (HSP70), and (4) the actin gene, can differentiate between *C. parvum* and *C. hominis*.

2.2 Classification of *Cryptosporidium* species

Cryptosporidium species are classified as eukaryotes in the Phylum Apicomplexa (Most apicomplexan parasites are characterized by the presence of an organelle called an apicoplast. In addition, the invasive forms have an apical

complex, comprising polar rings, rhoptries, micronemes, and conoid and sub-pellicular microtubules, which is involved in host-parasite interactions. Interestingly, *Cryptosporidium* species seem to have lost functional apicoplasts and mitochondria, as shown by genome sequence analyses. 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 Eimeriida (independent micro and macrogamy development), Family Cryptosporiidae (four naked sporozoites within oocysts) (Tzipori and Widmer, 2000).

The relative uniform appearance of these organisms and their 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* species. To date, over 40 species of *Cryptosporidium* have been identified, some of which are host specific, whereas others are more promiscuous regarding host infectivity. Furthermore, infection with some species of *Cryptosporidium* tend to be associated with little or no illness, others are particularly pathogenic with severe symptoms, which may even result in mortality. However, whether infection manifests as disease (Cryptosporidiosis), and the severity of that disease, also depends on host factors, particularly those associated with host immunity and other health challenges (Robertson *et al.,* 2020).

2.3 Morphology of the *Cryptosporidium* Oocysts

Cryptosporidium spp. oocysts are rounded and measure 4.2 to 5.4 µm in diameter. Sporozoites are sometimes visible inside the oocysts, indicating that sporulation has occurred (CDC, 2019). Oocyst is the spore phase that can survive

for lengthy periods outside a host and it is the infective stage. The oocysts (thin- or thick-walled) with 4 haploid sporozoites (sporulated oocysts) develop inside the parasitophorous vacuole. Thin-walled oocysts (about 20%) excystate in the host intestinal tract, leading to endogenous autoinfection, and the thick walled oocysts (about 80%) are extremely resistant to several disinfectants, are excreted with the faeces to the environment and can survive outside the host for a long time (Yosra and Hafez, 2022). The thick-walled oocysts represent the exogenous stage of the *Cryptosporidium* parasite. *Cryptosporidium* oocysts are spherical to ovoid shape, have a residual body, and four banana-like or comma-shaped sporozoites with a pointed front end and a stubbed hind end, where the nucleus is localized (Yosra and Hafez, 2022). The oocyst cell wall is tripartite with electroluscent middle zone within two electron dense layers. The oocyst stain readily with acid- fast but not with iodine (Otubanjo, 2013).

Figure 2.1 is a cross section of *Cryptosporidium* oocyst showing the four banana-shaped sporozoites each with a nucleus in blue colour. It also revealed some of the chemical constituents of the oocyst wall and the breaking point (suture) where the sporozoites exit from the oocyst to initiate infection in the host.

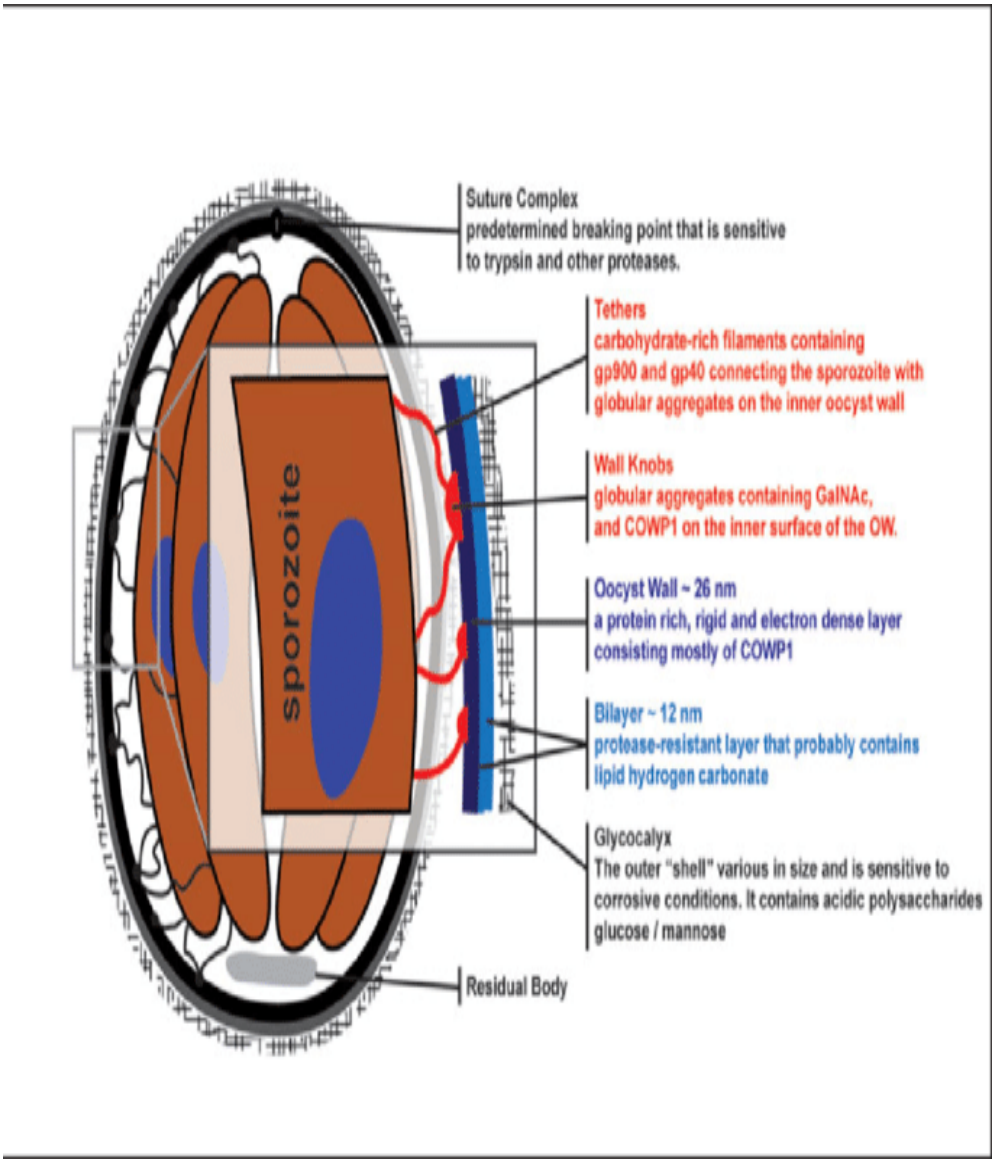


Figure 2.1: Morphology of the Oocyst of *Cryptosporidium* species
Source**:** Mathias and Arwid, 2014

2.3.1 Life Cycle of *Cryptosporidium* Species

Based on the developmental phases, the life cycle of *Cryptosporidium* can be sub-divided into six stages. The first stage is the excystation phase that releases infectious sporozoites, leading to the second stage of asexual proliferation within the host cell, called merogony. The third stage of gametogony refers to the formation of the micro and macro-gametes, followed by the fourth phase of

fertilization of these gametes. The fifth stage constitutes the formation of oocyst walls to create an environmentally tolerant stage for the transition of the infection from one host to the next. The sixth and final stage of sporogony refers to the formation of infectious sporozoites (Miyamoto and Eckmann, 2015; Dumaine *et al.*, 2020)

Cryptosporidium species do not multiply outside the host (Checkley *et al.*, 2015; White, 2020). Infection is initiated by ingestion of oocysts, which are activated in the stomach and upper intestines to release four infective sporozoites. These motile sporozoites bind to the receptors on the surface of the intestinal epithelial cells and are ingested into a parasitophorous vacuole near the surface of the epithelial cell, separated from the cytoplasm by a dense layer. *Cryptosporidium* oocysts are round and measure 4.2-5.4 µm in diameter. Each oocysts contains 4 sporozoites that hatch at the intestinal level, releasing infectious sporozoites (Gerace *et al.*, 2019). After excystation, these sporozoites are ingested into a modified host membrane separated from the cytoplasm by a dense layer; then, the location of parasites within the host is not intracellular but extracytoplasmic. Within the parasitophorous vacuole, the parasite undergoes asexual or schizogony reproduction, leading to the production of 8 merozoites within a type I meront. The merozoites can invade the neighbouring epithelial cells and propagate the infection to other sites of the intestines. During this stage, the merozoites can undergo 2 distinct replicative cycles: an asexual stage characterized by multiplication of merozoites (type I meront) and production of thin-walled oocysts that autoinfect the host and/or a sexual stage with formation of type II meront, which, after differentiation in microgametocytes and macrogametocytes, will unite to form the zygote. The diploid zygote, through a

process called sporogony, will form 4 sporozoites within thick or thin-walled oocysts. The thick-walled oocyst, protected by a resistant wall, after releasing in the faeces is shed into the environment, ready to infect a new individual.

Figure 2.2 is a chart showing the life cycle of *Cryptosporidium* with all the developmental stages in the host as indicated from ingestion of oocysts to the stage when the sporulated oocysts exit the host. Human and animal served as sources of infection in this chart.

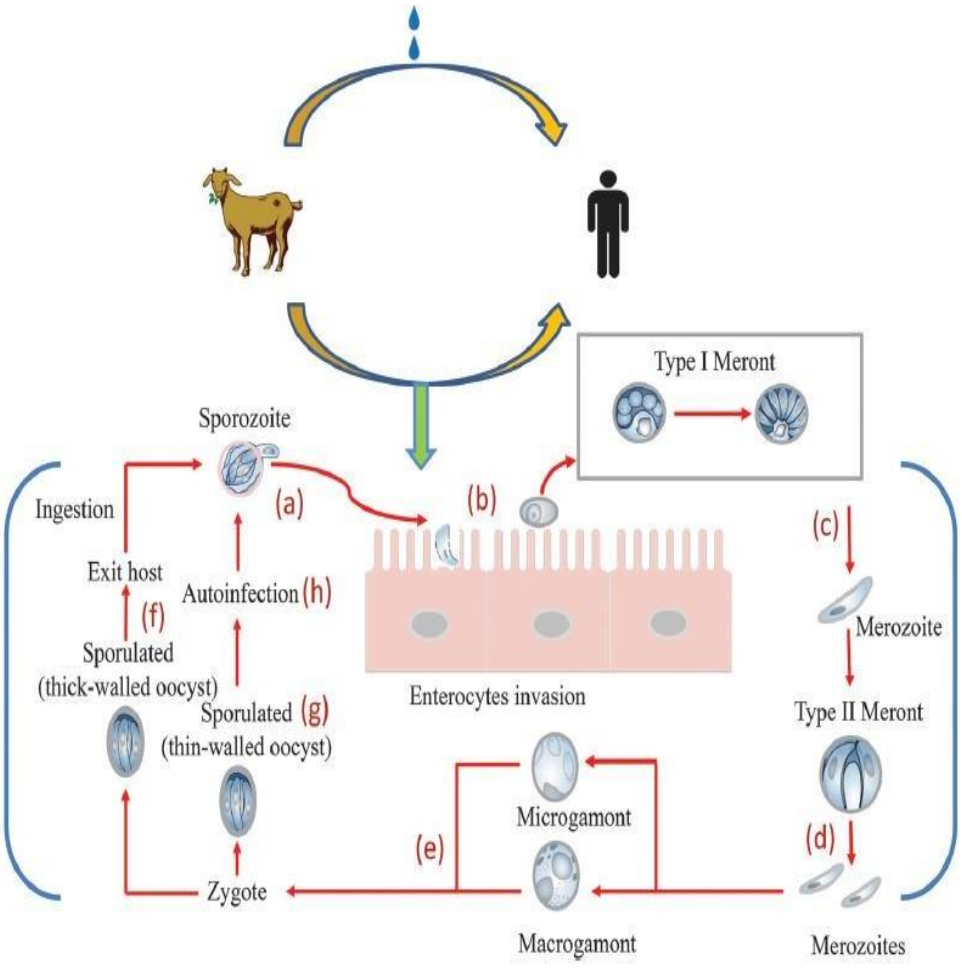


Figure 2.2: Life cycle of *Cryptosporidium* species. Source: Gerace *et al.*, 2019.

2.4 Epidemiology of Cryptosporidiosis in Human

Although the number of worldwide reported cases of cryptosporidiosis in the last years has increased with a number of 3 cases per 100,000 populations, numerous indicators (i.e., clinical symptoms) indicate that the frequency of infection is likely to be 100-fold higher than the number of reported cases (Shrivastava *et al.*, 2017). The prevalence of *Cryptosporidium* infection is significantly lower in industrialized countries compared to developing countries since, in the latter, there are many people that still lack a basic level of drinking water and sanitation (Bouzid *et al*., 2018; Shoultz *et al.*, 2016). In developing countries, cryptosporidiosis is rarely reported in immunocompetent persons, while this infection causes approximately 10–15% of cases of severe diarrheal illness mostly in malnourished children less than five years old (Bouzid *et al*., 2018). *Cryptosporidium* spp. have been isolated worldwide, and outbreaks of cryptosporidiosis associated with swimming pool or contaminated drinking water have been reported in several countries. Although there are more than 40 species of *Cryptosporidium*, only few species like *C. hominis, C. parvum, C. meleagridis, C. felis, and C. canis* are commonly found in people (Ryan *et al.*, 2014, Ayinmode *et al.*, 2018). Among these species, *C. hominis and C. parvum* are most frequently found in intestinal infections in humans, although they differ in host range, with the former that infects exclusively humans, while the latter has an infectious cycle involving humans and ruminants, and usually *C. parvum* infects people who have close contact with large numbers of animals (Ehsan *et al.*, 2015). Although the transmission from animals is possible, it is very uncommon, and *Cryptosporidium* infection transmitted from sheep, horses, goats, and rodents has been rarely reported (Thomson *et al.*, 2017). *C. canis* and

C. felis, the dog- and the cat-adapted species, respectively, have been reported to cause infection in individuals without being sick at all (Gerace *et al.*, 2019). However, human infections with *Cryptosporidium* species from pets are very rare.

2.4.1 World Statistics of Cryptosporidiosis

Cryptosporidiosis is a notifiable disease at the European Union level, and surveillance data are collected through the European Basic Surveillance Network (Ajjampur *et al.,* 2008). The crude incidence rate was similar to that in the United States, although considerable differences in the rates of cryptosporidiosis were observed between countries and over time. In resource-limited countries, most infections occur in children. According to National Notifiable Disease Surveillance (NNDS) in the US, an estimated 748,000 cryptosporidiosis cases occur annually, this means that less than 2% are nationally notified (CDC, 2018).

In sub-Saharan region and the Asia, an estimated 2.9 million cases occur annually in children less than 2 years of age. In spite of this enormous public health burden, there is only suboptimal treatment, and no vaccine is available currently (Sow *et al.,* 2016). In a large multicentre study of moderate to severe diarrhoea in sub-Saharan Africa and South Asia, *Cryptosporidium* was second only to rotavirus as a cause of diarrhoea in children younger than 2 years and was associated with 200,000 deaths. A multicentre birth cohort study from Asia, Africa, and Latin America (the malnutrition and enteric disease study) also found *Cryptosporidium* to be among the top causes of diarrhoeal disease, and non-diarrheal infection was associated with malnutrition. Studies suggest that

the burden of disease related to malnutrition may be greater than that due to diarrhoea. (Khalil *et al.,* 2018). In persons with AIDS, cryptosporidiosis is more common in developing countries, affecting 12%-48% of persons with AIDS who have diarrhoea (Naseer *et al.,* 2018; White *et al.,* 2020).

2.4.2 Cryptosporidiosis in Nigeria

In Nigeria, several researches have been conducted on human and animals. Only a few works have been documented on fresh vegetable samples. Odeke *et al.* (2019) reported 31% occurrence of *Cryptosporidium* oocysts on fresh vegetables in Zaria. A prevalence of 38.5% was 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 the authors noted that infection was higher among females (27%) than males (17%) and among adult (29%) than children (8%).

In North-Central Nigeria, Nwabuisi (2009) 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, 2009). Egberongbe *et al*. (2010) reported a prevalence of 21.4% among children of Ijebu and Remo areas of Ogun state Nigeria.

2.5 Transmission of Infection

In order to have proper understanding of this organism, its transmission route must be clearly stated. The transmission of *Cryptosporidium* is linked with the oocyst. The life cycle is predominantly faecal-oral, although often indirect with transmission by a vehicle such as water or food (Robertson *et al*, 2020).

2.5.1 Human to Human Transmission

In human population, transmission has been reported in homes and public settings where personal and sanitary hygiene are grossly inadequate causing increased cases of the disease in developing countries (Ahmed and Karanis, 2018). Direct contact with the infected host through faecal oral route is the main mechanism that enhances their transmission. The practice of anal oral sexual behaviour with the infected individuals especially HIV/AIDS patients is another risk factor that facilitate human to human transmission (Cameron, 2023)

2.5.2 Animal to Human Transmission

Animals play a vital role in human Cryptosporidiosis. About 60-90 percent of human infection is attributable to *C. parvum* in nations that are undergoing development. Livestock (mainly cattle) and wildlife (deer and migrating geese) were demonstrated as relevant contributors to zoonotic *Cryptosporidium* oocysts in source water catchments intended for human consumption (Widmer *et al.*, 2020).

Zoonotic transmission of merged species of *C. parvum* and *C. hominis* has enhanced the understanding of animal infection in human population as the major reason for the continuous presence of diarrhoeic condition in developing

nations. The South African water supply has been greatly threatened by this situation over the years (Efstratiou *et al*., 2017)

2.5.3 Transmission Through Water

The potential for water to be a transmission vehicle for *Cryptosporidium* is accepted globally, with communitywide outbreaks and smaller outbreaks reported from multiple countries. The scarcity of such outbreaks being reported from Africa probably represents limitations in technological capabilities and surveillance systems (Schaefer *et al.,* 2020). *Cryptosporidium* spp. are largely recognized as common causes of water- and food-borne outbreaks of diarrheal illness globally (Ryan *et al.,* 2018).

Waterborne transmission is a major route in the epidemiology of the parasite and is thus a serious public health concern. As a reflection of the heightened interest in the transmission of cryptosporidiosis, a significant number of studies presented at seventh International *Giardia* and *Cryptosporidium* Conference (IGCC) focused on the detection and quantification of *Cryptosporidium* oocysts in surface, Wells, recreational and waste water matric (Widmer *et al.,* 2020). The waterborne transmission of this infectious pathogen is well documented, neither the natural reservoir nor the exact infection route of *Cryptosporidium* species is well-known (Khalil *et al.,* 2018).

2.5.4 Transmission Through Food

When foods such as animal products and raw vegetables are contaminated with the cyst of the parasite are consumed, human Cryptosporidiosis occurs. More so, human infections were also linked to improper handling of food or washing with

contaminated water. Large-scale foodborne outbreaks of cryptosporidiosis have been documented globally in recent years (Ryan *et al.*, 2018). Therefore, increasing attention has to be paid to the detection of *Cryptosporidium* oocysts in fresh produce including lettuce, Cabbage, parsley, coriander, fruits, and sprout seeds (Widmer *et al.*, 2020).

Figure 2.3 is a chart that denotes transmission routes of *Cryptosporidium* species in the environment ranging from soil, water, animal, human, fruits and vegetables, diary product, raw meat, shellfish and unpasteurised milk.

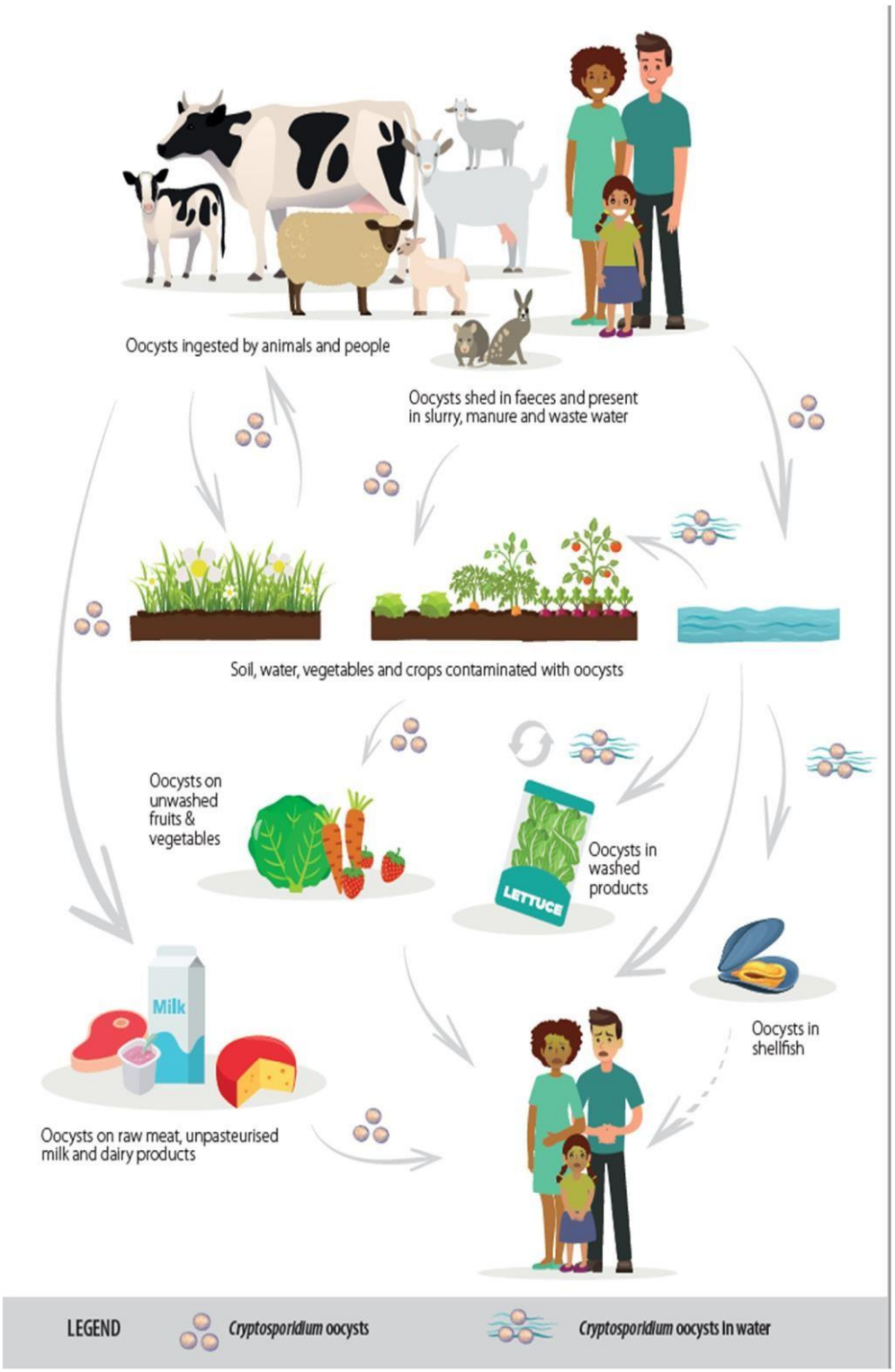


Figure 2.3: Transmission routes of *Cryptosporidium* spp.
Source: EFSA, 2018

2.6 Methods of Isolating *Cryptosporidium* on Fresh Vegetables

The isolation methods of *Cryptosporidium* from fresh vegetables are based on four basic steps: elution, release of oocysts from vegetables, concentration of the eluate, and separation of the oocysts from the debris. The concentration and separation steps are performed by centrifugation, followed by immunomagnetic separation (IMS).

Specific staining of the oocysts is applied for visualization and identification by microscopy. An alternative specific and sensitive method for *Cryptosporidium* detection and genotyping is based on polymerase chain reaction (PCR) that follows DNA extraction. Several elution solutions have been applied to release *Cryptosporidium* from vegetables. Deionized water is the basic solution used for the elution of *Cryptosporidium* from leafy vegetables (Shields *et al.*, 2012). The recovery efficiency of *Cryptosporidium* oocysts by using deionized water from artificially contaminated spinach leaves was 38.4%. An elution buffer on the basis of a solution of 1M glycine was found to be the cheapest and most effective (36.2%) for the release of *Cryptosporidium* oocysts from lettuce (Cook *et al.*, 2006). A detection method on the basis of the release of oocysts by a solution of 1M glycine, with a pH of 5.5, followed by concentration and separation by IMS, specific staining, and microscopic enumeration was adopted as an International Organization for Standardization method for the detection of oocysts from fresh produce and raspberries (ISO, 2016). Robertson and Gjerde adopted the elution solution used in Method 1623.1 of the U.S. Environmental Protection Agency for the release of *Cryptosporidium* from a membrane filter (Envirochek) that is applied to concentrate *Giardia* and *Cryptosporidium* from large volumes of water (US EPA, 2012). The filter elution solution consists of 10 mL of 10%

laureth-12, 10 mL of 1M Tris, pH 7.4 Tris solution, and 2 mL of 0.5 M EDTA, pH 8.0, and 150 µL of Antifoam A in 1,000 mL of reagent water.

Detection of concentrated and isolated oocysts can be accomplished by various methods, including direct light microscopy, Ziehl-Neelsen staining, immunofluorescence microscopy (IFM), PCR, or quantitative PCR (qPCR). Oocysts can be detected microscopically on the basis of the morphological features and specific fluorescent staining (Hohweyer *et al.*, 2016, Lalonde and Gajadhar, 2016). Concentrated and isolated oocysts of *Cryptosporidium* from vegetables may be detected by highly experienced personnel with microscopic detection of the oocysts following staining or without staining by direct light microscopy.

2.7 Human Health Impacts of Cryptosporidiosis

There is no doubt that cryptosporidiosis has a substantial health impact world-wide, particularly in lower-income countries. Most African countries are classified using World Bank definitions (World Bank, 2020), as having low-income or lower-middle income economies, with the exception of Algeria, Botswana, Equatorial Guinea, Gabon, Libya, Mauritius, Namibia, and South Africa, which are classified as upper-middle income, and Seychelles being high income. Out of the 31 countries globally classified as being in the lowest income group, 24 (77%) are in Africa.

One of the earliest studies investigating the impact of *Cryptosporidium* in an African country was from Guinea Bissau, and demonstrated that *Cryptosporidium* was associated with excess mortality in children younger than 12 months, with this excess mortality persisting into the second year of life

(Robertson *et al.,* 2020). Although this impact from cryptosporidiosis in particular countries has long been assumed, the first comprehensive data demonstrating this were produced relatively recently, from the Global Burden of Disease (GBD) and the Global

Enteric Multicenter Study (GEMS) outputs (GBD, 2017). These studies provided the first global estimates on impacts of cryptosporidiosis (among other diseases) in different age groups and different countries, in terms of mortality, morbidity, and disability-adjusted life-years (DALYs). A meta-analysis published in 2018 showed that earlier reports probably under-estimated the true burden by not taking into account impacts occurring after the acute phase of infection, such as decreased growth, particularly weight gain, and a greater risk of subsequent episodes of infection (Khalil *et al.,* 2018). As *Cryptosporidium* diarrhoea damages gut endothelial cells and microvilli, absorption of macronutrients, and micronutrients are impaired (Colombara *et al.,* 2016). In addition, *Cryptosporidium*-related malnutrition results in secondary impairment of cell-mediated immunity, which is associated with increased susceptibility to other infectious diseases. Other long-term abnormalities include reduced cognitive development, poor school performance, and elevated risk of cardiovascular and metabolic diseases later in life (Robertson *et al.,* 2020), all likely to have a disproportionate effect on the global poor.

Cryptosporidium infection in children under 5 years was estimated to be associated with 44.8 million diarrheal episodes and 48,300 deaths globally (Khalil *et al.,* 2018). Of these, the vast majority were from Africa, accounting for 75% of the diarrheal episodes and 88% of the deaths. In particular, the burden of *Cryptosporidium*-associated diarrhoea is greatest in Sub- Saharan Africa,

especially Nigeria and the Democratic Republic of the Congo (DRC) where about 48% of the under-5 associated deaths occur (Khalil *et al.,* 2018). When including downstream effects of growth shortfalls associated with cryptosporidiosis, it was estimated that the burden of this parasite could be 2.5 times higher than previous estimates (Khalil *et al.,* 2018), and recognized that accounting for the direct or indirect burden of asymptomatic infections could elevate these estimates even further.

2.8 Pathogenesis of *Cryptosporidium* species

In immunocompetent persons, *Cryptosporidium* infection usually produces a period of illness characterised by watery diarrhoea, although the infection in some persons may not lead to the symptoms (Shoultz *et al.,* 2016; Khalil *et al.,* 2018). The disease is likely underestimated, since the diarrhoea usually resolves without any treatment. Although even people who do not have direct contact with animals may be infected, those who have direct contact with infected animals (particularly calves) or swallow pool water or drink untreated water are at a higher risk of contracting cryptosporidiosis (Bouzid *et al.,* 2018). *Cryptosporidium* infections are also more common in individuals who are in poor health or who have weakened immune systems (e.g., human immunodeficiency virus (HIV)/ AIDS, cancer, and transplant patients (Florescu and Sandkovsky 2016; Wang *et al.,* 2018). Between the parasites that caused about 1 million deaths every year, cryptosporidiosis resulted in over 50,000 deaths (Wang *et al.,* 2018).

Moreover, *Cryptosporidium* is one of the most important protozoan pathogen that cause waterborne outbreaks worldwide. *Cryptosporidium* lives in the

intestines of the infected individuals and animals in the form of oocysts, which will be released in the faeces (Bouzid *et al.*, 2013). After infection, the parasite alters the function of the intestinal barrier, increasing its permeability, absorption, and secretion of fluid and electrolytes, and thereby, the severity, persistence, and outcome of the infection depend on the degree of the immunocompromised status (Kumar *et al*., 2018). The oocysts are very resistant to chlorine, chloramines, and chlorine dioxide, which are commonly used in methods of water system disinfection, and remain vital for infection in the environment for a long time. Humans become infected with *Cryptosporidium* by touching anything that has come in contact with contaminated faeces, although the most common mode of transmission is represented by ingestion of oocysts in contaminated food and water or air (Gerace *et al.*, 2019).

Recent studies indicate that cryptosporidiosis may be transmitted by inhalation of aerosolized droplets via respiratory secretions or by coughing, in addition to the well-documented faecal–oral transmission (Sponseller *et al.*, 2014). Pulmonary infections also have been reported (Reina *et al*., 2016).

Immunocompromised hosts are more susceptible to infection than people with a healthy immune system, and in the subjects with HIV/AIDS, the parasite often causes a chronic, prolonged form of a disease, which is difficult to treat and can even result in death (Wang *et al*., 2018). In these patients, fever and malabsorption are common, and the parasite can cause inflammatory disease of the biliary tree leading to biliary tract obstruction, sclerosis cholangitis, papillary stenosis, and pancreatitis (Wang *et al*., 2018). For this reason, cryptosporidiosis is considered one of the riskiest opportunistic infections for patients with acquired immunodeficiency syndrome (Wang *et al*., 2018).

2.9 Clinical Presentation of Cryptosporidiosis

After an incubation period of 5-10 days (range 2-28 days), an infected individual develops watery diarrhoea, which may be associated with abdominal cramps (White *et al.*, 2020; Robinson and Chalmers, 2020). In pattern less cases, fever may be low grade or non-existent; however, during a sudden increase of infection, fever may occur in 30%-60% of patients.

Diarrhoea, with or without cramp abdominal pain, may be intermittent and scant, or continuous, watery, and copious; sometimes, the diarrhoea is mucoid. It rarely contains blood or leukocytes. In individuals who are immunocompetent, the median duration of diarrhoea ranges from 5-10 days (mean of 10 days). Relapses may follow a diarrhoea-free period of several days to weeks. Diarrhoea can persist longer in individuals who are immunosuppressed.

The clinical manifestations of cryptosporidiosis in patients with HIV vary (White *et al.*, 2020; Wang *et al.*, 2018). In patients with CD4 cell counts of more than 200/μL, most infections are self-limited, similar to those in healthy hosts. Other patients develop chronic diarrheal illness with frequent, foul-smelling, bulky stools associated with significant weight loss. A minority of patients develop a profuse, cholera-like diarrhoea that can be complicated by malabsorption and volume depletion. The volume of fluid losses through diarrhoea may be extremely high, particularly in individuals with AIDS and CD4 cell counts below 50 cells/μL.

Biliary tract involvement is seen in persons with AIDS who have very low CD4 cell counts and is common in children with X-linked immunodeficiency with

hyper-immunoglobulin M (IgM). Biliary involvement may include cholecystitis that is not caused by inflammation of the gall bladder, sclerosing cholangitis, papillary stenosis, or pancreatitis. All are associated with right upper quadrant pain, nausea, and vomiting (Korpe *et al.,* 2016).

Although the main symptoms of cryptosporidiosis are related to the gastrointestinal (GI) tract, in immunocompromised patients, respiratory symptoms may also develop. Respiratory tract involvement is often asymptomatic, but it may manifest as bilateral pulmonary infiltrates with dyspnoea. Nonspecific respiratory symptoms, including shortness of breath, wheezing, cough, hoarseness, and harsh coughing, may be manifestations of respiratory infection. Rarely, conjunctival irritation is also present. In waterborne outbreaks, immunocompetent patients present with subclinical or milder illness that lasts for less than five days.

2.10 Clinical Diagnosis of Cryptosporidiosis

The diagnosis of cryptosporidiosis is usually made by microscopic detection of the parasite oocysts, oocyst antigens, or oocyst DNA in stool samples. Since the most common symptom of cryptosporidiosis is a watery diarrhoea, the differential diagnosis for *Cryptosporidium* includes bacterial, viral, and parasitic enteric pathogens associated with acute diarrhoea such as rotaviruses, coronaviruses, *Escherichia coli*, and *Salmonella* spp. (Khurana Chaudhary, 2018). However, gastrointestinal disorders may also have non-infectious causes, such as inflammatory bowel disease in humans (Omoruyi *et al*., 2014).

Diagnosis of cryptosporidiosis is usually made by microscopically identifying the presence of oocysts of 4 to 6 μm in diameter in the stool of the infected

subjects (Khurana and Chaudhary, 2018; Ahmed and Karanis, 2018). However, since the detection of *Cryptosporidium* oocysts can be difficult, three faecal samples collected on separate days should be microscopically examined for detection of oocysts prior to exclude a *Cryptosporidium* infection in subjects with severe diarrhoea. Besides, for detection of oocysts in stool, sample must be concentrated using the formalin-ether sedimentation method prior to microscopic examination (Pacheco *et al*., 2013). The oocysts of *Cryptosporidium* can also be observed by acid-fast (modified Ziehl–Neelsen method) or phenol–auramine staining on un-concentrated faecal smears, where the oocysts stain red and bright yellow, respectively (Omoruyi *et al*., 2014; Khurana and Chaudhary, 2018). However, much attention should be given to this staining since the oocysts may also appear as "ghost" cells (Vanathy *et al.*, 2017). Furthermore, the oocysts of *Cryptosporidium* are half the size of those of *Cyclospora cayetanensis* (about 4–5 μm in diameter vs. 9–10 μm in diameter), another coccidian protozoan parasite that infects the intestine of humans causing acute diarrhoea, much attention should be given when evaluating stool samples since the oocysts of both parasites are auto fluorescent and acid-fast (Pacheco *et al.*, 2013). In addition, although *C. cayetanensis* has a life cycle similar to *Cryptosporidium*, its oocysts are un-sporulated and not infective when shed in the faeces, and thereby, direct faecal–oral transmission cannot occur (Ahmed and Karanis, 2018). Although routine diagnosis of cryptosporidiosis is generally made by the microscopic identification of oocysts in faecal smears, this method, despite being easy to use and low cost, unfortunately has a low sensitivity (≤ 30%). Moreover, accurate diagnosis of cryptosporidiosis using this technique is largely dependent on the experience of the person using the microscope.

Sensitivity can be improved by performing modified acid fast stain, a staining generally performed if there are structures suspicious for *Cryptosporidium*, which has been reported to be associated with a sensitivity of 55%. However, these methods cannot distinguish between *Cryptosporidium* species (McHardy *et al*., 2014). Other methods for detecting *Cryptosporidium* includes**;** Enzyme immunoassay (EIA): It is highly sensitive and specific, and is useful for screening large numbers of specimens, Rapid immuno-chromatographic cartridge assays (ICCA) and genetic methods using Polymerase Chain Reaction (PCR) to amplify *Cryptosporidium* nucleic acids (Jennifer, 2021). For species identification, by PCR-restriction fragment length polymorphism (RFLP), PCR sequencing, or real-time PCR assays is usually performed as a reference test in specialist Laboratories and is practiced more often in some countries than in others (Xiao, 2010).

Standardised sub-typing method is currently not available, however, sequence analysis of GP60 gene has been applied to further characterise *C. parvum* and *C. hominis*; single-locust typing will undermine diversity, as it ignores the potential for genetic recombination within *Cryptosporidium* species population during reproduction (Widmer and Sullivan, 2012), and standardised multi locus subtyping schemes need to be validated to substantiate epidemiological studies (Robinson and Chalmers, 2020).

2.11 Treatment of Cryptosporidiosis

Nitazoxanide inhibits the growth of *Cryptosporidium parvum* and *Giardia lamblia* trophozoites (Cameron, 2023). It significantly shortens the duration of diarrhoea and can decrease the mortality risk in malnourished children (Khurana

and Chaudhary, 2018). Trials have also demonstrated efficacy in adults (CDC, 2019). Trials of antiparasitic drugs in patients with AIDS and cryptosporidiosis have been disappointing. Nitazoxanide, paromomycin, and azithromycin are partially active (Bhadauria *et al*., 2015). It is administered in a 3-day, twice-daily course of tablets or oral suspension (Chavez and White Jr, 2018; CDC, 2019). In clinical trials, nitazoxanide significantly reduced the duration of diarrhoea, increased the rate of parasitological clearance, and improved the mortality rate in malnourished children with *Cryptosporidium* infection who were HIV sero-negative (Khurana and Chaudhary, 2018). The most common adverse effects reported were abdominal pain, diarrhoea, vomiting, and headache; adverse effects were not significantly different from those reported with placebo. However, the use of nitazoxanide alone has not been successful in controlled trials in patients with AIDS (Khurana and Chaudhary, 2018; Nachipo *et al.*, 2018). In patients with HIV/AIDS and renal transplant recipients, studies have proposed off–label prolonged courses.

No antiparasitic drug has been proven to reliably cure cryptosporidiosis in patients that are immunocompromised. In patients with AIDS, cryptosporidiosis usually cannot be eradicated prior to restoration of the CD4 cell count in response to combination of antiretroviral therapy (White *et al.*, 2020). During early immune reconstitution, patients should generally continue antiparasitic therapy (e.g, nitazoxanide or paromomycin) and antimotility agents, as needed. In transplant recipients, reduction of immunosuppression, change from tacrolimus-based treatment to cyclosporine treatments, and combination antiparasitic therapy have proven satisfactory results (White *et al.*, 2020). Most recently, a case report of *Cryptosporidium* on a renal transplanted patient-

reported resolution of infection with a combination therapy of nitazoxanide, azithromycin, and rifaximin (Tomczak *et al*., 2022).

2.12 Novel Drug Targets Against Cryptosporidiosis

Despite the challenges of animal model-based research and *in vitro* culture, the availability of complete genome sequences of *Cryptosporidium* species has opened up new avenues for developing novel drug candidates. The streamlined metabolic pathways of the parasite allow greater opportunities for selective drug therapy (Funkhouser-Jones *et al.,* 2020). Various *in silico* approaches have been employed using comparative genomics analysis, homology modelling, prioritization parameters, epitope prediction, virtual screening, molecular docking, and simulation studies (Dhal *et al*., 2019). In the life cycle of apicomplexan parasites, the regulation of Ca^{2+} binding by calcium-dependent protein kinases (CDPKs) is necessary for parasite secretion, motility, and growth (Etzold *et al*., 2014). Therefore, CDPKs are thought to be potential therapeutic targets against the parasites (Su *et al*., 2022). After understanding the crystal structures of CDPKs having a gatekeeper glycine residue, specific bumped-kinase inhibitors (BKIs) were developed that inhibit CDPK1 functions through the hydrophobic pocket that opens up next to the glycine residue (Huang *et al.*, 2017; Van Voorhis *et al.,* 2021). Calcium-dependent protein kinase 6 (CpCDPK6) is a hypothetical protein of the CDPK family that regulates sporozoite invasion, gliding, and parasite egress (Zhang *et al.,* 2021). An *in silico* study that evaluated potential inhibitors against the CpCDPK6 reported the Tres Cantos Antimalarial Set (TCAMS) as potential inhibitors (Dhal *et al.,* 2020).

2.13 Prevention of Cryptosporidiosis

Water purification is the most important public health measure in the prevention of cryptosporidiosis (Love *et al.,* 2017). Because chlorination has little effect on the oocysts, water purification should involve flocculation and filtration (using filters with a pore size of 1-4 μm). Ultraviolet radiation and ozonisation are other means of disinfecting contaminated water. Decontamination can also be achieved by boiling water before use. Prompt, aggressive measures, including temporary closure of pools, must be carried out in cases of suspected faecal contamination of recreational water. People with diarrhoea should not use recreational water, and those with cryptosporidiosis should not use recreational waters for 2 weeks after symptoms resolve. Wearing gloves and hand washing after handling diapers can prevent person-to-person spread in day-care centres and hospitals. Endoscopes and similar instruments should be disinfected between uses. Prompt antiparasitic treatment of infected children decreases oocyst shedding. Individuals with AIDS or another immunosuppressive condition should avoid swimming in communal pools or recreational water that might be polluted with the parasite oocyst (Cameron, 2023). In hospitalized patients, contact precautions are strictly recommended in addition to standard precautions for patients who are incontinent or who use diapers. Vegetables should be properly washed before consumption. Contaminated water with animal and human faeces should not be used for irrigation purpose.

2.14 Risk Factors of Cryptosporidiosis

The following categories of individuals stand a higher chance or risk in the environment of ingesting *Cryptosporidium* oocysts and that can lead to the development of Cryptosporidiosis:

i. younger ones who attend day-care facilities.
ii. people who work with children, especially those who change diapers.
iii. the parents of children who carry *Cryptosporidium* oocysts.
iv. health workers that take care of Cryptosporidiosis patients.
v. tourists who may have exposure to unfiltered and untreated water.
vi. those that consume water from shallow wells that that are polluted.
vii. swimmers in non-private pool may ingest oocysts from polluted water.
viii. those who engage in unhealthy practise of anal sex.
ix. consumers of leaf salad where fresh vegetables are not necessarily cooked.
x. animal handlers that may carry cryptosporidiosis.

The parasites responsible for cryptosporidiosis occur in every part of the U.S. and all over the world. However, in countries with less effective water treatment and food safety, it can transmit to more people. This is because oocysts are more likely to remain in drinking water and food products and fresh vegetables.

In third world countries, cryptosporidiosis poses a serious health challenge. In a 2019 study of children in a Cameroon hospital, for example, doctors found *Cryptosporidium* in 8.9% of children overall and in 13.4% of those who had diarrhoea (Cameron, 2023).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Niger State is located in the north central region of Nigeria; it covers an area of 76,363 square kilometres. It is the largest Nigerian State by land area. The State shares boundaries with Kaduna State and Federal Capital Territory, Abuja in the East and South-East respectively, Kebbi and Zamfara in the North, Kwara and Kogi States in the South. The State also has an International Boundary with the Republic of Benin along Agwara and Borgu Local Government Areas to the North West. There are twenty-five (25) Local Government Area in Niger State. Generally, agricultural activities form the mainstay of the people's economy and engage directly or indirectly more than 80% of the population. According to the 2006 census, the population figure of Niger State is 3,950,249. Niger State is one of the richest in the country in terms of tourism. Some of the Tourist attractions are Zuma Rock, Gurara falls, Baro Empire Hill, Lord Lugard Colonial ruins at Zungeru, Nagwamatse Well and Kainji Lake National Park.

Niger State experiences distinct dry and wet seasons with annual rainfall varying from 1,100mm in the northern parts to 1,600mm in the southern parts. There are three Predominant ethnic groups (Nupe, Gbagyi, and Hausa) in the State, other tribal groups found in the State include; Kadara, Koro, Baraba, Kakanda, GanaGana, Dibo, Kambari, Kamuku, Pangu, Dukkawa, Gwada and Ingwai (Niger State Bureau of Statistics, 2011).

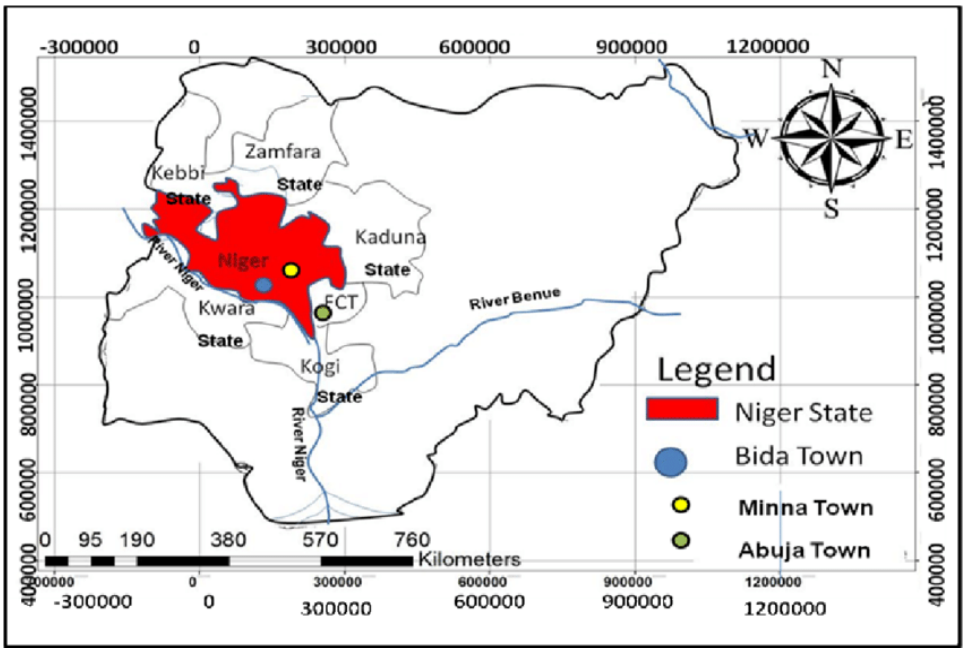


Figure 3.1: Map of Nigeria, showing Niger State.
Source: Niger State Bureau of Statistics, 2011.

3.2 Determination of Sample Size

The sample size was determined using a 31% prevalence of *Cryptosporidium oocysts* in fresh vegetables as reported by Odeke *et al*. (2019). The sample size was determined by using the equation described by Naing *et al*. (2006):

$$n = \frac{Z^2\ P(1-P)}{d^2} \tag{3.1}$$

Where n is the sample size;

P is the prevalence from a previous study = 31% = 0.31;

Z is the standard normal distribution at 95% confidence interval = 1.96;

d is the absolute desired precision at 5% = 0.05.

Therefore,

$$n = \frac{1.96^2 \times 0.31 \times (1-0.31)}{0.05^2}$$

$$n = \frac{0.8217}{0.0025} = 329$$

$\cong 400$ samples. Additional 200 samples were added to give 600 samples.

3.3 Sample Collection

Six hundred (600) vegetable samples were collected randomly from the selected markets (one hundred and fifty for each vegetable). Samples were collected during the morning hours into clean polythene bags and then taken to the Microbiology Laboratory of the Federal University of Technology, Minna. Thereafter, the Samples were processed for the detection of *Cryptosporidium* oocysts and other medically important parasites.

The following vegetables were selected for the study: Carrot, Lettuce, Tomatoes and Cabbage.

Table 3.1 contains basic information about the selected vegetables for the study that is, their common name, botanical name and the nature of the vegetables.

Table 3.1: **Common Name, Botanical Name and Nature of Vegetables selected for the Study**

Common Name	Botanical Name	Nature of Vegetable
Cabbage	*Brassica oleracea*	Leafy
Carrot	*Daucus carota*	Root
Lettuce	*Lactuca sativa*	Leafy
Tomato	*Lycopersicum esculentum*	Fruit

3.4 Laboratory Detection of *Cryptosporidium* Oocysts

Each sample was treated according to the protocol described by Ortega *et al.* (1997). One hundred grams of vegetables samples weighed and washed with distilled water by manual agitation for 10 minutes. The resulting washings was concentrated by centrifugation at 1500 rpm for five minutes.

Using modified Ziehl-Neelsen (ZN) staining technique (Chessbrough, 2005), a thin smear of the sediment was made on a clean microscope glass slide. The slide was air-dried, fixed into methanol for 2-3 minutes. The slide then was flooded

with cold carbol-fuchsin for 15 minutes. The slide was then rinsed thoroughly with tap water and decolourised in 1% HCl in methanol for 10 to 15 minutes. Slide was again washed with tap water, counter stained with 0.25% methylene blue for 30 seconds, then rinsed with tap water to remove the excess stain and air-dried. The slides were examined under the microscope using x10 and x40 objective lens.

3.5 Detection of *Cryptosporidium* Species by Microscopy

The prepared slides were taken for microscopy to detect *Cryptosporidium* oocysts using a light microscope. Magnification of x10, x40 and the oil immersion objective lens were used to detect the parasite oocysts. Photomicrographs of *Cryptosporidium* oocysts was used as positive slides which served as guide for proper identification.

3.6 Sedimentation Technique for Recovery of other Parasites from Vegetable Samples

A modified method (Abougrain *et al.,* 2010) was used as follows:

Each vegetable sample was weighed (250 g) and washed in tap water (1500 ml) in a plastic container. The resultant water was left in the plastic container for 10 minutes to sediment. The supernatant was discarded and the residue transferred into the centrifuge tube and spin at 1500 rpm for five minutes with the help of centrifugation machine. The supernatant was discarded and the residue allowed to settle again. A drop from this residue was placed on a clean microscopic slides and covered gently with cover slip. Microscopic examination of this prepared slide was carried out using 10x and 40x objective lens for the presence of other parasite eggs, larvae and adult stages respectively. The quantification of

parasites burden on the vegetables was carried out by direct smear egg/larva count.

3.7 Evaluation of Predisposing Factors

The risk factors such as source of water for washing and sprinkling of vegetables, duration of harvest and sale and means of transportation among others, were assessed with the aid of well-structured questionnaires. Data were collected from the respondents that is, the vegetables sellers or vendors in the selected markets after the objectives of the study were explained to them and their verbal consent was obtained.

3.8 Data Analysis

Data from the research findings were analysed using SPSS version 21 (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 statistically significant).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Occurrence of *Cryptosporidium* Oocysts Based on the Type of Vegetables Analysed

Five (0.83%) of the 600 vegetables sampled during the study were positive for *Cryptosporidium* oocysts. Only 3 out of 4 types of vegetable examined were positive for *Cryptosporidium* oocysts. Lettuce and cabbage had the highest prevalence of (1.33%) respectively followed by carrot (1%), while tomatoes showed no presence of oocysts completely. There was significant difference between occurrence of *Cryptosporidium* oocysts and type of vegetables examined as shown in Table 4.1

Table 4.1: **Occurrence of *Cryptosporidium* Oocysts Based on the Type of Vegetables Analyzed**

Kind of Vegetable	Number Examined	Number positive (%)	P value
Cabbage	150	2 (1.33)	
Carrot	150	1 (0.67)	
Lettuce	150	2 (1.33)	0.00
Tomatoes	150	0 (0)	
Total	**600**	**5 (0.83)**	

4.1.2 : Occurrence of *Cryptosporidium* Oocysts on the Vegetables Based on the Markets Surveyed

Three (1.5%) of the vegetables examined from Gwari Market and 2 (1.0%) from Kure Market were positive for *Cryptosporidium* oocysts respectively, and vegetables from Bosso Market showed no oocyst. There was significant difference between occurrence of *Cryptosporidium* oocysts and the markets in which the vegetables were sold as indicated in Table 4.2

Table 4.2: Occurrence of *Cryptosporidium* Oocysts on the Vegetables Based on the Markets Surveyed

Market	Number Examined	Number positive (%)	P value
Bosso	200	0 (0)	
Gwari	200	3 (1.5)	0.00
Kure	200	2 (1.0)	
Total	**600**	**5 (0.83)**	

4.1.3 : Occurrence of *Cryptosporidium* Oocyst Based on the Months of Survey

Table 4.3 reveals monthly variation of *Cryptosporidium* oocyst on vegetables examined from the selected Markets within Minna. Vegetable samples recorded the highest contamination with *Cryptosporidium* oocyst in the month of August (2.50%) and 0.83% contamination were recorded in the months of June and July respectively. The months of November and December recorded no oocyst contamination at all. There was significant difference between the months surveyed and the occurrence of *Cryptosporidium* oocyst.

Table 4.3: Occurrence of *Cryptosporidium* Oocyst Based on the Months of Survey.

Month	Number Examined	Number positive (%)	p value
June	120	1 (0.83)	
July	120	1 (0.83)	
August	120	3 (2.50)	0.00
November	120	0 (0)	
December	120	0 (0)	
Total	**600**	**5 (0.83)**	

The photomicrographs below (plate I and II) are oocysts of *Cryptosporidium* species, an intestinal protozoa parasite, belonging to the class coccidian. They were isolated from vegetable smear stained with modified Ziehl-Neelsen stain. The oocysts stained pink with this stain. They are spherical or oval in shape. Oocysts are environmentally resistant and become infective when ingested by an individual through water or consumption of uncooked vegetables.

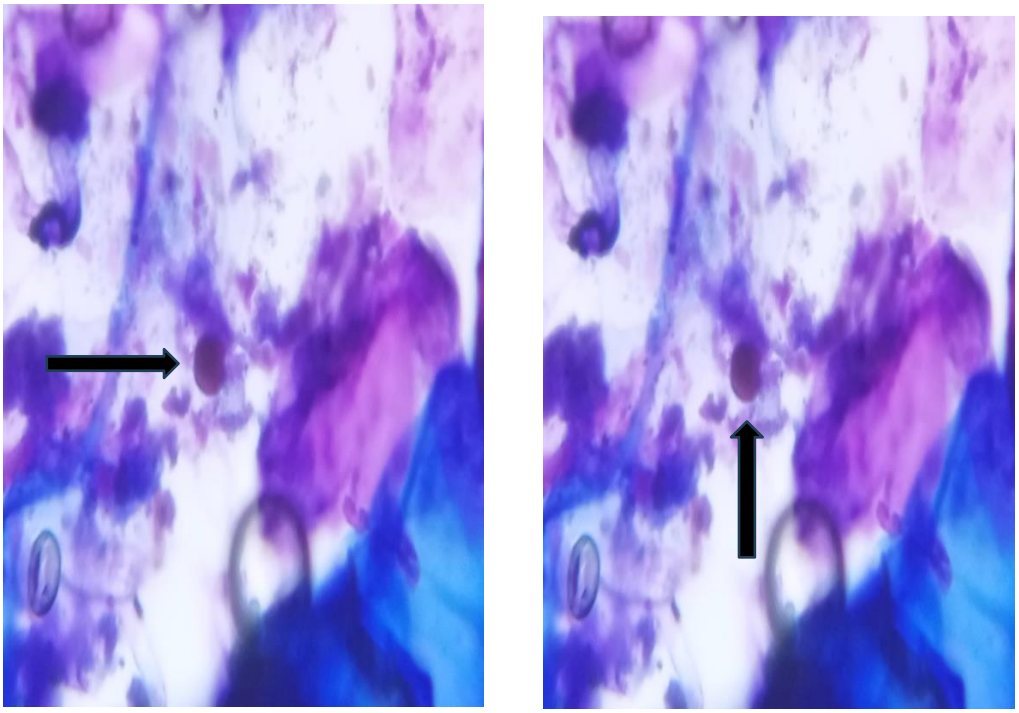


Plate I: Photomicrograph of *Cryptosporidium* oocyst (x 1000 magnification) isolated from cabbage

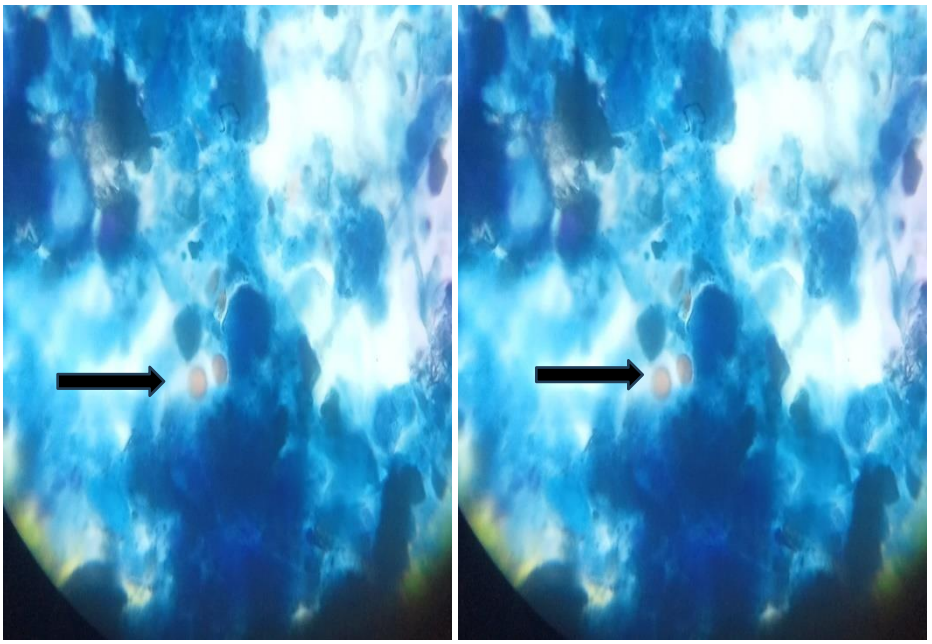


Plate II: Photomicrograph of *Cryptosporidium* oocyst (x 1000 magnification) isolated from lettuce

4.1.4 : Overall Occurrences of Other Parasites Identified from the Vegetable Samples

Table 4.4 shows parasites of medical importance other than *Cryptosporidium* oocysts that were also isolated from the vegetables. The highest species of parasite recorded was *Entameoba histolytica* representing 5.8% followed by *Ascaris lumbricoides* with 5.0% occurrence while *Trichiuris trichiura* was the lowest parasitic species recorded with 1.0% occurrence.

Table 4.4: Overall Occurrences of Other Parasites Identified from the Vegetable Samples

Parasite detected	Number	Occurrence (%)
Ascaris lumbricoides (ova)	30	5.0
Hookworm (ova)	15	2.5
Nematode larva (not identified)	18	3.0
Entameoba histolytica (cyst)	35	5.8
Enterobiusvermicularis (Egg)	8	1.3
Trichiuris trichiura (Egg)	6	1.0
Strongyloides species (Larva)	12	2.0
Rotifers * (larva)	10	1.6
Total	**134**	**22.3**

4.1.5 : Number of Parasites on the Basis of Vegetable Types

Table 4.5 shows total number of parasites identified from each vegetable samples. Carrot recorded the highest number of parasite with 8.0% occurrence followed closely by lettuce with 6.5% while tomatoes recorded the lowest number of 17 parasites representing 2.8% occurrence.

Table 4.5: Number of Parasites on the Basis of Vegetable Types

Type of Vegetable	Number	Occurrence (%)
Cabbage	30	5.0
Carrot	48	8.0
Lettuce	39	6.5
Tomatoes	17	2.8
Total	**134**	**22.3**

4.1.6 : Number of Parasites on the Vegetables Based on the Markets Surveyed.

Table 4.6 shows the number of parasites on the vegetable on the basis of Markets sampled. Sixty (60) Vegetable samples from Gwari market was the highest contamination load representing 10.0% occurrence. The second was vegetable samples from Kure market that had 49 positive cases representing 8.2%. only 25 vegetable samples from Bosso market were positive for some parasite with 2.5% rate of occurrence.

Table 4.6: Number of Parasites on the Vegetables Based on the Markets Surveyed.

Market	Number	Occurrence (%)
Bosso	25	4.2
Gwari	60	10.0
Kure	49	8.2
Total	**134**	**22.3**

4.1.7 : Percentage Occurrence of Helminthic Eggs on the Vegetable Samples with Respect to the Markets Surveyed.

Table 4.7 signifies the percentage occurrence of helminthic ova on the vegetable samples in the selected Markets. It shows the distribution pattern of the helminthic eggs across the markets. *Ascaris lumbricoides* eggs recorded the highest spread across the three markets; 8.5%, 14.1% and 19.7% in Bosso, Gwari and Kure markets respectively while the parasitic eggs with the least spread across the markets is *Trichiuris trichiura*; 0%, 5.6% and 0.3% in Bosso, Gwari and Kure markets respectively.

Table 4.7: Percentage Occurrence of Helminthic Eggs on the Vegetable Samples with Respect to the Markets Surveyed.

Market	*Strongyloides**	*Ascaris lumbricoides*	Hookworm	*Trichiuris trichiura*	*Enterobius Vermicularis*
Bosso	3 (4.2)	6 (8.5)	2 (2.8)	0 (0)	1 (1.4)
Gwari	5 (7.0)	10 (14.1)	4 (5.6)	4 (5.6)	2 (2.8)
Kure	4 (5.6)	14 (19.7)	9 (12.7)	2 (0.3)	5 (7.0)
Total	**12**	**30**	**15**	**6**	**8**

* = Larvae but not ova

4.1.8 : Parasitic Stage(s) as Seen on the Vegetables with their Percentage Contamination.

Table 4.8 indicates the parasitic stage(s) as observed on the vegetables. *Entamoeba histolytica* cystic stage dominated with 5.8% occurrence followed by the egg and larvae stages of *Ascaris lumbricoides* with 5.0% occurrence. The least parasitic stage is the ova of *Trichiuris trichiura* with 1.0% occurrence.

Table 4.8: Parasitic Stage(s) as Seen on the Vegetable Samples with their Percentage Contamination

Organisms	Life-stage (s)	Occurrence (%)
Ascaris lumbricoides	Ova, larvae	30 (5.0)
Hookworm	Ova	15 (2.5)
Nematodes (others)	Larvae	18 (3.0)
Entamoeba histolytica	Cyst,	35 (5.8)
Enterobius vermicularis	Ova	8 (1.3)
Trichiuris trichiura	Ova	6 (1.0)
Strongyloides species	Larvae	12 (2.0)
Rotifers *	Larvae	10 (1.6)

* = Non parasitic organisms.

4.1.9 : Descriptions of the Observed Parasitic Objects on the Vegetable Samples Analysed.

Table 4.9 shows the descriptive features of parasitic life-stages on the vegetable samples. These descriptive features aided in the easy identification of the suspected parasites as observed on the different vegetable samples.

Table 4.9: Descriptions of the Observed Parasitic Objects on the Vegetable Samples Analysed.

Vegetable	Parasitic-stage	Description	Probable parasite
CT, TM, LT	Ova	Brown in colour with lumpy outer shell	*Ascaris lumbricoides*
CT, LT	Ova	Lemon-shaped, elongated and bipolar plugs at each end	*Trichiuris trichiura*
CT, CB	Ova	Colourless with a thin shell that appears as black line microscopically	Hookworm
LT	Ova	Oval-shaped and flattened at one side	*Enterobius vermicularis*
CT, CB, LT, TM	Cyst	Spherical shaped with nuclei	*Entamoeba histolytica*

Key: CT - Carrot, CB - Cabbage, LT - Lettuce, TM – Tomatoes

This is a photomicrograph of *Ascaris*, common round worm immature stage (larva). The adult stage is the largest nematode parasitizing the human intestinal tract. When the parasite is in the body, the migrating larvae are capable of causing ascariasis and pneumonitis with eosinophilia.

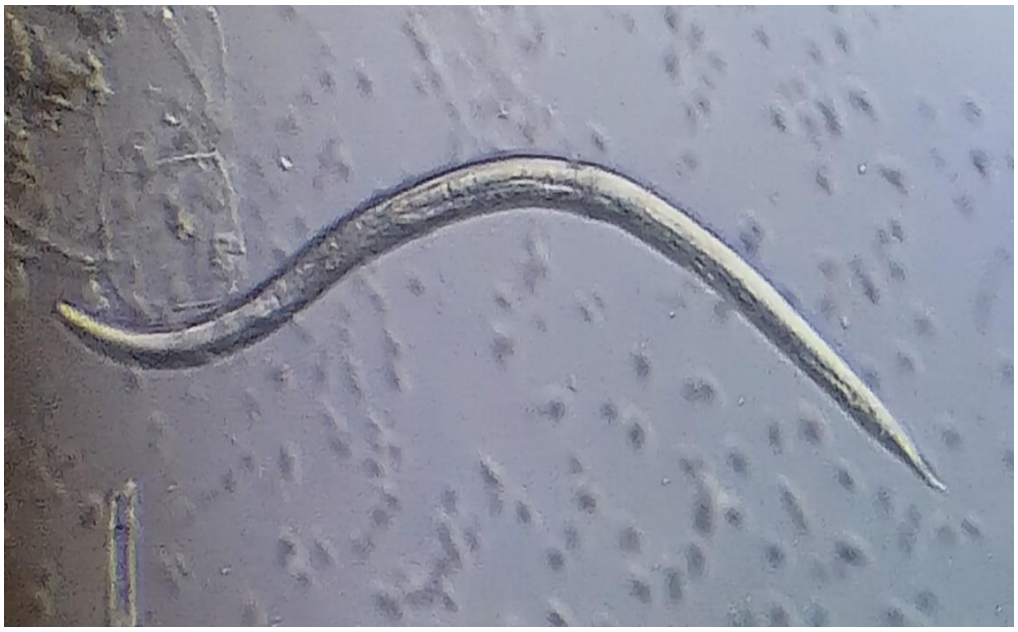


Plate III: Photomicrograph of *Ascaris* larva stage (x 1000 magnification) isolated from cabbage.

This is immature stage (larva) of nematode (round worm) showing the anterior (mouth region) and the posterior (anal region). The adult stage of this parasite inhabit the small intestine.

A **P**

Plate IV: Photomicrographs of Nematode larva stage, anterior (A) and posterior (P) view (x 1000 magnification) isolated from cabbage.

These are the photomicrographs (plate V and VI) of the larva stage of *Strongyloides* species, the smallest nematode known to cause infection in human. The adult stage of this parasite is found in the small intestine. The posterior and full view are shown below.

Plate V: Photomicrographs of *Strongyloides* spp larva stage, posterior view and full view (x 400 magnification) isolated from Tomatoes.

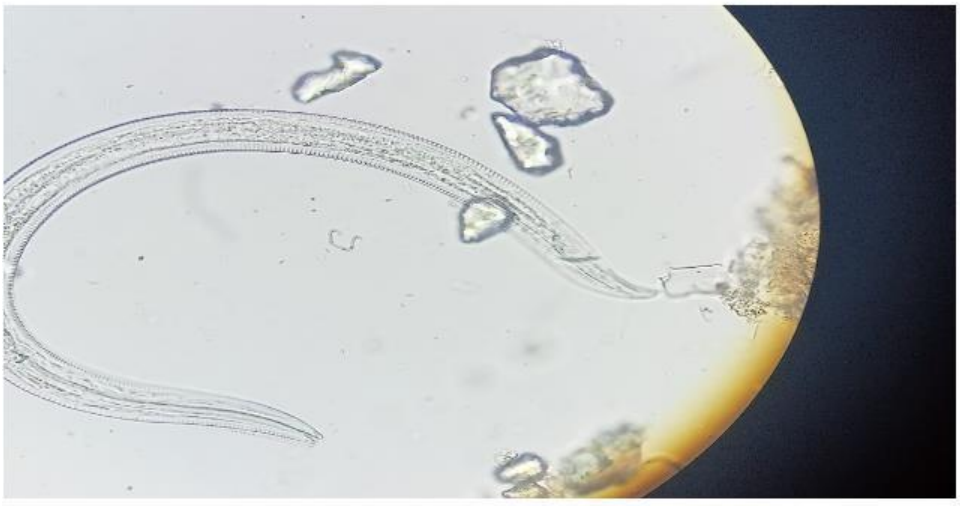


Plate VI: Photomicrograph of *Strongyloides* spp larva stage, anterior and posterior view (x 400 magnification) isolated from Carrot.

This is the cystic stage of *Entamoeba* species, a protozoa causing the disease known as amoebiasis. This is the infective stage of the parasite. The parasite inhabits the large intestinal tract of human.

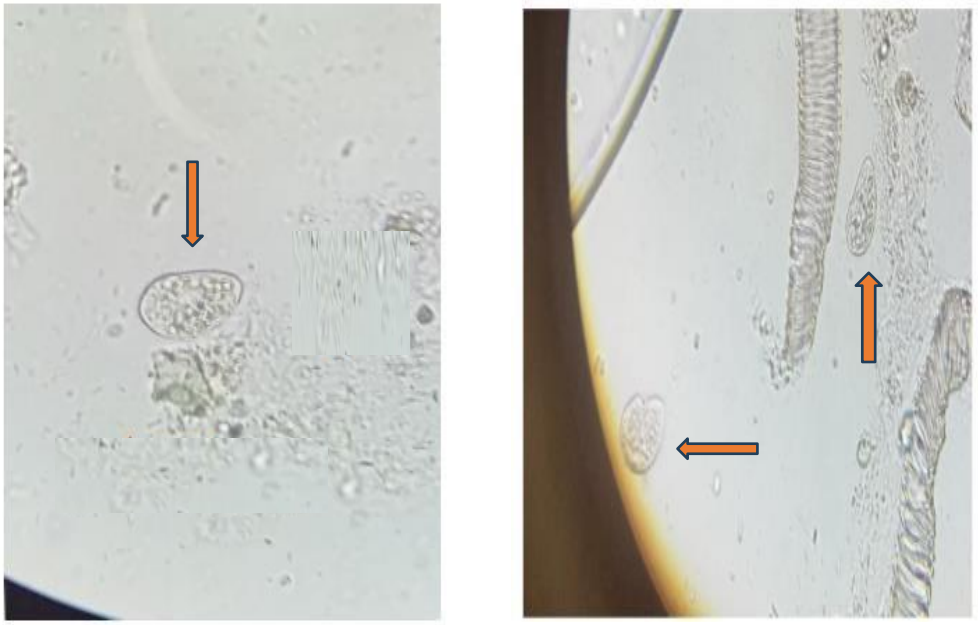


Plate VII: Photomicrographs of *Entamoeba* species cyst stage, (x 400 magnification) isolated from tomatoes.

The photomicrograph below is the oval stage of thread or pin worm, *Enterobius vermicularis*. It is flattened on one side (plano-convex). The ova are usually surrounded by thin, smooth and transparent shell. The adult stage of this parasite is found in the large intestine of human. It causes enterobiasis.

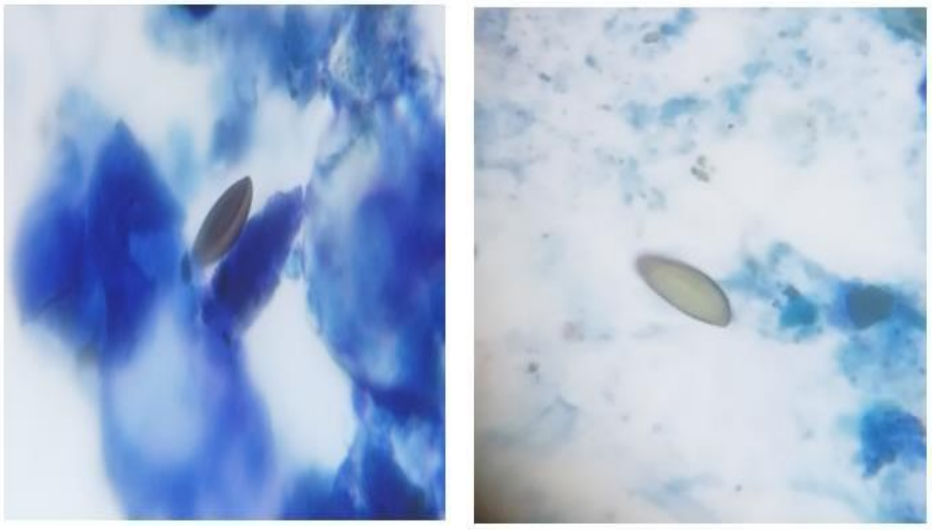


Plate VIII: Photomicrographs of *Enterobius vermicularis* ova stage, (x 1000 magnification) isolated from Lettuce and carrot.

This is the oval stage of whipworm, *Trichiuris trichiura.* The eggs are barrel-shaped or lemon-shaped with mucuos plug at each pole. The adult form of this parasite is found in the large intestine of human and is responsible for the disease called trichiuriasis.

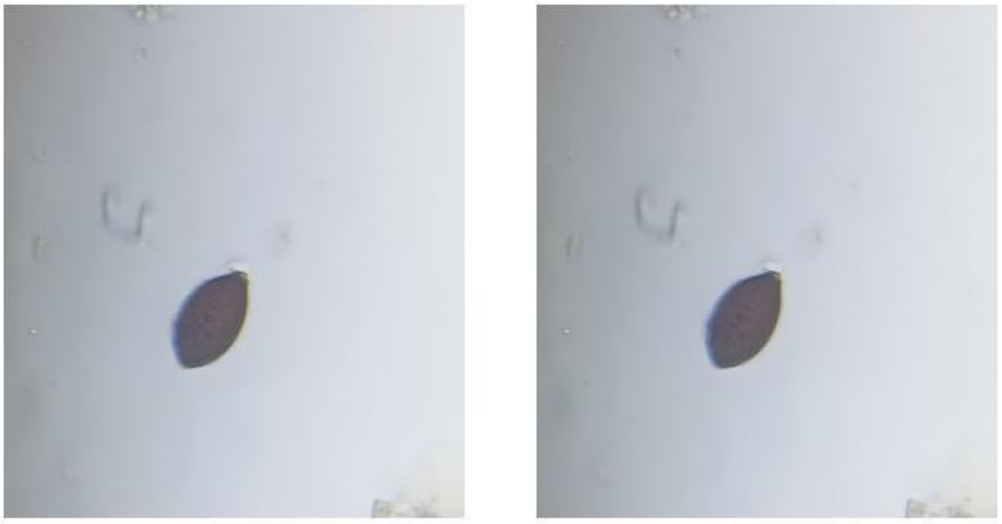
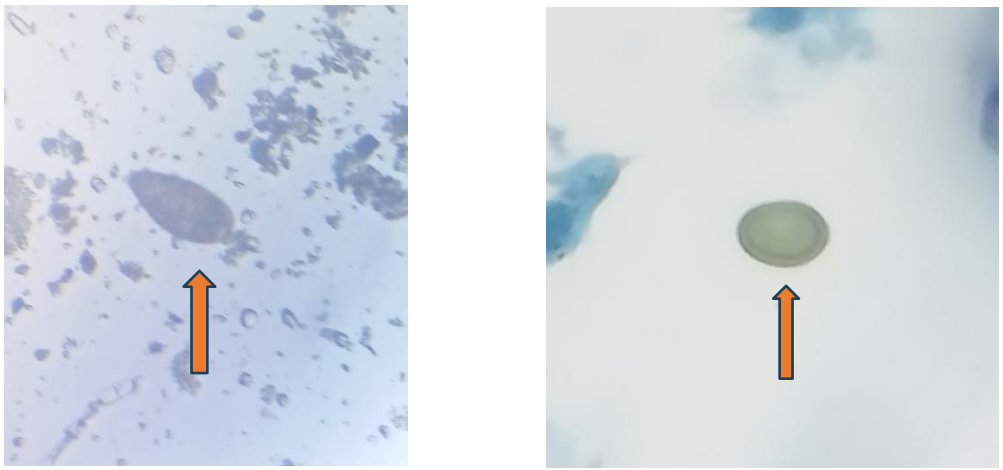


Plate IX: Photomicrographs of *Trichiuris trichiura* species ova stage, (x 400 magnification) isolated from Lettuce.

The egg stage of nematode species. The first one (A) has brown colour with lumpy outer shell while the other one (B) has a thin shell that appears as black line microscopically.



A **B**

Plate X: Photomicrographs of nematode species ova stage, (x 400 magnification) isolated from Carrot and Lettuce.

These photomicrographs are rotifers, thorny-headed worms. They are known for their cryptobiotic capabilities, that is, they are able to stop their metabolism, dehydrate their cell and enter into a state of dormancy. Although non-parasitic, they are indicators of biological pollution in the environment.

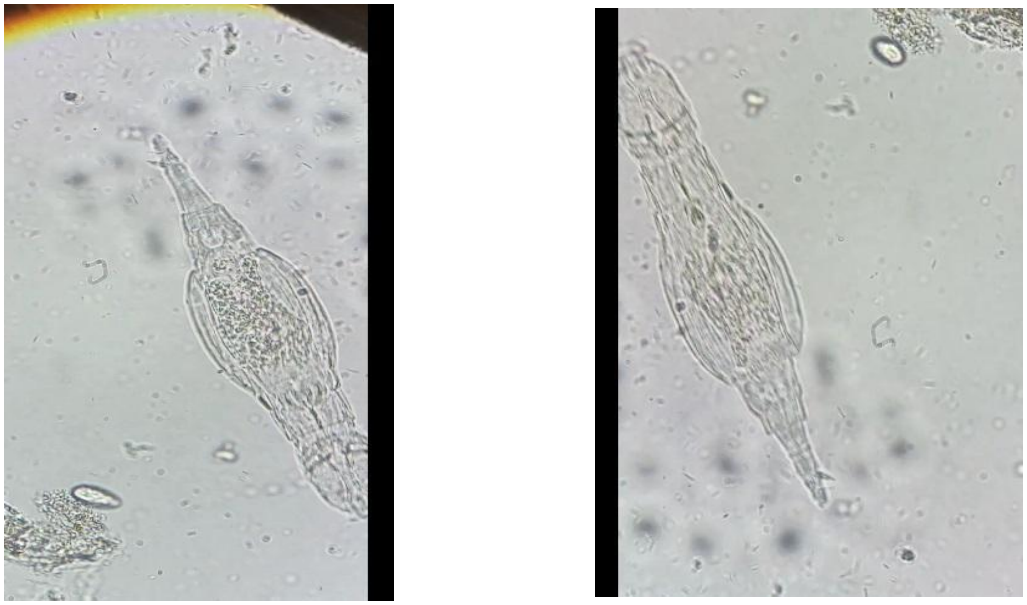


Plate XI: Photomicrographs of Rotifer species isolated from cabbage.

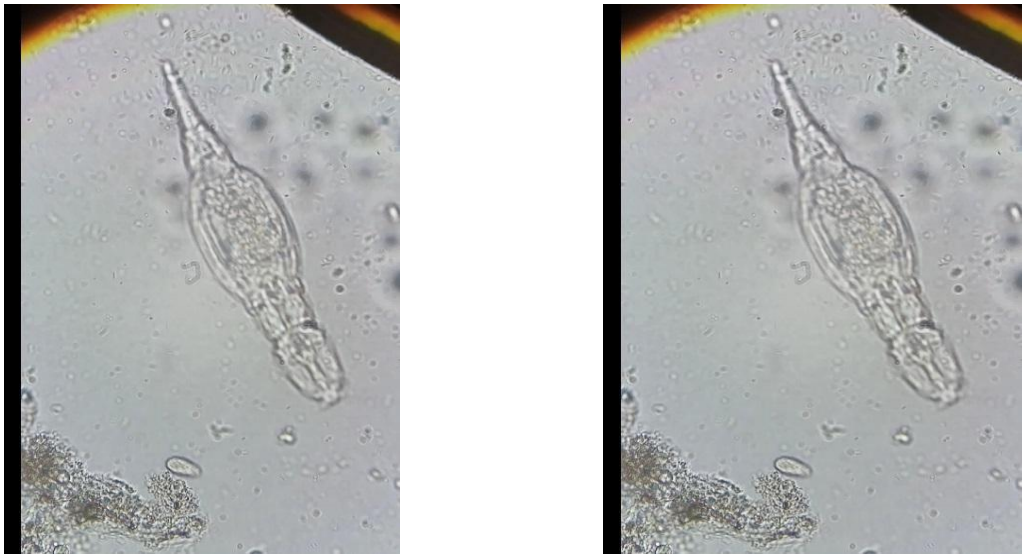


Plate XII: Photomicrographs of Rotifer species isolated from cabbage.

4.2 Possible Sources of Vegetable Contamination in the Markets

4.2.1 Means of Transporting Vegetables to the Markets

Figure 4.1 shows the means of transporting vegetable samples to the markets. Sixty vegetable vendors used vehicle as means of transportation representing 40% while 35 vendors used motorcycle accounting for 23.3% and 55 used tricycle representing 36.7%.

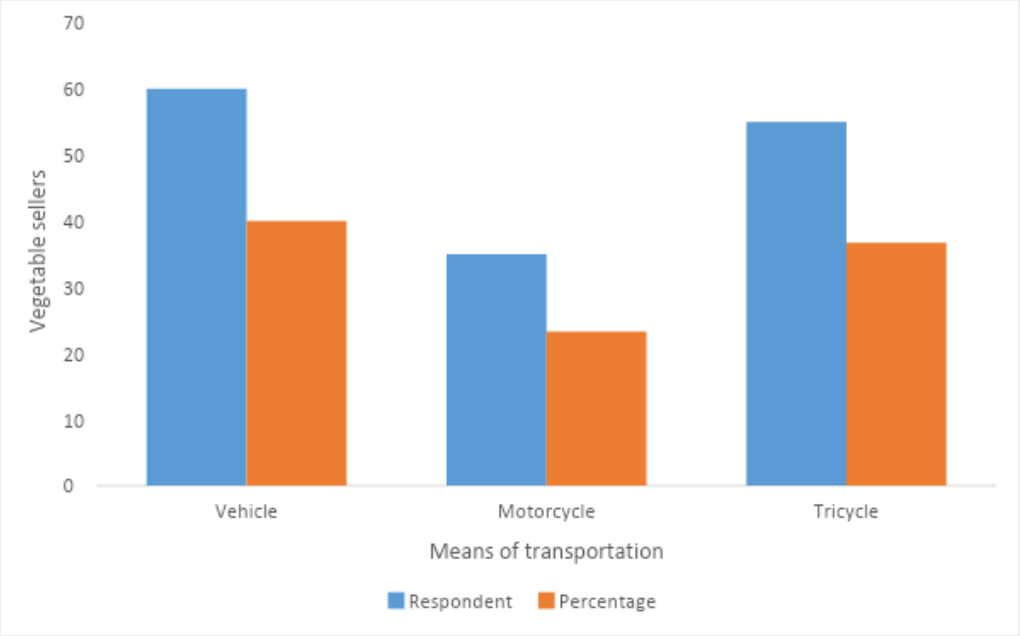


Figure 4.1: Means of transporting vegetables to the Markets.

4.2.2 Methods of Displaying Vegetables for Sale in the Markets

Figure 4.2 shows the various methods of displaying the vegetable samples for sale in the markets. Fifty-one respondents display their vegetables on the floor accounting for 34%, 95 sellers display their fresh produce on table/wheel barrow accounting for 63.3% and only 4 of them display their produce inside the shops representing 2.7%.

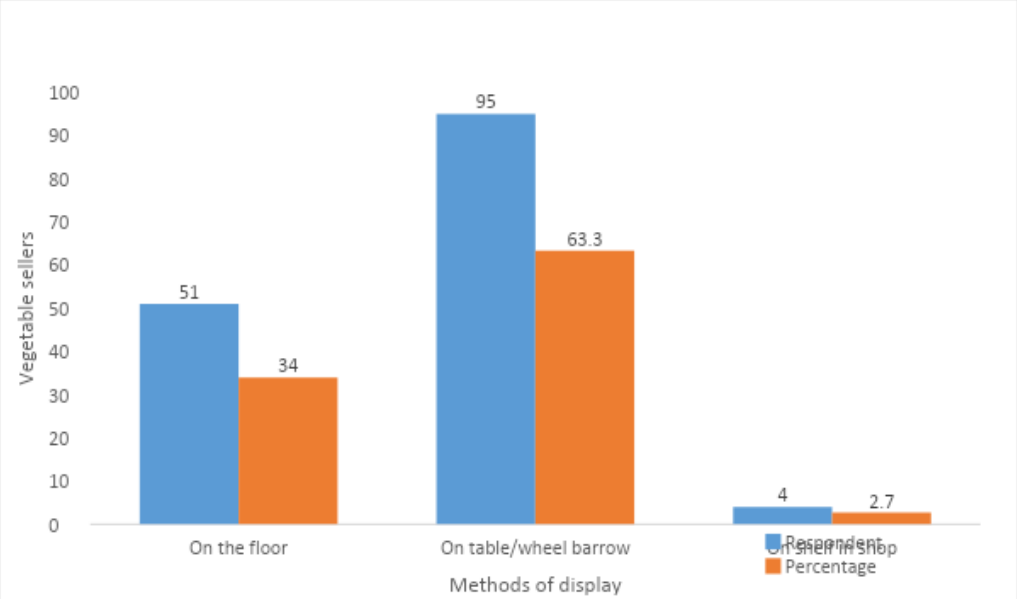


Figure 4.2: Methods of displaying vegetables for sale in the markets.

4.2.3 Sources of Water for Washing and Sprinkling of the Vegetables in the Markets

Figure 4.3 indicates sources of water used for washing and sprinkling of the vegetables in the markets. Thirty vegetable sellers used well water representing 20%, 40 of them used tap water accounting for 26.7% and 80 vegetable sellers used borehole water which represents 53.3% as the most frequently used source of water.

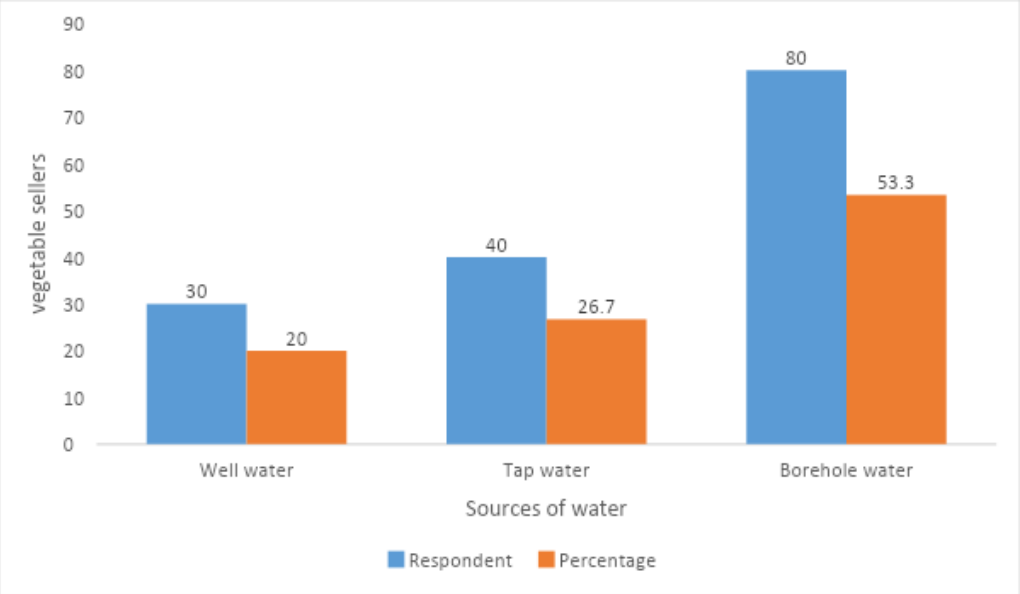


Figure 4.3: Sources of water for washing and sprinkling vegetables in the Markets.

4.2.4 Educational Status of the Vegetable Vendors

Figure 4.4 denotes the educational status of the vegetable vendors in the selected markets. Seventy of them had primary education accounting for 46.7%, 43 vegetable vendors acquired secondary education representing 28.7% and 35 of them had no formal education representing 23.3%.

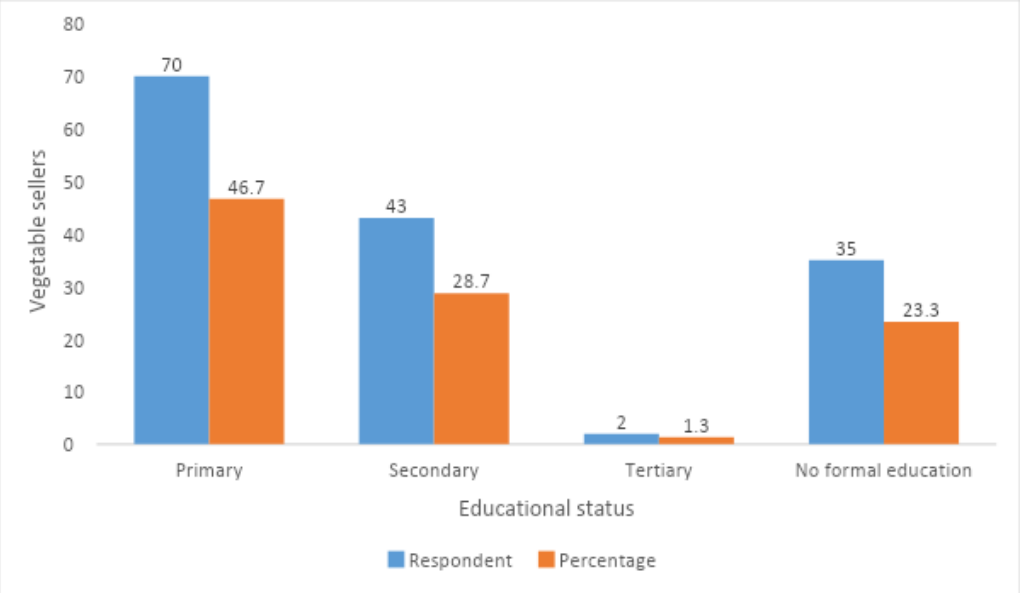


Figure 4.4: Educational status of the vegetable sellers in the Markets.

4.3 Discussion

The outcome of this study showed that 5 (0.83%) samples of vegetables were contaminated with *Cryptosporidium* oocyst out of 600 vegetable samples from three different Markets situated in Minna, Niger State. This rate is far lower than the 31% recorded in Zaria, Kaduna State as reported by Odeke, 2019 from vegetable farm irrigated with treated wastewater. The reason for this variation may be due to factors, such as environmental conditions, nature and choice of the vegetables utilized and the months when the samples were taken. Out of the four different vegetables assessed for *Cryptosporidium* oocyst, Lettuce and Cabbage had 2 (1.33%) positive samples each. The nature of the leaves of these vegetables might be a significant variable. The broadness of lettuce leaves confers large surface area to the plant which may facilitate the trapping of parasitic oocyst from contaminated sources. The overlapping leaves of Cabbage may hold water contaminated with the parasite oocyst (Robertson, 2019). Carrot had a lower contamination rate of the oocyst, this may be due to its decreased surface area. The absence of the oocyst on Tomato is presumably because of its smooth surface making it challenging for the oocyst to attach with firmness (Said, 2012). Chi square analysis P value (< 0.05) indicated a significant with regards to contamination and vegetable types.

Vegetables from Gwari Market recorded 3 (1.5%) cases followed by vegetables from Kure Market, which recorded 2 (1.0%) cases. The selling points of the vegetables in these Markets had a close proximity to where fresh meat and fish are being sold. The abundance of house flies coupled with the dirty surrounding could contribute to contamination of the vegetables. Shockingly, vegetables

from Bosso Market had no occurrence of *Cryptosporidium* oocyst. This might be due to relatively clean surrounding of the majority of the selling points.

In the month of August, 3 (2.5%) samples were positive for the oocyst. This might be due to higher amount of rainfall in this month. Higher rainfall prompts expanded spill over water, overflow from river or stream which may interact with these vegetables submerged with faecal matters of grazing animals in the farm prior to being taken to the market (Omowaye and Audu, 2012). No contamination rates were recorded in the period of November and December. Reduced rainfall in this month might be a significant factor. Freshly harvested vegetable can transmit the oocyst of this protozoan (Idahosa, 2011). Episodes of disease brought about by intestinal parasites because of consuming raw vegetable were documented in advanced and non-industrial nations.

Other parasites that are of medical importance identified during this research includes; *Entamoeba histolytica* with contamination rate of 35 (5.8%) being the most prevalent. This is in agreement with the work conducted by Sai-su *et al*. (2012). The parasite with the least prevalence was *Trichiuris trichiura* having 6 (1.0%) rate of contamination. Others were *Ascaris lumbricoides*, hookworm, *Enterobius vermicularis* and *Strongyloides* spp. The identification of hookworm oval was not in agreement with the research conducted by Ebrahimzadeh *et al.* (2013). Geographical location might be an important factor. The presence of these parasites on vegetables has further revealed the risk of coming down with infections as a result of consuming raw vegetables. Most of the parasites become infective in soil which can easily be transmitted through foods and water (Tamirat *et al.*, 2014).

The risk factors that may contribute to the low occurrence of *Cryptosporidium* oocysts on the vegetables include the means of transporting the produce to the market. 60 (40%) respondents from this study conveyed their vegetables to the market by vehicle, this may prevent further contamination on transit by airborne oocyst. 95 (63.3%) displayed vegetables on table and wheelbarrow, this may reduce the occurrence of the parasite compared to when it is on the floor. Water source is another risk factor that contribute to the contamination of the foods. 80 (53.3%) used water from borehole to wash and moist the raw vegetables while on display in the market. Studies showed that *Cryptosporidium* oocysts can be found in all sources of water and their presence is more prominent in surface water than in ground water (Glaberman *et al*., 2002). According to Dixon (2014), good quality of water for washing and processing, monitoring and enforcing good personal hygiene in food handlers can serve as postharvest control measures against the parasite. From this study, 70 (46.7%) of the vegetable vendors have at least primary education, this may contribute to low occurrence of the oocyst because basic personal hygiene must have been practised in handling the produce. Despite the low occurrence of *Cryptosporidium* oocysts on vegetables, there is need for concern because the protozoa parasite is known to have very low infective dose and the younger ones are at high risk of infection (Guerrant *et al*., 2004).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The current study showed a low occurrence (0.83%) of *Cryptosporidium* oocysts on the vegetables sold within the selected Markets in Minna, Nigeria. The vegetables that were found to be more contaminated were cabbage and lettuce (1.33%) for each. Risk factors such as sources of water for washing and sprinkling the vegetables, means of transportation and method of displaying vegetables for sale in the Market are key to the occurrence of the parasites on the vegetables. Worthy of note, are the protozoa and helminths of medical importance that were identified on the vegetables, with carrot (8.0%) found to be more contaminated. The presence of these parasites is a great concern as they pose a high public health challenge to individuals particularly the Children and the immunocompromised persons.

5.2 Recommendations

Based on the findings of this research, the following recommendations are made:

i. Vegetables should be thoroughly washed with clean water before consumption.
ii. Fresh vegetables should be washed with salt and vinegar.
iii. Vegetables should be kept in low temperature storage for future use.
iv. Handlers and farmers should be educated on the need to maintain good hygienic practice.
v. Animal dungs should be dried to kill some parasites before application as fertilizer.

vi. Regular deworming of those who cultivate vegetables should be encouraged.

5.3 Contributions to Knowledge

This research work shows low prevalence of *Cryptosporidium* oocyst on the vegetables sold in the selected markets.

i. This information is important to the public health practitioners for policy making in terms of tropical disease control. The findings from this research point to the need for the general public to be more alert on how fresh vegetables such as cabbage and lettuce are handled and processed before consumption.

ii. The work has also demonstrated that fresh vegetables are vital routes for parasitic transmission to human in our environment.

iii. High load of other helminthic and protozoa parasites of medical importance on carrot as observed from this study is an eye opener to consumers for them to be more caution when consuming this fresh product especially the immunosuppressed individuals.

iv. Furthermore, another contribution to knowledge attributable to this research is the identification of *Rotifer* species from cabbage. Although, the organism is non-parasitic, it is an indicator of biologically polluted environment.

REFERENCES

Abougrain, A. K., Nahaisi, M. H., Madi, N. S., Saied, M. M., & Ghenghesh, K. S. (2010). Parasitological contamination in salad vegetables in Tripoli-Libya, *Food Control*, 21(5), 760–762.

Adamu, H., Petros, B., Zhang, G., Kassa, H., Amer, S., Ye, J., Feng, Y., & Xiao, L. (2014). Distribution and clinical manifestations of *Cryptosporidium* species and subtypes in HIV/AIDS patients in Ethiopia. *PLoS Neglected Tropical Disease*, 8, e2831.

Ahmed, S. A., & Karanis, P. (2018). Comparison of current methods used to detect *Cryptosporidium* oocysts in stools. *International Journal and Hygiene Environmental Health*, 221 (5), 743–63.

Ahmed, S. A., & Karanis, P. (2020). *Cryptosporidium* and Cryptosporidiosis: The Perspective from the Gulf Countries. *International Journal of Environmental Resource on Public Heath*, 17, 6824.

Ajjampur, S. S., Rajendran, P., Ramani, S., Banerjee, I., Monica, B., & Sankaran, P. (2008). Closing the diarrhoea diagnostic gap in Indian children by the application of molecular techniques. *Journal Medical Microbiology*, 57, 1364-8.

Almeida, A. J., Lima, V. S., Rodrigues, A. B., & Di Filippo, P. A. (2013). Contamination with *Cryptosporidium* spp. in manure used as fertilizer in urban gardens. *Review of Veterinary science*, 108, 23–27.

Ayinmode, A. B., Oliveira, B. C. M., Obebe, O. O., Dada-Adgebola, H. O., Ayede, A. I., & Widmer, G. (2018). Genotypic Characterization of *Cryptosporidium* Species in Humans and Peri-Domestic Animals in Ekiti and Oyo States, Nigeria. *Journal of Parasitology*, 104(6), 639–44.

Banwat, E. B., Egah, D. Z., Onile, B. A., Angyo, I. A., & Audu, E. S. (2003). Prevalence of *Cryptosporidium* infection among undernourished children in Jos, central, Nigeria. *Postgraduate Medical Journal*, 10 (2), 84-87.

Bhadauria, D., Goel, A., Kaul, A., Sharma, R. K., Gupta, A., & Ruhela, V. (2015). *Cryptosporidium* infection after renal transplantation in an endemic area. *Transplant Infectious Disease*, 17 (1), 48-55.

Bouzid, M., Kintz, E., & Hunter, P. R. (2018). Risk factors for *Cryptosporidium* infection in low and middle income countries: A systematic review and meta-analysis. *PLoS Neglected Tropical Disease*, 12(6), e0006553.

Bouzid, M., Hunter, P. R., Chalmers, R. M., & Tyler, K. M. (2013) *Cryptosporidium* Pathogenicity and Virulence, *Clinical Microbiology Review*, 26 (1), 115-34.

Cameron, W. (2023). What to know about cryptosporidiosis, a medical review updated on January 24, 2023 available at https://www.medicalnewstoday.com/articles/190664 accessed on 24 Jan. 2023.

Carrero, J. C., Reyes-López, M., Serrano-Luna, J., Shibayama, M., Unzueta, J., & León-Sicairos, N. (2020). Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. *International Journal of Medical Microbiology*, 151358 https://doi.org/10.1016/j.ijmm.2019.151358.

Centre for Disease Control and Prevention, CDC. (2019). Centre for Disease Control and Prevention. - Laboratory Identification of Parasites of Public Health Concern: Cryptosporidiosis. Laboratory identification CDC. Available at https://www.cdc.gov/dpdx/cryptosporidiosis/index.html. Accessed: November 6, 2019.

Centre for Disease Control and Prevention, CDC. (2018). Cryptosporidiosis summary report – National Notifiable Disease Surveillance System, United State, 2018. Atlanta Georgia U.S.

Centre for Disease Control and Prevention, CDC. (2020). Cryptosporidiosis. Available online: https://www.cdc.gov/dpdx/cryptosporidiosis/index.html (accessed on 1 August 2020).

Chalmers, R. M., Robinson, G., Elwin, K., & Elson, R. (2019). Analysis of the *Cryptosporidium* species and gp60 subtypes linked to human outbreaks of cryptosporidiosis in England and Wales, 2009 to 2017. *Parasite Vectors*, 12 (1), 95.

Chavez, M. A., & White, A. C. Jr. (2018). Novel treatment strategies and drugs in development for cryptosporidiosis. *Expert Review on Anti Infection Ther*apy, 16 (8), 655-661.

Checkley, W., Epstein, L. D., Gilman, R. H., Black, R. E., Cabrerea, L., & Sterling, C. R. (2015). Effects of *Cryptosporidium parvum* infection in Peruvian children: Growth faltering and subsequent catch-up growth. *American Journal of Epidemiology*, 148, 497-506.

Cheesbrough, M. (2005). *District Laboratory Practice in Tropical Countries*, Low Price 2nd ed. Cambridge University press, UK. P. 206.

Colombara, D. V., Khalil, I. A., Rao, P. C., Troeger, C., & Forouzanfar, M. H. (2016). Chronic health consequences of acute enteric infections in the developing world. *American Journal of Gastroenterology*, 3(4), 225-235.

Cook, N. C., Paton, N., Wilkinson, R. A., Nichols, K. B., & Smith, H. V. (2006). Towards standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries. Part 1: development and optimization of methods. *International Journal Food Microbiology*,109, 215–221.

Dhal, A. K., Young, G. M., Yun, S. I. L., & Mahapatra, R. K. (2022). Computational analysis of elongation factor 2 (EF-2) of *Cryptosporidium parvum* for identification of therapeutics. https://doi.org/10.1007/s11756-022-01030

Dhal, A. K., Pani, A., Mahapatra, R. K., & Yun, S. I. L. (2019). An immunoinformatics approach for design and validation of multi-subunit vaccine against *Cryptosporidium parvum*. Immunobiology 224(6),747–757. https://doi.org/10.1016/j.imbio.2019.09.001

Dhal, A. K., Chinmaya, P., Yun S. I. L., & Mahapatra, R. K. (2020). An update on *Cryptosporidium* biology and therapeutic avenues; *Journal of Parasitic Disease*, 46(3), 923–939 https://doi.org/10.1007/s12639-022-01510-5.

Dixon, B., Parrington, R., Cook, A., Pollari, F., & Farber, J. (2013). Detection of *Cyclospora*, *Cryptosporidium*, and *Giardia* in ready-to eat packaged leafy greens in Ontario, Canada. *Journal of Food Protection*, 76, 307–313.

Dixon, B. R. (2014). Encyclopaedia of food safety Pp18-22. https://doi.org/10.1016/B978-0-12-378612-3.

Dumaine, J. E., Tandel, J., & Striepen, B. (2020). *Cryptosporidium parvum*. *Trends Parasitology*, 36(5), 485–486. https://doi.org/10.1016/j.pt.2019.11.003.

Ebrahimzadeh, A., Jamshidi, A., & Mohammadi, S. (2013). The parasitic contamination of raw vegetables consumed in Zahedan, Iran," *Health Scope*, 1(4), 205–209.

Efstratiou, A., Ongerth, J. E., & Karanis, P. (2017). Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2011–2016. Water Research, 114, 14–22.

Egberongbe, H. O., Agbolade, O. M., Adesetan, T. O., Mabekoje, O. O., & Olugbode, A. M. (2010). Cryptosporidiosis among children in relation to toilet facilities and water sources in Ijebu and Remo areas southwestern Nigeria. *Journal of Medicine and medical science*, 10, 485-489.

Ehsan, A. M., Geurden, T., Casaert, S., Parvin, S. M., Islam, T. M., & Ahmed, U. M. (2015). Assessment of zoonotic transmission of *Giardia* and *Cryptosporidium* between cattle and humans in rural villages in Bangladesh. PLoS One, 10(2), e0118239.

El Sherbini, G. T., Kamel, O. H., Geneedy, M. R., & Temsah, A. G. (2016). A comparative study of the occurrence of *Cryptosporidium parvum* oocysts found on fresh fruits and vegetables sold in supermarkets and open-aired markets. *International Journal on Current Microbiology*, 5, 760–768.

Etzold, M., Lendner, M., Daugschies, A., & Dyachenko, V. (2014). CDPKs of *Cryptosporidium parvum*—stage-specific expression in vitro. *Parasitology Resource*, 113(7), 2525–2533. https://doi.org/10.1007/s00436-014-3902-0.

European Food Safety Authority, EFSA. (2018). Scientific Opinion on the public health risks associated with food-borne parasites. *European Food Safety Authority Journal*, 16, 5495. Doi: 10.2903/j.efsa.2018.5495.

Fallah, A. A., Pirali-Kheirabadi, K., Fatemeh, S., & Saei-Dehkordi, S. (2012): Prevalence of parasitic contamination in vegetables used for raw consumption in Shahrekord, Iran: Influence of season and washing procedure. *Iran Food Control*, 25(2), 617–620.

Florescu, D. F., & Sandkovsky, U. (2016). *Cryptosporidium* infection in solid organ transplantation. *World Journal of Transplant*, 6(3), 460–71.

Food and Agriculture Organization of the United Nations and World Health Organization. (FAO-WHO) (2004). Fruit and vegetables for health: report of the Joint FAO/WHO Workshop on Fruit and Vegetables for Health, Kobe, Japan, 1 to 3 September 2004. FAO/WHO, Geneva.

Food and Agricultural Organisation of the United Nations, FAO-UN. (2020). Fruits and vegetables- your dietary essentials. The International Year of Fruits and Vegetables, 2021, background paper. Rome. https://doi.org/10.4060/cb2395en.

Funkhouser-Jones, L. J., Ravindran S., & Sibley, L. D. (2020). Defining stage specific activity of potent new inhibitors of *Cryptosporidium parvum* growth in vitro. *Molecular biology*, 11(2), e00052-e120 https://doi.org/10.1128/mBio.00052-20.

Gerace, E., Di Marco, V., Presti, Lo., & Biondo, C. (2019). *Cryptosporidium* Infection: Epidemiology, Pathogenesis, and Differential Diagnosis, *European Journal of Microbiology and Immunology*, 9(4), 119–123, DOI: 10.1556/1886.2019.00019.

Glaberman, S., Moore, J. E., & Lowry, C. J. (2002). Three drinking water associated Cryptosporidiosis outbreaks. Northern Ireland. *Emerging Infectious Disease*, 8, 631-633.

Global Burden of Disease, GBD. (2017). Diarrhoeal Diseases Collaborators. Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study. *Lancet Infectious Disease*, 17, 909–48. doi: 10.1016/S1473-3099(17)30276-1.

Gharpure, R., Perez A., Miller, A. D., Wikswo, M. E., Silver, R., & Hlavsa, M. C. (2017). Cryptosporidiosis Outbreaks - United States, 2009-2017. *Morbidity and Mortal Weekly Report*, 68 (25), 568-572.

Guerrant, D. I., & Moore, R. L. (2004). Association of early childhood diarrhoea and Cryptosporidiosis with impaired physical fitness and cognitive function 4-7 years later in a poor urban community in Northeast Brazil. *American journal of Tropical Medicine and Hygiene*, 61, 707-713.

Gupta, S., Satpati, S., Nayek, S., & Garai, D. (2010) Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes *Environmental Monitoring Assessement*, 165(1–4), 169–177.

Helmy, Y. A., Krucken, J., Abdelwhab, E. M., Von Samson-Himmelstjerna, G., & Hafez, H. M. (2017). Molecular diagnosis and characterization of *Cryptosporidium* spp. in turkeys and chickens in Germany reveals

evidence for previously undetected parasite species. PLoS ONE, 12, e0177150.

Helmy, Y. A., Krucken, J., Nockler, K., Von Samson-Himmelstjerna, G., & Zessin, K. H. (2013). Molecular epidemiology of *Cryptosporidium* in livestock animals and humans in the Ismailia province of Egypt. *Veterinary Parasitology*, 193, 15–24.

Hohweyer, J. C., Cazeaux, E., Travaillé, E., Languet, A., Dumètre, D., Aubert, C., Terryn, J. P., Dubey, N., Azas, M., Houssin, F., Loïc, I., Villena, D., & Carbona, S. L. (2016). Simultaneous detection of the protozoan parasites *Toxoplasma*, *Cryptosporidium* and *Giardia* in food matrices and their persistence on basil leaves. *Food Microbiology*, 57, 36–44.

Huang, W., Choi, R., Hulverson, M. A., Zhang, Z., McCloskey, M. C., & Schaefer, D. A. (2017). 5-aminopyrazole-4-carboxamide-based compounds prevent the growth of *Cryptosporidium parvum*. Antimicrobial Agents Chemotherapy, 61(8), e00020-e117. https://doi.org/10.1128/AAC.00020-17.

Idahosa, O. T. (2011). Parasitic contamination of fresh vegetables sold in Jos Markets, *Global Journal of Medical Research*, 11(1), 20–25.

International Organization for Standardization, IOS. (2016). Microbiology of the food chain—detection and enumeration of *Cryptosporidium* and *Giardia* in fresh leafy green vegetables and berry fruits. ISO 18744:2016. ISO, Geneva.

Jennifer, K. O., Roy, D. S., & Brigid, L. (2021). *Cryptosporidium* species. Diagnosis and Research in the 21st Century. *Food & waterborne parasitology*, 24 (1), 12-25.

Khalil, I. A., Troeger, C., & Rao, P. C. (2018). Morbidity, mortality, and long-term consequences associated with diarrhoea from *Cryptosporidium* infection in children younger than 5 years: a meta-analyses study. *Lancet Global Health*, 6 (7), 758-768.

Khurana, S., & Chaudhary, P. (2018). Laboratory diagnosis of cryptosporidiosis. *Tropical Parasitology*, 8 (1), 2–7.

Korpe, P. S., Haque, R., Gilchrist, C., Valencia, C., Niu, F., & Lu, M. (2016). Natural History of Cryptosporidiosis in a Longitudinal Study of Slum-Dwelling Bangladeshi Children:Association with Severe Malnutrition. *PLoS Neglected Tropical Disease*, 10 (5), e0004564.

Kotloff, K. L., Nasrin, D., Blackwelder, W. C., Wu, Y., Farag T., & Panchalingham, S. (2019). The incidence, aetiology, and adverse clinical consequences of less severe diarrhoeal episodes among infants and children residing in low-income and middle-income countries: a 12-month case-control study as a follow-on to the Global Enteric Multicentre St. *Lancet Global Health,* 7(5), 568– 584.

Kumar, A., Chatterjee, I., Anbazhagan, A. N., Jayawardena, D., Priyamvada, S., & Alrefai, W. A. (2018). *Cryptosporidium parvum* disrupts intestinal epithelial barrier function via altering expression of key tight junction and adherence junction proteins. *Cell Microbiology*, 20(6), e12830.

Kwaga, J. K, Umoh, J. U., & Odoba, M. B. (1988). *Cryptosporidium* infection in humans with gastroenteritis in Zaria, Nigeria. *Epidemiology and Infection*, 101 (1), 93-97.

Lalonde, L. F., & Gajadhar, A. A. (2016). Optimization and validation of methods for isolation and real-time PCR identification of protozoan oocysts on leafy green vegetables and berry fruits. *Food Waterborne Parasitology*, 2, 1–7.

Love, M. S., Beasley, F. C., Jumani, R. S., Wright, T. M., Chatterjee, A. K., & Huston, C. D. (2017). A high-throughput phenotypic screen identifies clofazimine as a potential treatment for cryptosporidiosis. *PLoS Neglected Tropical Disease*, (2), e0005373.

Mamedova, S., & Karanis, P. (2020): *Cryptosporidium* spp. Infections in Livestock and Wild Animals in Azerbaijan Territory. *Environmental Science Proceedings*, 2 (44).

Mathias, L. & Arwid, D. (2014). *Cryptosporidium* infections: Molecular advances, *Parasitology*, 14(11), 1-22 Doi: 10. 1017/S003118201400023.

McHardy, I. H., Wu, M., Shimizu-Cohen, R., Couturier, M. R., & Humphries, R. M. (2014). Detection of intestinal protozoa in the clinical laboratory. *Journal of Clinical Microbiology*, 52(3), 712–20.

Miyamoto, Y., & Eckmann, L. (2015). Drug development against the major diarrhoea-causing parasites of the small intestine, *Cryptosporidium* and *Giardia*. *Front Microbiology*, 5, 4.

Nachipo, P., Hermann, D., Quinnan, G., Gordon, M. A., Van Voorhis, W. C., & Iroh Tam, P. Y. (2018). Evaluating the safety, tolerability, pharmacokinetics and efficacy of clofazimine in Cryptosporidiosis

(CRYPTOFAZ): study protocol for a randomized controlled trial. *Trials*, 19 (1), 456.

Naing, L., Winn, T., & Rushi, B. N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Science*, 1, 9-14.

Naseer, A. M. (2022). Transmission of *Cryptosporidium* by Fresh Vegetables, *Journal of Food Protection,* 85(12), 1737–1744.

Naseer, M., Dailey, F. E., Juboori, A. A., Samiullah, S., & Tahan, V. (2018). Epidemiology, determinants, and management of AIDS cholangiopathy: A review. *World Journal of Gastroenterology*, 24 (7), 767-77.

Niger State Bureau of Statistics (2011). Niger State Planning Commission; Niger State of Nigeria, Statistical year book. P 6.

Nime, F. A., Burek, J. D., Page, D. L., Holscher, M. A., & Yardley, J. H. (1976). Acute enterocolitis in a human being infected with the protozoan cryptosporidium. Gastroenterology, 70(4),592–598.https://doi.org/10.1016/S0016-5085(76)80503-3

Nwabuisi, C. (2009). Childhood cryptosporidiosis and intestinal parasitic infection in association with diarrhoea in Kwara State, Nigeria. *West Africa Journal of Medicine,* 20(2), 165-168.

Odeke, E. H., Abe, A. S., Esew, O., Idio, U. I., Okiezie, V. C., Idowu, O. O., & Ogabidu, O. A. (2019). Occurrence of *Cryptosporidium* Oocysts on Vegetable Samples from a Vegetable Farm Irrigated with Treated Wastewater in Samaru, Zaria, Kaduna State; *Nigerian Research Journal of Chemical Sciences*,7.

Omoruyi, B. E., Nwodo, U. U., Udem, C. S., & Okonkwo, F. O. (2014). Comparative diagnostic techniques for *Cryptosporidium* infection. Molecules, 19 (2), 2674–83.

Omowaye, O. S., & Audu, P. A. (2012). Parasites contamination and distribution on fruits and vegetables in Kogi, Nigeria," *Cibtech Journal of Bio-Protocols*, 1(1), 44–47.

Otubanjo, A. O. (2013) *Textbook of parasitology, Parasites of Man and Animals*. Lagos Nigeria, Concept Publication Limited. Pp 163-164.

Orlandi, A.P., Dan-my, T., Bier, J. W., & George, J. J. (2002). Parasites and the food supply. *Food Technology magazine*, institute of food Technologists' expert panel on food safety and nutrition.

Ortega, Y. R., Roxas, C. R., Gilman, R. H., Miller, N. J., Cabrera, L., Taquiri, C., & Sterling, C. R. (1997). Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *American Journal of Tropical Medicine and Hygiene*, 57, 683–686.

Pacheco, F. T., Silva, R. K., Martins, A. S., Oliveira, R. R., Alcantara-Neves, N. M., & Silva, M. P. (2013). Differences in the detection of *Cryptosporidium* and *Isospora* oocysts according to the faecal concentration or staining method used in a clinical laboratory. *Journal of Parasitology,* 99(6), 1002–1008.

Pam, V. A., & Onwuliri, C. O. (2009). *Cryptosporidium* and cryptosporidiosis in calves at Jos, Northern Nigeria. *Animal Production Research*, 5, 70-76.

Platts-Mills, J. A., Babji, S., Bodhidatta, L., Gratz, J., Haque, R., & Havt, A. (2015). Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Global Health, 3(9), e564-e575.

Reina, F. T., Ribeiro, C. A., Araujo, R. S., Matte, M. H., Castanho, R. E., & Tanaka, I. I. (2016). Intestinal and Pulmonary Infection by *Cryptosporidium parvum* in Two Patients with HIV/AIDS. *Review Institute Medical Tropics* Sao Paulo, 58:21.

Robertson, L. J., Johansen, ØH., Kifleyohannes, T., Efunshile, A. M., & Terefe, G. (2020). *Cryptosporidium*; Infections in Africa—How Important Is Zoonotic Transmission? A Review of the Evidence. *Frontier Veterinary Science,* 7:575881.

Robertson, L. J. (2019). Documented foodborne outbreaks of cryptosporidiosis, Pp 13–23. In *Cryptosporidium* as a foodborne pathogen. Springer, New York.

Robinson, G., & Chalmers, R. M. (2020): *Cryptosporidium* Diagnostic Assays: Microscopy. *Methods Molecular Biology*, 2052, 1-10.

Ryan, U., Hijjawi, N., & Xiao, L. (2018). Foodborne cryptosporidiosis. *International. Journal. Parasitology,* 48,1–12.

Ryan, U., Fayer, R., & Xiao, L. (2014). *Cryptosporidium* species in humans and animals: Current understanding and research needs. *Parasitology*, 141, 1667–1685.

Said, D. E. (2012). Detection of parasites in commonly consumed raw vegetables. *Alexandria Journal of Medicine*, 48, 345–352.

Shields, J. M., Lee, M. M., & Murphy, H. R. (2012). Use of a common laboratory glassware detergent improves recovery of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from lettuce, herbs and raspberries. *International Journal of Food Microbiology*, 153, 123-128.

Schaefer, L. M., le Roux, W. J., & Abia, A. L. (2020). Reservoirs of *Cryptosporidium* and *Giardia* in Africa. *Current Microbiological Research in Africa*. Cham: Springer

Shoultz, D. A., de Hostos, E. L., & Choy, R. K. (2016). Addressing *Cryptosporidium* Infection among Young Children in Low-Income Settings: The Crucial Role of New and Existing Drugs for Reducing Morbidity and Mortality. *PLoS Neglected Tropical Disease*, 10(1), 0004242.

Shrivastava, A. K., Kumar, S., Smith, W. A., & Sahu, P. S. (2017). Revisiting the global problem of Cryptosporidiosis and recommendations. *Tropical Parasitology*, 7(1), 8–17.

Sia-Su, G. L., Mariano, C. M., Matti, N. S., & Ramos, G. B. (2012). Assessing parasitic infestation of vegetables in selected markets in Metro Manila, Philippines," *Asian Pacific Journal of Tropical Disease*, 2(1), 51–54.

Sow, S. O., Muhsen, K., & Nasrin, D. (2016). The burden of *Cryptosporidium* diarrhoea disease among Children less than 24 months of age in moderate/high mortality regions of sub-Saharan Africa and Asia, utilising data from the global enteric multicentre study (GEMS) PLoS *Tropical Neglected Disease*, 10, 0004729.

Sponseller, J. K., Griffiths, J. K., & Tzipori, S. (2014). The evolution of respiratory Cryptosporidiosis: evidence for transmission by inhalation. *Clinical Microbiology Review*, 27(3), 575–86.

Su, J., Shen, Y., Li N., Li Y., Zhang, Z., & Xiao, L. (2022). Comparative characterization of CpCDPK1 and CpCDPK9, two potential drug targets against cryptosporidiosis. *Microorganisms*, 10(2), 333. https://doi.org/10.3390/microorganisms10020333.

Sudfeld, C. R., McCoy, D. C., Danaei, G., Fink, G., Ezzati, M., & Andrews, K. G. (2015). Linear growth and child development in low-and middle-income countries: a meta-analysis. *Paediatric*, 135, 1266-75. doi: 10.1542/peds.2014-3111.

Tamirat T., Abdissa, B., Zeleke, M., & Teferi, E. (2014). Parasitic Contamination of Fruits and Vegetables Collected from Selected Local Markets of Jimma Town, Southwest Ethiopia, *International Scholarly Research Notices*, Article ID 382715, https://dx.doi.org/10.1155/2014/382715.

Thomson, S., Hamilton, C. A., Hope, J. C., Katzer, F., Mabbott, N. A., & Morrison, L. J. (2017). Bovine Cryptosporidiosis: impact, host-parasite interaction and control strategies. *Veterinary Resources*, 48(1), 42.

Tomczak, E., McDougal, A. N., & White, A. C. Jr. (2022). Resolution of Cryptosporidiosis in Transplant Recipients: Review of the Literature and Presentation of a Renal Transplant Patient Treated with Nitazoxanide, Azithromycin, and Rifaximin. *Open Forum Infectious Disease*, 9.

Tyzzer, E. E (1907). A sporozoan found in the peptic glands of the common mouse. *Proceedings of the Society for Experimental Biology and Medicine;* 5(1):12–13.

Tzipori, S., & Widmer, G. (2000). Identification of genetic heterogeneity in the *Cryptosporidium parvum* ribosomal repeat. *Applied Environmental Microbiology*, 62, 712–716.

United State Environmental Protection Agency, EPA. (2012). United State Environmental Protection Agency method EPA 1623.1 on *Cryptosporidium* oocysts and *Giardia* Cysts Analysis, a *memorandum* from the office of ground Water and Drinking Water. USA.

Utaaker, K. S., A. Kumar, H., Joshi, S., Chaudhary D., & Robertson, L. J. (2017). Checking the detail in retail: occurrence of *Cryptosporidium* and *Giardia* on vegetables sold across different counters in Chandigarh, India. *International. Journal of Food Microbiology*, 18, 1–8.

Valenzuela, O., Gonzalez-Diaz, M., Garibay-Escobar, A., Burgara-Estrella, A., Cano, M., Durazo, M., Bernal, R. M., Hernandez, J., & Xiao, L. (2014). Molecular Characterization of *Cryptosporidium* species in Children from Mexico. *PLoS ONE*, 9, e96128.

Van Voorhis, W. C., Hulverson, M. A., Choi, R., Huang, W., Arnold, S.L.M., & Schaefer, D. A. (2021). One health therapeutics: Target-Based drug development for cryptosporidiosis and other apicomplexa diseases. *Veterinary Parasitology*, 289, 109336.https://doi.org/10.1016/j.vetpar.2020.109336

Vanathy, K., Parija, S. C., Mandal, J., Hamide, A., & Krishnamurthy, S. (2017). Detection of *Cryptosporidium* in stool samples of immunocompromised patients. *Tropical Parasitology,* 7(1), 41–46.

Walter, E. M., Charles, M., Elick, O., Manfred M., & Domitila, K. (2021). Prevalence of Zoonotic *Cryptosporidium* spp. isolates in Njoro Sub County, Nakuru County, Kenya. *African Journal of Infectious Disease,* 15(2), 3–9. doi:10.21010/ajidv15i2.2

Wang, R. J., Li J. Q., Chen, Y. C., Zhang, L. X., & Xiao, L. (2018). Widespread occurrence of *Cryptosporidium* infections in patients with HIV/AIDS: Epidemiology, clinical feature, diagnosis, and therapy. *Acta Tropica*, 187, 257–263.

White, A. C. Jr., Bennett, J. E., Dolin, R., & Blaser, M. K. (2020). Cryptosporidiosis. *Principles and Practice of Infectious Diseases*. Philadelphia: Elsevier Inc, 3410-3420.

Widmer, G, & Sullivan, S. (2012). Genomics and population Biology of *Cryptosporidium* Species. *Parasite Immunology,* 34, 61–71.

Widmer, G., Carmena, D., Kváč, M., Chalmers, R. M., Kissinger, J. C., Xiao, L., Sateriale, A., Striepen, B., Laurent, F., Lacroix-Lamandé, S., Gargala, G., & Favennec, L. (2020). Update on *Cryptosporidium* spp.: highlights from the Seventh International *Giardia* and *Cryptosporidium* Conference. *Parasite*, 27, 14.

World Bank. (2020). Country Classification: World Bank Country and Lending Groups. Available online at: https://datahelpdesk.worldbank.org knowledgebase/articles/906519-world-bank-country-and-lending-groups [accessed 22 June 2020].

Xiao, L. (2010). Molecular epidemiology of *Cryptosporidium*: An update: *Experimental parasitology*, 124 (1), 80-89.

Yosra, A. H., & Hafez, M. H. (2022). Cryptosporidiosis: From Prevention to Treatment, a Narrative Review *Microorganisms*, *10* (12), 2456; https://doi.org/10.3390/microorganisms10122456.

Zahedi, A., Paparini, A., Jian, F., Robertson, I., & Ryan, U. (2016). Public health significance of zoonotic *Cryptosporidium* species in wildlife: Critical insights into better drinking water management, *International Journal of Parasitology*. Parasites, 5, 88–109.

Zhang, H., Guo, F., Zhou, H., & Zhu, G. (2021). Transcriptome analysis reveals unique metabolic features in the *Cryptosporidium parvum* oocysts associated with environmental survival and stresses. BMC Genomics, 13(1), 1–15. https://doi.org/10.1186/1471-2164-13-647.

Appendix A

Photos of all the four (4) kinds of Vegetable samples used for the study.

Plate XIII: Photographs of Carrot used for the study

Plate XIV: Photographs of Cabbage used for the study

Plate XV: Photographs of Lettuce used for the study

Plate XVI: Photographs of Tomatoes used for the study

Appendix B

Structured Questioner for Assessing Possible sources of Vegetable Contamination in the Markets.

1. Sources of the produce (vegetable): Farmers ☐ Middlemen ☐ Private ☐
2. Nature of manure used: Animal dung ☐ Plant humus ☐ Inorganic ☐
3. Means of transportation to the market: Vehicle ☐ Motorcycle ☐ Tricycle ☐
4. Means of display in the market: On the floor ☐ On shelf in the shop ☐ On table/wheelbarrow ☐
5. Wash before display: Yes ☐ No ☐
6. Water source for washing purpose: Well water ☐ Tap water ☐ River/stream water ☐
7. Source of water for sprinkling in the market: Well water ☐ Tap water ☐ Stream water ☐
8. Produce handled by seller who has: No formal education ☐ Primary education ☐ Secondary education ☐
9. Neatness of the surrounding selling point: Tidy ☐ Untidy ☐
10. Number of days between transport and sale: One day ☐ Two days ☐ Three days and above ☐

Appendix C

Tables of Result Generated from Administered Questioners on the Vegetable Sellers (Some Parameters were not having responses from the Vegetable Sellers)

Table 4.10: Means of transporting vegetable samples to the Markets

Means of transportation	Respondent	Percentage (%)
Vehicle	60	40.0
Motorcycle	35	23.3
Tricycle	55	36.7
Total	**150**	

Table 4.11: Methods of displaying vegetable for sale in the Market

Method of display	Respondent	Percentage (%)
On the floor	51	34.0
On table/wheel barrow	95	63.3
On shelf in shop	4	2.7
Total	**150**	

Table 4.12: Sources of water for washing and sprinkling vegetables bin the Markets

Sources of water	Respondent	Percentage (%)
Well water	30	20.0
Tap water	40	26.7
Borehole water	80	53.3
Total	**150**	

Table 4.13: Educational status of the vegetable sellers in the Market

Education status	Respondent	Percentage (%)
Primary	70	46.7
Secondary	43	28.7
Tertiary	2	1.3
No formal education	35	23.3
Total	**150**	

Appendix D

Life Cycle of *Cryptosporidium* species with Footnotes on all the Lettered Stages.

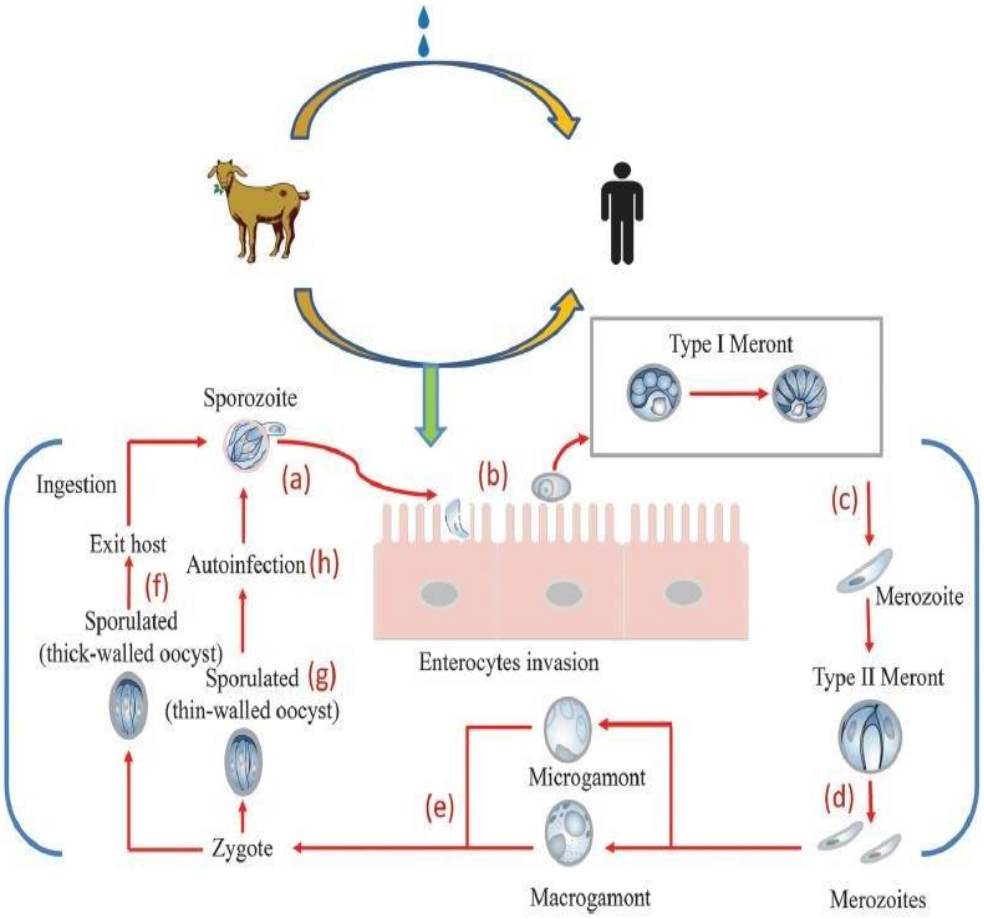


Figure 2.2: Life cycle of *Cryptosporidium* species. Source: (Gerace *et al.*, 2019).

a. Ingested oocyst that releases 4 sporozoites;
b. Trophozoite invades the epical portion of the cell;
c. Merozoite formed from the asexual division of the trophozoite through merogony;
d. Sexual stage of merozoite formed from type II meront;
e. Male (microgamont) and Female (macrogamont);
f. Resistant thick-walled oocysts (exit the host);
g. Thin-walled oocysts (excyst within host);
h. Autoinfection.

Appendix E

STATISTICAL ANALYSIS REPORT

Table 4.1: Occurrence of *Cryptosporidium* oocyst based on the type of Vegetable

Sample		Response (%)		
	n	Positive	Negative	*p* value
Cabbage	150	1	99	0.00
Carrot	150	1	99	0.00
Lettuce	150	1	99	0.00
Tomatoes	150	0	100	0.00
Total	600	1	99	0.00

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.2: Occurrence of *Cryptosporidium* oocyst based on the Market surveyed

Sample		Response (%)		
	n	Positive	Negative	*p* value
Bosso	200	0	100	0.00
Gwari	200	2	99	0.00
Kure	200	1	99	0.00
Total	600	1	99	0.00

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.3: Occurrence of *Cryptosporidium* oocyst based on the Months of survey

Sample		Response (%)		
	n	Positive	Negative	*p* value
June	120	1	99	0.00
July	120	1	99	0.00
August	120	3	98	0.00
November	120	0	100	0.00
December	120	0	100	0.00
Total	600	1	99	0.00

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.5: Parasitic load on the basis of Vegetable type

Sample		Response (%)		
	n	Positive	Negative	*p* value
Cabbage	30	5	95	0.00
Carrot	48	8	92	0.00
Lettuce	39	7	94	0.00
Tomatoes	17	22	78	0.00
Total	134	1	99	0.00

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.6: Parasitic load on the Vegetable based on the Markets surveyed.

Sample		Response (%)		
	n	Positive	Negative	*p* value
Bosso	25	4	96	0.00
Gwari	60	10	90	0.00
Kure	49	8	92	0.00
Total	134	22	78	0.00

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.10: Means of transporting vegetable samples to the Markets

Means of transportation	*N*	Percentage (%)	*p* value
Vehicle	60	40.0	
Motorcycle	35	23.3	0.09
Tricycle	55	36.7	

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.11: Methods of displaying vegetable for sale in the Market

Method of display	*N*	Percentage (%)	*p* value
On the floor	51	34.0	
On table/wheel barrow	95	63.3	0.00
On shelf in shop	4	2.7	

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.12: Sources of water for washing and sprinkling vegetables bin the Markets

Sources of water	*N*	Percentage (%)	*p* value
Well water	30	20.0	0.00
Tap water	40	26.7	
Borehole water	80	53.3	

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference

Table 4.13: Educational status of the vegetable sellers in the Market

Education status	*N*	Percentage (%)	*p* value
Primary	70	46.7	0.00
Secondary	43	28.7	
Tertiary	2	1.3	
No formal education	35	23.3	

p value < 0.05 indicates significant difference

p *value* > 0.05 implies non-significant difference