OCCURRENCE AND RISK FACTORS ASSOCIATED WITH FAECAL SHEDDING OF *CRYPTOSPORIDIUM* OOCYSTSIN AVIAN SPECIES IN KANO METROPOLIS, NIGERIA

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BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN AVIAN MEDICINE

DEPARTMENT OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY
ZARIA, NIGERIA

MARCH, 2016

DECLARATION

I declare that the work in this Dissertation titled **"Occurrence and Risk Factors Associated with Faecal Shedding of *Cryptosporidium* Oocysts in Avian Species in Kano Metropolis, Nigeria"**has been carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in thetext and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other institution.

Mahmud Alhaji MUSTAPHA _____ _____
Signature Date

CERTIFICATION

This Dissertation titled, "OCCURRENCE AND RISK FACTORS ASSOCIATED WITH FAECAL SHEDDING OF*CRYPTOSPORIDIUM* OOCYSTS IN AVIAN SPECIES IN KANO METROPOLIS, NIGERIA" by Mahmud Alhaji MUSTAPHA meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, Zaria and has approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this humble effort, to my parents and lovingGrandmother Hajiya Khadija (May her gentle soul rest in perfect peace) for the moral upbringing and guidance throughout my life. You will remain in my memories forever.

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I am grateful to Almighty Allah who gave me health, strength and capability to start and complete this research work. To my family indeed,particularly my son (Jibreel Mahmud), I am overwhelmed by the support, love and care they have shown to me throughout my studies. May Almighty Allah reward them abundantly.

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This research could not have been completed without the assistance of the technical staff of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. I thank you all.

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ABSTRACT

Cryptosporidium is a coccidian parasite found infecting a wide range of mammals including man, birds and lower vertebrates. The aim of this study was to determine the occurrence and risk factors associated with faecal shedding of *Cryptosporidium* oocysts in avian species in Kano Metropolis, Nigeria. Faecal samples from three hundred and thirty avian species were examined for *Cryptosporidium* oocysts using Modified Ziehl-Neelsen staining technique. The overall prevalence was 6.4%. Live bird markets had the highest prevalence rate of 10%, slaughter slabs and commercial farms had 4.55% and 4.55% respectively. The difference in the prevalence rates between sampling points was not significant (P>0.05). Based on species, turkeys had the highest prevalence rate of 18.75%, followed by ducks (9.09%), chickens (6.61%), guinea fowls(0%) and pigeons(0.0%).The prevalence of *Cryptosporidium*among the different avian species was not significant (P>0.05). The occurrence of *Cryptosporidium* oocysts in avian species based on Local Government Areas (LGAs) were also studied. Flocks in Dala LGA recorded the highest prevalence rate of 12.12%, while those in Kumbotso had the lowest prevalence of 1.52%. The association between *Cryptosporidium* oocysts and LGAs was not significant (P>0.05). Risk factors for the spread of *Cryptosporidium* oocysts by avian species were also studied and analyzed; source of birds (interstate OR=1.314; p=0.735), frequency of cleaning (not daily OR=1.859; p=6.756), source of feeds (not commercial OR= 2.604; p=0.126), source of water (well water OR=1.770; p=0.521) and types of cage (wooden cage OR=1.186, p=0.533) were found to have a high risk estimate for the spread of the *Cryptosporidium*, though not significant (p>0.05). This study has shown the presence of *Cryptosporidium* oocysts in poultry sampled in Kano Metropolis, Nigeria, which is of veterinary and public health significance. It is therefore imperative that public awareness should be embarked upon

on the importance of biosecurity, so that the veterinary and public health significance of the disease can be prevented.

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LIST OF ABBREVIATIONS

Abbreviation or symbol	Terms
AICP	Avian Influenza ControlProject
AIDS	Acquired Immuno Deficiency Syndrome
AFB	Acid Fast Bacilli
BUK	Bayero University Kano
C	*Cryptosporidium*
DNA	Deoxyribonucleic acid
EIAs	Enzyme immune assays
ELISA	Enzyme Linked Immunosorbant Assay
FITC	Flourescein isothiocyanate
ICLF	Immunochromatographic lateral flow
IFA	Immunofluorescence assay
KMC	Kano Municipal
LBM	Live bird market
LGAs	Local Government Areas
mZN	modified Ziehl Neelsen
NBLS	National baseline survey
OR	Odds ratio
PCR	Polymerase chain reaction
P-value	Probability value
rRNA	ribosomal ribonucleic acid
Spp	Species
SPSS	Statistical Package for social sciences
UV	Ultra violet

CHAPTER ONE

INTRODUCTION

1.1Background of the Study

Cryptosporidiosis is caused by *Cryptosporidium* species, which has a broad range of hosts and worldwide distribution. *Cryptosporidium* species can infect human beings as well as other animals. It is mainly located in the epithelial cells of the gastrointestinal tract and likely in the respiratory tract (Gomes *et al*., 2012). The main clinical manifestation is persistent diarrhoea and respiratory disturbances can occasionally be observed (Langsley *et al*., 2008; Das *et al*., 2011). *Cryptosporidium* from birds was first described in 1929 by Tyzzer, in the ceaca of chickens. In 1955, Slavin reported a structurally similar parasite in turkeys and named it *C. meleagridis* (Slavin, 1955).

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

Kingdom: Protozoa,

Phylum: Apicomplexa:

Class:Conoidasida

Subclass: Coccidiasina,

Order: Eucoccidiorida

Suborder: Eimeriorina

Family: *Cryptosporidiidae*.

Cryptosporidium is one of the most common and important parasites in poultry and this pathogen can infect more than 30 avian species. Traditionally, three different species of *Cryptosporidium* (*C. baileyi, C. meleagridis* and *C. galli*) were considered the major species affecting birds (Xiao*et al*., 2004; Fayer, 2010) and out of these, only *C. meleagridis*, which infects turkeys and parrots, is a known threat to human beings. Nevertheless, *C. baileyi* is probably the most common avian species because it is able to infect chickens, turkeys, ducks, cockatiel, quails and ostriches whereas *C. galli* infects domestic chickens, capercaille and pine grosbeaks (Xiao *et al*., 2004).

Transmission of infection to humans and other mammals is thought to be caused by ingestion of the oocysts of *C. parvum* (Fayer *et al.,* 2000). Both modified Ziehl Neelsen and Acid Fast Staining are effective for detecting *Cryptosporidium* oocysts in faeces (Casemore, 1991; Smith, 2008). Modified Ziehl Neelsen stained slides should be screened under the ×40 objective lens and putative oocysts confirmed and measured under the ×100 objective lens (morphology and morphometry) using a bright-field microscope with a ×10 eyepiece. These oocysts are resistant to most common disinfectants and are not readily killed by routine chlorination of water supplies (LeChevallier *et al.,* 1991).

1.2 Statement of Research Problems

Cryptosporidiosis is a zoonotic disease and is transmitted by faeco-oral route. The disease caused by *Cryptosporidium* species is known to cause heavy losses in poultry

industry. *Cryptosporidium meleagridis*, a poultry based organism is also known to have zoonotic importance.

Cryptosporidiosisis not always confined to gastrointestinal tract, in some cases respiratory Cryptosporidiosis, cholecystitis, hepatitis and pancreatitis have been observed (Arora and Arora, 2005).

It is highly probable that the disease is misdiagnosed in Nigeria since it is not routinely diagnosed and so the condition can persist in the farm and even be confused with similar conditions like coccidiosis.

1.3 Justification of the Study

Poultry is an essential component of the Nigerian economy and a good source of high quality protein for the rapidly growing population of the country. In livestock production, poultry occupies a prominent position for supply of animal protein and this accounts for about 25% of local meat production in Nigeria (Ekeren*et al.*,1995).

Cryptosporidiosis is an emerging zoonotic disease, resulting in intestinal and extra-intestinal disorders in both humans and animals. The disease is well known in veterinary medicine and it has been recognized as a leading protozoal cause of diarrhoea in humans (GoldSmith*et al*.,1989).

In Nigeria, the few available studies on bovine Cryptosporidiosis were based on microscopic detection of the oocysts on acid-fast stained faecal smears with reports of prevalence rates of 23.4% in Oyo State (Ayinmode and Fagbemi, 2010), 28.0% were reported in calves in Plateau State (Pam *et al.,* 2013) and 33.0% in Sokoto State (Faleke *et al.,* 2014).

There is paucity of information on the occurrence and risk factors associated with faecal shedding of avian*Cryptosporidium* oocysts in Kano Metropolis and limited data on its molecular characterization.

The results obtained from this study could serve asbaseline information in planning for the surveillance and control of the disease and its importance in Kano Metropolis by the veterinarians and other health workers.

1.4Aim of the Research

The aim of the study was to determine the occurrence and risk factors associated with faecal shedding of *Cryptosporidium* oocysts in live bird markets, slaughter slabs and commercial poultryfarms in Kano Metropolis, Nigeria.

1.5 Objectives of the Research

The objectives were to:

1. determine the occurrence of *Cryptosporidium* oocysts in avian species in live bird markets, slaughter slabsand commercial poultry farms in Kano Metropolis.
2. evaluate the associated risk factors for the spread of avian *Cryptosporidium* in Kano Metropolis.

1.6 Research Questions

1. What is the occurrence of *Cryptosporidium* oocysts in live bird markets, slaughter slabs and commercial poultry farms in Kano Metropolis?
2. What are the risk factors associated with faecal shedding of*Cryptosporidium* oocystsin live bird markets, slaughter slabs and commercial poultry farms in Kano Metropolis?

CHAPTER TWO

LITERATURE REVIEW

2.1 Preamble

Cryptosporidium is a coccidian parasite found infecting a wide range of mammals (including man, birds and lower vertebrates). Cryptosporidiosis is considered to be of socio-economic and/or public health importance (OIE, 2004). The infection is self-limiting except in the immunodeficient hosts, such as those with the acquired immunodeficiency syndrome (AIDS) in humans, tuberculosis in cattle and neonates of various animal hosts (Current and Garcia, 1991), in which it may cause protracted diarrhoea.

Cryptosporidiosis is caused by *Cryptosporidium*, which has a broad range of hosts and worldwide distribution. The *Cryptosporidium* species can infect human beings as well as other animals, it is mainly located in the epithelial cells of the gastrointestinal tract, and is also likelyfound in the respiratory tract(Gomes*et al.*, 2012), particularly in the case of infection by *Cryptosporidium meleagridis*, a bird species.

It is now recognised that *Cryptosporidium* species differ principally in their host range (Adriana *et al.*, 2010). While some *Cryptosporidium* species appear to be restricted to particular types of hosts, others have broad host range, including man and are therefore of zoonotic significance (Thompson *et al.*, 2008). *Cryptosporidium bovis*, *C. ryanae* and *C. andersoni* are the commonly encountered speciesaffecting cattle in Nigeria (Ayinmode and Fagbemi, 2010; Maikai *et al.*, 2011). *Cryptosporidium parvum* is however, recognized as an important agent in life-threatening neonatal diarrhoea in calves (Current and Garcia, 1991; Miron *et al.*, 1991). Transmission of infection to humans and other mammals is thought to be caused by ingestion of the oocysts of *C.*

parvum (Fayer *et al.,* 2000). These oocysts are resistant to most common disinfectants and are not readily killed by routine chlorination of water supplies (LeChevallier *et al.,* 1991).

The main clinical manifestation are persistent diarrhoea but respiratory disturbances can occasionally be observed (Langsley *et al*., 2008; Das *et al.,* 2011). *Cryptosporidium* is one of the most common and important parasites in poultry and this pathogen can infect more than 30 avian species. Traditionally, three different species ofCryptosporidium (*C. baileyi, C. meleagridis, and C. galli*) were considered the major species in birds (Current*et al.,* 1986; Ryan *et al.,* 2003). Recently, more than 11 novel genotypes have been described which are genetically different from the above three species by means of molecular analysis, including avian genotypes (I–V), goose genotypes (I–IV), the Eurasian woodcock genotype and the black duck genotype (Abe and Makino,2010). *Cryptosporidium meleagridis, C. parvum, and C. hominis* carried by poultry may infect human beings through the oocysts in contaminated water or vegetables eaten uncooked (Majewska*et al.,* 2009). Considering that the possibility of exposure to *Cryptosporidium* ofhuman beings still exists, the prevention of Cryptosporidiosisis important for public health (Karanis *et al.,* 2007).

Although *Cryptosporidium* was first described in the laboratory mouse by Tyzzer in 1907, the medical and veterinary significance of this protozoan was not fully appreciated for another 70 years. The interest in *Cryptosporidium* escalated tremendously over the last two decades, as reflected in the number of publications, which increased from 80 in 1983 to 2850 currently listed in MEDLINE. The early history of *Cryptosporidium* is extensively documented in several review articles and book chapters published (Tzipori, 1983; O'Donoghue, 1995).

Taxonomically, *C. parvum* belongs to Phylum Apicomplexa (which possess an apical complex), Class Sporozoasida (which reproduce by asexual and sexual cycles, with oocyst formation), Subclass Coccidiasina (with a life cycle involving merogony, gametogeny and sporogeny), Order Eucoccidiida (in which schizogony occurs), Suborder Eimeriina (in which independent micro- and macrogamy develop), Family *Cryptosporiidae* (contain four naked sporozoites within oocysts, but with no sporocyst)(Levine *et al.,* 1985). Like other enteric coccidia of vertebrates, *Cryptosporidium* has a monoxenous life cycle that is primarily completed within the gastrointestinal tract of a single host. There are however, many unique features that distinguish *Cryptosporidium* from other coccidian, of which, the relative lack of host and organ specificity, resistance to antimicrobial agents, ability for autoinfection and the curious location itoccupies within the host cell membrane are the mostobvious (Tzipori, 1983).Between 1980 and 1993, three broad entities ofCryptosporidiosisbecame recognized (Tzipori and Griffiths, 1998). The first was therevelation in 1980 that *Cryptosporidium* was, in fact, acommon, yet serious primary cause of outbreaks as well assporadic cases of diarrhea in certain mammals (Tzipori, 1983). From1983 onwards, with the onset of the AIDS epidemic,*Cryptosporidium* emerged as a life-threatening disease inthis subpopulation (Anonymous, 1982; Mackenzie *et al.,* 1994).In 1993,it reached the publicdomain when it became widely recognized as the mostserious and difficult to control, cause of waterborne-relateddiarrhea (Mackenzie*et al.,* 1994). The first glimpse of the seriousness of*Cryptosporidium* in mammals, mainly in calves, was providedin the late 1970s (Morin *et al.,* 1976; Pohlenz*et al.,* 1978). Until then, *Cryptosporidium*was mostly identified histologically in infected gut sectionsor in biopsy specimens (Nime *et al.,*

1976; Meisel *et al.,* 1976).Moreover, it was considered opportunistic protozoan that caused a few or no symptoms.

2.2 Morphology of the*Cryptosporidium* oocysts

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

2.3 Taxonomy of *Cryptosporidium*

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

Kingdom	Protozoa
Phylum	Apicomplexa
Class	Conoidasida

Subclass	Coccidiasina
Order	Eucoccidiorida
Suborder	Eimeriorina
Family	*Cryptosporidiidae*

2.4 Life Cycle of *Cryptosporidium*

Cryptosporidium is an apicomplexan parasite of public health and veterinary importance. This phylum contains other medically important parasites such as *Plasmodium* and *Toxoplasma*. 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 subpellicular microtubules, which is involved in host-parasite interactions. Interestingly, *Cryptosporidium* spp. seems to have lost functional apicoplasts and mitochondria, as shown by genome sequence analyses. The *Cryptosporidium* life cycle can be divided into six major developmental phases (Current and Garcia, 1991), excystation (the release of infective sporozoites), merogony (asexual multiplication within host cells), gametogony (the formation of micro- and macrogametes), fertilization (the union of micro- and macrogametes), oocyst wall formation, (to produce the environmentally resistant stage responsible for the transmission of the infection from one host to another), and sporogony (the formation of infective sporozoites).

Cryptosporidium species have a monoxenous life cycle completed within the gastrointestinal tract of a single host. During the whole cycle, the different forms are confined to the apical surfaces of the host cells. The infective forms attach to the apical

surfaces by a poorly understood process and become internalized within an intracellular but extracytoplasmic compartment separated from the cytoplasm by an electron-dense layer that appears to be predominantly of host origin, (Barta and Thompson, 2006). The parasite is located within a closed compartment of the host cell plasmalemma, described as the parasitophorous vacuole (Thompson *et al.,*2005), where it is protected from the hostile gut environment and is supplied with energy and nutrients by the host cell through a feeder organelle, which is unique among apicomplexan parasites (Tzipori and Ward, 2002). It has also been reported that *C. parvum* may have extracellular gregarine-like life stages (Rosales *et al.,* 2005), although no subsequent studies have so far confirmed this. The established life cycle begins with the ingestion of the sporulated oocysts by the susceptible host, the oocysts undergo excystation and release four infective sporozoites. The excystation of the oocysts has been reported to be triggered upon ingestion by various factors, including reducing conditions, carbondioxide, temperature, pancreatic enzymes, and bile salts (Robertson *et al.,* 1993). Excystation allows the emergence of the four infectious sporozoites through a suture in the oocyst wall (Reduker and Speer, 1985). The released sporozoites glide over the intestinal cell, releasing material from the apical complex (Okhuysen and Chappell, 2002). Gliding motility allows the sporozoites to migrate across the surface of host cells and to actively invade them (Wetzel, 2005). This form of motility is conserved among different apicomplexan parasites. *C. parvum* sporozoites undergo circular and helical gliding movements, which are actinomyosin- tubulin dependent (Wetzel, 2005). During gliding motility, sporozoites deposit trails of proteins, which are involved in the attachment to and invasion of host cells (Wanyiri *et al.,*2006).

Cryptosporidium sporozoites possess an apical complex composed of micronemes, a single rhoptry, and dense granules. These secretory organelles are also present in other

apicomplexan parasites, such as *Toxoplasma*, *Plasmodium*, and *Eimeria*, and are involved in host cell attachment and invasion (Okhuysen and Chappell,2002). The pellicle mediates movement and attachment to the host cell membrane via molecules exposed on the surface that drive the movement of the sporozoite, while the rhoptries and micronemes enable the sporozoite to adhere to and invade the cell, inducing the cell membrane to enclose the parasite in the parasitophorous vacuole (Boulter *et al.*, 2007). Each sporozoite develops into a spherical trophozoite, which undergoes merogony and forms a type I meront containing eight merozoites (Dupont *et al.*, 1995). These merozoites are released and attach again to the surface of an epithelial cell, where they undergo merogony once more and form either a further type I meront or a type II meront. A type II meront contains four merozoites. These merozoites, when released, attach again to the epithelial cell, but instead of developing into further meronts, they initiate gametogony. Individual merozoites produce either microgamonts or macrogamonts (Gobel and Brandler, 1982). Each microgamont undertakes nuclear division and differentiates to form up to 16 microgametes, which, when released from the parasitophorous vacuole, locate and fertilize aunicellular macrogametocyte that has developed from a macrogamont. The product of fertilization, the zygote, undergoes two asexual cycles of sporogony to produce an oocyst with either a thick wall or a thin wall, containing four sporozoites (Current and Reese, 1986). The thick-walled oocysts are released into the lumen of the intestine, are excreted from the host in the faeces, and are immediately infective, allowing the spread of infection to other susceptible hosts (Smith and Rose, 1998). In addition, unlike other parasites belonging to the same subclass (*Coccidia*), *Cryptosporidium* is able to auto infect the same host. Autoinfection occurs through the thin-walled oocysts, which excyst once they are separated from the epithelium, and the cycle starts again.

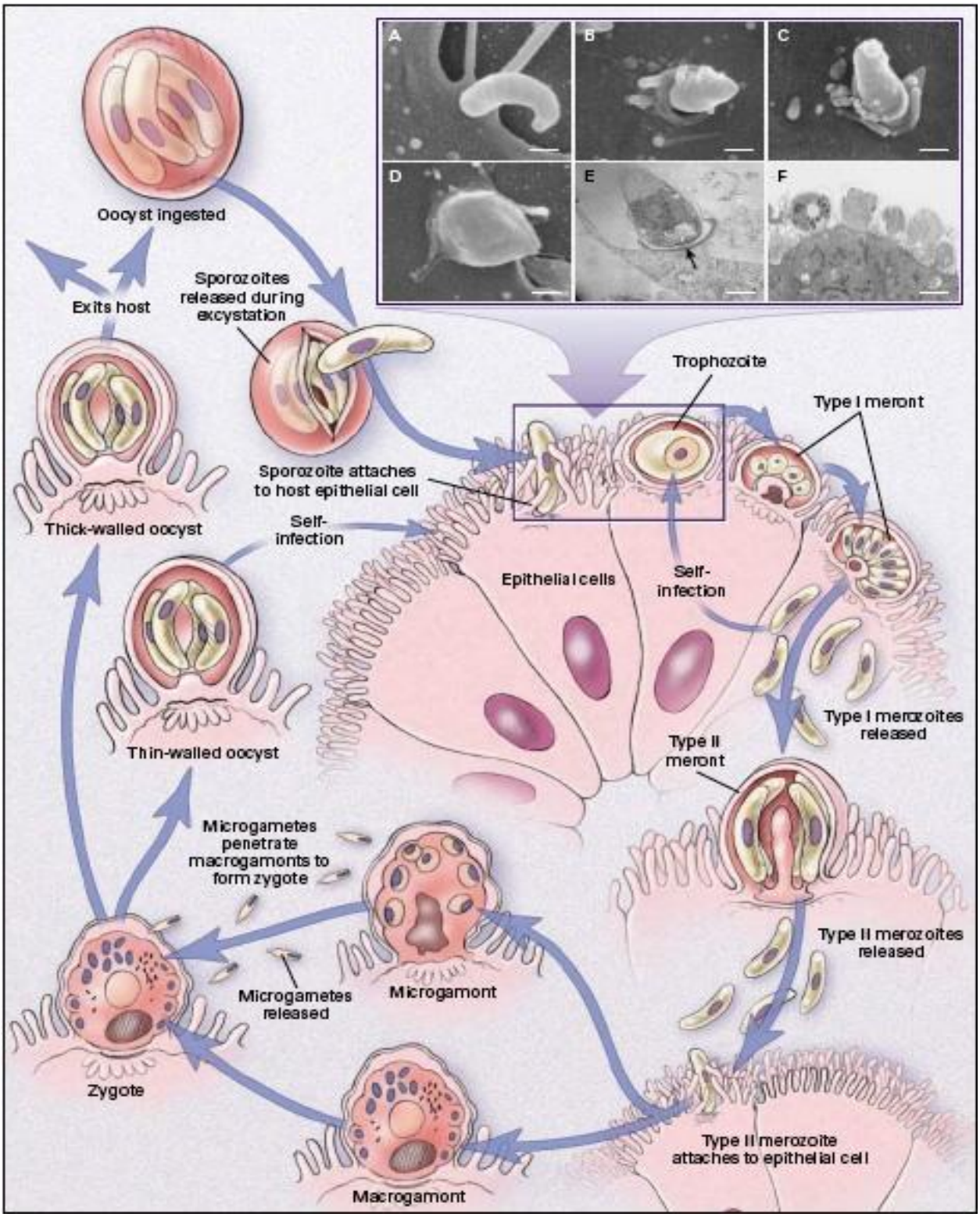


Figure 2.1: Life cycle of *Cryptosporidium*. Retrieved from web.standardford.edu Access at 5:00pm on 11 May, 2015

2.5Pathogenesis of Cryptosporidiosis

The pathogenic mechanisms by which *Cryptosporidium* causes diarrhoea, malabsorption and wasting are poorlyunderstood. Whatever these mechanisms may be, the initial host–parasite interactions of attachment and invasion arecritical primary events in pathogenesis. The ultra-structuralcharacteristics of attachment and invasion and variousfactors influencing attachment have beendescribed(Theodos, 1998). However, little is known about specific parasite and host molecules involved in these processes. Knowledge of such molecules is crucial for understanding the pathogenic mechanisms employed by this parasite. The initial host–parasite interactions of attachment, invasionand parasitophorous vacuole formation are complex processes that involve multiple parasite ligands and host receptors. These interactions have been best studied inapicomplexans such as *Toxoplasma*, *Plasmodium* and *Eimeria*. Invasive "zoite" stages of apicomplexans possess specialized secretory organelles (rhoptries, micronemes and dense granules) collectively known as the apical complex. During initial host–parasite interactions, these organelles secrete and successively exocytose proteins, which facilitate attachment, invasion and parasitophorous vacuole formation.

Many micronemal proteins have adhesive "modules"that are conserved among apicomplexan parasites, whereas others express unique domains (Ward and Cevallos,1998). Increasing recognition of *Cryptosporidium* as an emerging human pathogen has led to the identification of surface and/or apical complex proteins (such as CSL, GP900, p23/27, TRAP C1, GP15, CP 15, CP60/15, cp47, gp40/45 and gp15/Cp17) that have features in common with those of other apicomplexans and that are implicated in mediating these interactions. Many ofthese proteins have been reviewed previously (Tomley and Soldati, 2001).

2.6 Transmission of *Cryptosporidium* species

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

2.6.1 Routes of Transmission

Collectively, the various species of *Cryptospodium* have a broad host range affecting gastro-intestinal epithelial cells. The durability of oocysts, along with their diminutive size, lend them11selves to the incurable transmittable diseases via municipal drinking water, as they can survive many disinfectants, thus having the potential to cause community-wide outbreak (Robertson and Gjerde, 2007). Outbreaks illustrate different routes of the transmitted diseases namely person-person contact with infected patients (family members, health care workers, users of communal swimming pools and travelers), human-animal contact (zoonosis) (veterinarians, farmers), or via indirect transmission through the environment (particularly by water) (Chen *et al.,* 2002). The guidelines of World Health Organization (WHO, 1991) for drinking water, classifies *Cryptosporidium* as a pathogen of significant public health importance (WHO, 2006).

2.7 Hosts and Species Affected

2.7.1 *Cryptosporidium* species that infect mammals

Mammals represent the largest group of animals infected with *Cryptosporidium* spp. The taxonomy of *Cryptosporidium* in mammals has been the subject of dispute and for some time only two species (*C. parvum* as the intestinal species and *C. muris* as the gastric species) were recognized (Tzipori and Griffiths, 1998). There are now considered to be 6 valid species in mammals, reported previously, and described in more detail below.

2.7.1.1 Cryptosporidium parvum

Over 150 species of mammals have been identified as hosts of *C. parvum or C. parvum*-like parasites. Most descriptions, however, have been based solely on microscopy, with no careful morphometric measurements or genetic or other biological data. Recent molecular characterizations, however, have shown that there is extensive host adaptation in *Cryptosporidium* evolution, and many mammals or groups of mammals have host-adapted *Cryptosporidium* genotypes, which differ from each other in both DNA sequences and infectivity.

Currently, it is considered that *C. parvum* consists of nearly 20 genotypes that have been associated with mouse, pig, bear, deer, marsupial, monkey, muskrat, skunk, cattle, and ferret, some of which may represent different species (Xiao *et al.,* 2002). Some genotypes are clearly being delineated as distinct species and include *C. hominis* (human genotype or genotype 1), *C. parvum* (bovine genotype or genotype 2), and *C. canis* (the dog genotype). It has therefore been suggested that the name, *C. parvum* be used for the *Cryptosporidium* parasites previously known as bovine genotype and avoid the use of *C. parvum* broadly for *Cryptosporidium* in mammals.

C. parvum bovine genotype or genotype 2 is therefore known to infect mainly ruminants (cattle, sheep, goats, and deer) and humans (Morgan *et al.*, 1998).

2.7.1.2Cryptosporidium muris

The type species of *Cryptosporidium* was first described in the gastric glands of laboratory mice, but not wild mice (Tyzzer, 1907). A more detailed description of each life cycle stage was later provided, and all stages were found to localize in the gastric glands of the stomach (Tyzzer, 1910). The pathology appeared to be slight and infection appears to be non-pathogenic in mice.

Based solely on morphology, *C. muris or C. muris*-like oocysts have been found in the faeces of cattle in the several countries (Anderson, 1991; Bukhari and Smith, 1996; Kaneta and Nakai, 1998) and camels in Iran (Nouri, 2002). Because species identification was not confirmed genetically or experimentally, many of these authors qualified their findings by calling the organism *C. muris*-like. Recent molecular characterizations of *C. muris* and *C. muris*-like parasites have indicated that all bovine isolates are *C. andersoni*.

Cryptosporidium muris can infect a wide range of additional hosts, including hamsters, squirrels, Siberian chipmunks, wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), maras (*Dolichotis patagonum*), rock hyrax, bactrian camels, mountain goats, humans, and cynomolgus monkeys (Chalmers *et al.*, 1997; Morgan *et al.*, 2000, Torres *et al.*, 2000 Dubey *et al.*, 2002; Gatei *et al.*, 2002).

2.7.1.3Cryptosporidium andersoni

Cryptosporidium andersoni infects the gastric glands of the abomasum of older calves and adult cattle. Oocysts of *C. andersoni* are not infectious for mice, chickens or goats.

There are conflicting cross-transmission data on species specificity of *C. andersoni* and *C. muris*-like isolates, such that molecular methods are usually employed in cross-transmission studies to confirm species identification. Genetically confirmed *C. andersoni* infection has so far been found only in cattle, bactrian camels, and sheep (Xiao*et al.*, 1999).

2.7.1.4Cryptosporidium canis

Cryptosporidium oocysts have been observed in the faeces of dogs worldwide. The oocysts are morphologically indistinguishable from those of *C. parvum* and can also infect humans and bovines but not mice (Fayer *et al.,* 2001). Confirmed *C. canis* infections have been found in dogs, coyotes, foxes, and humans.

2.7.1.5Cryptosporidium felis

Cryptosporidium oocysts observed in the faeces of cats were reported to be *C. felis*based onoocyst morphology, host specificity and pathogenicity (Iseki, 1979). Molecular characterizations support the concept of *C. felis* as a valid species. All *Cryptosporidium* isolates from cats characterized have so far shown significant sequence differences from other known *Cryptosporidium* spp. and genotypes, but similarity to each other, even though they were from different geographic regions (Morgan *et al.,* 1998). Confirmed *C. felis* infections have been found in cats, humans, and cattle (Morgan *et al.,* 1998; Bornay-Llinares *et al.,* 1999; Caccio *et al.,* 1999; Pedraza-Diaz, 2001).

2.7.1.6Cryptosporidium wrairi

Cryptosporidium wrairi has been described from the guineapig (*Cavia porcellus*) (Vetterling *et al.,* 1971). Infection was not associated with diarrhoea or overt signs of coccidiosis, but only with enteritis in small guineapigs weighing 200 to 300g (Jervis *et*

al., 1966; Vetterling *et al.,* 1971). Initially, cross-transmission studies and oocyst morphological studies suggested that *C. parvum* and *C. wrairi* might actually be the same species (Tilley *et al.,* 1991). Molecular characterizations have identified significant differences between *C. parvum* and *C. wrairi* at multiple genetic loci (Chrisp and LeGendre, 1994; Spano *et al.,* 1997; Morgan-Ryan *et al.,* 2001). These combined data, along with the fact that naturally occurring *C. wrairi* infections have been found only in guineapigs, strongly indicate that this organism is a different species from *C. parvum*.

2.7.1.7Cryptic speciesin mammals

Recent research has revealed that mice harbour a genetically distinct form of *Cryptosporidium*, which is different from *C. parvum* (bovine genotype 2). Only rarely is*C. parvum* (bovine genotype) found naturally in mice, since it is predominantly a parasite of ruminants and humans (Morgan *et al.,* 1998; Morgan *et al.,* 1999).

Therefore, it is likely that the species described by Tyzzer in 1912 was not *C. parvum* (bovine genotype) but was, in fact, the mouse genotype and it is suggested that this will be named as a new species in the foreseeable future (Xiao *et al.,* 2004).

There are probably many other cryptic *Cryptosporidium* species in mammals, all of which were previously assumed to be *C. parvum*. Limited cross-transmission studies have shown biological differences among some of the genotypes (Enemark *et al.,* 2002).

2.7.2*Cryptosporidium* species of birds

Cryptosporidium is a primary pathogen in chickens, turkeys and quails, causing respiratory and/or intestinal disease, leading to morbidity and mortality. Although

infections have been found in over 30 species of birds, only three avian *Cryptosporidium* species have been named (Lindsay and Blagburn, 1990; Sreter and Varga, 2000). The three species of *Cryptosporidium* (*C meleagridis, C. baileyi*and *C galli*) can each infect a broad range of birds, but they differ in predilection sites. *C. meleagridis* and *C. baileyi* are found in turkeys and chickens infecting the small and large intestine and cloacal bursa, but they differ significantly in oocyst size. *C. baileyi* is also found in the respiratory tissues such as the sinuses and trachea and can be a significant cause of respiratory disease. *C. galli* infects only the proventriculus of chickens and finches.

2.7.2.1 Cryptosporidium meleagridis

Developmental stages of *C. meleagridis* are found on the villi epithelia in the small intestine of turkeys and chicken. It is also the third most common *Cryptosporidium* parasite in humans (Pedraza-Diaz *et al.*, 2001). The oocysts are indistinguishable from those of *C. parvum.* The parasite can infect other avian hosts such as parrots (Morgan *et al.*, 2000; Morgan *et al.*, 2001). Molecular analysis has demonstrated the genetic uniqueness of *C. meleagridis*(Xiao *et al.,* 1999 and Morgan *et al.,* 2000), although several subtypes of *C. meleagridis* have been described based on multilocus analysis (Glaberman *et al.*, 2002).

2.7.2.2Cryptosporidium baileyi

A second species of avian *Cryptosporidium*, originally isolated from commercial broiler chickens, was named *C. baileyi*, based on its life cycle and morphological features (Current *et al.*, 1986). *C. baileyi* is probably the most common avian *Cryptosporidium sp*ecies and can infect a wide range of birds. It has so far been found in chicken,

turkeys, ducks, cockatiels, a brown quail, gulls and an ostrich (Lindsay, 1997; Pavlasek, 1999; Morgan *et al.,* 2001).

2.7.2.3 Cryptosporidium galli

A third species of avian *Cryptosporidium* was first found in the proventriculus of chickens on the basis of biological differences, including oocyst size and morphology (Pavlasek, 1999, 2001; Ryan *et al.,* 2003). Confirmed hosts of *C. galli* include finches (Fringillidae), domestic chickens, capercaille (*Tetrao urogallus*), and pine grosbeaks (*Pinicola enucleator*) (Ryan *et al.,* 2003). Morphologically similar oocysts have been observed in a variety of exotic and wild birds including members of the Phasianidae, Passeriformes, and Icteridae (Ryan *et al.,* 2003). Future studies are required to determine the extent of the host range for *C. galli* (Xiao *et al.,* 2004).

2.7.2.4Cryptic species in birds

Based on limited biological and molecular studies, it appears that there are several other distinct avian *Cryptosporidium* species in ostriches and quail and other species of birds (Lindsay, 1997;Gajadhar, 1994). Infection with a *Cryptosporidium* species in bobwhite quails infects the entire small intestine causing severe morbidity and mortality (Hoerr *et al.,* 1986; Ritter *et al.,* 1986; Guy *et al.,* 1988). Several other new *Cryptosporidium* spp. have been found in birds by molecular analysis, such as a duck genotype in a black duck and two goose genotypes in Canada geese, all of which are related to intestinal *Cryptosporidium* species (Morgan *et al.,* 2001; Xiao *et al.,* 2002).

2.7.3*Cryptosporidium* species of reptiles

Even though a high prevalence of *Cryptosporidium* infections has sometimes been found in captive reptiles, few studies have attempted to identify the species of parasite involved. For quite some time, one species, *C. serpentis* (Levine *et al.,* 1985), was the

only species identified in snakes. However, *C. saurophilum* was described in lizards (Koudela and Modry, 1998). Of all the animals, reptiles, especially snakes, are the most severely affected due to the chronic nature of Cryptosporidiosis in these animals.

2.7.3.1Cryptosporidium serpentis

Cryptosporidium was initially reported as causing a severe chronic gastroenteritis belonging to three genera and four species of snakes (*Elaphe guttata*, *Elaphe subocularis*, *Crotalus horridus*, and *Sansinia madagascarensis*) (Brownstein *et al.*, 1977). This species was subsequently named *C. serpentis* (Levine, 1985), and its taxonomic status subsequently validated by morphological and other biological data (Tilley *et al.*, 1990). All developmental forms of this species were identified by ultrastructure in the gastric mucosa. It causes postprandial regurgitation and firm, mid-body swelling. Unlike avian and mammalian cryptosporidiosis, infections appear to occur in mature snakes, with a protracted clinical course, and, once infected, most snakes remain infected (Brownstein *et al.*, 1977). In a subsequent study, a number of snakes, representing eight genera and 11 species from 3 continents were found to be infected with *Cryptosporidium* isolates that were considered to fall into five separate groups (Upton *et al.*, 1989), possibly representing other species. So far, most isolates from snakes characterized by molecular analysis appear to be related to other gastric *Cryptosporidium* spp. *(C. muris, C andersoni* and *C galli*) found in mammals and birds (Morgan *et al.*, 1999). *C. serpentis* has also been isolated from savanna monitors (*Varanus exanthematicus*) and the isolates shown be genetically related to *C. serpentis* based on sequence analysis (Xiao *et al.*, 1999).

2.7.3.2Cryptosporidium saurophilum

C saurophilum was named following an extensive study of the faeces or intestinal contents from 220 wild and captive lizards of 67 species (Koudela and Modry, 1998). Six species of lizards in five genera were found to be shedding oocysts, and was designated the type host. The site of infection in Schneider's skink (*Eumeces schneideri*) was the intestine and cloaca. No pathological changes were found in the intestine and cloaca of adult lizards, but weight loss, abdominal swelling, and mortality occurred in some colonies of juvenile geckos *(Eublepharis macularius*) (Taylor *et al.,* 1999). Molecular characterizations support the existence of *C. saurophilum* and evidence suggests that *C. saurophilum* can also infect snakes (Xiao *et al.,* 2004).

2.7.3.3Cryptic species in reptiles

More *Cryptosporidium* species are likely to be present in reptiles, based on the study in snakes referred to earlier (Upton *et al.,* 1989). Some of the reported isolates may represent oocysts of *C. muris,* the *Cryptosporidium* mouse genotype, or other species from ingested and infected prey (pseudoparasites), and such findings are frequent in snakes showing no clinical signs of infection (Morgan, 1999a). Turtles and tortoises are also known to be infected with distinct gastric and intestinal forms of *Cryptosporidium* (Graczyk *et al.,* 1998), and geckoes are known to be infected with a distinct cloacal form (Upton and Barnard, 1987). Another new species, *C. varanii* was found in an Emerald monitor (*Varanus prasinus*) (Pavlasek *et al.,* 1995), but it is unclear at this stage whether this species is actually *C. saurophilum.*

2.8 Clinical Signs of Cryptosporidiosis

Cryptosporidium parvum is a cause of scour in young, unweaned farmed livestock including calves, lambs, goat kids and alpaca. Endogenous stages infect enterocytes of the

distal small intestine, caecum and colon. Villous atrophy, shortening of microvilli and sloughing of enterocytes are the major pathological changes associated with disease and affectedanimals usually recover within 2 weeks of showing signs of illness(Taylor, 1995).Scouring is most common in young animals but weaned and adult animals can also become infected. Signs can range from a mild to inapparent infection in older animals to severe scouring in young animals. Mortality is low unless occurring as a mixed infection with other enteric pathogens such as rotavirus. Older animals can remain infected and excrete oocysts that can be transmitted to other susceptible hosts. *Cryptosporidium parvum* infections of cattle can cause varying degrees of dehydration, dullness, anorexia, fever and loss of condition. Mortality may be high. Rarely do they cause the acute dehydration, collapse and high mortality seen with enterotoxigenic *Escherichia coli* or rotavirus, which can occur at a similar time. Oocysts can be detected in clinically normal and clinically ill hosts. Scouring calves and lambs can excrete between 106 and 108 oocysts per gramme of faeces. Infected adult cattle excrete far fewer oocysts, yet subclinical infections of adult cattle can generate similar numbers of oocysts over a 12-month period (Lindsay and Blagburn, 1991).

Cryptosporidium andersoni colonises the digestive glands of the abomasum of older calves and adult cattle. The microvilli of peptic glands are destroyed by endogenous stages, which may account for the elevatedconcentrations of plasma pepsinogen detected in infected hosts. Some infected animalsexhibit reduced weight gain compared with uninfected controls. Infected cattle do not develop diarrhoea, but can excrete oocysts for several months(Lindsay *et al*., 2000).

Cryptosporidium is a primary pathogen in chickens, turkeys and quails, causing respiratory and/or intestinal disease, leading to morbidity and mortality.

Twospeciesinfect chickens and turkeys (*C. baileyi* and *C. meleagridis*) (Morgan *et al.*, 2001), and a third, unnamed species infects quail (*Cryptosporidium* spp). *Cryptosporidium* spp. are common intestinal infections in broiler chicken in the USA and Japan.

Cryptosporidium baileyi Cryptosporidiosis is a disease of the epithelial lining of the bursa of Fabricius and cloaca of chicken, although the trachea and the conjunctiva are lesser sites of infection. *Cryptosporidium baileyi* intestinalCryptosporidiosis of chicken does not normally result in gross lesions or result in overt signs of disease. Villous atrophy, shortening of microvilli and enterocyte detachment are the major pathological changes associated with disease. *Cryptosporidium baileyi*respiratory Cryptosporidiosis of chicken can result in severe morbidity and, on occasion, mortality. Initially, severe disease isaccompanied by sneezing and coughing, followed by head extension to facilitate breathing. Epithelial cell deciliation and hyperplasia, mucosal thickening and discharge of mucocellular exudate into the airways are major pathological changes associated with disease in young broilers. Severe signs of respiratory disease can last up to 4 weeks post-infection. *Cryptosporidium baileyi* Cryptosporidiosis of turkeys is similar to that of chicken. Chicken isolates of *C. baileyi* cause infection in other birds (Current, 1997).

Cryptosporidium meleagridis Cryptosporidiosis is a disease of the ileum of turkey and other poults and humanbeings. *Cryptosporidium meleagridis* Cryptosporidiosis can cause severe diarrhoea in turkey poults. Villous atrophy, crypt hyperplasia and shortening of microvilli are major pathological changes associated with disease (Current, 1997). Transmission of a turkey isolate of *C. meleagridis* to chicken and domestic ducks has been reported.

Cryptosporidium galli Cryptosporidiosis is a disease of adult hens and some wild and exotic birds (Pavlasek, 2001). Unlike the life cycle stages of either *C. meleagridis* or *C. baileyi*, the life cycle stages of *C. galli* are limited to the epithelial cells of the proventriculus. Histology reveals *Cryptosporidium* parasites in the proventriculus (surface, ductal, and glandular epithelium) (Blagburn *et al.,* 1990). Clinical signs include puffed plumage with head held under the wing, responsiveness to external stimuli, and failure to thrive (Ryan *et al.,* 1999). Histopathology of haematoxylin and eosin stained sections from finches demonstrated necrosis and hyperplasia of proventricular glandular epithelial cells, and a mixed inflammatory cell infiltration into the lamina propria of the proventriculus associated with large numbers of *Cryptosporidium* oocysts attached to the surface of glandular epithelial cells (Ryan *et al.,* 1999). *Cryptosporidiumgalli* oocysts (8.0–8.5 × 6.2–6.4 μm) are larger than those of *C. baileyi*. *Chapter 2.9.4.* –Cryptosporidiosis1196 OIE *Terrestrial Manual* 2008 Respiratory and intestinalCryptosporidiosis have been reported in commercially grown quails caused by *Cryptosporidium* of an inadequately described species (*Cryptosporidium* spp).Whoseoocysts are smaller than those of *C. baileyi* and are not infectious to chicken or turkeys. Pathological changes are similar to those described for *C. baileyi* respiratory and intestinal Cryptosporidiosisof chicken (Current, 1997).

2.9 Clinical and Pathological features of Cryptosporidiosis

Cryptosporidiosis in birds manifests itself mainly in two clinical forms: respiratory and intestinal disease. Generally, only one form of the disease is present in an outbreak (Lindsay and Blagburn, 1990).

2.9.1 Respiratory Disease

Respiratory disease caused by *C. baileyi* has been reported in chickens, turkeys, ducks, pheasents, quails, pheasants, gulls and some pet bird species (Lindsay and Blagburn, 1990; Goodwin and Davis, 1993; Fayer *et al.*, 1997). Cryptosporidia can infect the nasal turbinates, nasopharynx, sinuses, larynx, trachea, lungs, air sacs, and conjunctiva in birds. Respiratory Cryptosporidiosis can be a major cause of morbidity and mortality in the poultry industry. The factors responsible for naturally occurring outbreaks of respiratory Cryptosporidiosis are unknown (Current, 1991). Studies indicate that *C. baileyi* plays an important role in the (Sr´eterand Varga,2000)pathogenesis of respiratory diseases of broilers and production losses can be unexpectedly high (Goodwin and Waltman,1996). There appear to be two different manifestations of respiratory disease: an upper respiratory involvement that included sinusitis ("swollen head"), and a lower respiratory involvement that included colonization of the trachea, bronchi, air sacs, and lungs. In the lower respiratory form of the disease, increased mortality, depression, lethargy, anorexia, unthriftiness, coughing, sneezing, gurgling, dyspnoae and conjunctivitis are the common clinical symptoms. Reported gross lesions include excessive mucoid exudate in the conjunctival sacs or nasal passage, sinuses and trachea, chemosis, hyperemia, swollen sinuses, mottled grey-red lungs, cloudy air sacs, bursal atrophy, mottled liver, swollen spleen, and emaciation. In addition to the presence ofCryptosporidialight microscopic lesions in sections of oculonasal and respiratory tract organs show increased surface mucus and cellular detritus, mucus gland distension or cystic hypertrophy/hyperplasia and necrosis, congestion, and purulent inflammation (Dhillon *et al.*, 1981; Goodwin and Waltman, 1996).

Several bacterial and viral pathogens were isolated from birds infected with *Cryptosporidium* spp. (Dhillon *et al.*, 1981; Tham *et al.*, 1982; Itakura *et al.*, 1984;

Tarwid *et al.,* 1985). In other cases, attempts to find organisms other than Cryptosporidia were not successful (Tham *et al.,* 1982; Whittington and Wilson, 1985; Current *et al.,* 1986). Experimentally induced respiratory infections in broiler chickens have been established the primary pathogenic potential of this parasite. *Cryptosporidium baileyi*, isolated from chickens and turkeys, caused severe respiratory clinical signs, gross and microscopic lesions (Blagburn*et al.,* 1987; Hoerr *et al.,* 1987; Lindsay *et al.,* 1987). Interaction of *C. baileyi* and otherrespiratory pathogens predisposes birds to secondary invasion by *Escherichia coli* because of disruption of the mucociliary elevator. Infectious bronchitis virus and *E. coli* also enhance the severity of respiratory Cryptosporidiosis in chickens (Blagburn *et al.,* 1991). Oral inoculation with the same isolates of *C. baileyi* failed to cause clinical signs, although organisms did colonize the nasopharynx, larynx, ileum, cloaca, and bursa of Fabricius (Blagburn *et al.,* 1987; Hoerr *et al.,* 1987; Lindsay *et al.,* 1987).

2.9.2 Gastrointestinal Disease

Cryptosporidium species can invade salivary and esophageal glands, proventriculus, small intestine, cecum, colon, cloaca, and bursa of Fabricius in birds. Clinical disease has been reported in turkeys, quails, pigeons, gulls, and pet birds (Lindsay and Blagburn, 1990; Fayer *et al.,* 1997). Lethargy, decreased bodyweight gain, lower pigmentation, and diarrhoea are the most common clinical signs. Grossly, intestinal distension due to mucoid intestinal contentsand gas can be seen in the small intestine. Microscopically lesions are as follows:detachment of enterocytes, villus atrophy in small intestine, crypt hypertrophy, microvillus atrophy, bursal epithelial cell hypertrophy and hyperplasia, purulent inflammation, lymphofollicular atrophy, and necrosis in the bursa of Fabricius (Randall, 1982; Tham *et al.,* 1982; Itakura *et al.,* 1984; Current *et al.,* 1986; Goodwin and Brown, 1988). In some cases, attempts to

isolate 268 infectious agents (Sr´eter andVarga,2000),other infectious agents were not made or were unsuccessful (Tham *et al.,* 1982; Hoerr *et al.,* 1986). In other cases, intestinal parasites (*Hexamita*, *Eimeria* and *Ascaridia* spp.) or viruses (reovirus, chicken anemia virus) were isolated from infected birds (Wages, 1987; Goodwin and Brown, 1988;Dobos-Kovács*et al.,* 1994). Oral inoculation of chickens or turkeys with *C. baileyi* oocysts does not generally produce overt disease or weight gain reduction. If present, lesions are generally confined to the bursa of Fabricius and cloaca (Current *et al.,* 1986; Lindsay *et al.,* 1987, 1988;Varga *et al.,* 1995). Concomitant infection with reovirus, infectious bursal disease virus and chicken anemia virus resulted in overt disease, a greater density of parasites and enhanced oocyst shedding (Guy *et al.,* 1988; Levy *et al.,* 1988; Hornok *et al.,* 1996). In contrast with *E. tenella*, F-2 and T-2 fusariotoxins had no effect on the course of *Cryptosporidium* infection in chickens (Varga and Ványi, 1992; Békési *et al.,* 1997). The histological lesions caused by*C. baileyi* indicate that the bursitis may suppress the development of humoral immunity, and the impairment of the bursa of Fabricius by Cryptosporidiosis may have an immunosuppressive effect on vaccination and render chickens vulnerable to other pathogens (Lindsay and Blagburn, 1990; Rhee *et al.,* 1997).

2.9.3OtherManifestations

Cryptosporidia have been seen in kidneys of chickens (Fayer *et al.,* 1997), a finch (Gardiner and Grimes, 1984), and a jungle fowl (Randall, 1986). Clinical signs of illness were not apparent before death. At necropsy, kidneys were enlarged and pale. Microscopically, ureteral branch and collecting duct epithelium were hyperplastic. Purulent nephritis was present only in the finch. Other infectious agents were not isolated from the genito-urinary tract. Heavy infection of the cloaca and bursal tissue

with an unclassified *Cryptosporidium* spp. can be responsible for prolapse of the phallus and cloaca in ostriches (Penrith *et al.,* 1994).

2.10 Clinical Diagnosis

The symptoms of Cryptosporidiosis are not pathognomonic, laboratory verification is required to confirm the diagnosis. This is usually done by the detection (presence or absence) of oocysts infaecal samples by a microscopic examination of smears stained with tinctorial stain (usually acid fast, such as modified Ziehl Neelsen stain), fluorescent stain (such as auramine O), or immunofluorescent stain (Jex *et al.,* 2008). Alternatively, oocyst antigen capture methods such as enzyme immune assays (EIAs) or immunochromatographic lateral flow (ICLF) assays may be used (Jex *et al.,* 2008), and positive reactions must be confirmed by using a suitable confirmatory test (Chalmers *et al.,* 2010).

The detection of *Cryptosporidium*DNAby PCR is also used in some diagnostic laboratories (Bouzid *et al.,*2008) usually as partof a multiplex gastrointestinal pathogen panel or parasite panel,for example, with *Giardia duodenalis, Entamoeba histolytica*, and*Dientamoeba fragilis* (Bruijnesteijn *et al.,* 2009). Commercial assays are becoming available, for which the target loci are not always revealed by the manufacturer, but for in-house *Cryptosporidium* detection assays, these include unspecified regions (Laxer *et al.,* 1991) and the small-subunit (SSU) rRNA gene (Amar *et al.,* 2007). The diagnostic sensitivities of routine tests have been compared, demonstrating the superior performance of immunofluorescence microscopy and PCR over comparable performances of EIAs and fluorescent stains, which in turn were better than acidfast staining and ICLF assays. The specificity of microscopy depends largely on the skill of the microscopist to differentiate oocysts from other bodies in smears stained with

nonspecific tinctorial and fluorescent reagents; immunofluorescent stains are more specific (Jex *et al.,* 2008). Test selection will also depend on additional multiple attributes and the resources available to meet them, including technical expertise, turnaround time, performance in batch mode, hands-on time, consumables cost per test or batch, and specialist equipment (Chalmers*et al*., 2011).

2.10.1 ConventionalStaining Methods

Both modified ZiehlNeelsen and Acid Fast are effective for detecting *Cryptosporidium* oocysts in faeces (Casemore, 1991; Smith, 2008). Modified Ziehl Neelsen stained slides should be screened under the ×40 objective lens and putative oocysts confirmed and measured under the ×100 objective lens (morphology and morphometry) using a bright-field microscope with a ×10 eyepiece. Acid Fast stained slides require to be read using an epifluorescence microscope equipped with a fluorescein isothiocyanate (FITC) filter set (excitation 490 nm; emission 510 nm). A UV filter set (excitation 355 nm, emission 450 nm) can assist in visualising Acid Faststained sporozoites. AF-stained slides can be screened under the ×20 objective lens and oocysts with typical morphology can be confirmed under the ×40 objective lens. The ×100 objective lens must be used for all morphometric (size) measurements. AF-stained oocysts visualised under either the FITC or UV filters can be measured by slowly increasing the voltage (light intensity) of the bright-field light source so that both fluorescent and bright-field images can be seen concurrently. The object can then be measured with the eye-piece graticule.

2.11 Treatment of Cryptosporidiosis

Treatment of *Cryptosporidium* in both humans and animals remains problematic. A wide variety of compounds have been tested, although few have been found to be effective, likewise attempts at developing vaccines have met mixed success (Jenkins

2004).However, some encouraging results following the use of supportive therapy remains the preferred option for the management of symptoms arising from the disease. An acknowledged report suggests that paromomycin is an antimicrobial drug that can alleviate Cryptosporidiosis symptoms with a reduction of oocysts excretion (Steiner *et al.*, 1997). Paromomycin is typically administered at a dose of 500 mg four times daily for 4-8 weeks, with a recommended maintenance dose of 500 mg twice daily following the initial treatment period (Hoepelman, 1996). However, clinical evaluations of these drugs have been disappointing, mainly because they failed to clear the parasite from patients with HIV/AIDS. To date, nitazoxanide, also known as Alinia, is an antiprotozoal drug believed to interfere with the pyruvate ferredoxin oxidoreductase-mediated electron transfer reaction that is essential for anaerobicenergy metabolism in the parasite (Herbert and Hoffman, 2002). Presently, nitazoxanide is the only drug approved by the use of USA Food and Drug Administration (FDA) for the treatment of diarrhoea caused by *Cryptosporidium* in children.

2.12 Prevention and Control of Cryptosporidiosis

Poultry farm litters should always be kept dry and special attention given to litter near water fonts or feeding troughs. Fonts that prevent water reaching the litter should always be used and should be placed on drip trays or over the droppings pit. Feeding and watering utensils should be of such a type and height that droppings cannot contaminate them. Batch rearing of birds, depopulation and adequate disinfection procedures should help limit levels of infection.

Good hygiene and management are important in preventing disease fromCryptosporidiosis. Feed and water containers should be high enough to prevent faecal contamination. Young animals should be given colostrum within the first 24 h of

birth and overstocking and overcrowding should be avoided. Dairy calves should be either isolated in individual pens or kept in similar age groups and cleaned out daily. On calf rearing farms with recurrent problems with *C. parvum*, the prophylactic use of halofuginone can be considered by treating for seven consecutive days commencing at 24-48 h after birth (AU-IBAR, 2013).

2.13 Cryptosporidiosis in Nigeria

Previous works have been carried out to determine the prevalence of *Cryptosporidium* species in faecal samples in cattle (Ayinmode and Fagbemi, 2010), ruminants (Pam *et al.*, 2013), calves (Faleke *et al.*, 2014), in pigs (Kwaga *et al.*, 1988;Ayeni *et al.*, 1998; Maikai *et al.*, 2008), calves (Kwaga *et al.*, 1988b), humans (Kwaga *et al.*, 1988; Wellington *et al.*, 2009), birds (Bamaiyi *et al.*, 2013),captive wild animals and birds ,(Ibrahim *et al.*, 2007) and water samples (Njoku *et al.*, 2005; Uneke and Uneke, 2008).

2.14 Live Bird Markets

Poultry is said to be the most numerous species of farm animals according to African Livestock Population Statistics (1995) and more than 80% of the Nigerian poultry are free roaming village poultry under extensive system, consisting of over 143 million birds (Adene and Oguntade, 2006; NBLS, 2007). Village chicken production is an extensive production system where birds survive by scavenging for food around their environment with little inputs from the owners who are mostly women, children and aged people (Abubakar *et al.*, 2007). They contribute substantially to annual egg and meat production and are mainly sold at live bird markets (Akinwumi*et al.*, 1979; Sonaiya, 1997; Pagani*et al.*, 2008).

The Nigerian, LBMs are of traditional structure in which poultry and poultry products are displayed in an open system for buyers and sellers (AICP, 2008). They are preferred

places for many people to purchase poultry for consumption throughout the world and are located within village markets or in major markets of towns and cities in Nigeria (Senne *et al.,* 2003; AICP, 2008). The birds are usually bought from multiple producers of free roaming chickens and backyard flocks around the neighbouring communities. Species such as pigeons, guinea fowls,ducks, chickens and a wide range of wild caught birds are usually mixed together at the markets (AICP, 2008; Pagani*et al.,* 2008). Village poultry may be transported to market in locally made cages, tied by legs, held in the hand, by foot or in a lorry (Pagani *et al.,* 2008).

There are two types of LBMs in Nigeria according to the AICP (2008), namely daily and weekly LBMs. Weekly markets are the LBMs that operate once or twice a week and are mostly located in rural or urban areas of local governments. They drain village poultry and serve as source of birds to daily market. Villagers and some final consumers also buy birds for their consumption, home rearing and/or for sacrifices (AICP, 2008; Pagani*et al.,* 2008). Daily LBMs are mostly located at the urban and cities of the country. Direct interaction between producers and final consumers is rare as the birds are moved to cities from distant and nearby villages. Processing of the poultry take place in most of the markets under poor hygienic conditions and they are serve as last destination (AICP, 2008). Some of the markets are colonized with lice and ticks, which are parasites of the birds and vectors of various diseases (Buriro, 1983; Bunza *et al.,* 2008).

The report of AICP (2008) suggested a biosecurity plan for LBMs in order to have a safer and better poultry business in Nigeria. They are as follows;

1. The LBMs must be isolated and confined within a controlled area; distant from human dwellings and farms.

2. There should be a mandatory routine disinfection and cleanup of the market every forth night.
3. The LBMs are to be sited in well-drained locations that are far away from wild birdsconcourse.
4. The LBM operators must avoid inter-market visitation and contact with dead birds. They should use mobile phones for contacts and business.
5. All visitors and customers must be restricted to the Reception/Bird view and car park areas only.
6. Trucks bringing in birds must be thoroughly disinfected after every delivery.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Kano Metropolis is located in the Central Western part of Kano State between latitude 11059”59.57 and 12002”570N of the equator and between longitudes 8033”19.69 and 8031” 59.690E. It lies in the NorthernCentral boundary of Nigeria and is located some 840 km away from the edge of the Sahara desert and 1,140 km from the Atlantic Ocean (Oseiki, 2009). Its population is the secondlargest in Nigeria after Lagos. Kano State has a mean altitude of about 472.5 m above sea level. The Kano urban area covers 137 sq. km and comprises Nine Local Government Areas (LGAs)-Municipal, Gwale, Dala, Tarauni, Nassarawa, Fagge, Ungogo and Kumbotso. Figure 3: Map of Kano Metropolis showing sampling sites (KSMI, 2005).

3.2 Sampling Units

Five live bird markets from Dala (Kurmi LBM), Kombotso (Sheka LBM),KMC (Sharada yan kaji LBM), Nasarawa (Yan kaba LBM) and Tarauni (Tarauni LBM), five slaughter slabs and some commercial farmswere sampled based on convenience and availability.

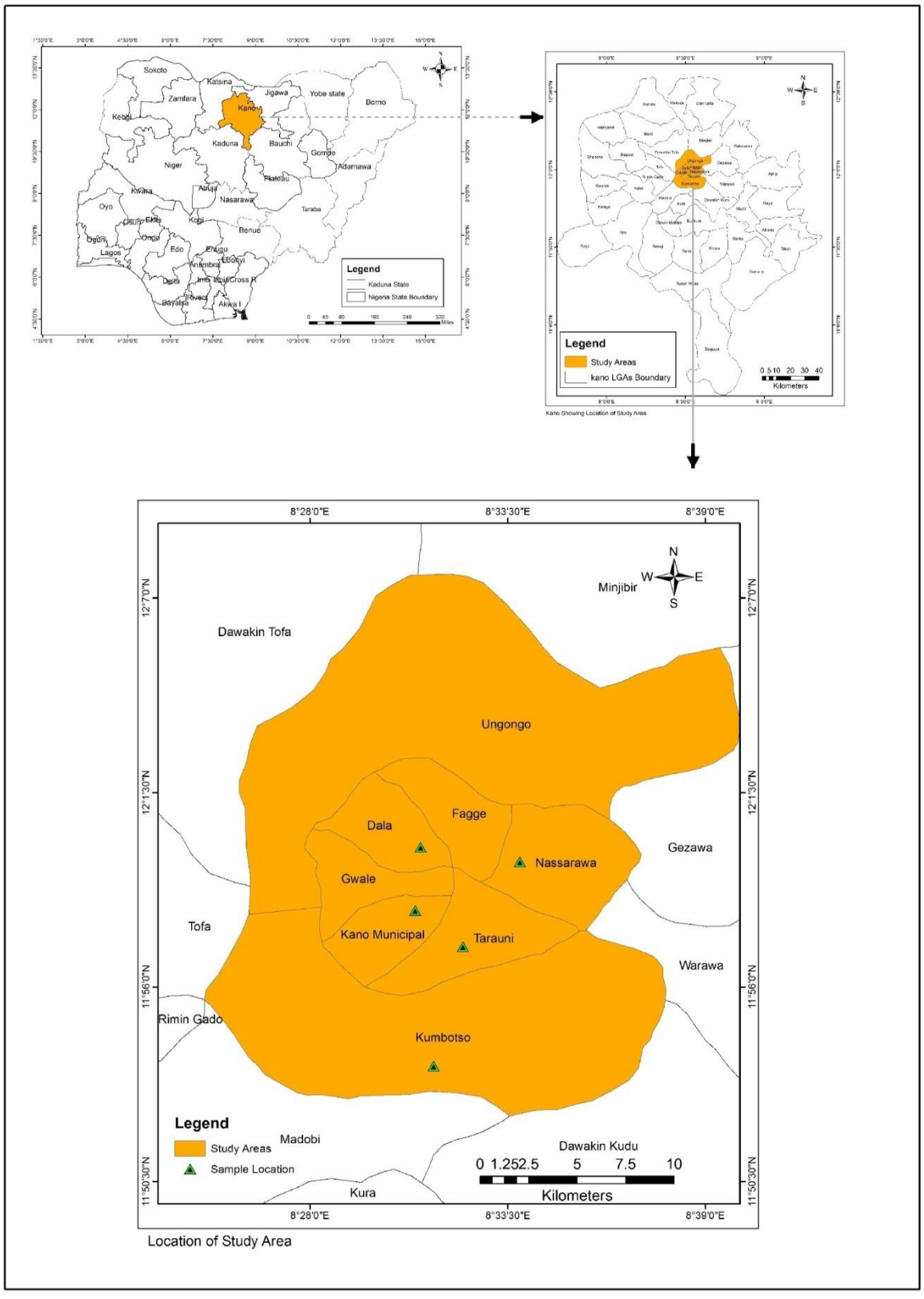


Figure 3.1:Map of Kano Metropolis and its eight Local Governments Areas of the Municipality.Source: Modified fromIsa and Yusuf, (2011).

3.3 Sample Size Determination

Sample size was determined with an established prevalence of 7.4% (Bamaiyi*et al.,* 2013) using the formula as described by Thrusfield,(1997).

$$N = \frac{Z^2pq}{d^2}$$

Where:

N= Sample Size

Z= Standard normal deviation of 95% confidence interval

P= Prevalence of 7.4% (Bamaiyi*et al.,* 2013)

q = 1-p

d = Level of Significance

Therefore, $N = \frac{Z^2pq}{d^2}$

$$N = \frac{1.96^2 \text{ X } 0.074 (1-0.074)}{0.05^2}$$

$$\frac{0.2843 \text{ X } 0.926}{0.0025}$$

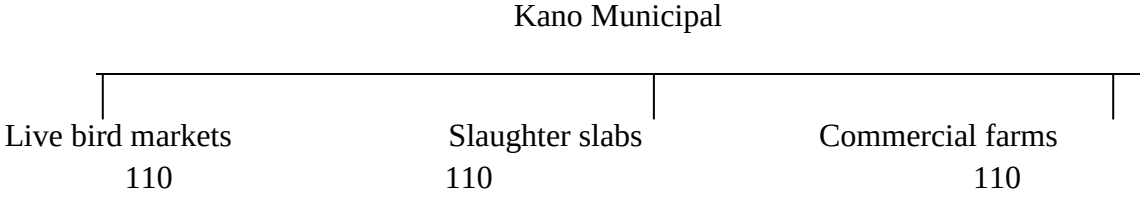
$$\frac{0.2632618}{0.0025}$$

$$= 105.305$$

One hundred and ten faecal samples were collectedfromeach of the three study locations in order to increase precision.

3.4Study Design

Below is a schematic diagram of where the samples were collected.



3.5Sample Collection and Transportation

Three hundred and thirty faecal samples were collected by means of convenience sampling technique. Freshly voided faecal samples were taken by use of disposable plastic bags. Faecal samples collected were labeled with location, species and then transported in a cold box to the Protozoology Laboratoryof the Department of Parasitology and Entomology, Ahmadu Bello University, Zaria for analysis.

3.6 Detection of *Cryptosporidium* oocysts

3.6.1Floatation Technique

Five millilitre of flotation medium (specific gravity 1.18-1.2) was added to 2 g of faeces in a centrifuge tube. The faeces were broken into the medium using a glass rod. The suspended faecal sample was sieved through a gauze into a test tube. More medium were added until a convex meniscus was formed. A coverslip was carefully placed on the tube containing the suspended faecal sample preparation and allowed to stand for about 3-5 minutes. The coverslip was then carefully removed and placed on a glass slide and viewed under the microscope at x10 and x40 magnifications. Oocysts seen were identified with the aid of an Atlas Encyclopedia of Parasitology and reference photographs (Jeffery and Leech, 1975; Bowman, 2009) and manual of parasitology

(Jeffery and Leech 1975; Cheesbrough, 1992). Positive oocysts were subjected to modified Ziehl Neelsen stain for confirmation of *Cryptosporidium* oocysts.

3.6.2 Modified Ziehl Neelsen Staining Technique

One to two drops of faecal specimens were smeared lightly on a glass slide and allowed to air-dry. The slide was placed on a heating block (70^{o}C) for 5 minutes before transferring onto a staining rack. It was then flooded with carbol fuchsin after which a Bunsen burner was used to steam the slide gently. Once the stain begins to steam, heating was discontinued. The staining was allowed for 5 minutes, more stain was added without additional heating, it was then rinsed thoroughly with water, drained and decolorized with 5% sulfuric acid for 30 seconds. After which it was rinsed again and drained. The slide was then flooded with methylene blue for 1 minute, before being rinsed with water, drained and air dried. This was examined with low or high dry objective to observe for internal morphology of the *Cryptosporidium* oocysts using x100 oil objectivelens (Lynne and Garcia, 1999). The Cryptosporidial oocysts appeared as pink to red to deep purple on a bluebackground.

3.7 Questionnaire Design and Administration

A structured questionnaire (Appendix 1) was designed for this study. Thequestionnaire contain some variables covering information about live bird markets location, feed and water sources, source of birds, frequency of cleaning of cages, presence of trees within the market and type of cages. The questionnaire was administered to poultry sellers through interviews during on-site visits (Appendix III). Information obtained from the respondents was recorded. Personal observations were used to validate the responses of the respondents during the questionnaire administration.

3.8 Data Analyses

Results were presented in form of tables.Statistical Package for Social Science (SPSS version 20.0) was used to analyze the data obtained. Chi-square tests, Odds ratio and 95% confidence interval were used to determine the association between *Cryptosporidium* oocystsand risk factors such as sampling sites, source of feed, source of drinking water, type of cages, method of cleaning, P value < 0.05 was considered statistically significant.

$$\text{Prevalence} = \frac{\text{Number of positive samples}}{\text{Total number of samples}} \times 100$$

CHAPTER FOUR

RESULTS

4.1 Occurrence of *Cryptosporidium* Oocysts in Live Bird Markets, Slaughter Slabs and Commercial Poultry Farms in Kano Metropolis, Nigeria

Out of 330 faecal samples collected from live bird markets (LBMs), slaughter slabs and commercial farms in Kano Metropolis,(10%) were positive for the *Cryptosporidium* oocysts in LBMs (Plate I), (4.55%) each from specimens collected in slaughter slabs (Plate II) and commercial farms (Plate III).The overall prevalence of *Cryptosporidium* oocysts was 6.4%. The difference in prevalence of *Cryptosporidium* oocysts according to units was found not to be statistically significant (df= 2; P=0.210) (Table 4.1).

4.2 Occurrence of *Cryptosporidium* Oocysts in Avian Species, in Kano Metropolis, Nigeria

Occurrence of *Cryptosporidium* oocysts in avian species was observed. Guinea fowls and pigeons were found not to harbour any *Cryptosporidium* oocysts, while turkeys had the highest prevalence rate (18.75%) of *Cryptosporidium* oocysts, followed by ducks (9.09%), with chickens being the least (6.61%). There was no significance difference in the prevalence of *Cryptosporidium* oocysts between different species of birds sampled (df=4; P=0.094) (Table 4.2).

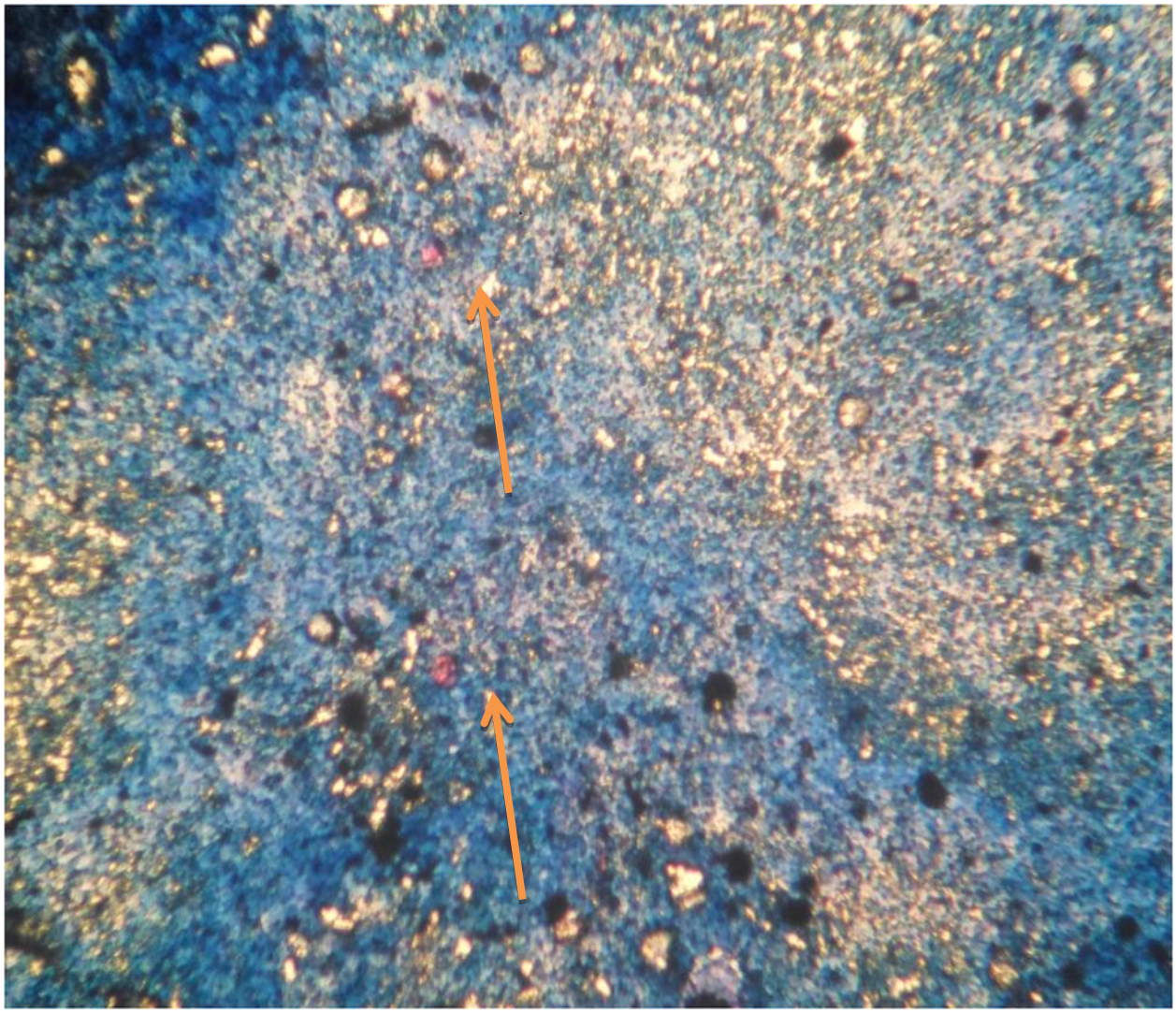


Plate I: Photomicrograph of *Cryptosporidium* oocysts (arrow) recovered from faecal sample of turkey at Sharada Live Bird Market in Kano Metropolis (Light microscope X40 magnification).

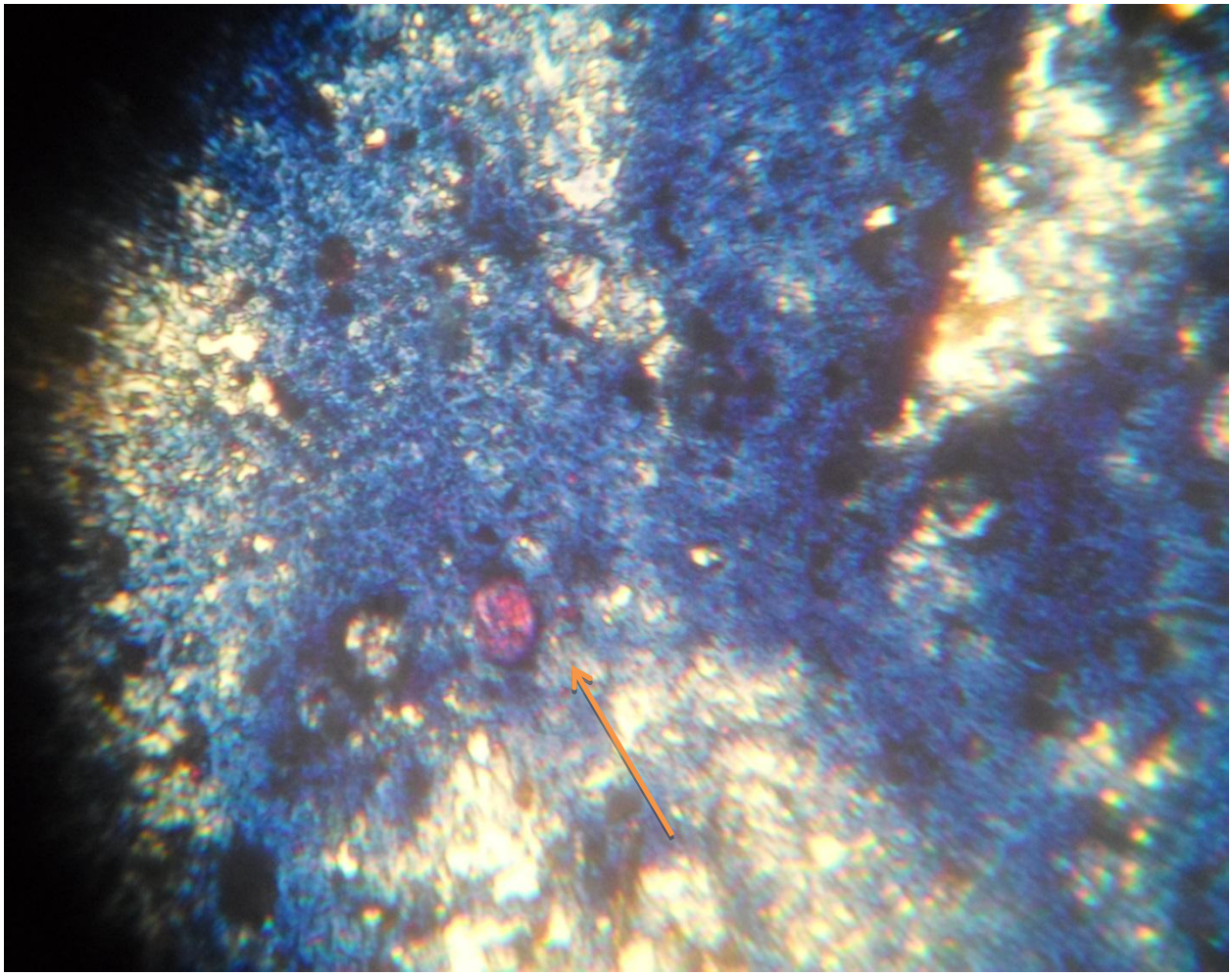


Plate II: Photomicrograph of *Cryptosporidium* oocyst (arrow) recovered fromfaecal sample of chicken at Kurmi Live Bird Market in Kano Metropolis (Light microscope X100 magnification).

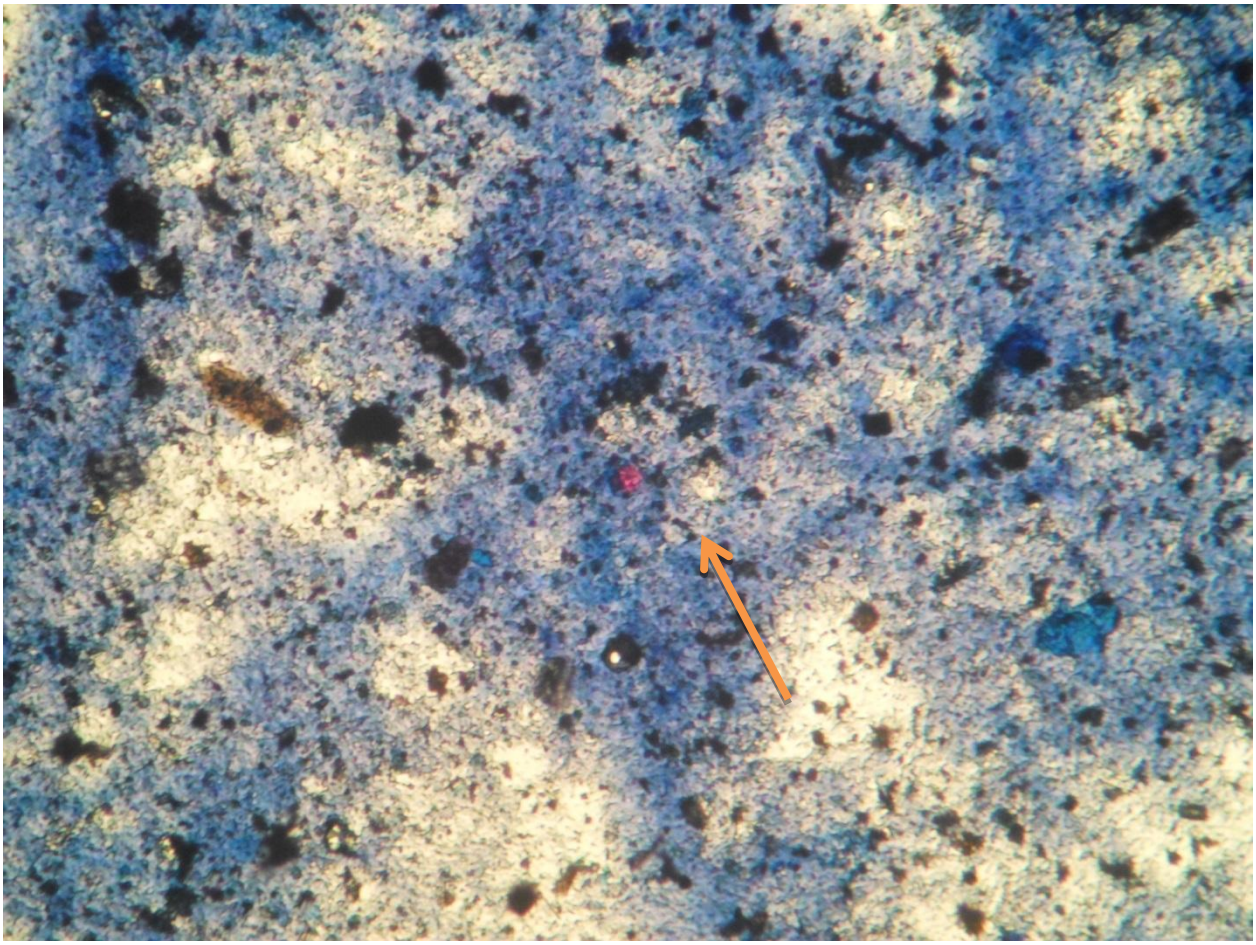


Plate III: Photomicrograph of *Cryptosporidium* oocyst (arrow) recovered from feacal sample of duck at Tarauni Live Bird Market in Kano Metropolis (MagnificationX40).

4.3 Occurrence of *Cryptosporidium* Oocysts Based on Local Government Areas (LGAs)

Occurrence of*Cryptosporidium* oocysts based on LGAs in Kano, revealed that Dala had 8 (12.12%) positive samples, followed by Tarauni 6 (9.09%), Nasarawa 4 (6.06%), Kano Municipal 2 (3.03%) and Kumbotso 1 (1.52%). There was no significant difference in the prevalence of *Cryptosporidium* oocysts between the LGAs studied (df=4; p=0.084) (Table 4.3).

4.4 Risk Factors Obtained from Questionnaire Survey of *Cryptosporidium* Oocysts

Table 4.4 shows the relationship between the risk estimate of *Cryptosporidium* oocysts and various variables used(market location within conventional market, source of birds, frequency of cleaning, source of feeds, source of water, presence of trees and types of cage) in different Local Government Areas of Kano State. The risk estimate was high among those that sourced their birds outside the state, (OR= 1.314; 95% CI= 0.358-4.828; p=0.735) than those that sourced within the state. Daily cleaning had low risk estimate than those that do not clean their cages daily (OR=1.859, 95% CI=0.512-6.756; p=0.263). In relation to source of feeds, the risk estimate was higher in other sources than commercially sourced feeds (OR=2.604; 95% CI=0.729-9.298; p=0.126). With respect to source of water, the risk was higher in those that drank well water than borehole (well OR=1.770; 95% CI=0.0506-6.196; p=0.521). However, there is no risk estimate associated with presence of trees within market (OR=1.000; 95% CI=0.247-4.050; p=0.653). There is high-risk estimate associated with metallic cages than wooden cages (metallic OR=1.186; 95% CI=0.326-4.320;p=0.533). However, these associations were not statistically significant.

Table 4.1: Occurrence of *Cryptosporidium* oocysts in live bird markets, slaughter slabs and commercial farms in Kano Metropolis, Nigeria

Serial number	Units	Total number of faecal samples examined	Number of positive samples	% positive
1	Live bird markets	110	11	10.00
2	Slaughter slabs	110	5	4.55
3	Commercial farms	110	5	4.55
Total		330	21	6.4

X^2 =3.662,df= 2, p=0.210

Table: 4.2: Occurrence of *Cryptosporidium* oocysts in avian species in Kano Metropolis, Nigeria

Serial number	Specie of bird	Total number of faecal samples examined	Number positive	% positive
1	Chicken	257	17	6.61
2	Turkey	16	3	18.75
3	Duck	11	1	9.09
4	Guinea fowl	38	0	0.00
5	Pigeon	8	0	0.00
Total		330	21	6.4

Fisher‘s exact =6.650,(df=4; p=0.094)

Table: 4.3: Occurrence of *Cryptosporidium* oocysts in avian species based on Local Government Areas

Serial Number	Local Government Areas	Number of samples examined	Number of Positive	% positive
1	Kumbotso	66	1	1.52
2	Kano Municipal	66	2	3.03
3	Nasarawa	66	4	6.06
4	Dala	66	8	12.12
5	Tarauni	66	6	9.09
Total		330	21	6.4

X^2=8.340, df=4, p=0.084

Table:4.4 Risk factors associated with faecal shedding of *Cryptosporidium*oocysts in Kano Metropolis, Nigeria

Variables	No. of samples	Positive (%)	P-value	OR	95% C.I
Local Government				1 ref	
Kano Municipal	22	1 (4.5)	0.618		
Kumbotso	22	1 (4.5)		1.000	0.059-17.065
Nasarawa	22	2 (9.1)		0.476	0.040-5.671
Tarauni	22	3 (13.6)		0.302	0.029-3.152
Dala	22	4 (18.8)		0.214	0.022-2.095
Live bird market within theconventional market?					
Yes	100	11 (11.0)	0.594	1 (ref)	
No	10	0 (0.0)		0.890	0.831-0.953
Source of birds					
Intrastate	34	4 (11.8)	0.735	1 (ref)	
Others	76	7 (9.2)		1.314	0.358-4.828
Cleaning frequency					
Daily	55	7 (12.7)	0.263	1 (ref)	
Others	55	4 (7.3)		1.859	0.512-6.756
Source of feed					
Commercial	29	5 (17.2)	0.126	1 (ref)	
Others	81	6 (7.4)		2.604	0.729-9.298
Source of water					
Borehole	46	6 (13.0)	0.521	1 (ref)	
Well	74	5 (7.8)		1.770	0.0506-6.196
Trees located within the LBM					
Yes	30	3 (10.0)	0.653	1 (ref)	
No	80	8 (10.0)		1.000	0.247-4.050
Type of cage					
Metallic	66	7 (10.6)	0.533	1 (ref)	
Wooden	44	4 (9.1)		1.186	0.326-4.320

CHAPTER FIVE

DISCUSSION

The prevalence of *Cryptosporidium* species recorded in this study was low(6.4%), though important,as it could pose a risk for the spread of infection in the area through contamination of feed, water, e.t.c. This finding is similar to a previous study conducted byBamaiyi*et al.*(2013) who reported a prevalence of 7.4% in birdsand further highlighted possible association between presence of *Cryptosporidium* oocysts and water contamination on the one hand and outbreak on the other. However, the slight differences in the prevalence rates may be due to differences in staining techniques employed. Mackenze*et al.*(1994) also reported that cryptosporidiosishas been associated with drinking water and this may lead to outbreak of the disease. Results from this study did not concur with the findings of Ibrahim*et al.* (2007), who reported a higher prevalence of 22.7% in wild captive animals and birds using modified Ziehl Neelsen staining technique. Dissimilarities in the prevalence recorded might also be due to concurrent mixed inter-species co-habiting same environment, althoughJohn*et al.* (1982) and Brent *et al.*(1982) argued that stress such as those obtained in animals in captivity compromised their reservoir status.

Even though the species of *Cryptosporidium*isolated from birds of different species were not identified in this study, it is important to note that most cases diagnosed in chickens were *C. baileyi*which can slow down growth rate in broilers and cause decrease in egg production in layers (Wang *et al.,* 2014). However, *C. meleagridis* may cause deadly disease in immunocompromised humans (Ryan, 2010).

The prevalence recorded was higher in live bird markets (10%), than slaughter slabs and commercial farms (4.55%). The higher prevalence rate recorded in LBMs may be

due to multiple species sampled,unlike in slaughter slabs and commercial farms, where only chickens were sampled. The finding that live bird markets had the highest prevalence of *Cryptosporidium*oocysts implies that LBMs might be potential reservoirs for the spread of *Cryptosporidium* oocysts to different places. It is also important to note that LBM is a place where people gather and are in close or direct contact with the *Cryptosporidium* oocysts, and this has highlighted the risk of exposure to both bird owners and the buyers (Ryan, 2010).

The prevalence of 4.55% recorded from commercial farms in this study is not in agreement with the findings of Baroudi*et al.* (2013) who obtained an overall prevalence of 38% in commercial farms in Algeria. However, the variation may be associated with the highly sensitive nature of molecular detection technique (Polymerase Chain Reaction) used in that study and possibly geographical differences.

The highest prevalence rate was reported among turkeys than the other species.The higher prevalence of *Cryptosporidium* oocysts observed in turkeys is also a matter of concern. Although, the oocysts recovered from the turkeys were not speciated, the possibility of these oocysts being *C. meleagridis* should not be overlooked. This is in view of the fact that *C. meleagridis*has zoonotic potential (Ryan, 2010). This finding also complements the report that cryptosporidiosis may be more severe in turkeys than other species (Wang *et al.*,2014). However, in comparison with the findings of Gharazoulou*et al.*(2006), who reported prevalence rate of 35% in Iran, the prevalence in this study is therefore low.In addition, Woodmansee *et al.*(1988) and Pavlasek,(1994) reported prevalence rates of 34% and 43% in turkeys in United State and Czech Republic, respectively.Bomfim *et al.*(2013) reported a prevalence of 76.6% in ducks, a higher percentage when compared with 9.09% in this study. However, the difference

might be attributed to the higher sensitivity of the molecular diagnostic techniqueused in that study which is more sensitive and specific than conventional microscopy. As in turkeys, early age affects the maternal immune defense mechanism in chickens, so their rapid growth mechanism dilute the maternal antibodies, but with relatively high degree of severity than in turkeys as reported by Baroudi *et al.* (2013). Yet, chickens in this study showed a lower prevalence of 6.61% than turkeys. Bamaiyi *et al.*(2013) reported a prevalence rate of 7.8% in chickens, which is higher than the prevalence rate recorded in this study, which might be due to different staining techniques employed and possibly the geographical locations.

Dala Local Government had the highest prevalence rate (12.12%) among the LGAs studied. This may be due to high stocking density of different avian species in the market within the Local Government. Also the finding that Dala LGA (when compared with other LGAs where commercial poultry predominate) appeared to have the highest prevalence of *Cryptosporidium* oocysts may not be unconnected with the fact that the poultry in this area is predominantly the local breeds that are usually maintained on free range or semi-intensive system of management. This finding is in agreement with the findings of Wang*et al*. (2014) in China who reported inter-regional variations in infection rates and attributed this variation, among other reasons to density of animals.

However, Kumbotso recorded the lowest prevalence rate (1.52%). This is not surprising because of the relative biosecurity measures in the market because of combined factors of trained personnel operating the poultry business and moderate stocking density compared to Dala. This finding agreed with the finding of Jellison *et al*. (2004)who recorded low infection rate and attributed this to efficient management in the aviaries.

Bamaiyi *et al*. (2013) also discovered variations in prevalence rates among different locations in Zaria, Nigeria.

Association between infection rates of *Cryptosporidium* and many variables was also analyzed. Variables such as source of birds, cleaning frequency, source of feeds, source of water, presence of trees within the live bird market and types of cages did not show any significant association with infection rate. However, there was variation in the odds ratio among the variables studied. For instance, feed sourced from other sources (toll mill, self-milling etc.) other than commercial source had higher odds ratio, which implies higher risk estimate. This is followed by high risk estimate from periodic cleaning frequency than daily cleaning, well as a source of water, birds from other sources than intrastate and metallic types of cage. The higher risk estimated associated with cleaning frequency could be due to lack of periodical cleaning of cages. This is in agreement with the findings of Bomfim *et al*. (2013), who recorded a higher infection rates and associated this findings with lack of daily cleaning of cages.

High risk estimate was also associated with well water. The finding that *Cryptosporidium* is more likely to be spread to poultry through well water is a matter of concern, given that well water is the most common source of drinking water for most poultry in the area. The implication is that, the contamination of well water by *Cryptosporidium* might result in major water-borne outbreaks of cryptosporidiosis not only in poultry but even in humans (Putignani and Manichella, 2010). It is also common for people to defaecate in the bush and with rain water flooding around it could easily run into such wells leading to contamination with *Cryptosporidium* oocysts. The fact that Dai and Boll. (2003) reported that these parasites are small and they could find their way into tap and well water and therefore could serve as a source

of contamination. Although not specifically reported based on source of feed, yet the finding of Behzadi *et al*. (2009) who associated feed with high risk estimate and a major predisposing factor to *Cryptosporidium* as was found in this study. The variation for high risk estimate associated with source of birds might be due to the fact that the birds were sourced from contaminated areas or where the disease is more prevalent. The high risk estimate associated with wooden cage may also be attributed to the nature of the wood which can provide more conducive environment for*Cryptosporidium*oocysts survival in that, the wood will be difficult to wash and clean than metallic cage. In addition wooden cage may retain high level of moisture that is known to be a predisposing factor for infection.

In view of the fact that *Cryptosporidium* is one of the causes of diarrhoeal illnesses in man and animals worldwide, close monitoring of the infection in farm animals and live bird markets is required since there can be zoonotic transmission of the infection which is not only of public health significance but also of an economic importance.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the findings of this study, the following conclusions were arrived at:

1. The study established an overall prevalence of 6.4% with 10%, 4.55% and 4.55% prevalences in live bird markets, slaughter slabs and commercial farms, respectivelyin Kano Metropolis.
2. Occurrences of *Cryptosporidium* oocysts in avian species were also established, in which turkeys, ducks and chickens had prevalence rates of18.75%, 9.09% and 6.61%,respectively.
3. Risk factors associated with the occurrence of *Cryptosporidium* oocyst were frequency of cleaning, source of birds, source of feeds, source of water and type of cages.

6.2 Recommendations

1. Furtherstudies should be carried outtodetermine the prevalence of avian *Cryptosporidium* oocysts and the species prevalent in birds in other parts of the State.
2. Poultry sellers and farmers should be encouraged to adhere to strict biosecurity practices in live bird markets, slaughter slabs and commercial farms as stipulated by Kano Government.
3. Poultry sellers and farmers should be enlightened on the importance of cryptosporidiosis and other poultry diseases by Veterinarians in the Kano State.

REFERENCES

Abe, N. and Makino, I. (2010). Multilocus genotypic analysis of *Cryptosporidium* isolates from cockatiels. *Japan Parasitology Research*, 106:1491–1497.

Abubakar, M.D.,Ambali, A.G. and Tamjdo, T. (2007). Rural chicken production: effects on gender on ownership and management responsibilities in some parts of Nigeria and Cameroon. *International Journal of Poultry Science*, 6:413-416.

Adene, D.F. and Oguntade, A.E. (2006). The importance and structure of commercial and rural based poultry industry in Nigeria. *Nigeria Poultry Sector Report*. Food and Agriculture Organization (Rome) study. Http:/www.fao.org.docs/

Adriana, T., Viorica, M., Achelaritei, D. and Cozma, D. (2010). Prevalence of *Cryptosporidium* species in asymptomatic dogs by ELISA and risk factors associated with infection. *Lucrari stiinlifice Medicina Veterinara*, 43: 12-16.

African Union-Interafrican Bureau for Animal Resources. Animal Health and Production Compendium(© CAB International 2013).

Avian Influenza Control Project (AICP) (2008). *The Development of Live Bird Markets in Nigeria*, pp. 1-77.

Amar, C.F.L., East, C.L., Gray, J., Iturriza-Gomara, M., Maclure, E.A. and McLauchlin, J. (2007). Detection by PCR of eight groups of enteric pathogens.*European Journal of Clinical MicrobiologyInfectious Disease*, 26:311–323.

Anderson, B.C. (1991). Prevalence of *Cryptosporidiummuris* like oocyts among cattle populations of the United States preliminary report. *Journal of Protozoology*, 38(6): S14-S15.

Anonymous (1982) Human Cryptosporidiosis: Alabama, Morbid. Mortal. *Weekly Representative*, 31: 252–254.

Arora, D.R. and Arora, B. (2005), *Medical Parasitology*: 2nd edition. CBS Publishers and Distributors. New Delhi, Bangalore, India, pp. 213-216.

Ayeni, A.O., Olubunmi, P.A. and Ade, J.O. (1998). The occurrence of *Cryptosporidium* in faeces in livestock in Ile – Ife, Nigeria. *Tropical Veterinarian*, 3: 96-100.

Ayinmode, A.B. and Fagbemi, B.O. (2010). Prevalence of *Cryptosporidium* infection in cattle from southern Nigeria. *Veterinarski Archiv*e, 80(6): 723-731.

Bamaiyi, H.P., Umoh, J. U., Abdu, P.A. and Idris, A. L. (2013). Prevalence of *Cryptosporidium* oocysts in birds in Zaria, Nigeria. *Borneo Journal of Resource Science Technology*, 2(2): 52-59.

Barta, J. R. and Thompson, R.C. (2006). What is *Cryptosporidium*? Reappraising its biology and phylogenetic affinities. *Trends in Parasitology*,22:463– 468.

Behzadi, M. A., Razavi, H. Yazdanpoor, A. Mirzaei, A. Tamadon, M.and Javdani, G. (2009). Epidemiology of *Cryptosporidium* infection in ostriches (*Struthiocamelus*) in Iran. *Bulgaria Journal of Veterinary Medicine*, 12: 55−61.

Blagburn, B. L., Lindsay, D. S., Hoerr, F. J., Atlas, A. L. and Toivio-Kinnucan, M. (1990). *Cryptosporidium* sp. infection in the proventriculus of an Australian diamond firetail finch (*Staganoplura bella*: Passeriformes, Estrildidae). *Avian Dis*eases, 34:1027–1030.

Blagburn, B. L., Lindsay, D. S., Hoerr, F. J., Davis, J. F. and Giambrone, J. J. (1991). Pathobiology of Cryptosporidiosis, (*C.* baileyi) in broiler chickens. *Journal of Protozoology*, 38: 25–28.

Blagburn, B., Lindsay, D.S., Giambrone, J. J., Sundermann, C.A. and Hoerr, F.J. (1987). Experimental Cryptosporidiosis in broiler chickens. *Poultry Sciences*,66:442–449.

Bomfim, T.C.B., Gomes, R.S., Huber, F. and Couto, M.C.M. (2013). The Importance of Poultry in Environmental Dissemination of *Cryptosporidium* spp. *The Open Veterinary Science Journal*, 7: 12-17.

Bornay-Llinares, F.J., do Silva, A.J., Moura, I.N.S., Myjak, P.,Pietkiewicks, H., Kruminis-Lozowska, W.and Pieniazek, N.j. (1999). Identification of *Cryptosporidium felis* in cow by Morphologic and Molecular Methods. *Applied and Environmental Microbiology*, 65: 1455-1458.

Baroudi, D., Khelef, D., Goucem, R., Adjou, K.T., Adamu H., Zhang, H. and Xiao, L. (2013). Common occurrence of zoonotic pathogen *Cryptosporidium meleagridis* in broiler chickens and turkeys in Algeria. *Veterinary Parasitology*, pp. 5

Bouzid, M., Steverding, D. and Tyler, K.M. (2008). Detection and surveillance of waterborne protozoan parasites. *Current Opinion Biotechnology*, 19:302–306.

Boulter-Bitzer, J., Lee, H. and Trevors, J.T. (2007). Molecular targets for detection and immunotherapy in *Cryptosporidium parvum*. *Biotechnology Advance*, 25:13– 44.

Brent, L., Carlson, B. S. and Svend, W. N. (1982). Cryptosporidiosis in a raccoon.*Journal of American Veterinary Medical Association*, 181: 1405-1406.

Brownstein, D.G., Strandberg, J.D., Montali, R.J., Bush, M. and Fortner, J.(1977). *Cryptosporidium* in snakes with hypertrophic gastritis. *Veterinary Pathology*, 14:606-617.

Bruijnesteijn, van Coppenraet, L.E.S., Wallinga, J.A., Ruijs, G.J.H.M., Bruins, M.J. and Verweij, J.J. (2009). Parasitological diagnosis combining an internally controlled real-time PCR assay for the detection of four protozoa in stool samples with a testing algorithm for microscopy. *Clinical Microbiology Infection*, 15:869–874.

Bukhari, Z. and Smith, H.V. (1996). Detection of *Cryptosporidium muris* oocysts in the faeces of adult dairy cattle in Scotland. *Veterinary Record*, 138: 207-208.

Bunza, M.D.A., Yahaya, M.M., Muhammad, A.S. and Saidu, A.R. (2008). A survey on tick species infecting domestic birds sold at Sokoto central market.*Sokoto Journal of Veterinary Science*, 7:52-54.

Buriro, S.N. (1983). Relative abundance of different species of bacteria isolated from *Argas persicus*.*Pakistan Veterinary Journal*, 3:126-128.

Casemore, D.P. (1991). Laboratory methods for diagnosingBroadsheet 128. *Journal of Clinical Pathology*, 44: 445–451.

Cevallos, A.M., Bhat, N., Verdon, R., Hamer, D.H., Stein, B., Tzipori, S. and Ward, H.D. (2000). Mediation of *Cryptosporidiumparvum* infection in vitro by mucin-like glycoproteins defined bya neutralizing monoclonal antibody. *Infectection and Immunology*,68:5167–5175.

Chalmers, R.M., Sturdee, A.P. and Timpson, P. (1997). *Cryptosporidium* parvum in environmental samples in the Sligo area, Republic of Ireland: a preliminary report. *Letters inApplied Microbiology*, 25:380-384.

Chalmers, R.M., Campbell, B., Crouch, N. and Davies, A.P. (2010). Clinical laboratory practices for detection and reporting of *Cryptosporidium* in community cases of diarrhoea in the United Kingdom, 2008. *European Surveillance*, 15(48):19731. Retrieved from http://www.eurosurveillance.org /ViewArticle.aspx?

Chalmers, R.M., Campbell, B.M., Crouch, N., Charlett, A. and Davies, A.P. (2011). Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the United Kingdom. J*ournal of Medical Microbiology*,60:1598 – 1604.

Chen, X.M., O'Hara, S.P., Huang, B.Q., Nelson, J.B., Lin, J.J., Zhu, G. and Larusso, N.F. (2002). Apical organelle discharge by *Cryptosporidiumparvum* is temperature, cytoskeleton, and intracellular calcium dependentand required for host cell invasion. *Infection andImmunity*, 72:6806–6816.

Chrisp, C.E. andLeGendre, M.(1994). Similarities and differences between DNA of *Cryptosporidium parvum* and *Cryptosporidium wrairi* detected by the polymerase chain reaction. Folia *Parasitologica*, 41(2):97-100.

Current, W. and Reese, N.C. (1986). A comparison of endogenous development of three isolates of *Cryptosporidium* in suckling mice. *Journal of Protozoology*, 33: 98–108.

Current, W.L., Upton, S.J. and Haynes, T.B. (1986). The life cycle of *Cryptosporidiumbaileyi* n. sp. (Apicomplexa, *Cryptosporidiidae*) infecting chickens. *Journal of Protozoology*, 33: 289–296.

Current, W. and Garcia, L.S. (1991). Cryptosporidiosis. *Clinical Microbiology Reviewed*, 4:325–358.

Current, W.L., Calnek, B.W., Barnes, H.J., Beard, C.W., Reid, W.M. and Yoder, H.W. (1991) Cryptosporidiosis. In: (Eds.), *Diseases of Poultry*. Iowa State Press, Ames, IA, pp. 797–804.

Current, W.L., Calnek, B.W., Barnes, H. J., Beard, C.W., McDougald, L.R. and Saif, Y.M. (1997). Mosby-Woolfe, Mosby International, Lynton House, 7– 12 Tavistock Square, London WC1H 9LB, UK, Cryptosporidiosis. *In*: *Diseases of Poultry*, Tenth Edition, 883–890.

Dai, X. and Boll, J. (2003). Evaluation of attachment of *Cryptosporidium parvum* and *Giardia lamblia* to soil particles. *Journal of Environmental Quality*, 32: 296-304.

Das, G., Changkija, B., Sarkar, S. and Das, P. (2011). Genotyping of *Cryptosporidiumparvum* isolates in bovine population in Kolkata and characterization of new bovine genotypes. *Research in Veterinary Science*, 91:246–250.

Dhillon, A.S., Thacker, H.L., Dietzel, A.V. and Winterfield, R.W. (1981). Respiratory Cryptosporidiosis in broiler chickens. *Avian Diseases*, 25:747–751.

Dobos-Kovács, M., Varga, I., Békési, L., Drén, Cs.N., Németh, I. and Farkas, T.(1994). Concurrent Cryptosporidiosisand chicken anaemia virus infection. *Avian Pathology*, 23: 365–368.

Dubey, J.P., Markovits, J.E. and Killary, K.A. (2002). *Cryptosporidiummuris*-like infection in stomach of cynomolgus monkeys (*Macaca fascicularis*). *Veterinary Pathology*, 39:363-371.

Dupont, H., Chappell, C.L., Sterling, C.R., Okhuysen, P.C., Rose, J.B. and Jakubowski, W. (1995). The infectivity of*Cryptosporidium* parvum inhealthy volunteers. *North England Journal of Medicine*,332: 855– 859.

Ekeren, N., Mass, A., Saatkanpp, H. and Verschuur, M. (1995). Small scale poultry production in the tropics. *Wageninger Agromisa Agrodok*, No4.

Enemark, H.L., Ahrens, P. Juel, C.D., Petersen, E. Petersen, R.F., Andersen, J.S., Lind, P. and Thamsborg, S.M.(2002). Molecular characterization of Danish *Cryptosporidium parvum* isolates. *Parasitology*, 125:331-341.

Egger, M., Mausezahl, D., Odermatt, P., Marti, H.P. and Tanner, M. (1990) Symptoms and transmission of intestinal Cryptosporidiosis. *Archive Child*, 65: 445–447.

Faleke, O.O., Yabo, Y.A., Olaleye, A.O., Dabai, Y.U. and Ibitoye, E.B. (2014). Point prevalence of *Cryptosporidium* oocysts in calves grazing along river Rima bank in Sokoto, Nigeria, *Pakistan Journal of Biological Sciences,* 17: 443-446.

Fall, R.C.A., Thompson, R.P., Hobbs, and Morgan-Ryan, U.M. (2003). Morphology is not a reliable tool for delineating species within *Cryptosporidium*. *Journal of Parasitology Today*, 89: 399-402.

Fayer, R., Speer, C.A. and Dubey, J.P. (1997) (Eds.), *Cryptosporidium* andCryptosporidiosis, *Chemical Rubber Company Press*, Boca Raton, Florida, USA.

Fayer, R., Speer, C.A. and Dubey, J.P. (1997). General biology of *Cryptosporidium*. *Cryptosporidium* andCryptosporidiosis. *Chemical Rubber Company Press*, Boca Raton, Florida USA, pp. 1–242.

Fayer, R., Morgan, U. and Upton, S.J. (2000). Epidemiology of *Cryptosporidium*: transmission, detection and identification. *International Journal of Parasitology,* 30: 1305–1322.

Fayer, R., Trout, J.M., Xiao, L., Morgan, U.M., Lal, A.A. and Dubey, J.P. (2001). *Cryptosporidiumcanis* sp. from domestic dogs. *Journal of Parasitology*, 87: 1415-1422.

Fayer, R., Santin, M. and Xiao, L. (2004). *Cryptosporidium bovis* (Apicomplexa *Cryptosporidiidae*) in cattle (*Bos taurus*). *Journal of Parasitology*, 91: 624-629.

Fayer, R. (2010). Taxonomy and species delimitation in *Cryptosporidium*. *Experimental Parasitology*, 124: 90–97.

Gajadhar, A.A.(1994). Host specificity studies and oocyst description of a *Cryptosporidium* sp. isolated from ostriches. *Parasitology Research*, 80(4): 316-319.

Gardiner, C.H. and Grimes, G.D. (1984). *Cryptosporidium* sp. in the kidney of a black-throated finch. *Journal of American Veterinary Medical Association*, 185: 1401–1402.

Gatei, W.,Suputtamongkol, Y., Waywa, D., Ashford, R.W.,Bailey, J.W., Greensill, J. and Hart, C.A.(2002). Zoonotic species of *Cryptosporidium* are as prevalent as the anthroponotic in HIV-infected patients in Thailand. *Annals of Tropical Medicine and Parasitology*, 96: 797-802.

Gharazouglou, M.J., Desfoulian, O., Rahbari, S., Bokaie, S., Jahanzad, I. and Razavi, A.N.E. (2006). Intestinal Cryptosporidiosis in turkeys in Iran. *Journal of Veterinary Medical Service*, 53: 282-285.

Glaberman, S., Moore, J.E., Lowery, C.J., Chalmers, R.M., Sulaiman, I., Elwin, K. and Xiao, L.H.(2002). Three drinking-water-associatedCryptosporidiosis outbreaks, Northern Ireland. *Emerging Infectious Diseases*, 8(6):631-633.

Gobel, E. and Brandler, U. (1982). Ultrastructure of microgametogenesis, microgametes and gametogony of *Cryptosporidium* sp. in the small intestine of mice.*Protistologica,*18:331–344.

GoldSmith, R.S., Schroeder, S.A., Krapp, M.A., Tierney, L M. and Mepha, S.J. (1989). Infectious Diseases: Protozoology In: (Eds.),Current Medical Diagnosis and Treatment. *Appleton and Lange Medical Book,* pp. 947.

Gomes, R.S. Huber, F., da Silva, S. and do Bomfim, T.C.(2012). *Cryptosporidium* spp. parasitize exotic birds that are commercialized in markets, commercial aviaries, and pet shops. *Parasitology Research*, 110: 1363–1370.

Goodwin, M.A. and Brown, J.(1988). Light-microscopic lesions associated with naturally occurring bursal Cryptosporidiosis in chickens. Avian Diseases, 33: 74–78.

Goodwin, M.A. and Davis, J.F. (1993). Fatal bronchopneumonia associated with *Cryptosporidium* sp. in a wood duck (*Aix sponsa*). *Journal of Association of Avian Veterinarian* 7: 77–78.

Goodwin, M.A. and Waltman, W.D.(1996). Transmission of *Eimeria* viruses, and bacteria to chicks. Darkling beetles (*Alphitobius diaperinus*) as vectors of pathogens. *Journal of Applied Poultry Resources*, 5: 51–55.

Graczyk, T.K., Cranfield, M.R., Mann, J. and Strandberg, J.D.(1998). Intestinal *Cryptosporidium* sp. infection in the Egyptian tortoise, Testudo kleinmanni. *International Journal for Parasitology*, 28(12): 1885-1888.

Guy, J.S., Levy, M.G., Ley, D.H., Barnes, H.J. and Gerig, T.M. (1988). Interaction of reovirus and *Cryptosporidiumbaileyi* in experimentally infected chickens. *Avian Disease,s* 32: 381–390.

Hoerr, F.J., Current, W.L. and Haynes, T.B. (1986). FatalCryptosporidiosis in a quail. *Avian Diseases*, 30: 421–425.

Hoerr, F.J., Blagburn, B.L., Lindsay, D.S. and Giambrone, J.J. (1987). Interactions of *Cryptosporidium* with other infectious pathogens. In: Proc. 124th *Annual* Meeting*of American Veterinary Medical Association* Chicago, pp. 134.

Hornok, S., Sréter, T., Békési, L., Széll, Z. and Varga, I. (1996). Attempts to immunize chickens with *Cryptosporidiumbaileyi* oocyst extract. *Journal of Parasitology*, 82: 650–652.

Ibrahim, U.I., Mbaya, A.W., Mahmud, H. and Mohammed, A. (2007). Prevalence of Cryptosporidiosis among captive wild animals and birds in the arid region of Northeastern Nigeria. *Veterinary Archive*, 77: 337-344.

Isa, U.F. and Yusuf, A.Y. (2011). Spatial distribution and utilization of intercontinental bank automated teller machines in Kano metropolis. *European Journal of Scientific Research*, 52 (1): 44-51.

Itakura, C.,Goryo, M. and Umemura, T. (1984). Cryptosporidial infection in chickens. *Avian Pathology*, 13: 487-499.

Iseki, M. (1979). *Cryptosporidium fel`is* sp. n. (Protozoa: eimeriorina) from the domestic cat. *Japanese Journal of Parasitology*, 28: 285-307.

Jellison, K.L., Distel, D.L., Hemond, H.F. and Schauer, D.B. (2004). Phylogenetic analysis of the hypervariable region of the 18S rRNA gene of *Cryptosporidium* oocysts in faeces of Canada geese (Branta canadensis): evidence for five novel genotypes. *Applied and Environmental Microbiology*, 70: 452–458.

Jenkins, M.C. (2004). Present and future control of *Cryptosporidium* in humans and animals. *Experimental Reviews and Vaccines*, 3: 669-671

Jervis, H.R., Merrill, T.G. and Sprinz, H.(1966). Coccidiosis in the guinea pig small intestine due to a *Cryptosporidium*. *American Journal ofVeterinary Resources* 27: 408-414.

Jex, A.R., Smith, H.V. Monis, P.T. Campbell, B.E. and Gasser, R.B. (2008). *Cryptosporidium* biotechnological advances in the detection, diagnosis and analysis of genetic variation. *Biotechnology Advances*, 26:304 –317.

John, P. S., David, H. and MichaelJ. R. (1982): Cryptosporidiosis in a gray squirrel. *Journal of American Veterinary Medical Association*, 181: (11): 420-422.

Kaneta, Y. and Nakai, Y. (1998). Survey of *Cryptosporidium* oocysts from adult cattle in a slaughter house. *Journal of Veterinary Medical Science*, 60: 585-588.

Kano State, Ministry of Information. (2005). Two years of Shekarau Administration, Ministry of Information Youth, Sports and Culture, Kano. In: Barau, A.S. *an account of the high population in Kano state*, federal college of education, Kano, Nigeria. Retrieved June 25, 2014 from: *www.ciesin.columbia.edu/repository/pern/papers/Barau.pdf.*

Karanis, P.,Kourenti, C.andSmith, H. (2007). Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health*, 5: 1–38.

Koudela, B. andModry, D. (1998). New species of *Cryptosporidium* (Apicomplexa: *Cryptosporidiidae*) from lizards. *Folia Parasitologica*, 45(2): 93-100.

Kwaga, J.K., Umoh, J.U. and Odoba, M.B. (1988a). *Cryptosporidium* infections in humans with gastroenteritis in Zaria, Nigeria. *Epidemiology and Infection*, 101(1): 93-7.

Kwaga, J.K., Uzor, E.I. and Umoh, J.U. (1988b). *Cryptosporidium* infection in calves and piglets in some part of Kaduna state, Nigeria. *Zariya Veterinarian*, 3(2): 86-89.

Langsley, G., van Noort, V., Carret, C., Meissner, M., de Villiers, E.P. Bishop, R. and Pain, A. (2008). Comparative genomics of the Rab protein family in Apicomplexan parasites. *Microbes and Infection*, 10: 462–470.

Laxer, M.A., Timblin, B.K. and Patel, R.J. (1991). DNA sequences for the specific detection of *Cryptosporidium parvum* by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, 45:688–694.

LeChevallier, M.W., Norton, W.D. and Lee, R.G. (1991). *Giardia* and *Cryptosporidium* spp. in filtered drinking water supplies. *Applied and Environmental Microbiology*, 57: 2617-2621.

Levine, N.D., Lee, R.R., Hutner, SH. and Bovee E.C. (1985), An Illustrated *Guide to the Protozoa*, Phylum II Apicomplexa, in:Allen Press, Lawrence, pp. 322–374.

Levy, M.G., Ley, D.H., Hunter, L., Corbett, W. and Barnes, H.J. (1988). Experimental Cryptosporidiosisand infectious bursal disease virus infection of specific-pathogen-free chickens. *Avian Diseases*, 32: 803–811.

Lindsay, D.S., Blagburn, B.L., Hoerr, F.J.(1987). Experimentally-induced infections in turkeys with *Cryptosporidium baileyi* isolated from chickens.*American Journal of Veterinary Resources*, 48: 104–108.

Lindsay,D.S. and Blagburn, B.L.(1990). Cryptosporidiosis in birds. In Cryptosporidiosis of *Man and Animals*. *Chemical Rubber Company Press*, Boca Raton, Florida USA, pp. 133–148.

Lindsay, D.S. and Blagburn, B.L.(1991). *Cryptosporidium parvum* infections of swine. *Compendium on Continuing Education for the Practicing Veterinarian*, 13(5): 891-894.

Lindsay, D.S.(1997). Laboratory models of Cryptosporidiosis. In: Fayer, R. (Ed.), *Cryptosporidium* and Cryptosporidiosis. *Chemical Rubber Company Press*, Boca Raton, Florida USA,pp. 210–224.

Lindsay, D.S., Upton, S.J., Owens, D.S., Morgan, U.M., Mead, J.R. and Blagburn, B.L. (2000). *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from cattle (*Bos Taurus*). *Journal of Eukaryotic Microbiology*, 47(1): 91-95.

Lynne, S. and Garcia, M.S. (1999). American Society for Microbiology. *Practical Guide to Diagnostic Parasitology*,pp. 102-104.

Mackenzie, W.R., Hoxie, N.J., Proctor, M.E., Gradus, M.S., Blair, K.A. and Peterson, D.E. (1994). A massive outbreak of *Cryptosporidium* infection transmitted through public water supply. *New England journal of Medicine*, 331:161-167

Maikai, B.V., Umoh, J.U., Kwaga, J.K.B., Lawal, I, . Maikai, V.A., Camae, V. and Xiao, L. (2011). Molecular characterization of *Cryptosporidium* spp. in native breeds of cattle in Kaduna State, Nigeria. *Veterinary Parasitology*, 17: 241–245.

Majewska, A.C., Graczyk, TK., Slodkowicz-Kowalska, A., Tamang, L., Jedrzejewski, S., Zduniak, P. and Nowosad, P. (2009). The role of free-ranging, captive, and domestic birds of Western Poland in environmental contamination with *Cryptosporidiumparvum* oocysts and *Giardia lamblia* cysts. *Parasitology Research*, 104: 1093–1099.

Meisel, J.L., Perera, D.R., Meligro, C. and Rubin, CE. (1976). Overwhelming watery diarrhoea associated with a *Cryptosporidium* in an immunosuppressed patient, *Gastroenterology*, 70: 1156–1160.

Miron, D., Kenes, J. and Dagan, R. (1991). Calves as a source of an outbreak of among young children in an agricultural closed community. *Paediatric Infectious Disease Journal*, 10: 438-441.

Morgan, U.M., Sargent, K.D., Elliot, A. and Thompson, R.C.A. (1998). *Cryptosporidium* in cats-additional evidence for C. felis. *Veterinary Journal*, 156:159-161.

Morgan, U.M.R., Buddle, R., Armson, A. and Thompson, R.C.A. (1999). Molecular and biological characterization of *Cryptosporidium* in Pigs. *Australia Veterinary Journal*, 77: 44-47.

Morgan, U., Weber, R., Xiao, L.H., Sulaiman, I., Thompson, R.C.A., Ndiritu, W. and Deplazes, P. (2000). Molecular characterization of *Cryptosporidium* isolates obtained from human immunodeficiency virus-infected individuals living in Switzerland, Kenya, and the United States. *Journal of Clinical Microbiology*, 38: 1180-1183.

Morgan, U.M., Monis, P.T., Xiao, L., Limor J., Irshad S., Raidal S. and Thompson, R.C.A. (2001). Molecular and phylogenetic characterization of *Cryptosporidium* from birds. *International Journal of Parasitology*, 31: 289–296.

Morgan-Ryan, U., Monis, Possenti, P.A., Crisanti, A. and Spano, F.(2001). Cloning and phylogenetic analysis of the ribosomal internal transcribed spacer-1 (ITS1) of *Cryptosporidium wrairi* and its relationship to *Cryptosporidium parvum*genotypes. *Parasitologia*, 43:159-163.

Morgan-Ryan, U.M., Fall, A., Sulaiman, I.R., Olson, M., Lal, A. and Xiao, L. (2002). *Cryptosporidium hominis* (Apicomplexa; *Cryptosporidiidae*) from humans.*Journal of Eukaryotic Microbiology*, 49: 433-440.

Morin, M., Lariviere, S. and Lallier, R. (1976). Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhoea. *Canada Journal of Comparative Medicine*, 40: 228.

National Baseline Survey Final Report, (2007). Federal Republic of Nigeria Avian Influenza and Control and Human Pandemic Preparedness and Response Project; pp. 1-100

Nime, F.A., Burek, J.D., Page, D.L., Holscher, M.A. and Yardley, J.H. (1976). Acute enterocolitis in a human being infected with the protozoan.*Cryptosporidium*, *Gastroenterology*, 70: 592–598.

Njoku, O.M., Uneke, C.J., Omale, S., Inyama, P.U., Jagun, B.I. and Duhlinska, D.D. (2005). Occurrence of *Cryptosporidium* species in surface water in the Jos-Plateau. *Journal of Science, Agriculture, Food Technology and Environment*, 56-63.

Nouri, M. (2002). A *Cryptosporidiummuris* - like parasite in cattle and camels in Iran. Indian *Veterinary Journal*, 79:558-559.

O'Donoghue, P.J. (1995). *Cryptosporidium* and Cryptosporidiosis in man and animals, *International Journal of Parasitology*, 25: 139–195.

Office International des Epizooties, (OIE) (2004). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees)*. 5th edition, pp. 330.

Okhuysen, P.C. and Chappell, C.L. (2002). *Cryptosporidium* virulence determinants *International Journal of Parasitology*,32:517–525.

Oseiki, A.O. (2009).The influence of gender on intra-urban transportation. A case study of Kano Metropolis. *Unpublished thesis* submitted to the Department of Geography, Ahmadu Bello University, Zaria.

Pagani, P., Abimiku, J.E. and Emeka – Okolie, W. (2008). *Assessment of the Nigerian poultry market chain to improve biosecurity* by Food and Agriculture Organization, pp. 1-60

Pam, V.A., Dakul, D.A., Karshima, NS., Bata, S.I., Ogbu, K.I., Danie,L.N.and Hassan, A.A. (2013). Survey of *Cryptosporidium* species among ruminants in Jos, Plateau State, North-Central Nigeria. *Journal of Veterinary Advances*, 3: 49-54.

Pavlásek, I. (1994). The black-headed gull (*Larus ridibundus*), a new host for *Cryptosporidium baileyi* (Apicomplexa: Cryptosporidiidae). *Veterinary MedicinePraha*, 38: 629–638.

Pavlasek, I.,Lavickova, M., Horak, P., Kral, J. and Kral, B.(1995). *Cryptosporidium varanii* n. sp. (Apicomplexa: Cryptosporidiidae) in Emerald monitor (*Varanus prasnius* Schegel, 1893) in captivity at Prague Zoo. *Gazella*, 22:99-108.

Pavlasek, I. (1999). Kryptosporidie: biologie, diagnostika, hostitelské spektrum, specificita a vztah kživotnímu prostředí (Cryptosporidia: biology, diagnosis, host spectrum, specificity and the environment). *Remedia Klin.Microbiology*, 3: 290–30.

Pavlasek, I. (2001). Nálezy kryptosporidií ve žlaznatém žaludku u slepic a u volně žijících a exotických ptáků odchycených z volné přírody (Findings of *Cryptosporidia* in the proventriculum of hens and in wild and exotic birds). *Veterinářství*, 3:103–108.

Pedraza-Diaz, S., Amar, C.F.L., McLauchlin, J., Nichols, G.L., Cotton, K.M., Godwin, P. and Youngs, E.R. (2001). *Cryptosporidium meleagridis* from humans: Molecular analysis and description of affected patients. *Journal of Infection*, 42(4):243-250.

Penrith, M.L., Beznidenhout, A.J., Burger, W.P. and Putteril, J.F. (1994). Evidence for *Cryptosporidial* infection as a cause of prolapse of phallus and cloaca in ostrich chicks (*Struthio camelus*) Onderstepoort. *Journal of Veterinary Resources*, 61: 283–289.

Pohlenz, J., Moon, H.W.,Cheville, N.F. and Bemrick, W.J. (1978). Cryptosporidiosis as a probable factor in neonatal diarrhea of calves, *Journal of American Veterinary Medical Association*, 17: 24-52.

Putignani, L. and Menichella, D. (2010). Global distribution, public health and clinical impact of the protozoan pathogen *Cryptosporidium*. Interdisciplinary Perspectives on Infectious Diseases, 2010: 1-39.

Randall, C.J.(1982). Cryptosporidiosis of the bursa of Fabricius and trachea in broilers. *Avian Pathology*, 11: 95–100.

Randall, C.J. (1986). Renal and nasalCryptosporidiosis in a jungle fowl (*Gallus sonneratii*). *Veterinary Record*, 119: 130–131.

Reduker, D.andSpeer, C.A. (1985). Factors influencing excystation in *Cryptosporidium* oocysts from cattle. *Journal of Parasitology*,71:112–115.

Rhee, J.K., Kim, H.C. and Park, B.C. (1997). Effects of *Cryptosporidiumbaileyi*infection on the bursa of Fabricius in chickens. *Korean Journal of Parasitology*, 35: 181–187

Ritter, G.D., Ley, D.H., Levy, M., Guy, J. and Barnes, H.J.(1986). Intestinal Cryptosporidiosis and reovirus isolation from bobwhite quail (*Colinus virginianus*) with enteritis. *Avian Diseases*, 30(3): 603-608.

Robertson, L., Campbell, A.T. and Smith, H.V. (1993). In vitro excystation of *Cryptosporidiumparvum*. *Parasitology*,106:13–19.

Robertson, L.J. and Gyerde, B. (2007). Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in raw waters in Norway. *Journal of Public Health*, 29:200-207.

Rosales, M.J., Cordon, G.P., Moreno, M.S. and Sanchez, C.M. (2005). Extracellular like-gregarine stages of *Cryptosporidiumparvum*. *Acta Tropic*, 95:74 –78.

Ryan, U.M., Xiao, L., Read, C., Sulaiman, I.M., Monis, P., Lal, A.A.and Pavlasek, I. (2003). A redescription of *Cryptosporidiumgalli* Pavlasek,(Apicomplexa: *Cryptosporidiidae*) from birds.*Journal of Parasitology*, 89: 809–813.

Ryan, U. (2010). *Cryptosporidium* in birds, fish and amphibians. *Experimental biology*, 124: 113-120

Senne, D.A., Pearson, J.E. and Panigraphy, B. (2008).Live poultry markets: In:proceedings of the third *International Symposium on Avian Influenza*Madison,Wisconsin, USA *Animal Health Association*, St. Joseoph, Missouri, USA.*A missing link in the Epidemiology of Avian Influenza*, pp 50-58.

Slavin, D. (1955). *Cryptosporidiummeleagridis* (sp. nov.). *Journal of Comparative Pathology*, 65: 262–266.

Smith, H. and Rose, J.B. (1998). Waterborne Cryptosporidiosis current status. *Parasitology Today*, 14: 14 –22.

Smith, H.V. (2008). Diagnostics. In: *Cryptosporidium* and Cryptosporidiosis, Second Edition,

Sonaiya, E.B. (1997). *African Network on Rural Poultry Development*:I'rogress report, November 1989 to june 1995.Proceedings of African Network for Rural Poultry Development Workshop, Adis Ababa, Ethiopia, Pp.134-143.

Spano, F., Putignani, L., McLauchlin, J., Casemore D.P. and Crisanti, A.(1997). PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum*, and between *C. parvum* isolates of human and animal origin. FEMS *Microbiology Letters*, 150(2): 209-217.

Sreter, T. and Varga, I.(2000). Cryptosporidiosis in birds - a review. *Veterinary Parasitology*, 87(4): 261-279.

Steiner, T.S., Thielman , N.M. and Guerrant, R.L. (1997) Protozoal agents: What are the dangers for the public water supply?*Annual Reviewed Medicine* , 48: 329-340.

Tarwid, J.N., Cawthorn, R.J. and Riddel, C. (1985). Cryptosporidiosis in the respiratory tract of turkeys in Saskatchewan.*Avian Diseases*, 29: 528–532.

Taylor, M.A. (1995). Protozoan parasites and water: Veterinary Aspects. In: Protozoan Parasites and Water (eds WB Betts, D. Casemore, C. Fricker, H. Smith. and J. Watkins), The Royal Society of Chemistry, Cambridge, United Kingdom.*The Royal Society of Chemistry*, Cambridge, pp. 246-249.

Taylor, M.A., Geach, M.R. and Cooley, W.A. (1999). Clinical and pathological observations on natural infections of Cryptosporidiosis and flagellate protozoa in leopard geckos (*Eublepharis macularius*). *Veterinary Record*, 145(24): 695-699.

Tham, V.L., Kinesberg, S. and Dixon, B.R. (1982). Cryptosporidiosisin quails. *Avian Pathology*, 11: 619–626.

Theodos, C. (1998). Innate and cell mediated immune responses to *Cryptosporidium parvum*. *Advances Parasitology*, 40: 88–119.

Thompson, R.C., Palmer, A.C.S. and O'Handley, R. (2008). The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *The Veterinary Journal*, 177: 18-25.

Thompson, R.C., Olson, M.E., Zhu, G., Enomoto, S., Abrahamsen, M.S. and Hijjawi, N.S. (2005). *Cryptosporidium* andCryptosporidiosis. *Advance Parasitology*, 59:77–158.

Thrusfield, M. (1997). *Veterinary Epidemiology*, second edition. *Blackwell Publications*, Oxford, pp. 182-183.

Tilley, M., Upton, S.J. and Freed, P.S.(1990). A comparative study of the biology of *Cryptosporidium serpentis* and *Cryprosporidium parvum* (Apicomplcxa: *Cryptosporidiidae*) *Journal of Zoo Wild Medicine*, 21:463-467.

Tilley, M., Upton, S.J. and Chrisp, C.E(1991). A comparative study on the biology of *Cryptosporidium* sp. from guinea pigs and *Cryptosporidium parvum* (Apicomplexa). *Canadian Journal of Microbiology*, 37(12): 949-952.

Tomley, F.M. and Soldati, D.S. (2001). Mix and match modules: structure and function of microneme proteins in apicomplexan parasites, *Trends in Parasitology*,17: 81–88.

Torres, J., Gracenea, M., Gomez, M.S., Arrizabalaga, A. and Gonzalez-Moreno, O. (2000). The occurrence of *Cryptosporidiumparvum* and *C. muris* in wild rodents and insectivores in Spain. *Veterinary Parasitology*, 92(4):253-260.

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

Tyzzer, E. (1910). An extracelluar coccidium, *Cryptosporidiummuris* of the gastric glands of the common mouse. *Journal of Medical Resources*, 18: 487 509.

Tyzzer, E. (1912).*Cryptosporidium parvum* (sp. nov.), a coccidium found in the small intestine of the common mouse. *Archive Protisenkd*, 26:394-412.

Tyzzer, E.E. (1929). Coccidiosis in gallinaceous birds. *American Journal of Hygiene* 10: 269–383

Tzipori, S., Campbell, I., Sherwood, D., Snodgrass, D. R. and Whitelaw, A. (1980). An outbreak of calf diarrhoea attributed to *Cryptosporidial* infection.*Veterinary Record*, 107:579 –580.

Tzipori, S. (1983). Cryptosporidiosis in animals and humans.*Microbiology Reviews* 47:84–96.

Tzipori, S., Baker, J.R., Muller, R. and Rollinson, D. (1998). Opportunistic Protozoa in Humans, Academic Press, London.*Advances in Parasitology*, pp. 40.

Tzipori, S. and Griffiths, J.K. (1998). Natural history and biology of *Cryptosporidiumparvum, Advances in Parasitology*, 40:5-36.

Tzipori, S. and Ward, H. (2002). Cryptosporidiosis: biology, pathogenesis and disease. *Microbes Infection*,4:1047–1058.

Uneke, C.J. and Uneke, B.I.(2008). Occurrence of *Cryptosporidium* species in surface water in South-Eastern Nigeria; The public health implication. *The Internet Journal of Health*, 7(2): 1-9.

Upton, S.J., McAllister, C.T., Freed, P.S. and Barnard, S.M.(1989). *Cryptosporidium* spp. in wild and captive reptiles. *Journal of Wildlife Diseases*, 25(1): 20-30.

Upton, S.J. and Barnard, S.M.(1987). Two new species of coccidia (Apicomplexa: Eimeriidae) from Madagascar Gekkonids. *Journal of Protozoology*, 34(4): 452-454.

USFDA/CCFSAN, (2005). Bad-bud book-*Cryptosporidiumparvum*. http//vm.fda.gov/mow/chp24-html. Accessed 160hrs 21/12/2009.

Varga, I. and Ványi, A.(1992). Interaction of T-2 fusariotoxin with anticoccidial efficacy of lasalocid in chickens. *International Journal of Parasitology*, 22: 523–525.

Varga, I., Sréter, T. and Bekesi, L. (1995). Potentiation of ionophorous anticoccidials with duokvin: Battery trials against *Cryptosporidiumbaileyi* in chickens. *Journal of Parasitology*, 81: 777–780.

Vetterling, J.M., Jervis, H.R., Merrill, G. and Sprinz, H.(1971). *Cryptosporidium wrairi* sp. n. from the guinea pig *Cavia porcellus*, with an emendation of the genus. *Journal of Protozoology*, 18:243-247.

Wages, D. P.(1987). Cryptosporidiosis and Viral hepatitis in turkey poults. In: Proc. 124th Annual Meeting, *American Veterinary Medical Association*, Chicago, pp. 121.

Wang, L., Xue, X., Li, J., Zhou, Q., Yu, Y. and Du, A.(2014). Cryptosporidiosis in broiler chickens in Zhejiang Province, China: *molecular characterization of oocysts detected in fecal samples*. *Parasite*, 21: 36.

Wanyiri, J. and Ward, H. (2006). Molecular basis of *Cryptosporidium*-host cell interactions: recent advances and future prospects. *Future Microbiology*, 1:201–208.

Ward, H. and Cevallos, A.M. (1998). *Cryptosporidium*: molecular basis of host–parasite interaction, *Advanced Parasitology*,40: 151–185.

Wellington, O., Chika, O., Teslim, O., Oladipo, O., Adetayo, F. and Godswill, I. (2009). Intestinal protozoans in children with diarrhoea in Lagos, Nigeria. *The Internet Journal of Tropical Medicine*, 5(2): 1-8.

Wetzel, D.M., Schmidt, J., Kuhlenschmidt, M.S., Dubey, J.P. and Sibley, L.D. (2005). Gliding motility leads to active cellular invasion by *Cryptosporidiumparvum* sporozoites. *Infection and Immunity*, 73:5379 –5387.

Whittington, R.J. and Wilson, J.M., (1985). Cryptosporidiosis of the respiratory tract in a pheasant. *Australia Veterinary Journal* 62, 284–285.

Woodmansee, D.B., Pavlásek, I., Pohlenz, J.F.L. and Moon, H.W. (1988). Subclinical Cryptosporidiosis in turkeys in Iowa. *Journal of Parasitology*, 74: 898–900.

World Health Organization (WHO) (1991). Basic laboratory methods in medical parasitology. *World Health Organization*, Geneva, pp. 16.

World Health Organization (WHO) (2006). *Guidelines for drinking water quality*, 3rd edition, WHO, Geneva, Switzerland.

Xiao, L., Escalante, L., Yang, Chunfu, Sulaiman, I., Escalante, A.A., Montali, R.J., Fayer, R. and Lal, A.A. (1999). Phylogenetic analysis of *Cryptosporidium*

parasites based on the small-subunit rRNA gene locus. *Applied and Environmental Microbiology*, 65(4):1578-1583.

Xiao, L.H., Bern, C., Arrowood, M., Sulaiman, I., Zhou, L., Kawa, V., Vivar, A. Lal, A.A. and Gilman, R.H. (2002). Identification of the *Cryptosporidium*pig genotype in a human patient.*Journal of Infectious Diseases*, 185(12):1846-1848.

Xiao, L., Fayer, R., Ryan, U. and Upton, S.J. (2004). *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clinical Microbiology Review*, 17: 72–97.

APPENDIX 1

QUESTIONNAIRE FOR LIVE BIRD MARKET EVALUATION OF ASSOCIATED RISK FACTORS WITH FAECAL SHEDDING OF*CRYPTOSPORIDIUM OOCYSTS* IN AVIANS SPECIES IN IN KANO METROPOLIS, NIGERIA.

Respondent Bio data **Date:……..**

Name:	Age:
Gender: Male ( ) Female ( )	Main occupation:
Educational qualification:	

LIVE BIRD MARKET CHARACTERISTICS

Located within a market: Yes ( ) No ( )

Source of birds: Intrastate ( )Others ( )

Source of feed: Commercial ( ) Others ( )

Source of water: Well ( ) Borehole ( )

Presence of trees**:**Yes ( ) No ( )

Frequency of cleaning:Daily ( ) Others ( )

Types of cage: Metallic ( ) Wooden ( )

APPENDIX II

ENCLOSED SHARADA LIVE BIRD MARKET IN KANO METROPOLIS

APPENDIX III

A FOWL SELLER BEEN INTERVIEWED WITH REGARDS TO RISK FACTORS ASSOCIATED WITH FAECAL SHEDDING OF*CRYPTOSPORIDIUM* OOCYSTS IN AVIAN SPECIES AT SHARADA LBM

APPENDIX IV

METALLIC CAGES LOCAL AND EXOTIC CHICKEN AT TARAUNI LIVE BIRD MARKET