CRYPTOSPORIDIUM INFECTION AMONG FARM WORKERS AND LIVESTOCK IN MINNA, NIGER STATE

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ABSTRACT

Cryptosporidiosis is a gastrointestinal infection caused by an obligate coccidian protozoan parasite, *Cryptosporidium* species. The protozoan constitutes a significant risk to humans and animals causing a self-limiting diarrhoea in immune-competent hosts and a life-threatening disease in immune-compromised hosts. There is a dearth of information on the distribution of the disease in most parts of Nigeria. This study was conducted between June and August, 2019 to evaluate the distribution and determine the risk factors for the transmission of the disease in Minna, Niger State. In total, 471 faecal specimens were collected from 146 cattles, 132 sheep, 150 broiler birds and 43 humans. The faecal specimens were screened using the modified ZiehlNeelsen staining technique. A total of 163 (34.6 %) tested positive for *Cryptosporidium* in one host or the other. Highest prevalence was recorded in cattles - 69/146 (47.3 %) and the least among the birds - 19/150 (12.7 %). Age distribution among the animals was similar ($P < 0.05$). In humans however, the infection was significantly higher in younger children than older individuals (17/25 (68 %) and 5/18 (27 %) respectively). Also, humans whose water sources were shallow wells and rivers recorded a higher prevalence 28/43 (65.17 %) than those whose water sources were the public piped water and borehole 15/43 (34.88 %). The results also showed that there was a significant association between the disease and risk factors such as contact with livestock and hand washing practices in the study area ($P < 0.05$). This study therefore shows that cryptosporidiosis is an emerging threat in this area and so control strategies such as education of the public on the risk factors and routine diagnosis should be put in place in other to curb the spread of the disease.

CHAPTER ONE

1.0. INTRODUCTION

1.1 Background to the Study

Cryptosporidiosis, otherwise known as *Cryptosporidium* infection is a gastrointestinal infection caused by an obligate coccidian protozoan parasite, *Cryptosporidium* species belonging to the sub-phylum apicomplexa (Heyneman, 2004). The disease affects the microvillus regions of epithelial cells lining the respiratory and digestive organs of vertebrate animals (Fayer, 2004; Helmy *et al.*, 2017).The protozoan constitutes a significant risk to humans and animals causing a self-limiting diarrhea in immune-competent hosts and thus, a life-threatening illness/disease in immune-compromised hosts such as children below age five, HIV/AIDS patients and young animals (Kotloff *et al.*, 2013; Paul and Isaiah, 2019), elderly individuals or patients who are receiving immune-suppressive drugs. (Chukwu *et al.*, 2019). Currently, thirty-eight (38) species and several valid genotypes of *Cryptosporidium* species have been described from a wide range of vertebrates including humans, mammals, wildlife, domestic livestock, reptiles, birds, amphibians and fish, causing asymptomatic, mild or severe gastro-intestinal disease in its host species (Ryan, *et al*, 2014; Kváč, *et al*, 2016; Holubova *et al.*, 2016; Huang *et al*., 2018).

Cryptosporidium genus has been reported to be the most common causative protozoan parasite responsible for about sixty percent waterborne disease outbreak worldwide between 2004 and 2010 (Baldursson and Karanis, 2011). *Cryptosporidium* is transmitted via the fecal-oral route. Most outbreak and spread have been associated with contaminated water supplies (Ponka *et al*., 2009). In endemic regions, infection is associated with poor sanitation, poverty, animal rearing and malnutrition (Mondal *et al.*, 2012).

Some recent studies aimed at improving household water supply and sanitation behaviour in Bangladesh showed reduction in overall diarrhea but it did not result in decreased *Cryptosporidium* infections or reduction in stunted growth, a known consequence of *Cryptosporidium* (Luby *et al.*, 2018; Lin, 2017). In the absence of effective behavioural and environmental interventions and drug therapies, understanding the factors involved in transmission and acquisition of infection is imperative (Poonum *et al.*, 2019).

In the United Kingdom, *Cryptosporidium* case numbers are higher in areas with a high estimate of *Cryptosporidium* oocysts applied to land from manure (Lake *et al.*, 2007). In the United States, the incidence of *Cryptosporidium* is the highest in mid-western states where dairy farming is most intensive. Indeed, massive slaughtering of farm animals and restriction of farm visits during foot and mouth disease outbreaks reduced sporadic human *Cryptosporidium* parvum infections in large communities in the United Kingdom (Hunter *et al*., 2004).

Humans and animals get infected when they ingest food and water containing the oocysts of the protozoan. Incidences of cryptosporidiosis are higher in less developed and developing countries where people have insufficient basic infrastructure or fundamental facilities to avoid food and drinking water contaminated by infectious oocysts in faeces. In addition, the oocysts play a potential role to contribute *Cryptosporidium* species because they are tolerant to several chemicals and disinfectants including chlorine which is commonly used to treat drinking water and processing swimming pools and water parks.

Studies show high report of *C. parvum* infection which indicates the importance of anthroponotic transmission of *Cryptosporidium* in Nigeria. Heterogeneity of subgroup

(regions, species) and risk factors (HIV status, age, gender, faecal type) analyses were determined. The pooled prevalence of *Cryptosporidium spp.* in different hosts were high and linked with several risk factors such as environmental contamination and animal contact (Paul and Isaiah, 2019).

In Nigeria, farm animal rearing is done mainly by extensive and semi intensive management systems which allow the animals to graze on natural pastures bringing them into close contact with farmlands and water bodies. Infective oocysts passed in the faeces of these animals contribute significantly to the contamination of the environment with potential for further transmission to other animals and humans (Okojokwu *et al*., 2016). Cryptosporidiosis in livestock is becoming the significant problem of animal health (both sub-clinical and clinical) and economic losses (Santin, 2013) because of increased veterinary services and labour costs increasing animal health-care cost and decreasing growth rate of animals and mortality of severe animals (Inpankaew *et al.,* 2014). There is a need for increased awareness on the prevalence of the disease to provide strategies that mitigate the disease in humans and animals.

1.2 Statement of the Research Problem

Cryptosporidiosis has a higher incidence in developing countries, causing outbreaks of diarrhoea which can result in high morbidity, economic impact and life-threatening illnesses/diseases in humans and farm animals (Kotloff *et al.*, 2013; Dora *et al.*,2016). Over time, *Cryptosporidium* has emerged as an important enteric pathogen with species that are adapted to a wide host range including farm animals (Nurul and Baha, 2013).In endemic regions, infection is associated with poor sanitation, poverty, animal rearing and malnutrition (Mondal *et al.,* 2012).World Health Organizationlisted Cryptosporidiosis

under the neglected tropical diseases in 2004, but despite the significant public health and veterinary importance of this disease, estimates of the prevalence of cryptosporidiosis in humans and animals are lacking in Nigeria (Paul and Isaiah, 2019) and the disease is not well recognized and well reported in several parts of the country including Minna, Niger State.

1.3 Aim and Objectives of the Study

The aim of this study was to determine *Cryptospodium* infection among farm workers and livestock in Minna, Niger state.

The specific objectives of this study are to determine:

i. the prevalence of cryptosporidiosis in farm workers, cattle, sheep and broiler birds in Minna, Niger state;
ii. the prevalence of cryptosporidiosis in relations to age, sex and breeding systems of farm animals;
iii. the risk factors for the disease such as age and sex of farm workers, contact with animals, water source, toilet facilities and hand washing among farm workers.

1.4 Justification for the Study

Cryptosporidium infection has been found to be associated with joint pains, eye pains, headaches, fatigue (Hunter *et al.*, 2004) and seronegative reactive arthritis has been reported in adults and children including one report of Reiter's syndrome (arthritis, conjunctivitis and urethritia). It has also been suggested that cryptosporidiosis infection may cause relapse in Crohn's disease and ulcerative colitis (Vadlamudi *et al.*,2013).

The recent Global Enteric Multicenter Study (GEMS) and other studies to identify the aetiology and population-based burden of paediatric diarrhoeal disease in Sub-Saharan

Africa, revealed that *Cryptosporidium* is second only to rotavirus as a contributor to moderate to severe diarrhoeal disease during the first 5 years of life (Kotloff *et al*., 2013). It has been estimated that 2.9 million *Cryptosporidium*- attributed cases occur annually in children < 24 months in Sub-Saharan Africa and infection is associated with a greater than two-fold increase in mortality in children aged 12 to 23 months (Sow *et al*., 2016).

Cryptosporidiosis in livestock is becoming the significant problem of animal health (both sub-clinical and clinical) and economic losses (Santin, 2013) because of increased veterinary services and labour costs increasing animal health-care cost and decreasing growth rate of animals and mortality of severe animals (Inpankaew *et al.,* 2014).

The public health and veterinary significance of cryptosporidiosis has been reported in several parts of the world. However, to the best of my knowledge, there seems to be very limited reports or knowledge on the public health and veterinary importance of cryptosporidiosis in this part of the country. This study, therefore, was designed to determine its prevalence and significance and also provide information on the risk factors for Cryptosporidiosis in Minna, Niger State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brief History of Cryptosporidiosis

The zoonotic intracellular protozoan parasite *Cryptosporidium* was first discovered in mice in 1907, but did not receive much interest from the scientific community for 75 years. However, *Cryptosporidium* research interest did intensify significantly in the 1980s due to increasing veterinary attention and the recognition of its impact on human health because of its association with the acquired immunodeficiency syndrome (AIDS) (Casemore *et al.*, 1985). There were no identified human cases until Nime *et al*. (1976) described cryptosporidiosis in a 3-year old girl who was "vomiting everything taken by mouth" and had severe diarrhoea. She was diagnosed with an abnormal x-ray that showed large amounts of gas in the colon and large amount of fluid levels present in both large and small bowels. To verify the cause of the signs and symptoms, light microscopy showed the parasite as "spherical or ovoid organism lying in the crypt lumen attached to the apical surface of the epithelial cell". Both sexual and asexual reproduction was identified. This 3-year old child had been infected from well water that supplied the community or numerous cats, dogs and non-dairy cattle nearby. However, the actual mode of transmission was not verified (Nime *et al.,* 1976). The increasing public health importance of *Cryptosporidium* has made the infection come to limelight of scientific investigations around the world.

Since 1976, there have been multiple outbreaks worldwide. One most notable outbreak was in 1993 in Milwaukee Wisconsin, where 403,000 became ill (Fayer, 2004).

2.2 Genus *Cryptosporidium*

Cryptosporidium is currently placed under the family Cryptosporidiidea, (family named by Legar, 1911) within the phylum Apicomplexa (Huang *et al*., 2018).

There has been an explosion of descriptions of new species of *Cryptosporidium* during the last two decades. This has been accompanied by confusion regarding the criteria for species designation, largely because of the lack of distinct morphologic differences and strict host specificity among *Cryptosporidium sp* (Lihua *et al.*, 2004).

According to the International Code of Zoological Nomenclature (ICZN), 16 species are currently recognized under the genus *Cryptosporidiu m*and these are found in mammals, birds, reptiles and fishes. The species are; *C. muris, C. andersoni, C. parvum, C. hominis, C. wrairi, C. felis, C. suis, C. bovis* and *C. cannis* in mammals. In birds, species isolated are; *C. baileyi, C. meleagridis and C. galli*. Species associated with reptiles are; *C. serpentis, C. varanii* and *C. saurophilum* while *C. molnari* is found in fish.Members of this genus have a direct life cycle. *Cryptosporidium* is traditionally considered a member of the coccidian, however, phylogenetic evidence shows it has closer affinity with gregarines (Zhu *et al.*, 2000; Leander and Keeling, 2003). Members of this genus develop under the surface membrane of the host cell. This location is often called intracellular but extracytoplasmatic. Their developmental stages within the host organism are attached to the cell with a specific organelle. Oocysts develop sexually into infective stages inside the host

cell and may sustain autoinfection. They appear to have secondarily lost the apicoplast and their mitochondrion is reduced to a minimum, the so called “mitosome” without any traces of DNA (Lihua *et al.,* 2004).

2.3 Structure and Morphology of *Cryptosporidium Sp*

2.3.1 Infective Oocyst:

The infective oocysts of *Cryptosporidium Spp* are nearly spherical in shape with a small size of about 4-6 µm on the average and have obscure internal structures. The hallmark characteristic of mature oocysts contains four sporozoites but no sporocyst.

An oocyst contains 4 sporozoites (Fayer and Ungar, 1986; O’Donoghue , 1995).

Tzipori and Ward (2002) stated that the infective oocyst have an infractile thick capsule. The thick-walled oocyst is appreciably resistant to natural decay in the environment as well as most disinfection processes. Plate 1 shows the original micrographs of the structure of the different stages of the oocyst development (Fayer & Ungar, 1986).

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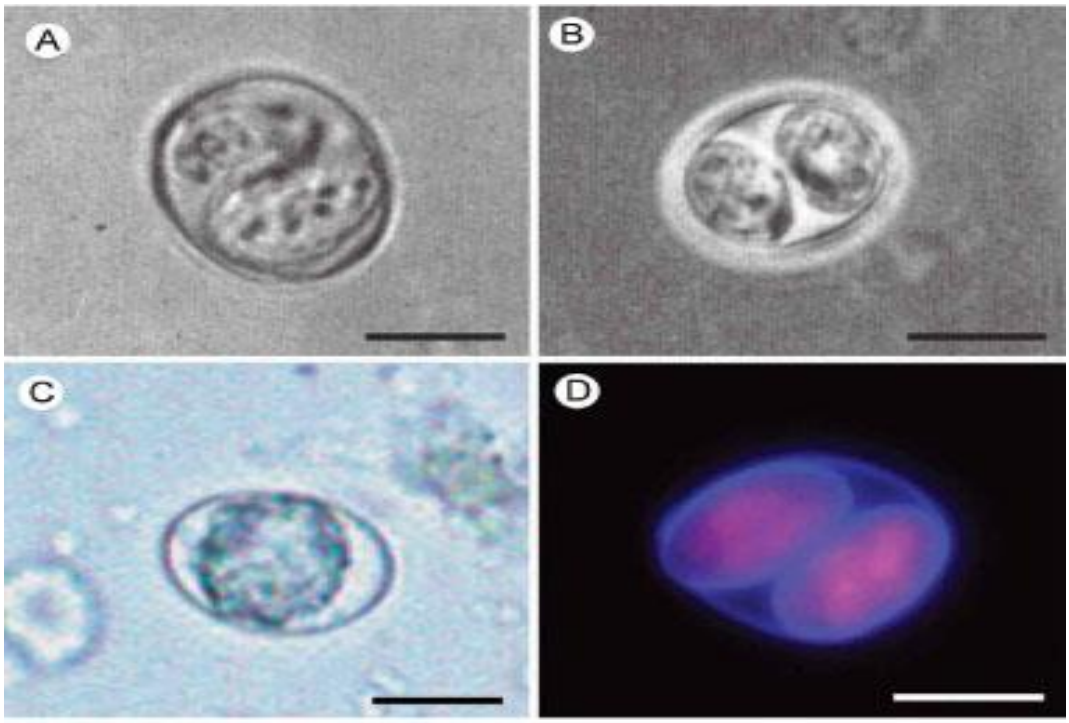


Plate 1: Oocysts of *Cryptosporidium spp.*

Source: Fayer & Ungar, 1986.

Keys:

A & B: micrographs showing new cyst (oocyst) of *Crptosoridium sp.*

C: Unsporulated oocyst showing the single cytoplasmic mass

D: Sporulated oocyst with two sporocysts showing the auto-flourescence of the oocyst and sporulated wall. Bars = 5µm

2.3.2 Adult Stage of *Cryptosporidium sp*.

The adult stage is the sporozoite stage.The sporozoites are nucleated, spindle shaped and measures 5.0 µm×0.5 µm in size. Invaded sporozoites are from parasitized vacuole surroundings where they can differentiate into trophozoite stage which are 1.5 - 2.5µm in diameter, within the parasitized vacuole, Laurence *et al.,* (1998) demonstrated an

ultrastructural analysis of the sporozoite of *Cryptosporidium parvum*. Their result suggests that sporozoites have a rhoptry containing an organized lamellar body, no mitochondria or conventional golgi apparatus, and one or two crystalline bodies. Micronemes were shown to be spherical, numerous and apically located and accounts for 0.8 % of the cell volume. Dense granules were less numerous, larger, accounted for 5.4 % of cell volume and were located more posteriorly than the micromeres. A structure juxtaposed to the nucleus with similarities to the acomplexa was observed. The apical complex plays the function of gliding motility to access the target cell. The nucleus is located at the center of the cell. Sporozoites recognize and penetrate to the target host cells including stomach (*C. muris* and *C. andersoni*) and intestine (such as *C. parvum* and *C. hominis*) (Inpankaew *et al.*, 2014).

The trophozoites commence three mitosis divisions (merogony) to produce type I meronts which contain 8 merozoites (morphologically similar to sporozoites which are rod-like 0.4 μm×1.0 μm). Type I merozoites can invade host cell again and reproduce asexual to from either type I meronts which contains eight merozoites or type II meronts which contain only four merozoites. Though, merozoites released from type II meronts are less uniform in shape, slightly larger and less active when compared to merozoites release from type I meronts. Type II merozoites can be developed to microgamont (male) or macrogamont (female) which undergoes sexual reproduction (gametogony). Each microgamont produces sixteen microgametes which are rod-like, non-flagellated and 1.4 μm×0.5 μm in size. It will fertilize with a unicellular adjacent macrogamont, a spherical to an oval structure of 4 to 6μm in diameter with a large central nucleus. After two mitosis divisions, zygote develops either a thin-walled oocyst covering only a single layer membrane or a thick-walled oocyst

containing two-layered membranes. The adult stages is shown in Plate 2 below (Lihua *et al.,* 2004)

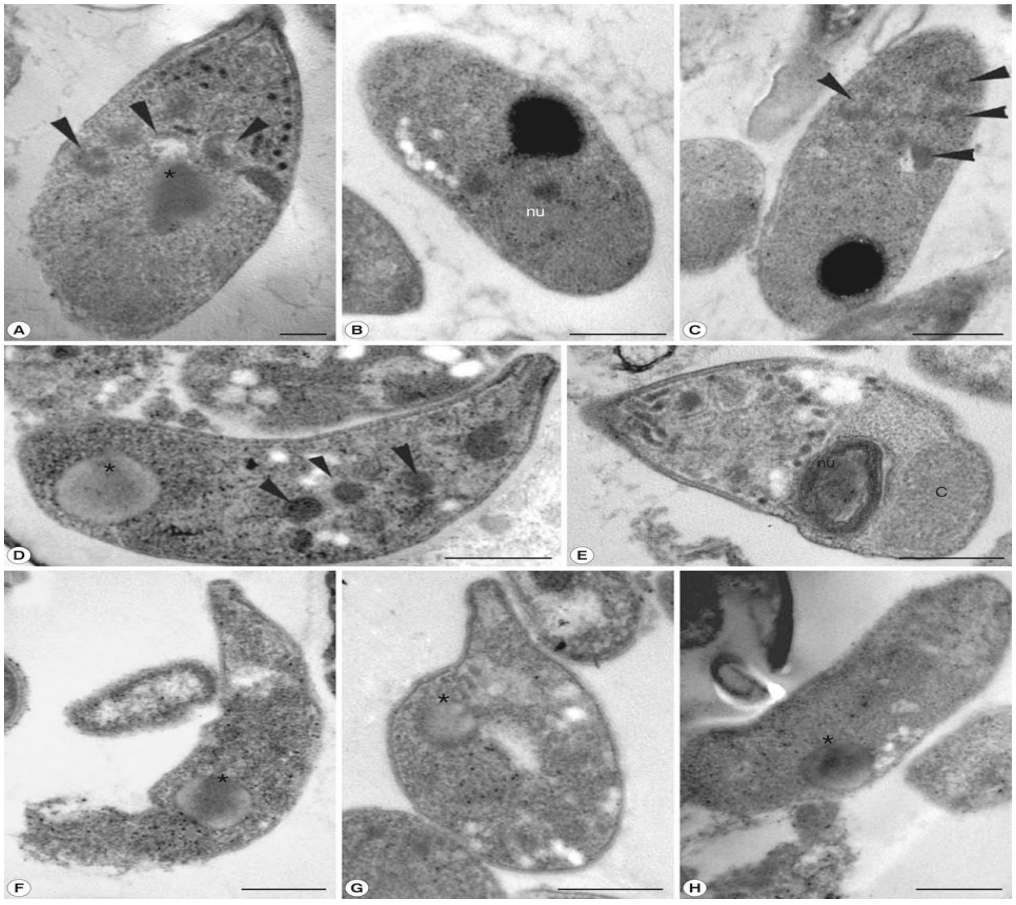


Plate 2: Sporozoites of *Cryptosporidium sp.*

Source: Lihua *et al.*, 2004.

2.4 Life Cycle of *Cryptosporidium sp.*

The genus *Cryptosporidium* has a complex life cycle involving both sexual (meiosis) and asexual replication (mitosis) but a monoxenous cycle (Bouzid *et al.*,2013). Oocysts are passed out into the environment by human and animals through faeces. The oocyst are of two types; the thin walled oocyst (one layer of a protein-lipid–carbohydrate matrix), and the thick walled oocyst (consisting of inner and outer oocyst walls). Only the thick walled oocyst is shed into the environment. The life cycle of *Cryptosporidiumsp.*begins with the

ingestion of the infective oocyst.Upon ingestion by the host,excystationoccurs to release four sporozoites.This progression occurs in the gastrointestinal tract triggered by Carbondioxide, at temperature of 37°C, pancreatic enzymes, and an acidic pH of bile salts (O'Donoghue, 1995). The sporozoites released adhere directly to the intestinal epithelial cells of the host. Cell invasion by sporozoites is followed by intracellular development to trophozoites. Trophozoite undergoes merogony to form meronts. A sexual replication occurs by re-infection of merozoites, released by type I schizont. Development of type II from type I meronts is the initial step of the asexual reproduction cycle. Merozoites are released from type II meronts and re-infect neighbouring cell where they develop into microgametocytes (male) or macrogametocytes (female). The macrogametocyte is fertilized by released microgametocytes and matures into an oocyst. Two types of oocysts are released. The thick-walled oocysts which are excreted in the faeces or the thin walled oocysts for endogenous re-infection (auto-infection) (Smith *et al.*, 2005; Sunnotel *et al.*, 2006) as shown in plate 3 (Fayer, 1986).

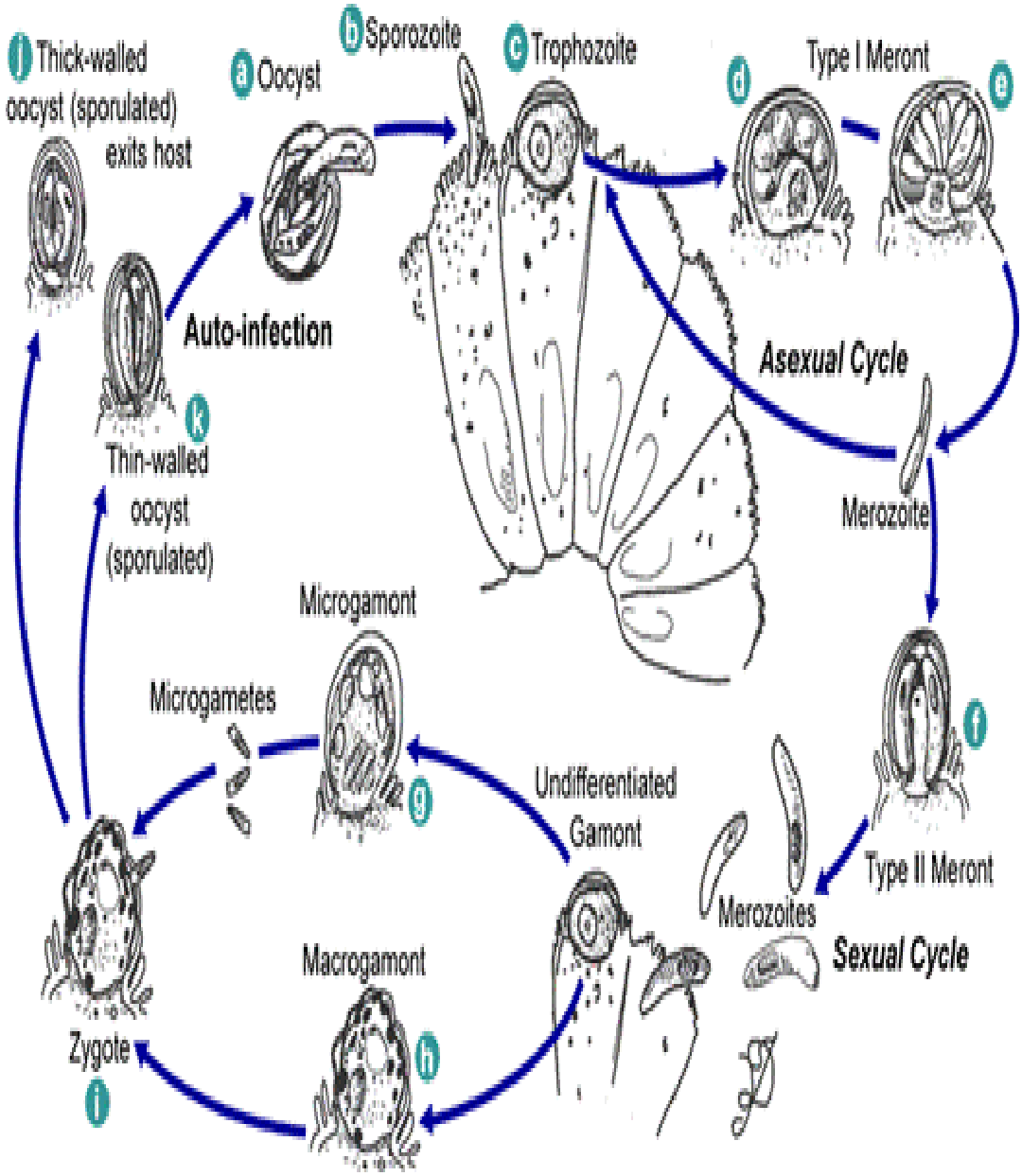


Plate 3:

Life Cycle of *Cryptosporidium sp.*

Source: Fayer, 1986.

2.5 Transmission of *Cryptosporidium* Infection

Transmission routes of this parasite can be divided into two: direct and indirect transmissions. Direct transmission occurs through a fecal-oral route by accidental ingestion, and the *Cryptosporidium* oocytes excreted in feces. The transmission could occur between animal to animal, animal to human (zoonosis), from human to animal (anthroponotic or reverse zoonosis) (Hubálek, 2003), and human-to-human transmissions which usually occur in swimming pools, water parks, day care centers, hospitals, and during anal sexual contact with human faeces. In addition, this process is frequently raised by sexual intercourse behaviour of men who have sex with men through faecal-oral route (Pedersen *et al.,* 1996). In addition, the direct transmission can occur through direct exposure to infected animals such as contact with infected calves by veterinarians or animal researchers who have a high chance of contact with infected animals. Indirect transmission can occur by cross-contamination of foodstuff, food materials, drinking water, and other personal effects such as clothes and footwear used in livestock farms or wildlife parks which have been exposed to the faeces of an infected human or animal. Stool containing oocysts when passed directly into the environment can contaminate the environment such as soil and water sources; pond, river, wastewater, sewage or slurry, even many water containers especially insufficiently treated public water supplies. The transmission and distribution are presented following a high rate of rainfall and flooding event (Jiang *et al.*, 2005). Other modes of transmission are poor hygienic practices which include not washing hands after using the toilet, changing diapers or handling animals and their wastes (Koch *et al.,* 1985; Smith *et al*., 2006). Thus, the infection of human and animal usually commences with the ingestion of an infective oocysts.

2.6 Epidemiology of *Cryptosporidium* Infection

The parasite *Cryptosporidium* is cosmopolitan. This protozoan parasite has been responsible for several outbreaks of cryptosporidiosis, affecting until half of 2001, approximately, 427,200 people worldwide (Teunis and Havelaar, 2002). It is the commonest cause of protozoal diarrhea in the UK, with nearly 6000 laboratory notifications in 2012. *Cryptosporidium* infection continues to be a significant health problem in both developed and developing countries (Harp, 2003). Cryptosporidiosis is an emerging infectious disease in many parts of sub-Saharan Africa, including Nigeria (Coker *et al*., 2000; Mor and Tzipori, 2008).

The importance of *Cryptosporidium spp* as a widespread cause of diarrhoea in humans is now increasing. Diarrhoea caused by these parasites accounts for more than 3.1 million deaths each year among children less than 15years of age, mostly in developing countries (Colford *et al.,* 2005).

A study conducted among Iranian farm workers and their household members indicated a high prevalence of *Cryptosporidium* species among calves and the farm workers in the area under study a high percentage of whom were affected by same spp ; *C. parvum*. The high prevalence of cryptosporidiosis detected in the study suggests that it may have a significant impact on livestock industry and that the close interaction between cattle and human played a role in zoonotic transmission to humans (Morteza *et al.,* 2014).

Obi *et al.* 2006 conducted a cross sectional study in a part of North Western Nigeria (Kebbi State) found out that cryptosporidiosis is common and is a significant zoonotic disease.

Lake *et al.,* (2007) in a literature implicated these protozoan parasites in three main epidemiological scenarios. The first is sporadic: this is often water related outbreaks of self-

limiting diarrhoea in otherwise healthy persons. The second is chronic. This is usually a life-threatening illness in immune-compromised patients, most notably those with HIV/AIDs and thirdly, diarrhoea and malnutrition in young children in developing countries. In industrialized countries, improved water management practices have resulted in the decline in cryptosporidiosis in the general population and anti-retrovirals have curbed the incidence and severity in patients with HIV/AIDs (Carr *et al.,* 1998; Maggi *et al.,* 2000). In the absence of these interventions, the burden of Crytosporidiosis continues to fall heavily on developing regions where infection is both ubiquitous and clinically consequential. In developing countries, cryptosporidiosis is most prevalent during early childhood with as many as 45 % of children experiencing the disease before the age of two years (Valentiner-Branth *et al.,* 2003). In Southeast Asia, cryptosporidiosis is frequently reported in both humans and animals from many countries such as Laos, Vietnam, Philippines, Myanmar, Indonesia, Cambodia, Malaysia, and Thailand (Lim *et al.*,, 2013).

In Thailand, 6 % of ocean water samples and 11 % of river samples are contaminated with *Cryptosporidium* spp. (Koompapong and Sukthana, 2012). Moreover, *C. muris, C. parvum, C. meleagridis, C. hominis, C. felis, andC.canis* have been isolated from HIV/AIDS cases in with the prevalence at 19-34 % from 1996 to 2009. In other regions, cryptosporidiosis is also recognized as a major concern by the increasing cases of immune-compromised individuals and children in many countries of the Americas such as Costa Rica, Argentina, Brazil, and the USA while the incidence of cryptosporidiosis in Europe is increases by climate changes as heavy rainfall or flood cause this pathogen to contaminate drinking water. Therefore, the reports of cryptosporidiosis cases in Europe are higher than

previous years, and *C. hominis* was identified as the most common pathogen (Bamaiyi and Redhuan, 2017).

Cryptosporidium spp. is associated with severe diarrhoea, mortality increase, and negative impact of development and growth to the children in Africa. More than that, HIV/AIDS outbreak and malnutrition status of some developing countries in Africa contribute to the high prevalence of the contagion in this region (Squire and Ryan, 2017).

Cryptosporidiosis in livestock is becoming the significant problems for animal health (both subclinical and clinical) and economic losses (Santin, 2013) because of increasing veterinary services and labour costs, increasing animal health-care cost, and decreasing a growth rate of animals and mortality of severe animals. Previous reports of cryptosporidiosis in livestock in Thailand were 31.5 %, 5.7 %, and 8.7 % in dairy farms, individual animals, and dairy herd, respectively (Jittapalapong *et al.*,, 2006). Moreover, 30 % of buffalo farms were infected with *Cryptosporidium* spp., and *C. parvum* was the most species contributed in livestock animal (Inpankaew *et al.,* 2014). For other animals, the prevalence of cryptosporidiosis showed 2.1 % in dog and 2.5 % in cat. For wildlife animal such as in long-tailed macaques which live closely and normally contact to human communities in Thailand, it showed a prevalence about 1 % but pose an important human health risk because infection in animals can be occur although less number of oocysts (Sricharern *et al.*,, 2016).

Similarly, outbreaks traceable to contaminated water have also been reported and several recent reports on the epidemiology and outbreaks of cryptosporidiosis in humans abound in literature from other parts of Africa and elsewhere (Endeshaw *et al.,* 2004; Jones *et al.,* 2006; Huh *et al.,* 2009; Ponka *et al.,* 2009). In Nigeria, human cryptosporidiosis has been earlier reported from the Northern (Kwaga *et al.,* 1988), Central (Banwat *et al.,* 2003; Udeh

et al., 2008), Eastern (Okafor and Okunji, 1994, 1996) and Western (Reinthaler *et. al.*, 1987; Nwabuisi, 2001) parts. More recent studies of the disease in humans and animals have been reported in several parts of Nigeria by Chukwu *et al*. (2019) who conducted a research on the trends and prevalence of *Cryptosporidium* infections in animals across Nigeria,

2.7 Pathology and Clinical Manifestationsof *Cryptosporidium* Infection

Cryptosporidiosis is acute but self-limiting in immune-competent individuals generally abating within 8 to 10 days (Fayer and Ungar, 1986). Conversely, the illness may manifest as gastrointestinal infection and eventually result to death in immuno-compromised individuals such as HIV patients, very young children and aged people.

The first signs and symptoms of *Cryptosporidium* infection usually appear within a week after infection and may include; watery diarrhoea with mucus, dehydration, lack of appetite, weight loss, stomach cramps or pain, fever, nausea and vomiting (Deb, 2018). The symptoms may last for up to two weeks, though they may come and go sporadically for up to a month, even in people with healthy immune systems. However, some people with cryptosporidiosis may have no symptoms (Deb, 2018).

Furthermore, In the UK, a case-control study of patients who had cryptosporidiosis found that infection with the species *C. hominis* (but not *C. parvum*) was associated with joint pain, eye pains, headaches and fatigue in the two months following infection (Hunter *et al.*, 2004). A seronegative reactive arthritis has been reported in adults (Ozgul *et al*.,1999) and children (Cron and Sherry, 1995) including one report of Reiter's syndrome (arthritis, conjunctivitis and urethritis) (Cron and Sherry, 1995). It has also been suggested that *Cryptosporidium*infection may cause relapse in Crohn's disease and ulcerative colitis

(Manthey *et al*., 1997; Collussi *et al*., 2010; Vadlamudi *et al*., 2013). A study in Sweden (Rehn *et al*., 2015) followed up 459 cases who suffered *C. hominis* infection in two waterborne outbreaks and controls, finding that outbreak cases were more likely to report diarrhea, abdominal pain and joint pain several months after infection than were controls. In terms of sequelae in developing countries, cryptosporidiosis is now recognized as being associated with stunting of growth, and with persistent diarrhea. In studies in Brazil (among others), early childhood diarrhea with *Cryptosporidium* was associated with impaired physical fitness and cognitive function 4–7 years later (Guerrant *et al*., 1999), and with increased diarrhoea morbidity in subsequent years (Agnew *et al*., 1998). It is therefore now believed that the effects of Cryptosporidiosis go beyond the initial acute diarrheal episode, possibly due to an effect on the gastrointestinal epithelium (for example, villous blunting with chronic inflammation, and an association with the poorly understood entity 'environmental enteropathy') (Bartelt *et al*., 2013).

2.8 Diagnosis of *Cryptosporidium* Infection

Diagnosis of *Cryptosporidium* has progressed from histologic identification in intestinal biopsies to microscopic examination of faecal specimens for infective oocysts to more complex molecular techniques such as; Enzyme Immunoassay (EIA) for parasite antigens and nucleic acid amplification assay as well as polymerase chain reaction (PCR) (Phillip *et al.,* 2008). There are different methods used in detecting *Cryptosporidium* oocyst in clinical and environmental samples (Tzipori and Ward, 2002).

2.8.1 Microscopic analysis

Microscopic analysis of stained faecal smears is the most widely used method for screening stool samples for *Cryptosporidium* in clinical diagnostic laboratories (Xiao *et al*., 2004). Microscopic diagnosis involves the morphological identification of the parasite in any

specimen. This depends totally on the skill, patience and experience of the microscopist. This method entails concentration of the samples and staining with an acid fast stain. The oocysts stain bright red when stained with Ziehl-Nelsen. Acid fast stain technique is more widely used than other staining techniques such as Giemsa and metheinamine silver stains (Xiao *et al.,* 2001; Pelayo *et al.,* 2008). The method has several advantages including provision of a simple procedure for the detection of *Cryptosporidium*, inexpensive nature, absence of false negative results, freedom from inhibitors and absence of technical complexity (Areeshi *et al.,* 2008). The system is however not without challenges as is made manifest by its time consuming procedures, tediousness, requirement of experienced microscopists for parasite identification (Areeshi *et al.,* 2008; Essid *et al.,* 2008).

2.8.2 Immunological based detection methods

Immunofluorescence Assay (IFA)

Considerable experience is often required with the concentration and staining methods to obtain an accurate diagnosis in microscopy method. For this reason, immunofluorescent assay (IFA) procedures, employing *Cryptosporidium* specific polyclonal and monoclonal antibodies was developed to aid in the identification of oocysts in stool and environmental samples (Garcia and Bruckner, 1998). Antibodies specific to *Cryptosporidium* have been detected. The IFA can either be direct or indirect. In direct IFA, samples are fixed into a microscope slide, then a drop of a pathogen specific antibody labeled with a suitable fluorochromee.gfluoesceinisothiocyanate (FITC) is applied and incubated. If the antibody binds to antigen, the sample will fluoresce against a green background while any unbound conjugates would be rinsed off when washed with water, the slide is then examined under an epifluorescent microscope (Garcia and Bruckner, 1998). In the case of indirect IFA, the

pathogen's specific – antibody is detected by a second anti-immunoglobulin antibody labeled with FITC. IFA technique is important since it can be done in combination with membrane exclusion/ permeability dyes to estimate oocyst visibility. However, important challenges facing the usefulness of this method in Africa is that it is time consuming, allows only few samples to be examined per day, necessity for expert or experienced personnel which may not be available, to interpret the result (Chapell and Okhuysen, 2002; Nair *et al.,* 2008).

Enzyme Immunoassay (Antigen Detection)

There are two commercially available enzyme immunoassays namely: The Prospect *Cryptosporidium*r apid assay and Colour PAC *Cryptosporidium* rapid assay. These two assays are used in detecting *Cryptosporidium* found in stool specimen which is fixed in 10 % formalin. The techniques combine the specimen antibody with the amplification of antibody-antigen interaction by enzyme catalysis, and therefore, can detect very small amounts of pathogen. Many assays are done in wells of microtitre plates in which the reactants are immobilized. Antigens in the sample may or may not be bound by specific antibody immobilized on the surface (Marques *et al.,* 2005). The enzyme immunoassay methods are the simplest and least labour intensive of the available immunoassays and rapid tests that can be done by relatively unskilled operators with little training. However, the cost of the enzyme immunoassay kits places them above the reach of many laboratories in developing countries and this may hamper their routine usage in diagnostic services in these parts of the world (Warren *et al.,* 2003; Miller *et al.,* 2006).

2.8.3 Polymerase chain reaction (PCR) based method

PCR is sensitive and has shown potentials for accurate diagnosis in patients unaware of the cause of their diarrhoea since it is highly sensitive. This genetic method of detecting *Cryptosporidium* oocysts has just been recently developed. It identifies and amplifies *Cryptosporidium* nucleic acid using the PCR (Abe *et al.,* 2002).

Several factors could complicate the PCR based detection of *Cryptosporidium* in stool samples. Morgan *et al.* (1998) reported that standard fixation in 10 % buffered formalin could reduce the sensitivity of PCR particularly if fixation occurs over an extended period. PCR is sensitive and has the potential for accurate diagnosis in patients who do not presently know the reason for their diarrhoea which could have considerable advantage in the treatment of immunosuppresant individuals, allowing for early diagnosis before the onset of symptoms. PCR technique is rapid and accurately obtained results are easy to interpret (Keegan *et al.,* 2003). PCR however has some challenges including its technical complexity and interference of results by inhibitors, its expensive nature and time-consuming procedures (Sulaiman *et al.,* 2005; Gatei *et al.,* 2006).

2.9 Treatment and Control of *Cryptosporidium* Infection

Recovery from Cryptosporidiosis depends largely upon the immune status of the host. Malnutrition, immune suppression, age and an increase in the preceding diarrhoea burdens are all risk factors for the development of persistent diarrhoea (Ochoa *et al.,* 2004). Immune competent patients usually have a spontaneous recovery within few weeks and parasitologic cure within a few months without requiring any specific therapy. When

therapy is required, nitazoxanid, a nitrothiazolebenzamide, is the preferred drug in children 1 to 11 years of age and has also been studied in adults (Abubakar *et al.,* 2007). Nitazoxanide is a new thiazolide antiparasitic agent that shows excellent invitro activity of protozoa and helminthes. At present, license for treatment of infection of *Cryptosporidium sp* in adults and for use in treating immune compromised hosts is pending (Abubakar *et al.,* 2007).

In immune compromised individuals such as HIV patients, antiretroviral therapy, supportive care, antimicrobial agents (such as nitazoxanide, paromomycin), combination therapy and other therapies are required. However, Rossignol *et al*. (2006) found out in a study that nitazoxanide is contraindicated for AIDs patients. Continuing antimicrobial agents may alleviate some of the diarrhea symptoms. Supportive care such as monitoring the hydration and supplemental nutrition are necessary. Antimotility agents like opiates or somatostatin and its analogues may also be helpful in preventing dehydration. Supportive therapy such as IV fluids is also necessary for *Cryptosporidium* infection (Kaplan *et al.,* 2009).

According to Yoder *et al.* (2010), some Preventive and control measures that will help prevent and control the spread of this infection includes; good hygiene, such as washing hands with soap and water before preparing and eating food and after using the toilet, after changing diapers or cleaning up a child who has used the toilet, before and after tending to someone who has diarrhea and after handling animals and animal wastes. Also, children with diarrhea should be excluded from child care settings until diarrhea has stopped. Furthermore, patients diagnosed with cryptosporidiosis should not use recreational water venues for at least two weeks after diarrhea stops. Showering before using public recreational water venues should be encouraged. Contact with animal faeces should be

minimized, disposable gloves should be used when cleaning up animal faeces, hands should be properly washed after wards and also wash hands after touching animals and their living area. Finally, the division also recommends the washing of fruits and vegetable before eating, drink properly treated water and avoid sexual activities that put one at risk of being infected (Yoder *et al.*, 2010).

According to the previous information, most patients and infected animals which have vigorous immune response can convalesce themselves without any treatments. Some clinical symptoms including watery fever, vomiting, nausea, stomach cramp, and dehydration can be managed by some supportive treatments as fluid and electrolyte replacement, anti-nausea drugs, antiemetic drugs, or analgesic drugs. Those medications can relieve the symptoms from cryptosporidiosis, but an antiprotozoal therapy is necessary in some cases. Nitazoxanide is the most effective drugs for treating patients who infected with *Cryptosporidium* spp., and it is the only anti-cryptosporidial agent which has been approved for the treatment of cryptosporidiosis in humans by the US Food and Drug Administration. However, it is not commercially available or not yet widely used at the present time (Chalmer *et al*., 2009). In addition, nitazoxanide cannot effective without a good immune response of the host so that it cannot be used effectively in immune-compromised patients (Hunter *et al*., 2004). For animals, they have few reports that studied the effect of nitazoxanide against clinical infections of *Cryptosporidium* in animals which demonstrated that nitazoxanide could decrease *Cryptosporidium* oocysts excretion (Robertson *et al*., 2002). Notwithstanding, it is also not yet generally used in animals.

Prevention of *Cryptosporidium* Infection

Cryptosporidium sp. can transmit and infect susceptible hosts by a major route of fecal to oral as cross-contamination in raw food and water from reservoir animals in community, farms, or abattoirs and some mechanical vectors such as cockroaches and flies (Kiang *et al*., 2006). Then, humans can get infected by their oocysts by ingesting this contaminated food and water with unprocessed or improper hygiene. Therefore, in a case of human prevention, the best strategy to prevent transmission of *Cryptosporidium sp*. is a practice of good personal hygiene including hand washing before preparing and consuming food, after using a toilet, and after contacting with diarrhea patients, children, and some animals or livestock. Raw food and water must be suitably cleaned, washed, heated, cooked, or boiled before consumption and drinking, respectively. More than that, patients who have diarrhea symptom should realize not to swim in a public swimming pool, public water park, or river for preventing a transmission to others and people who swim in a swimming pool, water park, or river should recognize a potential risk of disease infection if they swallow the water (Ondriska *et al*., 2013). Preventive measures that diminish transmission of *Cryptosporidium sp*. among animals, especially in livestock, should be emphasized on a primary prevention which reduce or eliminate causative risk factors by limiting the amount of animals density in the farms or stocks, minimizing a contraction between personnel, calves, and other herds, keeping young animals or susceptible hosts that have high risk of infection separated from adult animals, and keeping a short calving period of animals which may decrease the opportunities for *Cryptosporidium* spp. to spread within animal herds (Yakoob *et al*., 2010). In human living areas, livestock husbandry, and their drinking water, the destruction of *Cryptosporidium* oocysts can be managed by heat or chemical disinfection such as hydrogen peroxide, sterilization processes using steam, ethylene oxide, chlorine dioxide, ozone (O_3), and ultraviolet light (UV light) which have been used to

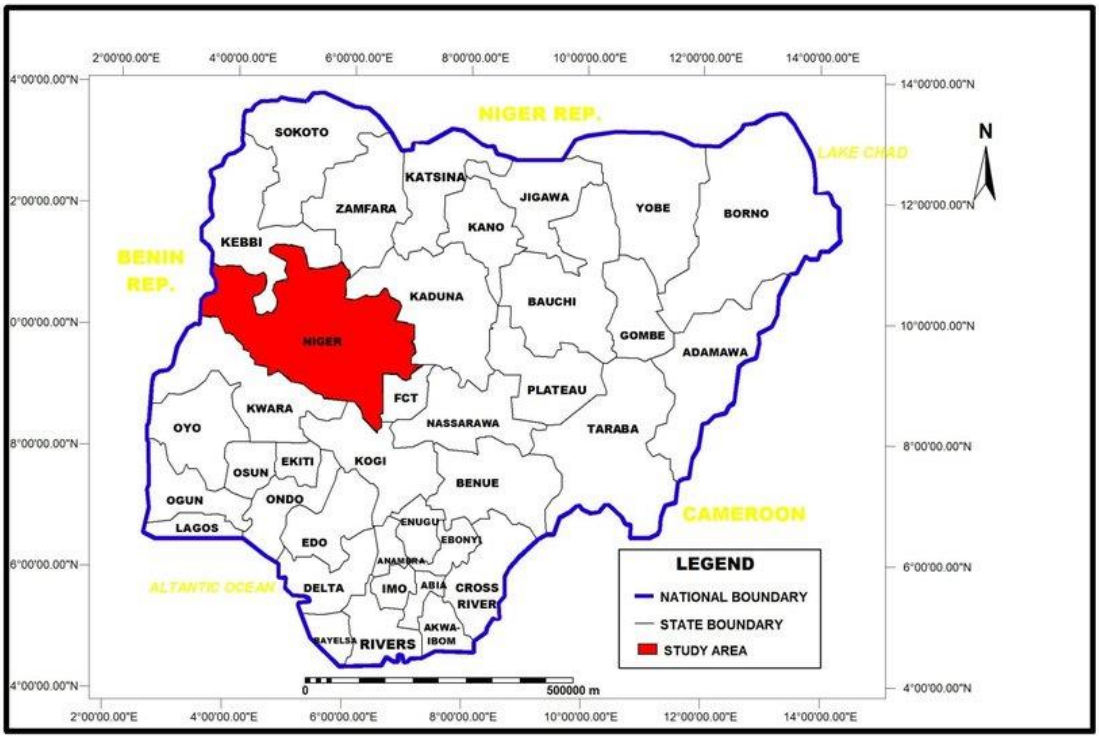
sterilize drinking water, but some of the disinfectants are not commonly practical procedure. However, all of the chemical disinfection can be used to prevent and control the occurrence of cryptosporidiosis and to reduce mortality and morbidity rate in both infected in both human and animals (Jafari *et al*., 2012).

CHAPTER THREE

3.0 MATERIALS AND METHOD

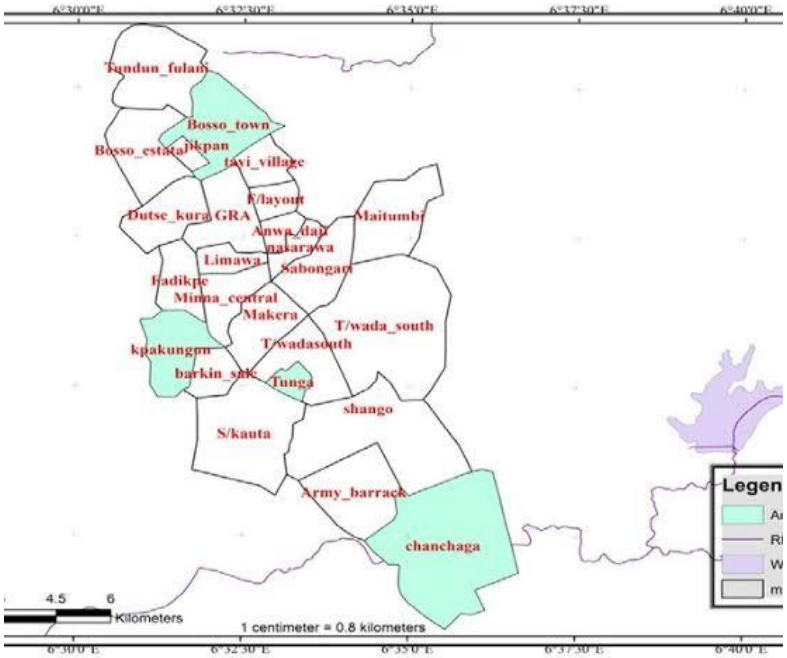
3.1 Study Area

The study area comprises Minna metropolis, Niger state, Nigeria. Minna lies between latitude 9.583555 and longitude 6.54636. It is a large city in Middle Belt Nigeria, with a population exceeding 300,000 people. It is the capital of Niger state. It consists of two major ethnic groups: the Nupe and the Gwari. It is an agricultural spot famous for plant farming and animal rearing.



Map of Nigeria

Source: Suleiman and Nma (2017)



Map of Minna, Niger State

Source: Suleiman and Nma (2017)

3.2 Study Population and Study Sites

A total of 471 farm worker and animals of different ages and sex which comprised 146 cattle, 132 sheep, 150 broiler birds and 43 humans within the study sites (Bosso, Kpakungun, Maikunkele and Chanchaga) participated in this study.

3.3 Sample Collection and Preservation

Fresh morning stool samples uncontaminated with urine were collected from the participants and taken for examination. The specimens were collected using sterile, dry stool sample containers. These sample containers where locked (stopped) tight and placed in a larger container containing formalin to preserve it while being transported to the laboratory where they are examined within 24 hours.

Adequate precaution was taken by preserving specimen in 10 % buffered formalin to render the oocysts nonviable. This is because the oocyst of *Cryptosporidium species*in stool specimens remain infective for an extended period of time.

Moreso, the usual safety measures of wearing hand gloves and avoiding oral contact in order to prevent being infected when handling infectious materials was also adopted.

3.4 Sample Analysis

The stool samples were analysed microscopically to note for the presence of *Cryptosporidium* oocysts. Stool specimens were concentrated prior to staining and microscopic examination in order to maximise oocyst recovery.

Formalin-ethyl acetate sedimentation technique was the stool concentration method used (Weber, R. *et al*, 1992). A drop of the deposit/sediment from the concentration technique

was placed on a glass slide, air-dried, fixed with alcohol and stained with an acid-fast stain (Ziehl-Neelsen) and examined under the microscope.

Oocysts of *Cryptosporidium species* appear as red round bodies against blue-green background.

3.5 Data Analysis

The suitable statistical methods were used in order to analyse and assess the results. These were used to accept or reject statistical hypothesis. Infectious rates and proportion were calculated for asymptomatic and symptomatic animals and farm workers. Az score tests used for infected participants. Values of less than 0.05 was considered significant. The prevalence of *Cryptosporidium* infection and prevalence of *Cryptosporidium* species in younger animals were compared with older ones, types of breeding systems, the rate of susceptibility of the different animals that were studied as well as for humans. Determination of the association between infection and risk factors like water source, toilet facility, hand washing and contact with farm animals was performed using chi-square or Fisher's exact test. Results were considered to be significant at$P< 0.05$.

Farmers were asked to complete a questionnaire on potential risk factors for *Cryptosporidium* infection including age, sex, contact with animals, cleaning shoes/boots after daily work, hand washing after work, contact with soil, their water source and the type of toilet used. A univariate analysis for the relation between *Cryptosporidium* infection and potential risk factors was performed (odds ratio) and the significance of variable association was tested using Wald's test. To appraise efficiently the association between exposure and consequence and to assess the effect of possible confounding variables, analysis was carried out using computer software SPSS ver.12 (SPSS Inc., USA). In the univariate analysis, associations were considered significant at $P< 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

In total, 471 faecal specimens were collected from 146 cattles, 132 sheep, 150 broiler birds and 43 humans. Of the 471 samples collected, 163 (34.6 %) tested positive for *Cryptosporidium* in one host or the other; (69/146), (53/132), 19/150) and (22/43) for cattle, sheep, birds and humans respectively. The highest prevalence was recorded in cattles (47.3 %) and the least among the birds (12.7 %). In humans however, the infection was significantly higher in younger children than older individuals (17/25 (68 %) and 5/18 (27 %) respectively). Also, humans whose water sources were shallow wells and rivers recorded a higher prevalence (65.5%) than those whose water sources were the public piped water and borehole (35.7 %).

4.1.1 Prevalence of *cryptosporidium* infection in humans based on their age and gender

The prevalence of *Cryptosporidium* infection among the examined humans with respect to sex and gender is shown in table 4.1. The highest prevalence of the infection was recorded amongst females less than ten years old 13/19 (68.42 %). The least prevalence was recorded amongst females also but above ten years old. 4/15 (26. 67 %).

The result showed no significant association of the infection in humans with respect to age and gender ($p>0.05$).This result is represented in table 4.1.

Table 4.1: Prevalence of *Cryptosporidium* Infection in Humans Based on Age and Gender

	Male		**Female**	
Age (Years)	**Number Examined**	**Number Infected (%)**	**Number Examined**	**Number Infected (%)**
0 – 10	6	4 (66.67)	19	13 (68.42)
11 – 20	0	0	6	1 (16.67 %)
21 – 30	1	1 (33.33)	4	3 (75 %)
31 – 40	2	0	4	0
41 – 50	0	0	1	0
50+	0	0	0	0
Total	**9**	**5 (55.56 %)**	**34**	**17 (50 %)**

4.1.2 Prevalence of *cryptosporidium* infection in cattle based on breeding systems and age

The prevalence of *Cryptosporidium* infection among cattle with respect to age and breeding system is shown in table 4.2. Of the 146 cattle examined for the infection, 69 (47.3 %) were positive. With regards to breeding system and age of the cattle, the highest prevalence was recorded amongst calves less than six months of age and reared under the semi extensive system 18 (78.25 %) while the lowest prevalence was amongst those above six months reared under the same breeding system 14 (29.79 %). The result shows no significant association between the disease and the age and breeding system of the animal ($P > 0.05$).

4.1.3 Prevalence of *cryptosporidium* infection in sheep based on breeding systems and age

The prevalence of *Cryptosporidium* infection among sheep with respect to age and breeding system is shown in table 4.3. A total of 132 sheep were examined for *Cryptosporidium* infection, of which 53 were infected. The highest prevalence of the infection amongst sheep with regards to age and breeding system was recorded among those less than three months of age and reared

under the extensive breeding system11/17 (64.71 %). The least was recorded amongst those reared under the semi extensive system and less than three months of age 5/16 (31. 25 %).

Host	Breeding System	Age	Number Examined	Number Positive	Prevalence (%)

Table 4.2: Prevalence of *Cryptosporidium* Infection in Cattle Based on Breeding Systems and Age

Cattle	Extensive	<6 months	11	8	72.73
		> 6 months	65	29	44.62
Cattle	Semi-intensive	< 6 months	23	18	78.25
		> 6 months	47	14	29.79

Host	**Breeding System**	**Age**	**Number Examined**	**Number Positive**	**Prevalence**

Table 4.3: Prevalence of *Cryptosporidium* Infection in Sheep Based on Breeding Systems and Age

Sheep	Semi-intensive	<3 months	16	5	31.25
		> 3 months	38	13	34.21
Sheep	Extensive	<3 months	17	11	64.71
		>3 months	66**132**	24	36.36
Total				**53**	**40.15**

4.1.4 Prevalence of *cryptosporidium* infection in birds based on breeding systems and age

The prevalence of *Cryptosporidium* infection in birds in relation to age and breeding system is shown in table 4.4. Among the 150 broiler birds examined for the infection, 19(12.7 %) tested positive.

Birds reared in housing with wood shavings on concrete floor and less than four weeks old recorded the highest prevalence 9/35 (25.71 %). The lowest prevalence was amongst those above four weeks reared in the battery cage housing system 2/40 (5.00 %). the result shows significant association between the disease and the age and breeding system of the birds (P> 0.05).

4.1.5 Prevalence of *cryptosporidiosis* in diarrhoeic and non-diarrhoeic farm animals and humans

Table 4.5 shows the prevalence of *Cryptosporidium* infection in diarrhoeic and non-diarrhoeic farm animals and humans. Of the 69 infected cattle, 37 (54 %) had diarrhoeal stool while 32 (49 %) had non-diarrhoeal stool. The prevalence of non-diarrheic sheep that were positive for the infection was higher (67 %) than those that had diarrhoeal (33 %). Infected birds that had diarrhoeal stool were more than those that had formed stool; 74 % and 26 % respectively. In humans, 68 % of the positive cases had non-diarrheic stools while 32 % had diarrhoeal stools. This result showed no significant association of the infection with stool consistency (p>0.05).

Table 4.4: Prevalence of *Cryptosporidium* Infection in Birds Based on Age and Breeding Systems

Host	Age	Housing	No. Examined	No. Positive	Prevalence (%)
Birds	< 4 wks.	Battery cage	35	3	8.51
	>4 wks.		40	2	5.00
Birds	< 4 wks.	Wood shavings on concrete floor	35	9	25.71
	> 4 wks.		40	5	12.50
Total			**150**	**19**	**12.7**

Table 4.5: Prevalence of *Cryptosporidiosis* in Diarrheic and Non-diarrheic Farm Animals and Humans.

Host	*Cryptosporidium*	Number Examined	Diarrheic* (%)	Non-diarrheic (%)
Cattle	+	69	37 (54)	32 (49)
	–	77	13 (17)	64 (83)
Sheep	+	53	18 (33)	35 (67)
	–	79	5 (6.0)	74 (94)
Birds	+	19	14 (74)	5.0(26)
	–	131	29 (22)	102 (78)
Humans	+	22	9.0 (32)	13 (68)
	–	21	3.0 (14)	18 (86)
Total		**471**	**128(27.18)**	**343(72.82)**

*Diarrhoea was defined as having≥1daywith>3 liquid or semi-liquid stools

4.1.6 Univariate analysis of association between potential risk factors and *cryptosporidium* infection in farm workers

Table 4.6 shows risk factors for the disease in farm workers. Of the risk factors examined for association with the disease, contact with animals and their direct product, hand washing and water source showed greater risk factors for the infection with p-values of 0.015, 0.019 and 0.048 respectively. With the highest risk factor being contact with animal products and the least risk factor being the sex of the animals or humans.

Table 4.6: Univariate Analysis of Association between Potential Risk Factors and *Cryptosporidium* Infection in Farm Workers

Risk factors	Odds Ratio	95 % CI	$\chi 2$	*P*-value
***Sex**	0.900	0.26–3.11	0.03	0.868
Toilet facilities	0.960	0.33–4.10	0.12	0.093
Contact with Animals and products	1.273	0.32–5.24	0.61	0.015
Water source(Use of piped water)	1.19	0.37–4.38	0.34	0.048
Hand washing	1.435	0.40–5.16	0.58	0.019

*Male versus female

4.1.7 Distribution of *cryptosporidium* infection with frequency of hand washing practices.

Figure 4.1 shows the infection rate based on different hand washing practices (those that wash their hands regularly after working with animals or handling their direct products, after toilet use and before eating and those who do not wash regularly). The blue colour represents the number of individuals examined and the red, the number of positive cases.

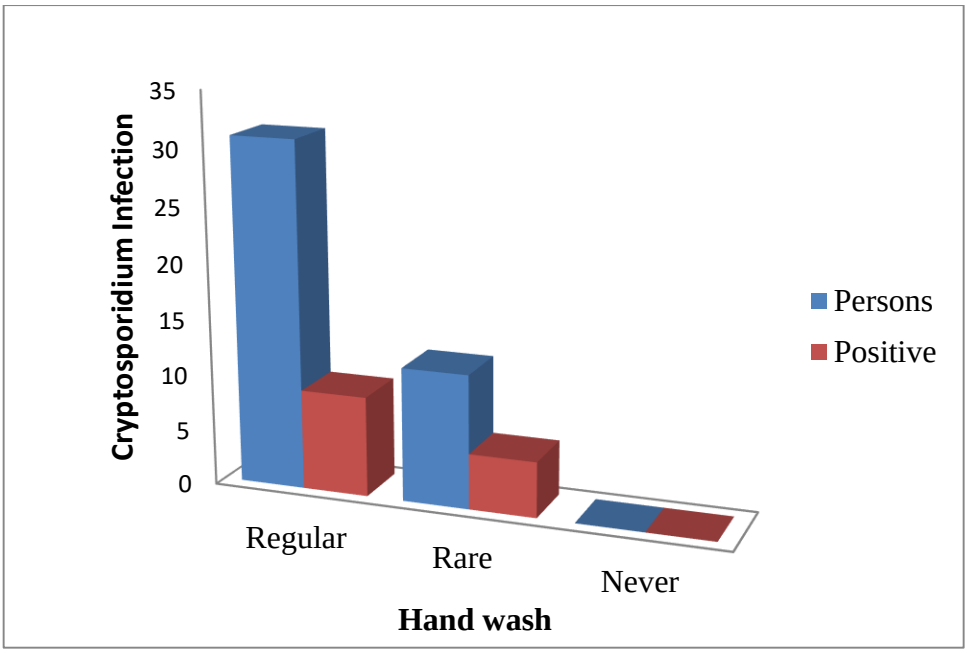


Figure 4.1: Distribution of *Cryptosporidium* Infection with Frequency of Hand Washing Practices.

4.1.8 Distribution of *cryptosporidium* infection with toilet types

Figure 4.2 shows the distribution of the infection based on the use of different toilet facilities. The blue colour represents the number of individuals examined and the red, the number of positive cases.

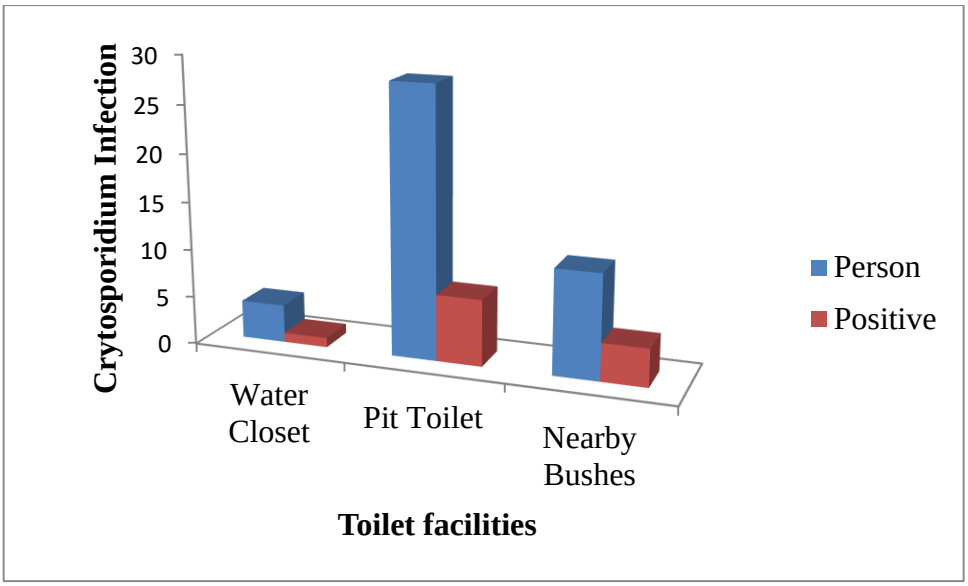


Figure 4.2: Distribution of *Cryptosporidium* Infection with Toilet Types

4.1.9 Distribution of *cryptosporidium* infection with regards with contact with different farm animals.

Figure 4.3 shows the distribution of the infection based on the type of animal that the farm workers had contact with. The blue colour represents the number of individuals examined and the red, the number of positive cases.

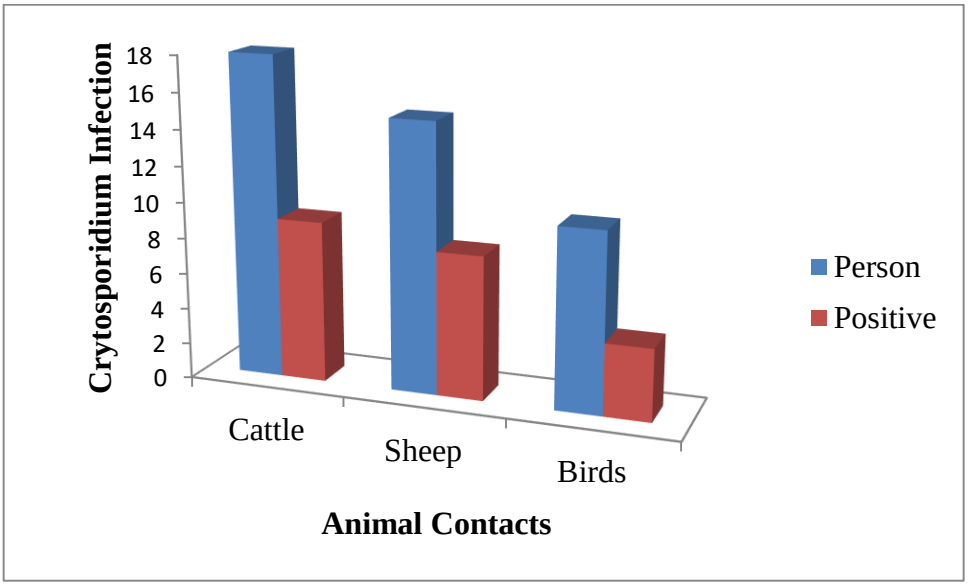


Figure 4.3: Distribution of *Cryptosporidium* Infection with Regards to Contact with Different Farm Animals.

4.1.10 Distribution of *cryptosporidium* infection with regards to water source

Figure 4.4 shows the rate of infection based on the drinking water source of the individuals. The blue colour represents the number of individuals examined and the red, the number of positive cases.

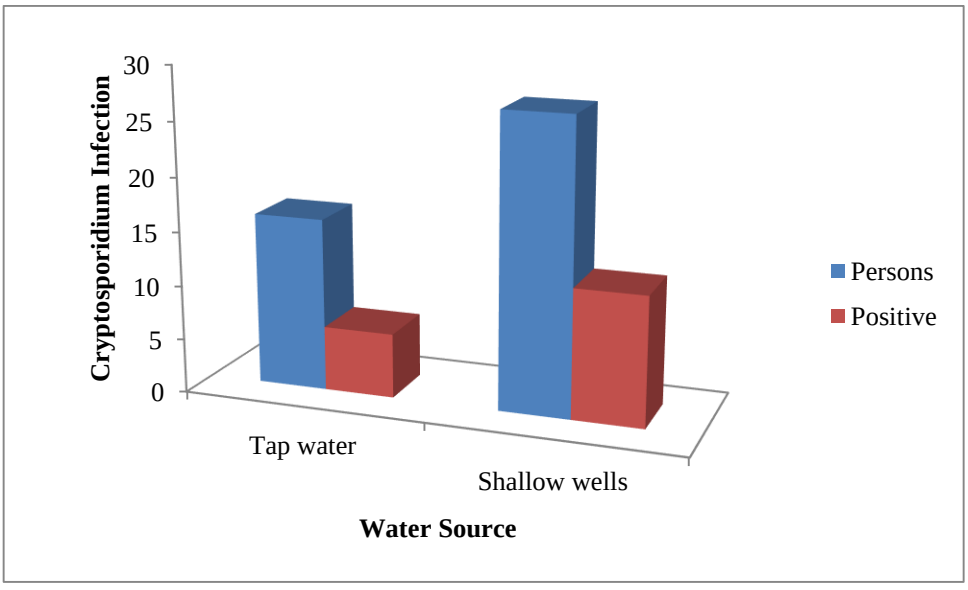


Figure 4.4: Distribution of *Cryptosporidium* Infection with Regards to Water Source.

4.1.11 Distribution of *cryptosporidium* infection and stool consistency

Figure 4.5 shows the rate of the infection among the animals and stool consistency based on the use of different toilet facilities. The blue colour represents the number of positive cases in animals that had diarrheic stool and the red, the number of positive cases in animals that had formed stool.

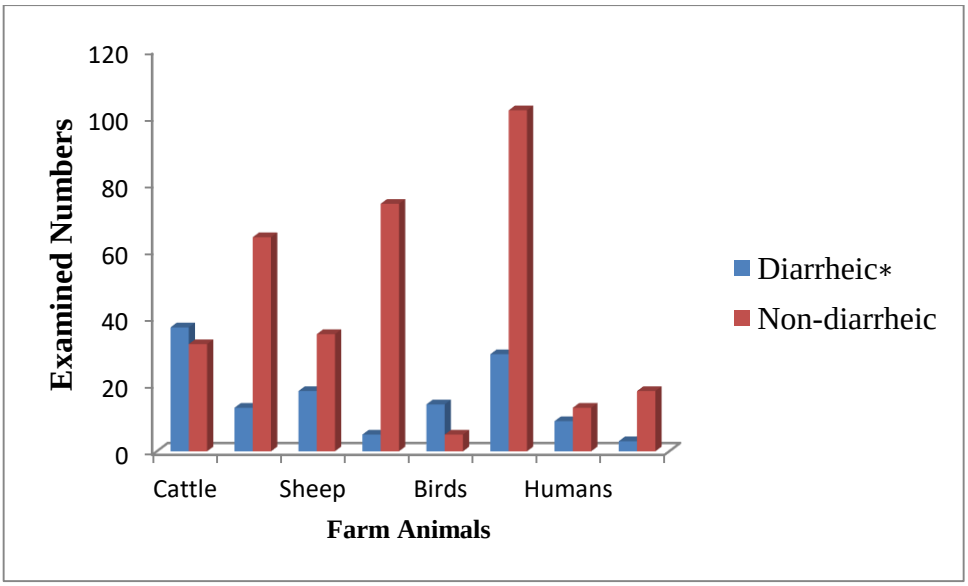


Figure 4.5: Distribution of *Cryptosporidium* Infection and Stool Consistency.

4.2 DISCUSSION

In this study, fecal samples of selected farm animals (cattle, birds and sheep) and farm workers and their household members were examined to establish *Cryptosporidium* infection as a zoonotic disease and to establish other risk factors for the infection in the study area. The high prevalence of *Cryptosporidium* infection among the farm animals and the farm workers shows a possible zoonotic transmission and a significant association with risk factors such as water source, age, and hand wash.

Cryptosporidium infection in the study area corroborates an earlier opinion that it is an emerging disease of concern in many parts of Sub- Sahara Africa including Nigeria and other parts of the world. (Morteza *et al.*, 2014; Obi *et al*., 2006). The high prevalence (34.6 %) of Cryptosporidiosis among the farm workers and the animals in the study area, Minna Metropolis, Niger State is worrisome but is consistent with the results gotten by Obi *et al*. (2006), who recorded an overall prevalence of 65.1 % among farm animals (goats, cattles and sheep) and humans in Kebbi State; Adamu *et al*. (2015) who also recorded an

overall prevalence of 22.3 % in Maiduguri, the North eastern part of Nigeria. A high prevalence of 23.4 % was also recorded from the south western parts of Nigeria (Ayinmode and Fagbemi, 2010). In other parts of the world, 20 % prevalence was recorded in Canada (O' donogue, 1995), 35 % in United States of America and 14.2 % in both cattle and humans in Isfahan, Iran (Morteza *et al*., 2014).

In this study, a total prevalence of *Cryptosporidium* infection of 34.6 % was recorded; (69/146), (53/132), 19/150) and (22/43) for cattle, sheep, birds and humans respectively. Morteza *et al*. (2014) got a prevalence of 14.2 % (31/218) and 8.5 % (36/422) in calves and humans respectively. The higher prevalence recorded in the present study may be as a result of the climatic difference and the breeding system and hygienic practices of the study population in this study area which generally favours the transmission of this disease.

Based on this study, it was observed that there was significant association between contact with farm animals and *Cryptosporidium* infection (OR =1.273; 95 % CI =0.32–5.24, $P<$ 0.015). This finding corroborates the results of Robertson *et al*. (2002) who reported calf contact away from home as the risk factor of *Cryptosporidium* infection in Melbourne (OR = 2.9; 95 % CI = 1.5–5.7) and Adelaide (OR = 5.1; 95 % CI =1.5-17. Roy *et al*. (2006) reported contact with livestock (OR=3.5;95 %CI=1.8–6.8; < 0.01) as risk factor in USA. Hunter *et al*. (2007) identified touching cattle (OR = 3.9; 95 % CI = 1.4–10.0) as risk factor of infection in Wales and New England and Morteza *et al*. (2014) was able to establish contact with calves as a risk a factor for *Cryptosporidium* infection among farm workers in Isfaham, Iran.

Furthermore, this study shows the infection is significantly higher in younger children than the older individuals (68 % and 27 % respectively) ($P < 0.05$). This is similar to the result gotten by Egberongbe *et al*. (2010) who recorded high infection among children less than 8years of age. This finding could be as a result of the lower immune systems of children and the fact that they may not be able to effectively avoid practices that put them at risk of this infection at this stage if not properly guided by an adult. A practice that is not so common within the study population.

It was also observed in this study that there is no significant association of the infection with risk factors such as toilet facilities, and sex but there was a significant negative association with hand washing (before eating, after toilet use and after work) and the use of public piped water source or borehole water. (OR = 1.19, 95 % CL =0.37–4.38; $P<0.048$) and (OR = 1.435, 95 % CL =0.40–5.16; $P<0.019$) respectively. This finding agrees with that of Morteza *et al*. (2014) who also recorded negative associations for hand washing and use of public piped water among farm workers in Isfahan, Iran. In other words, these factors were potentially protective against *Cryptosporidium* infection.

In addition, this study reveals a significant association ($P < 0.05$) between the age of the farm animals and *Cryptosporidium* infection. It shows that the younger animals are more susceptible to the infection as infection rate was higher among farm animals that were below 3 months of age. This result is in agreement with the findings of Ongerth and Stibbs (1989), Shobhamani (2005), Roy *et al*. (2006), Azami (2007) and Morteza *et al*. (2014) who recorded higher prevalence in farm animals younger than 2 months of age. Results of this study also indicate that farm animals reared under confinement or a semi intensive system recorded less prevalence than those reared in the extensive systems.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The results of this study clearly show that humans working with or in close contact with farm animals are more likely to be at risk of zoonotic infection with cryptosporidiosis. As reported earlier, the clear predominance of *Cryptosporidium* infection among the study population might be considered as a result of zoonotic transmission. However, more comprehensive studies of *Cryptosporidium* spp. both in humans in contact animals are needed to clarify accurately the zoonotic transmission of Cryptosporidiosis. Cryptosporidiosis is an emerging threat to both humans and livestock in this area and so control strategies such as education of the public on the risk factors and routine diagnosis and treatment should be put in place in other to curb the spread of the disease in this region.

5.2. Recommendations

1. *Cryptosporidium* infection is an emerging threat in the study area. Therefore, various methods should be used to create an increased awareness of the long-term impacts of the disease on people, animals, and our natural environment.
2. Opportunities should be made available for more collaborative work across the different sectors (including government and individuals) to tackle this global disease. A key target would be the development of a vaccine that could be used to help prevent disease and oocyst shedding in neonatal farm animals, as this would not only improve the health and welfare of the animals, it would also reduce environmental contamination as these animals are a main reservoir of C. parvum oocysts.

3. Another thing that can be done would be to apply an integrated genotyping approach that may be used in both veterinary and public health that would aid in source tracking and surveillance and inform epidemiology studies for the disease in the study area.
4. In addition, treatment of livestock and human faecal waste to reduce viability of Cryptosporidium oocysts would help to minimise contamination of the environment with infectious parasites and protect human and animal health. Also protecting Water Catchments in this area from Contaminated Livestock Faeces or providing a better source of drinkable water for farm workers can also help curb the spread of the disease.
5. Finally, rapid point-of-care detection of the disease in farm workers and livestock is needed to enable quick and effective intervention and treatment when infection occurs.

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