SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* INFECTION IN LOCAL CHICKENS SLAUGHTERED IN LIVE BIRD MARKETS IN KATSINA METROPOLIS KATSINA STATE, NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN VETERINARY EPIDEMIOLOGY

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MARCH, 2016

DECLARATION

I declare that the work in this Dissertation entitled **"Seroepidemiology of *Toxoplasma gondii* Infection in Local Chickens slaughtered in Live Bird Markets in Katsina Metropolis, Katsina State, Nigeria"** has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text 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.

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Name of Student	Signature	Date

CERTIFICATION

This Dissertation titled, **"SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* INFECTION IN LOCAL CHICKENS SLAUGHTERED IN LIVE BIRD MARKETS IN KATSINA METROPOLIS, NIGERIA"** by Mohammad Bashir ALIYU, meets the regulations governing the award of degree of Master of Science of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Toxoplasmosis is caused by a coccidial parasite, *Toxoplasma gondii*, which infects humans, livestock and birds. This study was to determine the sero-prevalence of *Toxoplasma gondii* antibodies in local chickens (*Gallus gallus domesticus*) in Katsina metropolis from January to March, 2015. A total of 460 sera were collected from two live bird markets (LBMs) at slaughter points and these were assayed with Indirect Enzyme Linked Immuno-Sorbent Assay (ELISA) test kits specific for avian toxoplasmosis. Structured questionnaires were administered to the workers at LBMs to evaluate their attitude and practice towards *Toxoplasma* infection. The overall prevalence for *Toxoplasma* antibodies was 7.83% (36/460). Gobarau yan kaji had a higher prevalence 9.06% (25) than Central market 5.98% (11). All (100%) respondents were males and with a mean age of 21.52±24.76 years. Mean score was 26.42±15.9 and 21.74±9.21 for attitude and practice respectively. There was a significant correlation ($p < 0.05$) between attitude and practice score of the respondents. The level of education and specific duty at the slaughter houses correlated significantly ($p < 0.05$) with attitude and practice mean ranks. There was no association (0.137) between experience on the job and practice score. Attitude and practice ranked as poor, fair and good in this study were 60% (30), 12% (6), 28% (14) and 80% (40), 6% (3), 14% (7) respectively. Biosecurity assessment of the LBM was in relation to fencing of the LBMs, infestation of the LBMs with rodents and presence of other animal species in the LBMs. The two LBMs were not fenced and rodents were seen in both of them. Other animal species were seen in both markets, notable among them was the presence of cats which were more in Gobarau 70% (7) than Central 30% (3) market. Most of the respondents (86%) do not allow cats to have access into the bird cages. Most birds kept in the two LBMs were caged (92%) with only 8% of the birds not caged. There were more dogs seen

in Central 62% (10) than in Gobarau 38% (6) market. The respondents participated more in environmental sanitation in Central market 68% (34) than Gobarau 32% (16) market. This result has shown the presence of *Toxoplasma gondii* antibodies in Local chickens slaughtered for human consumption in Katsina Metropolis and this warns on the public health safety problems. There is therefore the urgent need for the implementation of biosecurity measures, public awareness campaign for workers at LBMs in Katsina Metropolis.

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LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immuno-Deficiency Syndrome
CDC	Centres for Disease Control and Prevention
CFT	Compliment Fixation Test
CSF	Cerebrospinal Fluid
ELISA	Enzyme Linked Immuno-sorbent Assay
FAO	Food And Agriculture Organization
HIV	Human Immuno-deficiency Virus
HRP	Horse Radish Peroxidase
IFA	Immuno Flourescent Test
IHAT:	Indirect Haemagglutination Test
IU	International Unit
IgG	Immunoglobulin G
LAT	Latex Agglutination Test
LBM	Live Bird Market
MAT	Modified Agglutination Test
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
SP	Speficity

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Toxoplasmosis is a zoonotic parasitic infection caused by *Toxoplasma gondii* and is estimated to have infected about a third of the world's population (Montoya, 2004). *Toxoplasma gondii* is a protozoan parasite which belongs to the phylum *Apicomplexa*, subclass *Coccidiasina* and family *Sarcocystidae* (Hill *et al*., 2007; Pereira *et al*., 2010). This coccidian parasite infects mostly species of warm-blooded animals including man and birds. There are three (3) strains of *Toxoplasma gondii* (type I, type II and type III), of which type I is extremely virulent for mice and type II has been associated with the majority of toxoplasmosis cases in AIDS patients (Boothroyd and Grigg, 2002; Klaren and Kijlstra, 2004). Type III is present in animals and has been detected in AIDS patients, but does not seem to be associated with ocular toxoplasmosis (Boothroyd and Grigg, 2002).

Toxoplasmosis is generally transmitted to humans either congenitally, or via ingestion of undercooked or raw meat from infected animals, or ingestion of food or water contaminated with oocysts excreted by infected felids (Montoya, 2004). Ingestion of infected chicken meat can be a source of infection for *Toxoplasma gondii* infection in humans and other animals (Dubey, 2010b). Chickens are considered resistant to clinical toxoplasmosis. There are only a few reports of clinical toxoplasmosis in chickens worldwide (Dubey, 2008). There are several reports indicating that both chickens raised in backyard and commercial free-range systems harbour viable *Toxoplasma gondii* (Dubey *et al.,* 2004. 2005b).

Chickens play an important role in the epidemiology of *Toxoplasma gondii* in the rural environment, perhaps more than rodents, because they are clinically resistant to *Toxoplasma gondii* and live longer than rodents (Dubey, 2008). Chickens can harbour mouse-virulent *Toxoplasma gondii* without showing any clinical signs (Dubey *et al*., 2002). Most chickens raised under free range and backyard operations in developing countries are usually slaughtered at home without supervision. This can allow the transmission of *Toxoplasma gondii* infection to humans if care is not taken to wash hands thoroughly after cutting meat and during cooking of meat. Furthermore, the viscera of such chicken are usually not properly disposed but left for scavengers thereby encouraging the transmission of *Toxoplasma gondii* to reservoirs like rodents or even cats which are the final host (Ayinmode, 2012). The probability of humans getting infected from ingesting eggs from infected chicken is not yet substantiated, although *Toxoplasma. gondii* has been shown to survived in egg yolk and albumin of boiled egg and yolk of egg fried for 3 minutes, but there are evidences that showed that raw eggs are not likely to be a source of infection for humans (Dubey, 2010b). The detection of chronic infection with *Toxoplasma gondii* in animals relies primarily on serological assays. There is no gold standard test for the screening of the large diversity of *Toxoplasma* host species. The sensitivity and specificity of the techniques depend on the animal species. Several serological tests have been reported to be used in the diagnosis of chicken toxoplasmosis, they include Sabin-Feldman dye test, Indirect fluorescent antibody assay (IFA), Complement fixation test (CFT) and the Enzyme-linked immunosorbent assay (ELISA) (Hill and Dubey, 2002). Specific enzyme-linked immunosorbent assays (ELISA) have been developed for some domestic animal species such as goats and chickens (Dubey, 2011).

1.2 Statement of Research Problem

There is a high prevalence of toxoplasmosis throughout the world (20%–90%), as well as a high resistance and persistence of the parasite in a broad spectrum of biological matrixes (Vaz *et al*., 2010). Serological studies have indicated incidence of *Toxoplasma* infections ranging from less than 1% in young adults in some areas, to 90% among older persons in other places (Montoya and Remington, 1995). Toxoplasmosis is widespread biologically and geographically because of its simple mode of contraction. Infection can occur simply by ingestion of oocysts following the handling of contaminated soil with cat litter or the consumption of contaminated water or food. However, no direct association has been found between cat ownership and the risk of toxoplasmosis in people (Walker *et al.,* 2008).

Toxoplasmosis has long been reported to be widespread in West Africa (UNAIDS, 2004). In sub-Saharan Africa, toxoplasmosis often remains undetected and untreated due to insufficient diagnostic procedures (Lindstrom *et al*., 2006). Several studies have shown a consistently high *Toxoplasma gondii* seroprevalence for this region, ranging from 35% to 84% in different African countries south of Sahara (Tenter *et al*., 2000). Considering that around 30–50% of those coinfected with HIV and *Toxoplasma gondii* are expected to ultimately develop toxoplasmosis, the high seroprevalence combined with the HIV-pandemic indicates that 2.5–10 million people in this region may be at risk dying from toxoplasmosis (Lindstrom *et al.,* 2006). In Nigeria, toxoplasmosis has been reported both in man and some important animals. A work carried out among pregnant women attending Antenatal Clinics at University College Hospital, Ibadan, and St. Mary's Catholic Hospital, Ibadan, showed very high prevalence of *Toxoplasma* antibodies in the sera of both pregnant

(75.4%) and postpartum (80.5%) women (Onadeko *et al*., 1996). In Northern Nigeria, more work have been reported on veterinary toxoplasmosis. High seroprevalence of toxoplasmosis have been reported among some animals of economic importance, such as sheep (Okoh *et al*., 1981), pet dogs in Zaria (Aganga and Ortese, 1984), and dogs in Maiduguri (Kamani *et al*., 2010). These studies revealed the high preponderance and spread of veterinary toxoplasmosis in Northern Nigeria. They also show that these animals reported as having high prevalence may represent possible animal source of infection to humans in the region (Emmanuel *et al*., 2013).

Local breed of chickens in Zaria were surveyed serologically using indirect Haemagglutination test, the study reveals possible significant role of chickens in the epidemiology of animal and human toxoplasmosis in Zaria (Aganga, 1985). A review on the role of free-range chickens in the epidemiology of *Toxoplasma gondii* infection also reports a high prevalence of *Toxoplasma gondii* in free-range chickens in Oyo State, Nigeria (Ayinmode, 2012).

The risk-prone group of individuals including foetuses, new-born babies and immunologically impaired patients develop chorioretinitis, lymphadenitis, or rarely, mycocarditis and polymyositis (Jones *et al.,* 2003). Toxoplasmosis can cause abortion, birth defects (Ouermi *et al*., 2009) and reproductive disorders in pregnant women (Montoya and Liesenfeld, 2004). Children with acute congenital toxoplasmosis often die in the first month of life (Akyar, 2011). It causes tremendous losses of animals too, including valuable livestock. Infection of dairy goats with *Toxoplasma gondii* is widespread and constitutes a public health concern (Zhao *et al*., 2011), resulting in significant reproductive losses (Walsh *et al*., 1999; Dubey, 2010.

1.3 Justification for the Study

Toxoplasmosis is one of the most prevalent and most widespread parasitic infections, yet one of the most ignored of all human infections (Emmanuel *et al.*, 2013). In Nigeria, most studies are in pregnant women and immunocompromised individuals. Studies on means of transmission have focused mainly on sheep and goats as intermediate hosts, while a little is done on chickens as a means of transmission to humans. Toxoplasmosis is equally prevalent in HIV-infected patients and healthy adults from similar environments in Northern Nigeria. It is imperative to develop public health policies to prevent toxoplasmosis in Nigeria, especially in HIV-infected patients. Onadeko *et al*. (1996) observed an association between high prevalence of toxoplasmosis and overcrowding with poor environmental sanitation challenges, including considerable contamination with cat faeces. This tends to be common with most live bird markets which also have poor sanitary conditions as regards to poultry slaughter slabs.

Chickens are considered one of the most important hosts in the epidemiology of *Toxoplasma gondii* infection because they serve as an efficient source of infection for cats that excrete the environmentally resistant oocysts and also humans may become infected with this parasite after eating undercooked infected chicken meat (Dubey, 2010a). Domestic chickens feeding on the ground are known to be good indicators of the prevalence of *Toxoplasma gondii* in the environment and are used as sentinel animals in areas where the prevalence of human infection is high (Dubey, 2010b).

Recent studies showed high seroprevalence of *Toxoplamsa gondii* antibodies among the Nigerian population that do not keep pets (Kamani *et al.*, 2009; Akinbami *et al.*, 2010). Although these studies were unable to determine the infection sources, the role of poultry meat cannot be undermined as source of transmission of *Toxoplasma gondii* infection to humans, since most households keep free-range chickens in Nigeria. There is no information on the role of poultry meat and live bird markets, in assessing the level of environmental contamination by *Toxoplasma gondii* and in the transmission of the infection to humans, cats and livestock in Nigeria. Several seroprevalence studies on toxoplasmosis in Nigerian livestock were focused mainly on ruminants, while only scanty reports are available on canids (Kamani, 2010). Most of these studies showed that scavenging on refuse dumps where stray cats abound are the primary source of livestock infection that could subsequently become the infection source for humans. However, while there is increased investigation into the role of livestock and other domestic animals in the epidemiology of *Toxoplasma gondii* infection, the role of free-range chickens is overlooked.

1.4 Aim and Objectives of the Study

1.4.1 Aim of the study

The aim of the study was to determine the Seroprevalence of *Toxoplasma gondii* in local chickens at live bird markets in Katsina metropolis, Katsina State, Nigeria.

1.4.2 Objectives of the study

The objectives of the study were to:

i. determine the seroprevalence of *Toxoplasma gondii* in local chickens at live bird markets in Katsina metropolis, Katsina State.

ii. assess attitudes and practices of workers at the live bird markets towards *Toxoplasma gondii* infection.

iii. assess biosecurity practices at the live bird markets that will mitigate *Toxoplasma gondii* infection among birds.

1.5 Research Questions

i. What is the seroprevalence of *Toxoplasma gondii* in local chickens slaughtered for human consumption at live bird markets in Katsina metropolis?

ii. What are the attitudes and practices of workers at the live bird markets towards *Toxoplasma gondii* infection?

iii. What are the biosecurity practices at the live bird markets that will mitigate *Toxoplasma gondii* infection among birds?

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Toxoplasmosis

Toxoplasmosis was first described in 1908 when found in the blood, spleen, and liver of a North African rodent *Ctenodactylus gondii*. The parasite was named *Toxoplasma* (arc-like form) *gondii* (after the rodent) (Ryan and Ray, 2004). *Toxoplasma gondii* was first described in 1908 by Nicolle and Manceaux at the Pasteur Institute in Tunisia. While carrying out a research on leishmaniasis on the North African rodent, *Ctenodactylus gondi*, the researchers found *Toxoplasma gondii* merozoites in its blood, spleen and liver (Cox, 2002; Sukthana, 2006). In São Paulo, Brazil, *Toxoplasma gondii* was described in laboratory rabbit by Splendore, thus suggesting its worldwide distribution (Frenkel, 1973). The first human case of toxoplasmosis was described by Janků in 1923 (Cox, 2002; Sukthana, 2006). He observed tissue cysts in the retina of an 11-month-old infant in Prague (Sukthana, 2006). In 1939, Wolf *et al.* successfully isolated the parasite from tissue of a neonate with encephalitis by animal inoculation (Cox, 2002).

In 1954, Weinman and Chandler first investigated the possible transmission of the parasite through the consumption of undercooked meat and later substantiated by the work of Jacobs *et al.* (Frenkel, 1973). They found that *Toxoplasma gondii* cysts from pigs, sheep and other experimental animals were resistant to proteolytic enzymes and were readily infectious when swallowed (Frenkel, 1973). In 1970, more than 60 years after the first description of its asexual stage in intermediate hosts, the life cycle of *Toxoplasma gondii*

was completely elucidated by the discovery of a sexual stage in the small intestine of cats (Tenter *et al*., 2000).

Toxoplasmosis was first described by Hepding in a hen from Germany. A *Toxoplasma gondii*-like organisms were found in the retina and was named *Toxoplasma gallinarum* (Dubey, 2010b). This case was considered to be a primary infection with Marek's disease virus with secondary involvement of toxoplasmosis. Other reported cases of toxoplasmosis in chickens were in Italy and Switzerland (Dubey, 2008). Confirmed cases of toxoplasmosis were first reported in a flock of 40 White Leghorn hens from Norway (Dubey, 2010b).

2.2 Host Range of *Toxoplasma gondii*

Domestic and wild felines are definitive (final) hosts while humans and warm-blooded animals especially mammals and birds serve as intermediate hosts. Intermediate hosts acquire the infection by many routes like Ingestion of soil contaminated by oocyst which may be disseminated by means of transport hosts such as flies, cockroaches and worms; ingestion of cysts in raw or undercooked meat and transplacentary infection (Dubey, 2010b). Felids are the most important hosts in the life cycle of *Toxoplasma gondii* because they excrete environmentally resistant oocysts. Cats become infected with *Toxoplasma gondii* by eating infected tissues from intermediate hosts. *Toxoplasma gondii* infection in chickens are considered important as chickens are one of the best indicators for soil contamination with *Toxoplasma gondii* oocysts because they feed from the ground, and tissues of infected chickens are considered a good source of infection for cats (Dubey, 2002, 2006, 2008).

2.3 Distribution of Toxoplasmosis

Toxoplasmosis is a chronic infection with high prevalence that man has had to contend with (Jones *et al*., 2007). Toxoplasmosis is one of the most common parasitic zoonoses worldwide. It is estimated that around a third of the world's population are infected, with the majority of cases being asymptomatic (Innes, 2010; Pereira *et al*., 2010). Despite a large proportion of the population being seropositive for *Toxoplasma gondii*, scientific literature indicates that the seroprevalence is decreasing in several countries including France, Belgium, the United Kingdom and the United State (Rosso *et al*., 2008). The estimated seroprevalance for *Toxoplasma gondii* in domestic cats (*Felis catus*), worldwide, is 30–40% (Dubey, 2010a). Infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions (James, 2003). Sporulated *Toxoplasma gondii* oocysts are very resistant to environmental conditions, and remain infective in humid soil for more than 18 months. However, they do not survive long under cold or dry conditions (Dubey *et al.*, 2000). The distribution of this parasite depends on regions and weather condition where the oocysts survive in environment (Fayer, 1998; Dubey, 2004; Dubey and Jones, 2008). Prevalence of infection varies between countries. In France, for example, about 88% of the population are carriers, probably due to a high consumption of raw and lightly cooked meat (Adam, 2003). High prevalence rates of between 67% and 80% have been reported in Germany, the Netherlands and Brazil, in Britain and South Korea, about 22% and 4.3% respectively are carriers (Tenter, 2000).

2.4 Mode of Transmission of Toxoplamosis

Toxoplasmosis is generally transmitted to humans either congenitally, or via ingestion of undercooked or raw meat from infected animals, or ingestion of food or water contaminated

with oocysts excreted by infected felids (Montoya, 2004). Ingestion of infected chicken meat can be a source of infection for *Toxoplasma gondii* infection in humans and other animals (Dubey, 2010b). The principal modes of *Toxoplasma gondii* transmission are ingestion of faecal oocysts or tissue cysts, and the transplacental transmission of tachyzoites from mother to unborn child. Infection with faecal oocysts may occur by accidentally ingesting contaminated soil (e.g. not washing hands after gardening or eating unwashed fresh produce), drinking untreated contaminated water, eating shellfish grown in contaminated water, or contact with cat faeces (e.g. a cat litter box). Infection from tissue cysts may occur by consuming raw or undercooked meat, by accidentally consuming tissue cysts after handling raw meat and not washing hands thoroughly, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact with raw meat (Abu-Madi *et al*., 2010; Pereira *et al*., 2010). Oocyst-acquired infections in humans are clinically more severe than tissue cyst-acquired infections (Dubey, 2004). As tachyzoites are sensitive to environmental conditions they are usually killed rapidly outside the host and so are rarely involved in foodborne transmission of *Toxoplasma gondii* (Tenter, 2009). Organ transplant recipients can develop toxoplasmosis due to transmission of the parasite with the transplanted organ from a *Toxoplasma*-seropositive donor to a *Toxoplasma*-seronegative recipient. Heart transplantation is the most common type of organ transplantation procedure which occurs with cysts formed in the cardiac muscles (Martina *et al*., 2011; Derouin and Pelloux, 2012). It is also possible that parasite transmission could occur as a result of blood transfusion or haematopoietic stem cell transplantation. The chance of either of these occurring is very low and could only occur if the donor was recently infected with *Toxoplasma gondii* and so had tachyzoites present in their blood and bone marrow (Derouin and Pelloux, 2012). Overall, less than 1% of humans

and livestock acquire *Toxoplasma gondii* infection transplacentally. The proportion of the human population that becomes infected by ingesting *Toxoplasma gondii*-infected meat, food or water contaminated with oocysts is unknown and currently there are no tests to distinguish meat- versus oocyst-acquired infections. Most of the evidence is based on epidemiological investigations and prevalence studies in animals.

2.5 *Toxoplasma gondii* Infection in Man

Asymptomatic toxoplasmosis can occur pre or post-natally (Dubey, 2008). Most persons infected after birth are asymptomatic (Montoya and Liesenfeld, 2004; Remington *et al*., 2006), however, some develop a mild disease or in rare cases, a more severe systemic illness. Once infected, humans are believed to remain infected for life (Dubey, 2008). Unless immunosuppression occurs and the organism reactivates, people usually remain asymptomatic.

Symptomatic toxplasmosis do occur in healthy persons, especially during the first few weeks post exposure. It usually causes mild flu-like illness with signs such as fever, malaise and lymphadenopathy (Montoya and Liesenfeld, 2004; Remington *et al*., 2006). However, in rare cases, humans who were previously healthy have developed severe and even fatal disease, including pulmonary and multivisceral involvement, possibly from more virulent types of the organism (Carme *et al*., 2002; Demar *et al*., 2007). Retinochoroiditis can be due to congenital or post-natally acquired disease and can be associated with acute infection or reactivation (Montoya and Remington, 1996; Holland, 1999). Current thinking is that the majority of ocular toxoplasmosis comes from post-natally acquired disease

(Holland, 1999, 2003). Acute toxoplasmic retinochoroiditis results in pain, photophobia, tearing and loss of vision.

Congenital toxoplasmosis generally occurs when a woman is newly infected with *Toxoplasma gondii* during pregnancy (Remington *et al*., 2006), although rare exceptions have been reported in which women were infected just before pregnancy (Vogel *et al*., 1996). Toxoplasmosis may be associated with other diseases such as HIV/AIDS in humans or persons under immunosuppressive therapy (Akyar, 2011). *Toxoplasma* encephalitis reportedly develops in approximately 40% of individuals with AIDS, and is fatal in 10-30% of these cases (Patton, 1993). Among those chronically infected with AIDS, reactivated infection can result in encephalitis (inflammation of the brain), pneumonitis and neurologic diseases, and can affect the heart, liver, inner ears, and eyes (chorioretinitis) (Dimie, 2013).

2.6 Morphology of *Toxoplasma gondii*

There are three infective stages of *Toxoplasma gondii*: the sporozoite stage, the tachyzoites and bradyzoite stage (Jones *et al.*, 2001; Peterson and Dubey, 2001). Tachyzoites are crescent shape, found during rapidly dividing acute phase and approximately 6 μm long and 2 μm wide (Bhopale, 2003). They are coated by a three-unit membrane, namely a plasmalemma and an inner membrane consisting of two closely situated membranes, all of which form the pellicle (Dubey *et al.*, 1998; Bhopale, 2003). Other organelles in the tachyzoite include conoid, subpellicular microtubules, mitochondria, micropores, smooth and rough endoplasmic reticula, apical and polar rings, rhoptries, micronemes, Golgi complexes, ribosomes and a nucleus consisting of a chromatin mass and a nucleolus (Dubey *et al.*, 1998) (Figure 2.1). Bradyzoites are found in quiescent tissue cysts, usually

in the brain where cysts are spheroidal in shape, and in muscle tissue where they are elongated (Dubey *et al.*, 1998; Roberts and McLeod, 1999) (Figure 2.2). Tissue cysts are able to persist for the duration of the host's life and bradyzoites can be released from these cysts to form tachyzoites again, causing a reactivated infection in immunocompromised hosts (Montoya and Liesenfeld, 2004). Oocysts are 10 μm to 12 μm in size and are produced within the intestine of the definitive host. Four Sporozoites are found in two sporocyst within the oocysts, which are shed in the faeces of the definitive host (Peterson and Dubey, 2001).

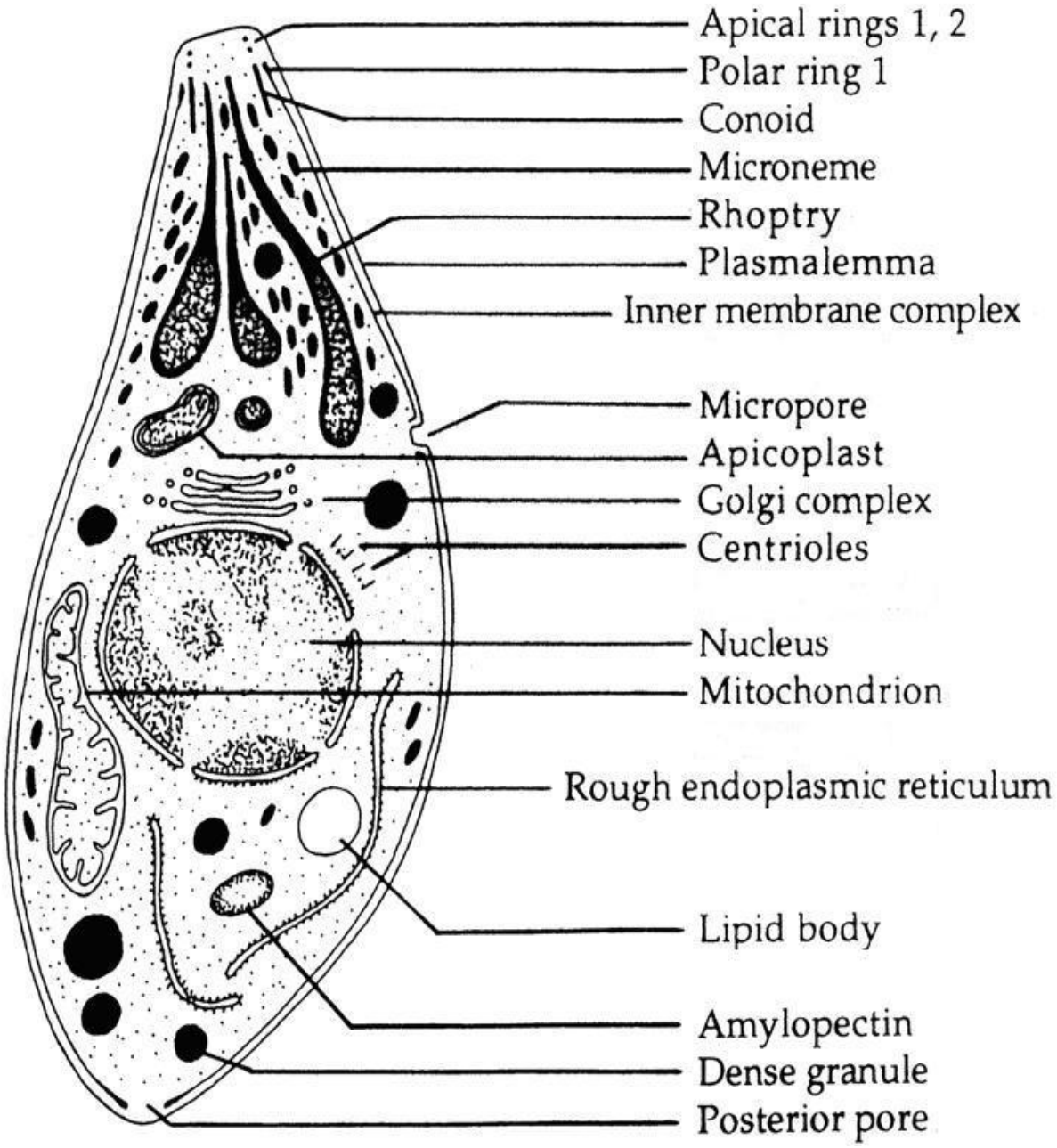


Figure 2.1: Diagram of a Tachyzoite of *Toxoplasma gondii* (Adapted from Dubey *et al*., 1998.).

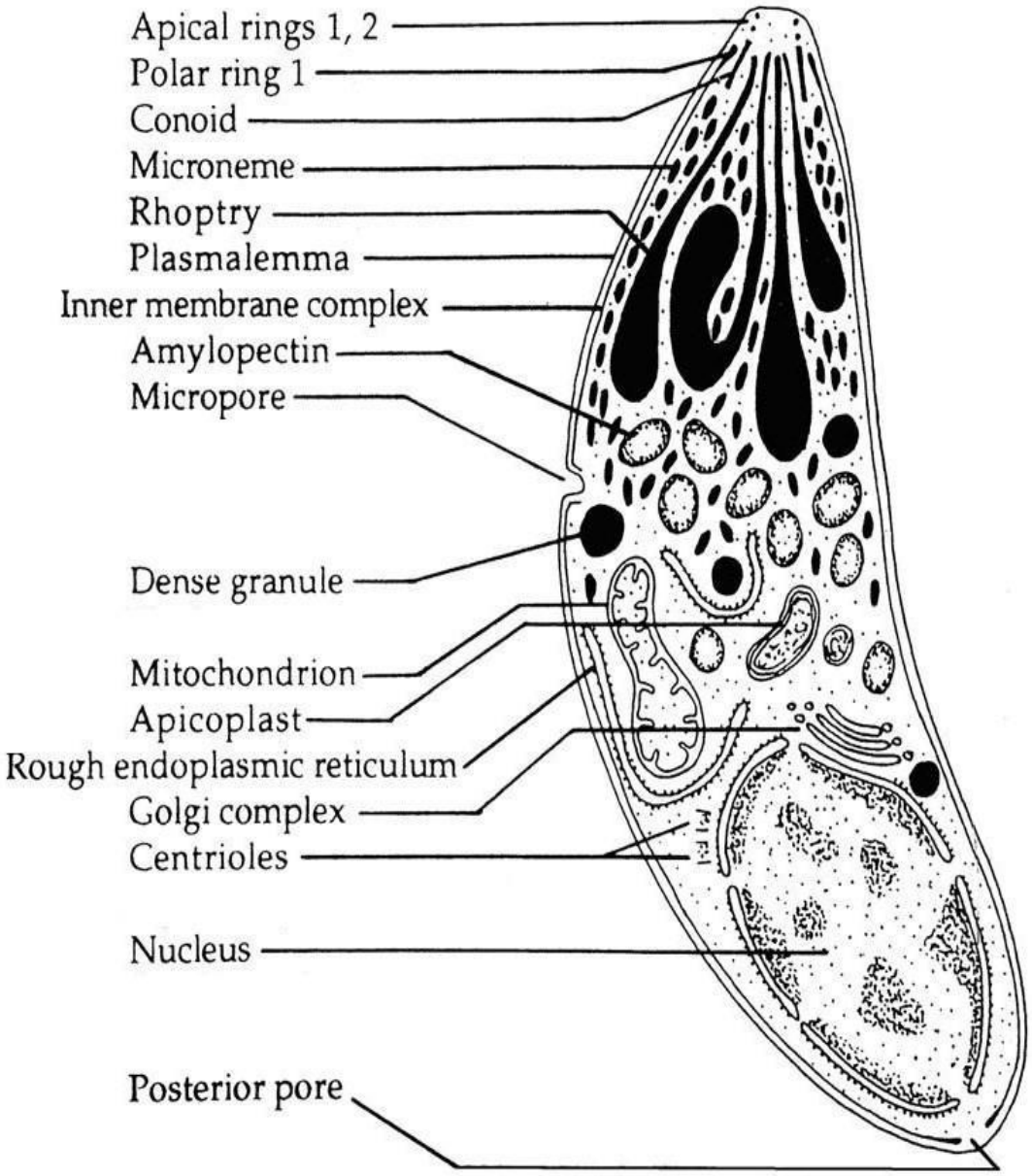


Figure 2.2: Diagram of a Bradyzoite of *Toxoplasma gondii* (Adapted from Dubey *et al*., 1998).

2.7 Life Cycle of *Toxoplasma gondii*

Toxoplasma gondii has a two-stage life cycle, consisting of a sexual phase in the definitive host (felidae), and an asexual phase occurring in the intermediate host (warm blooded animals). The definitive hosts can be infected either by ingestion of parasites within tissue cysts or oocysts. There are five distinct asexual phases that occur in enterocytes before gametogony begins. Microgametes fertilize macrogametes in the enterocyte forming fertilized zygotes. A wall is then laid around each zygote forming unsporulated oocysts, which are released into the intestinal lumen when enterocytes rupture. These oocysts are then released into the environment with the faeces (Peterson and Dubey, 2001). The prepatent period, which is the period between ingestion and shedding, is approximately three to ten days (Tenter *et al*., 2000; Peterson and Dubey, 2001). Infected cats can shed more than 100 million oocysts in their faeces, which may continue for up to 20 days (Dubey, 1972; Jone *et al*., 2010). Cats not previously infected with *Toxoplasma gondii* shed oocysts after ingestion of infectious stages, whereas cats that have been previously infected are generally immune against renewed oocyst shedding. This immunity does not, however, continue throughout the duration of the cat's life (Petersen, 2003). Depending on the temperature, aeration and humidity of the environment, oocysts begin to sporulate within five days, dividing into two sporocysts, each containing four sporozoites. These sporulated oocysts are very resistant to environmental condition and are infectious for months in the environment (Peterson and Dubey, 2001). Once ingested by an intermediate host, the outer walls of cysts or oocysts are disrupted by enzymatic degradation, releasing either bradyzoites or sporozoites into

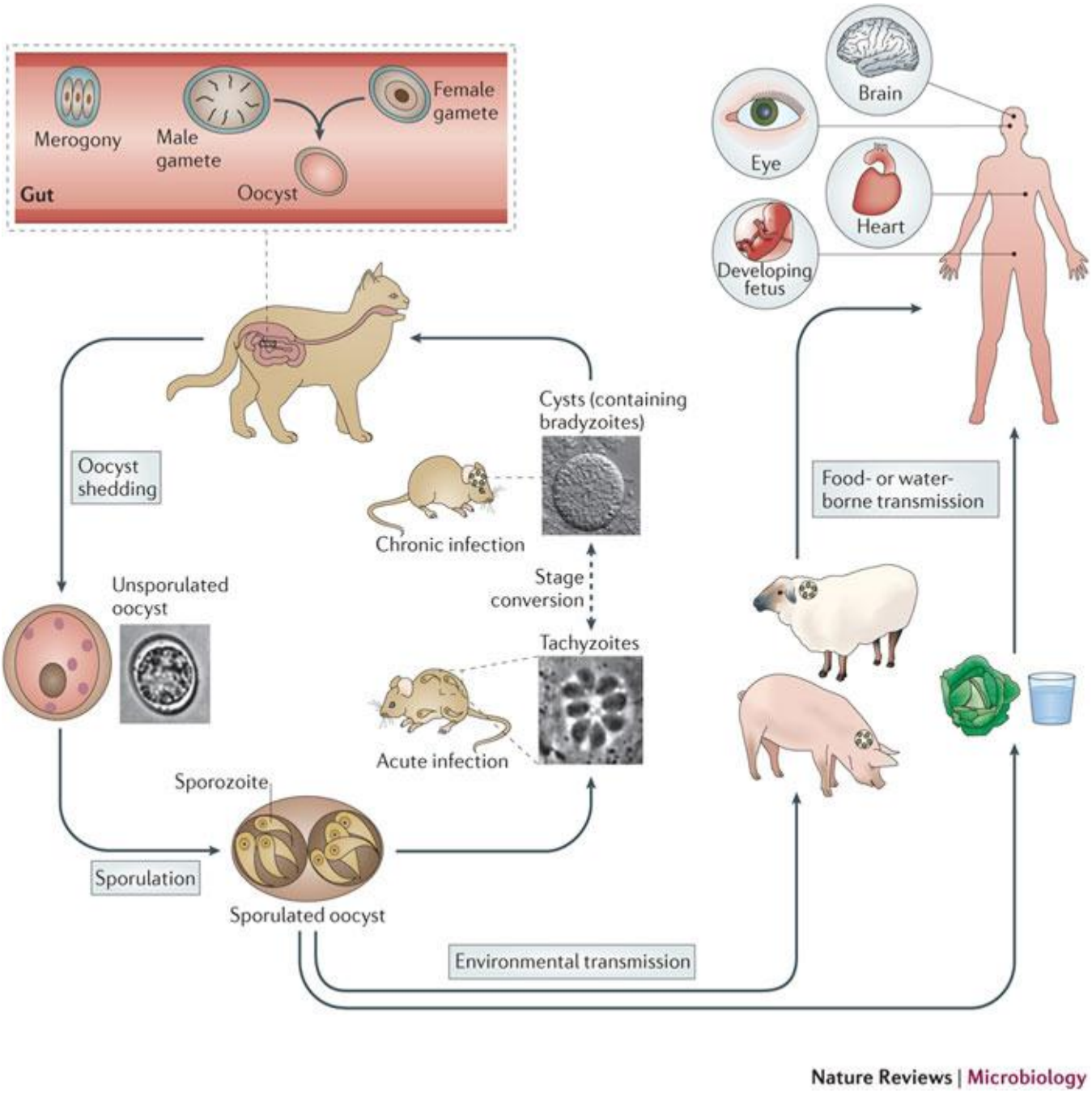


Figure 2.3: Life cycle of *Toxoplasma gondii* (CDC, 2010)

the intestinal lumen. These then actively invade surrounding cells and transform into tachyzoites. Upon entering the host cell, the parasite pulls the host cell membrane around itself and is then surrounded by a parasitophorous vacuole (Bhopale, 2003; Petersen, 2003) (Figure 2.3).

Tachyzoites rapidly divide within the host cell, leading to its rupture, releasing the parasites into the blood and lymphatic system, where they are carried to other cells which they then invade, repeating this process. Tissue cyst usually arise 7 to 10 days post-infection (Bhopale, 2003; Montoya and Rosso, 2005).

2.8 Adaptability of *Toxoplasma gondii*

Certain environmental conditions determine the time taken for oocysts to sporulate and become infectious, which usually ranges from one day to several weeks. Humidity, aeration and temperature are the major conditions that affect the length of time required for sporulation to occur, with lower temperatures slowing the sporulation rate (Lindsay *et al*., 2002; Jones *et al*., 2003; Hill *et al*., 2007). A study conducted by Lindsay (2002) demonstrated that unsporulated oocysts can survive in the environment at 4°C and retain their ability to sporulate for at least 3 months. Sporulated oocysts are more resilient than unsporulated oocysts. Once sporulated, oocysts maintain infectivity in moist soils for up to 18 months (Frenkel *et al*., 1981) and in water and seawater for several years at 4°C (Dubey, 1998a; Lindsay and Dubey, 2009). The duration of infectivity, however, decreases with increasing temperatures. Infectivity is maintained for at least 200 days in the temperature range of 10–25°C, for 1 month at 35°C, for 1 day at 45°C and sporulated oocysts become

non-infective after 1 minute at 60°C (Dubey, 1998a). Unsporulated oocysts die within 24 hours when stored at 37°C, whereas sporulated oocysts can survive for over a month at 35°C and 9 days at 40°C (Lindsay *et al*., 2002). Constant freezing at -21°C kills unsporulated and sporulated oocysts within 1 and 28 days, respectively (ESR, 2010).

Toxoplasma gondii tissue cysts remain viable in infected meat stored at refrigeration temperatures of 4°C for up to 19 days. Cooking infected meat to internal temperatures of 67°C or higher inactivates the tissues cysts (Dubey *et al*., 1990; Dubey 2004). Freezing meat at -10°C for 3 days or -20°C for 2 days or treatment with gamma irradiation at a dose of 75 krad is also sufficient to kill tissue cysts (El-Nawawi *et al*., 2008). Tachyzoites that may be found in the milk of intermediate hosts are inactivated by pasteurisation (Tenter *et al*., 2000). Bradyzoites found in tissue cysts are resistant to gastric digestion, whereas tachyzoites are usually destroyed by the acid and proteolytic enzymes of the stomach (Tenter, 2009). Experimental evidence indicates that tachyzoites may survive in acid-pepsin solution for up to 2 hours (Dubey, 1998b) and the type of meal eaten may also increase the pH of the stomach and allow tachyzoites to traverse the stomach into the small intestine in an infective state (Tenter, 2009).

2.9 Pathogenesis and Virulence of *Toxoplasma gondii*

The number of faecal oocysts or tissue cysts required to cause *Toxoplasma gondii* infection in humans has not been established (ESR, 2010). However, using a pig animal model it was demonstrated that as few as one sporulated oocyst was able to cause infection. These findings are biologically interesting because *Toxoplasma gondii* was considered a neurotropic parasite based on studies in rodents. However, there are now ample data from

chickens and other animals indicating that *Toxoplasma gondii* encysts in muscle more efficiently than in the brain (Dubey and Beattie, 1988).

Tachyzoites are spread via the blood system in lymphocytes, macrophages and free in the plasma and are able to infect almost any type of tissue, especially those in the eye, central nervous system, heart, placenta and skeletal muscle (Montoya and Liesenfeld, 2004). They are capable of crossing tissue boundaries, such as the blood-brain barrier and the placenta (Carruthers, 2002). They are able to multiply rapidly by endodyogeny and such replication leads to cell necrosis, when invaded cells can no longer hold these parasites and erupt. Replication of tachyzoites occurs during the first 8-12 days and accounts for the acute phase of infection (Carruthers, 2002). This stage is responsible for the clinical manifestations of the disease as it produces a strong inflammatory response (Montoya and Liesenfeld, 2004). As the host develops immunity, the parasite retains its overall size and shape but transforms into the bradyzoite stage and multiplies more slowly within tissue-cysts to establish a persistent infection. These microscopic tissue cysts are present most frequently in brain and skeletal muscle and represent the quiescent stage of the parasite within the host. Viable tissue cysts within muscle (meat) are a significant source of human infection. In animals that succumb to acute infection, tachyzoites may be demonstrated in ascitic fluid or in lung impression smears as well as in tissue sections of the liver and other affected organs.

2.10 *Toxoplasma gondii* infection in chickens

Chickens play an important role in the epidemiology of *Toxoplasma gondii* in the rural environment, perhaps more than rodents, because they are clinically resistant to *Toxoplama*

gondii and live longer than rodents. Cats fed naturally infected chicken tissues can shed millions of oocysts (Dubey *et al.,* 2002). Chickens fed *Toxoplasma gondii* oocysts were killed 1–2 weeks later and their tissues were fed to cats. Cats fed tissues of chickens that had been infected 10 days earlier shed oocysts (Ruiz and Frenkel, 1980). Chickens can harbour mouse-virulent *Toxoplasma gondii* without showing any clinical signs (Dubey *et al*., 2002).

2.11 Epidemiology of *Toxoplasma gondii*

Toxoplasma gondii has a worldwide distribution, but its prevalence varies greatly. Serological studies have indicated a rate of 10-17% in Western Europe, 34-93% in Central Europe, 16-40% in North America, 35-76% in Central America, 2.3-58% in Southeast Asia and 7-80% in Africa (Sukthana, 2006). Seropositivity rate also varies greatly between countries and within the same country (Jones *et al.,* 2001). The distribution of this parasite depends on regions and weather condition where the oocysts survive in environment (Fayer, 1998; Dubey, 2004; Dubey and Jones, 2008). Another factor that determines distribution of the disease is the cooking culture of the people. In France, for example, about 88% of the population are carriers, probably due to a high consumption of raw and lightly cooked meat (Adam, 2003). High prevalence rates of between 67% and 80% have been reported in Germany, the Netherlands and Brazil (Heckeroth, 2004), while in Britain and South Korea, and about 22% and 4.3% respectively are carriers (Weiss, 2004). Infection is reportedly highest in countries where undercooked meat is traditionally consumed (Dubey, 2008).

A worldwide survey was of *Toxoplasma gondii* infection in chickens was carried out with seroprevalence rate varying from 36.3-65.5% in Argentina, 40-66% in Brazil, 18.7-40.4% in Egypt, 64% in Ghana, 16.9-100% in USA, 27.1% in Portugal, 13.3% in Kenya. 13.7% in Italy (Dubey, 2008).

2.12 Clinical Manifestattion of Chicken Toxoplasmosis

Chickens are considered resistant to clinical toxoplasmosis. There are only a few reports of clinical toxoplasmosis in chickens worldwide. Historically, toxoplasmosis was first described by Hepding (1939) in a hen from Germany. Confirmed toxoplasmosis was first reported by Erichsen and Harboe (1953) in a flock of 40 White Leghorn hens from Norway. Clinical signs observed were anorexia, emaciation, diarrhoea, blindness and sudden death. Microscopically, lesions observed include sciatic nerve neuritis, chorioretinitis, encephalitis, myocarditis and pericarditis (Dubey, 2010b).

In the last 53 years, no literature has reported clinical toxoplasmosis with these aforementioned characteristics. Recently, a clinical toxoplasmosis was reported in three chickens from Georgia, showing peripheral neuritis and the diagnosis was made using immunohistochemical technique. (Goodwin *et al*., 1994; Dubey *et al*., 2007). Also, in Illinois, three birds died suddenly out of a group of 14 backyard chickens. Clinical manifestations were inability to stand, torticollis and lateral recumbency. At necropsy, lesions were limited to the brain which had multiple areas of necrosis, perivascular lymphocytic cuffs and gliosis. A strange finding was the presence of numerous tissue cysts and tachyzoites in the lesions. The protozoa in the brain reacted positively with *Toxoplasma gondii* antibodies (Dubey, 2008).

2.13 Diagnosis of Toxoplasmosis

Considering that toxoplasmosis is usually asymptomatic and clinical manifestations are non-specific and mimic other infections, hence making definitive clinical diagnosis is very difficult (Hill and Dubey, 2002; Kompalic-Cristo *et al.*, 2004). There are two major diagnostic techniques for *Toxoplasma gondii*; Identification of the agent and Serological test.

2.13.1 Identification of *Toxoplasma gondii*

Diagnosis is usually made by immunological testing, histological identification, isolation in tissue culture, recovery of the parasite DNA by the polymerase chain reaction (PCR) or by a combination of these techniques. Cerebral toxoplasmosis can also be diagnosed using computerized tomography and magnetic resonance imaging (Hill and Dubey, 2002; Markus, 2003; Sukthana, 2006).

i. Isolation: Isolation of *Toxoplasma gondii* from chicken tissue is best made by inoculation of laboratory mice. The tissues usually used for inoculation are brain and heart tissues, fresh and contamination free samples ensure better result. Samples must not be frozen at any stage, as this kills the parasite. Aseptically obtain brain tissue from the chicken and homogenize in sterile phosphate buffered saline (PBS), pH 7.4, with added antibiotics (100 International Units [IU]/ml penicillin and 745 IU/ml streptomycin). Inoculate each of three *Toxoplasma*-free mice intraperitoneally with the homogenate. Kill the mice 6–8 weeks after inoculation and harvest the brain. Homogenize the mouse brain and carry out Giemsa stain with the homogenate. Examine slides under a microscope for cyst in circular shape filled

with blue-staining, crescent-shaped bradyzoites. Isolation of *Toxoplasma gondii* from samples is expensive and slow (Dubey *et al*., 1995a)

ii. Tissue section: In animals that die with acute toxoplasmosis, focal mononuclear inflammation with or without focal necrosis may be seen in a number of tissues, including the liver, heart and lungs. The latter may be oedematous. Lymph nodes may have undergone expansion and there may or may not be focal necrosis with or without haemorrhage. Typically *Toxoplasma* tachyzoites may be demonstrable in association with necrosis and inflammation. Sometimes small numbers of intracellular and extracellular toxoplasms are visible, usually on the periphery of a necrotic area or in a villus that is in the early stages of infection. The *Toxoplasma* tachyzoites appear ovoid, 2–6 μm long, with nuclei that are moderately basophilic and located centrally or towards the posterior end. Immunohistochemistry is a sensitive and convenient method to confirm the identity of *Toxoplasma gondii*- structures in tissue sections , as well as from instances of acute toxoplasmosis. This is possible because it labels intact *Toxoplasma gondii* or antigenic debris. This method can be used with fixed tissues (including archive tissues) that may also exhibit a degree of decomposition, where isolation would not be appropriate or possible (Silva *et al*., 2001).

iii. Nuclei acid recognition method: Polymerase chain reaction is usually used to identify parasite DNA in tissues. Several polymerase chain reaction (PCR)-based assays have been developed for the detection of DNA from *Toxoplasma gondii*. The main target regions are the B1 repetitive sequence (Burg *et al*., 1989), the P30 (SAG1) gene (Savva *et al*., 1990) and 18S ribosomal RNA (rRNA) (Ellis, 1998). The sensitivity of the PCR is dependent on the copy number of the target sequence (P30: 1 copy; B1: 35 copies; rRNA: 110 repeat

units). Customised synthetic DNA oligonucleotides are commercially available. However, although the PCR is extremely sensitive, care should be taken if it is the only test available, as in many situations a more reliable diagnosis will be gained if it is used in combination with other diagnostic data. Real-time PCR allows for simultaneous quantification and amplification of DNA. It is very similar to existing PCR methods and can be carried out on 96-well microtitre plates. The real-time PCR is a highly sensitive and specific method, however it is expensive and requires specialized detection systems and therefore may only be cost-effective in laboratories where analysis of large numbers of samples is carried out. Immune status of the individual do not affect the detection of nucleic acid when PCR is been used. It is generally more sensitive and rapid than serological tests and diagnosis can be made from biopsies, blood, cerebrospinal fluid (CSF) and amniotic fluid. Disadvantages are that false positive results due to contamination may occur, it may be too sensitive in detecting nonviable *Toxoplasma gondii* remnants that do not cause disease, and may yield false negative results due to inhibition (Johnson *et al.*, 1993).

2.13.2 Serological test

Serological tests are most widely used, yet they have the greatest limitations as they often provide ambiguous results (Markus, 2003; Kompalic-Cristo *et al.*, 2004). Examples include the Sabin-Feldman dye test, which is the traditional gold standard, Indirect fluorescent antibody assay (IFA), Complement fixation test (CFT) and the Enzyme-linked immunosorbent assay (ELISA) (Hill and Dubey, 2002).

Criteria such as the given colour of tachyzoites under a microscope (Dye Test and IFA test),

the principle of agglutination of *Toxoplasma* tachyzoites (Direct agglutination test (DAT), red blood cells (indirect haemagglutination test (IHA) or latex particles (Latex agglutination (LA) test), also the degree of colour change which defines the quantity of specific antibody in a given

solution (ELISA).

i**.** The Dye Test is one of the most sensitive and specific tests for the diagnosis of toxoplasmosis in humans. However, it does not work with chicken sera or gives erratic results (Dubey, 2008). In addition, it is potentially hazardous as live parasite is used. It is expensive and requires a high degree of technical expertise. The failure to detect the antibodies in chicken sera by the Dye Test is not related to antigenic differences between mammalian and avian strains of *Toxoplasma gondii* and does not apply to all birds because pigeons develop high Dye Test antibody titres (Frenkel, 1981). The Dye Test is a complement-mediated test and erratic results with Dye Test in chickens may be related to fixation of the first component of complement (Frenkel, 1981).

ii. The Complement Fixation Test, like the Dye Test, did not detect antibodies to *Toxoplasma gondii* in chicken sera. Erichsen and Harboe (1953), Robertson (1963) developed a complement inhibition test to detect *Toxoplasma gondii* antibodies in chicken sera (Dubey, 2010b).

iii. The IHAT is easy to perform and kits are available commercially, hence it has been used in some surveys to detect antibodies to *Toxoplasma gondii* in chicken sera. However, it is an insensitive test (Dubey, 2008). There is limited information on the accuracy of the IFAT test for the detection of *Toxoplasma gondii* antibodies in chicken sera (Dubey, 2008).

The IFA test is a simple and widely used method. Whole, killed *Toxoplasma* tachyzoites are incubated with diluted test serum, the appropriate fluorescent antispecies serum is added, and the result is then viewed with a fluorescence microscope (Munday *et al*., 1971). Fluorescent-labelled antibodies are available commercially for a variety of animal species, the method is relatively inexpensive and kits are commercially available. However, the method requires a fluorescence microscope and the results are read by eye, so individual variation may occur. It may be difficult to find some species-specific conjugates and there is a risk of possible cross-reactivity with rheumatoid factor and anti-nuclear antibodies (Dubey, 2010b).

iv. The Direct Agglutination Test is both sensitive and specific. Formalinised *Toxoplasma* tachyzoites are added to U-shaped well microtitre plates and dilutions of test sera are then applied. Positive samples will produce agglutination that can be graded, while negative samples will produce a 'button' of precipitated tachyzoites at the bottom of the well (Desmonts and Remington, 1980). The test is simple and easy to perform although relatively large amounts of antigen are required. Kits are commercially available. The Direct Agglutination Test and Latex Agglutination Test are not species specific and are suitable for use in all species (Dubey, 2010b).

v. The ELISA test has the advantage that it can be automated and is convenient for large-scale surveys. Originally, ELISA uses a soluble antigen preparation made from *Toxoplasma* RH strain tachyzoites and layered into wells in a microtitre plate. Test sera (e.g. ovine in origin) are added, followed by an anti-species enzyme-labelled conjugate such as horseradish peroxidase-labelled anti-ovine-IgG. Any attached conjugate causes a colour change in the substrate that is directly related to the amount of bound antibody, and which

can be read with a spectrophotometer at the absorbance specific to the substrate used (Voller *et al*., 1976). The assay is simple, can readily test a large number of samples and is easy to perform with the chosen anti-species conjugate (Dubey, 2010b).

Defined anti-species conjugates, substrates and whole kits are commercially available. However, the assay does require a spectrophotometer. The ELISA is well suited to laboratories required to analyse large numbers of samples. Recently, a kinetics ELISA (KELA) has been developed. The KELA system measures the rate of reaction between bound enzyme and the substrate solution that leads to development of colour (Were *et al.*, 2002).

A problem with serological tests is that the detection of antibodies in immunocompromised individuals may be difficult due to the deterioration of the immune system (Schneider *et al.,* 1992). Avidity tests have helped to overcome this problem as they help differentiate between recently and distantly acquired infections (Lappalainen and Hedman, 2004; Montoya and Rosso, 2005). Avidity tests are based on the fact that during acute infections, IgG antibodies bind antigen relatively weakly and therefore have a low avidity. Chronic infections, however, have more strongly-binding antibodies and therefore have a high avidity (Lappalainen and Hedman, 2004; Montoya and Rosso, 2005).

2.14 Treatment and Control of Toxoplasmosis

Traditional drug therapy for clinical toxoplasmosis consists of a combination of pyrimethamine and sulfonamides. This combination can cause dose-related bone marrow suppression with resultant anemia, leucopenia, thrombocytopenia, and reversible acute

renal failure. Leucovorin (folinic acid) could be added to the combination to prevent bone marrow suppression and to reduce the severity of congenital infection and increase the proportion of infants asymptomatic at birth (Daffos *et al*., 1988). Spiramycin is one of the current drugs of choice for treatment of infected pregnant women. Treatment may decrease the severity of congenital toxoplasmosis or long term consequences, but possibly not the risk of transmission (Tenter *et al*., 2000).

2.15 Immunity against *Toxoplasma gondii*

Toxoplasma gondii induces a strong type 1 T-cell-mediated immune response, which promotes a self-limiting infection, ensuring the survival of its host and thus itself (Carruthers, 2002). As the immune response progresses, interferon-γ, secreted by antigen-specific T-cells, restricts tachyzoite replication. Tachyzoites are sensitive to proteolytic enzymes and can therefore be destroyed during gastric digestion. The pressure of the host's immune system on the parasite stimulate the formation of cysts and causes tachyzoites to be transformed into bradyzoites, marking the beginning of the chronic phase of the infection (Carruthers, 2002; Montoya and Liesenfeld, 2004). Bradyzoites, the slowly dividing stage of the parasite, are more resistant to proteolytic enzymes and are therefore able to cause infection if ingested as a tissue cyst by a host (Tenter *et al*., 2000).

2.16 Prevention of Toxoplasmosis

The risk of infection can be reduced by properly cooking meats to a temperature sufficient to kill *Toxoplasma gondii*. Beef, lamb and veal steaks or roasts should be heated to achieve internal temperature of at least 63°C (145°F), poultry meat should reach a temperature of

82°C (180°F) especially in the thigh. Freezing, salting, pickling and smoking do not reliably destroy *Toxoplasma gondii*. Fruits and vegetables should be peeled or washed thoroughly to remove oocysts. Good hygiene is important in preventing infections (Tenter *et al*., 2000). Kitchen items should be washed with hot soapy water after they have contacted raw meats, raw seafood or unwashed fruits and vegetables. Hands should be washed after contact with raw meat, soil or sand and before eating or touching the face. Pregnant women and others at risk should wear gloves when gardening and during other soil or sand contact (Hill and Dubey, 2002). To help prevent transmission of *Toxoplasma gondii* to humans, cats should be fed only commercial pet food or well-cooked meats. Indoor cats are less likely to transmit toxoplasmosis than outdoor cats. Litter boxes should be cleaned daily to reduce the risk of oocyst sporulation, and rinsed with boiling water. Pregnant women should avoid cleaning the litter box; if this is unavoidable, they should use gloves then wash their hands. Human vaccines are not available (Hill and Dubey, 2002). For adequate prevention in livestock, cats should be kept away from pastures and barns where possible. After an abortion, the placenta and abortion products should be removed, and the area cleaned and disinfected (Buxton, 2000). A modified live vaccine is also available for sheep in Europe and New Zealand. The only available vaccine is a commercially produced live preparation for sheep (Toxovax, Intervet BV, The Netherlands; Toxovax, AgVax, Ag Research, New Zealand), currently licensed for use in certain European countries and New Zealand. It consists of tissue culture grown S48 *Toxoplasma gondii* tachyzoites attenuated by over 3000 passages in mice. The vaccine stimulates effective protective immunity for at least 18 months following a single subcutaneous injection, but as it is unable to produce tissue cysts, sheep are not left with a persistent vaccinal infection. The vaccine has a short shelf life and is a potential risk to

immunosuppressed and pregnant female operatives. The vaccine should be stored and used strictly according to the manufacturer's instruction, thus it should not be frozen at any time, should be maintained cool (around 4°C) and not exposed to direct sunlight. The diluent provided should be added to the concentrated suspension of tachyzoites immediately prior to use (Buxton, 1993).

2.17 The Situation of Toxoplasmosis in Nigeria

In Nigeria, toxoplasmosis has been reported both in man and some animals. A study carried out among pregnant women attending Antenatal Clinics at University College Hospital, Ibadan, and St. Mary's Catholic Hospital, Ibadan, showed very high prevalence of *Toxoplasma* antibodies in the sera of both pregnant (75.4%) and postpartum (80.5%) women (Onadeko *et al*., 1996). In the Middle belt region of the country, high prevalence of toxoplasmosis has been reported among pregnant women from Benue State. Among women of the 39-42 age bracket, 71.4% presented with serological evidence of toxoplasmosis (Olusi *et al*., 1996). In Northern Nigeria, work has been reported on veterinary toxoplasmosis. High seroprevalence of toxoplasmosis have been reported among some animals of economic importance, such as sheep (Okoh *et al*., 1981), pet dogs in Zaria (Aganga and Ortese, 1984), and dogs in Maiduguri (Kamani *et al*., 2010). These studies reveal the high preponderance and spread of veterinary toxoplasmosis in Northern Nigeria. They also show that these animals reported as having high prevalence may represent possible animal sources of infection to humans in the region (Emmanuel *et al*., 2013). A study on Local breed of chickens in Zaria reveals possible significant role of chickens in the epidemiology of animal and human toxoplasmosis (Aganga, 1985). The role of free-range chickens in the epidemiology of *Toxoplasma gondii* infection was reported to have a high

prevalence of *Toxoplasma gondii* in free-range chickens in Oyo State, Nigeria (Ayinmode, 2012). A study on *Toxoplasma gondii* isolates from chickens in six different African countries (Nigeria, Congo, Egypt, Burkina Faso, Kenya and Mali) revealed four genotypes. Most isolates belonged to the clonal types II and III strains, with one Nigerian isolate having an atypical genotype (Velmurugan *et al*., 2008).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study Area

Katsina State is located on 12.5139°N 076114°E with altitude of 182.82 -457 metres above sea level. The population of Katsina State is 5,792,578 (NPC, 2006). It has 34 Local Government Areas and covers an area of 24,971 km^2 (Figure 3.1). It falls into the ecological zone of Sudan Savannah, characterized by a short rainy season with annual rainfall of about 500 to 800 mm from June to October. It has a minimum and maximum temperatures between 21°C and 35°C and it also has temperature variations, where it is cool in the morning, hot in the afternoon and cool again in the night in the month of December to March. The area has a relative humidity of 20% to 40% in January, rising from 60% to 80% in July (Sunmap, 2011).

3.2 Study Design

A cross-sectional study approach was used for determining the seroprevalence of *Toxoplasma gondii* in slaughtered chickens in the study area. Two major live bird markets within Katsina metropolis were used for sample collection between January to March, 2015. Majority of local chickens at these markets were sourced from village weekly markets across Katsina State by the sellers at the live bird markets. Live birds are sold at the markets, where they are slaughtered and dressed for prospective customers.

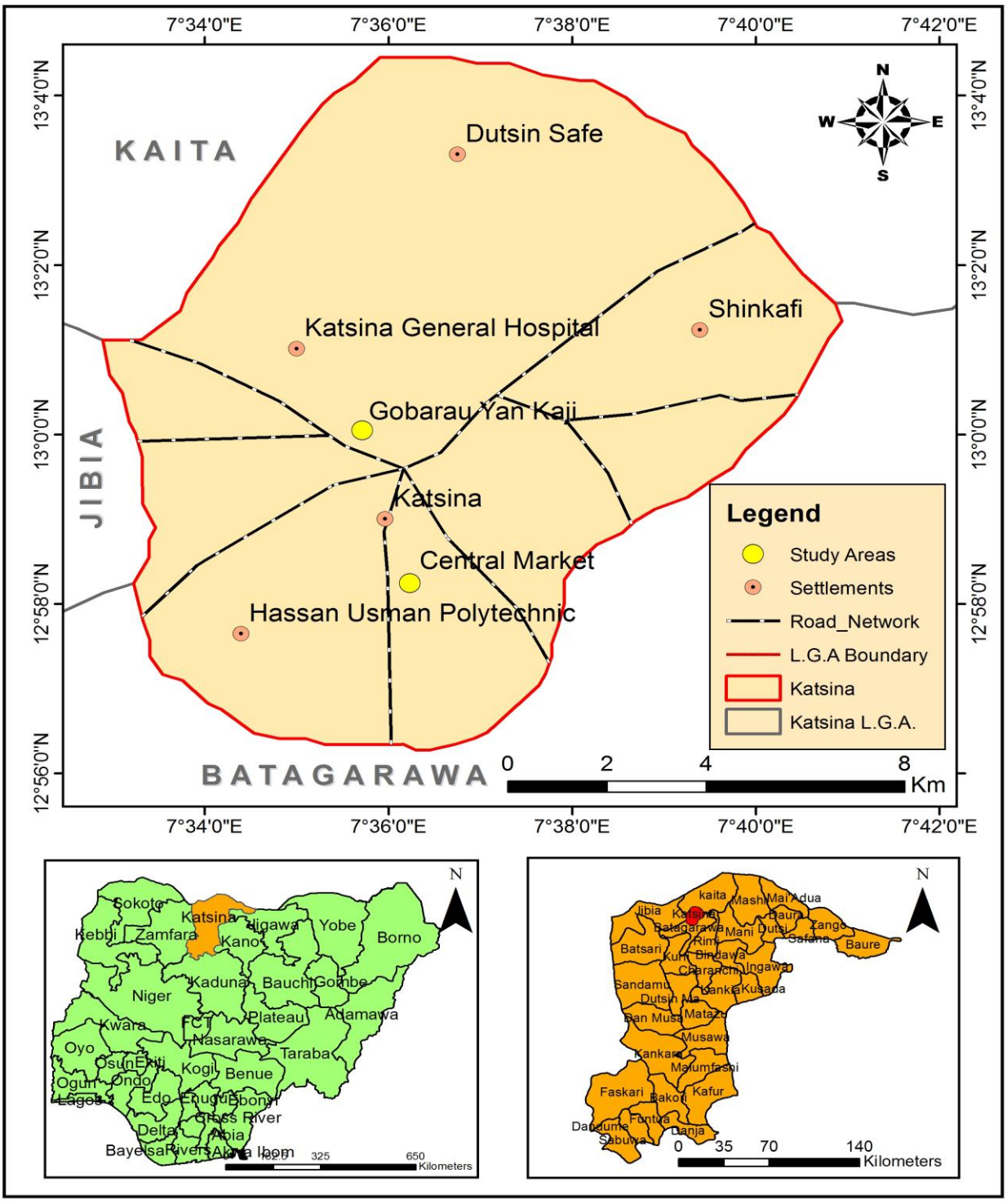


Figure 3.1; Map of Katsina State showing Study area and the 34 LGAs (Quick Bird Imagery; 2013)

3.3 Sample size

Sample size was determined using the formula by Thrusfield (2005).

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

N=sample size;

Z=standard normal deviation of 95% confidence level (1.96)

At the prevalence of 50% in chickens

q=1-p

d=level of significance or standard error (0.05)

Hence, N = $\frac{(1.96)^2 \times 0.5 \times 0.5}{(0.05)^2}$ = 0.9604/0.0025 = 384.16

The sample size was increased to 460 to increase precision and minimize sampling error. Therefore, a larger sample size of 276 birds was collected at Gobarau LBM because of availability and cooperation of the workers while 184 birds were collected from Central market.

3.4 Administration of Structured Questionnaire

Structured questionnaires were administered, of which 20 were administered at Central LBM while 30 were administered at Gobarau LBM giving a total of 50. It was administered through oral interview using Hausa language to sellers and processors of chickens at the markets. The respondents were selected based on willingness to participate. There were 28

close ended questions which were used to obtain information on respondent's demographic data, attitudes, practices towards *Toxoplasma gondii* infection and biosecurity practices.

3.5 Collection of Samples

The plunger of 10 ml syringe was removed while the syringe container was used to collect 5 ml of blood at slaughter point of chickens in the live bird markets . The syringe was kept slanted for two hours, the upper clear region (serum) was carefully decanted into a plain tube, it was labeled and immediately placed into an ice pack. At the end of sample collection from both markets, the samples were transported in ice pack to the Veterinary Public Health and Preventive Medicine Laboratory of Ahmadu Bello University for serological test. Sera were then frozen until analyzed.

3.6 Serological Test

Indirect Enzyme Linked Immuno-sorbent Assay (ELISA) was carried out for the detection of anti-*Toxoplasma* antibodies in chicken sera using TOXOS-AV ver0714GB (ID Screen®, ID.vet, Paris, France). This technique uses microwells coated with *Toxoplasma* antigen, in which the samples were added for testing. An anti-*Toxoplasma* antibody, if present in the sample, forms an antigen-antibody complex. An anti-chicken peroxidase (Horse radish peroxidase) conjugate is added after washing the wells so as to fix the antibodies, therefore forming an antigen-antibody-conjugate-HRP complex. The excess conjugate was eliminated by washing using substrate solution. The resulting coloration depends on the quantity of specific antibodies present in the sample to be tested. A blue solution indicates the presence of an antibody, which becomes yellow on addition of a stop solution.

The samples and reagents were allowed to cool to room temperature, the reagents were homogenized by repeated inversion. About 90 ul of dilution buffer14 was added to each microwell. 10 ul of the negative control and positive control were added to well C1, D1 and A1, B1 respectively, while 10 ul of sera sample were added to the remaining wells. It was then incubated for thirty minutes at room temperature. The wells were emptied and washed three times with 300 ul of wash solution. About 100 ul of a prepared conjugate was added to each well and incubated for thirty minutes at room temperature. The wells were emptied and washed three times with 300 ul of wash solution. 100 ul of substrate solution was added to each well and incubated for fifteen minutes at room temperature in the dark. 100 ul of stop solution was added to each well in order to stop the reaction. The plate was then placed on 96-well microplate reader to read and record the optical density (OD) at 450 nm.

3.7 Scoring of Questionnaire

The items in the domain of the questionnaire were scored by method adopted from Iyor (2005). In scoring of respondent attitude, each positive attitude attracted one mark. Negative or no response attracted zero marks. The total marks scored by each respondent was divided by 8 and multiplied by 100 to obtain a percentage for attitude. Similarly, correct responses on the items designed to evaluate their practices attracted one mark. Wrong or no response attracted zero marks. The total marks scored by each respondent was divided by 6 and multiplied by 100 to obtain a percentage score for practice. Attitude and practice scores were classified into three categories thus; 0 to 39 poor, 40 to 59 fair and 60 to 100 good.

3.8 Data Analyses

Data generated from microplate reader were entered into Microsoft Excel® (Microsoft Corporation, USA) so as to interpret the value of OD. Data generated from responses to the questionnaires were also entered into Microsoft Excel® (Microsoft Corporation, USA) and subjected to descriptive analysis using Statistical Package for Social Science (SPSS version 16 standard version, SPSS Chicago, Illinois).

Percentage based prevalence was calculated using the formula:

$$\text{Prevalence (\%)} = \frac{\text{Number of positive samples}}{\text{Total samples collected}} \times 100$$

Descriptive statistics were used to analyze the demographic data. Mean and standard deviations of attitude and practice scores were obtained. Kruskal Wallis H tests was applied to compare mean ranks of the domains with various demographic factors. Pearson's correlation test was used to test relationship between practice and attitude of the respondents. The value of $p < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Seroprevalence of *Toxoplasma gondii* infection in chickens slaughtered at Live Bird Markets in Katsina Metropolis, Nigeria

Out of a total of 460 sera of chickens collected from two major slaughter points in Katsina Metropolis, 36 (7.83%) had antibodies against *Toxoplasma gondii* at a specificity cut off of 50%. The seroprevalence of *Toxoplasma gondii* in local chickens in the two live bird markets showed that Gobarau LBM had higher prevalence of 25 (9.06%) out of the 276 samples tested than Central market LBM where 11 (5.98%) samples out of the 184 was positive (Table 4.1).

Specificity of positive samples from Central LBM shows that percentage specificity ranged from 53 – 115%, among which two samples had percentage specificity above 100% (105% and 115%) (Figure 4.1). Positive samples from Gobarau LBM indicates percentage specificity varied from 50 – 222%, with four samples having percentage specificity of over 100% (Figure 4.2).

Table 4.1; Seroprevalence of *Toxoplasma gondii* in local chickens slaughtered at live bird markets in Katsina Metropolis, Nigeria.

Market	Number of Samples	Number Positive for antibodies	Prevalence (%)
Central	184	11	5.98
Gobarau yan kaji	276	25	9.06
Total	460	36	7.83

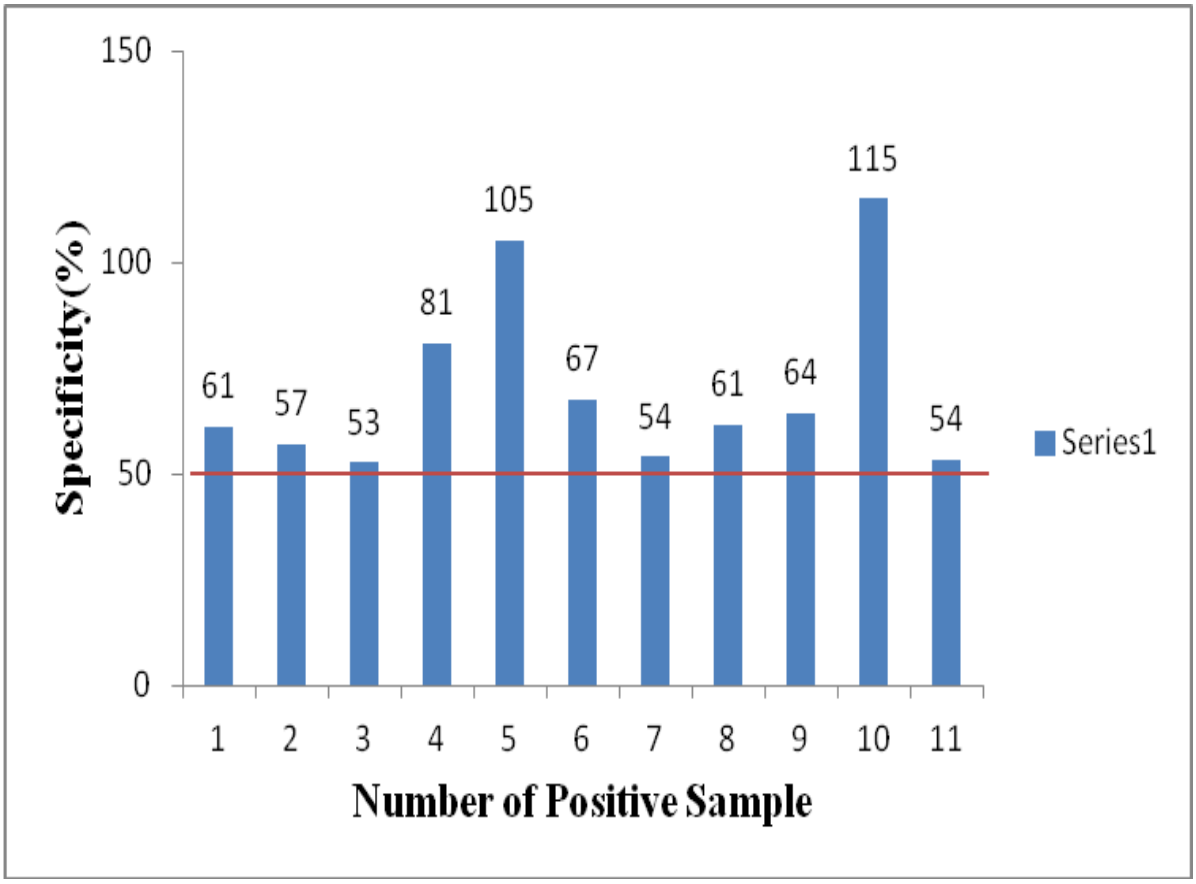


Figure 4.1 Specificity of positive samples from Central Live Bird Market, Katsina Metropolis, Nigeria.

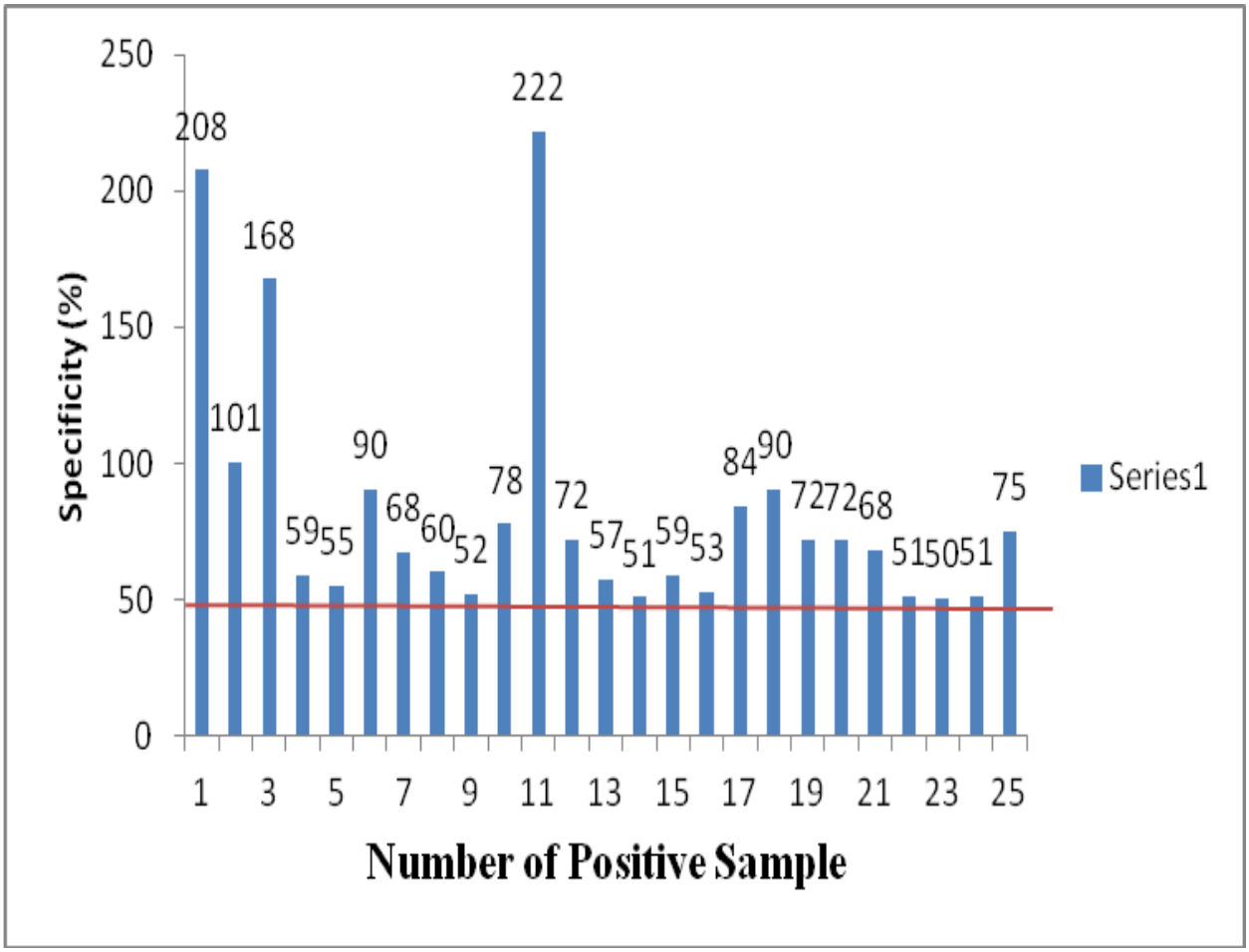


Figure 4.2; Specificity of positive samples from Gobarau Live Bird Market, Katsina Metropolis. Nigeria.

4.2 Demographic Characteristics of Respondents

The demographic characteristics of the respondents including level of education, specific duty and experience on job are shown in Table 4.2 Majority (66%) of the respondent had education up to secondary school level, while 6% had no formal education and another 6% were educated to tertiary level. Those that had primary education were 11(22%). Most of the respondents (78%) had been working at the live bird market for over five years, while those with less than one year experience were 4(8%). Those that had worked for between 1 to 5 years were 7 (14%). Most of the respondents interviewed were within 21-30 age group (42%) while the least number of respondents was in the age group that are less than 20 years (12%). Other age groups included 31-40 (22%) and those greater than 41 years (24%). The mean age was 21.52± 24.76 years. All the 50 respondents interviewed were males (100%).

4.3 Attitudes and Practices of Live Bird Markets Workers in Relation to Demographic Factors

Respondents with tertiary level of education had mean scores of 29.5 and 33.0 for attitude and practice which was higher than mean scores for respondents with secondary education (17.50, 17.18) and was statistically significant ($p < 0.05$). Higher mean scores were observed for poultry processors (31.63, 28.56) regarding attitude and practices, whilst Live Bird marketers had mean scores of 15.50 and 20.50. Respondents that have been working at the market for 1 – 5 years had mean score of 13.50 for attitude, 18.50 for practice, which was statistically insignificant. Mean score for respondents working for over 5 years was higher with attitude (25.29) and less with practice (24.40) (Table 4.3).

Table 4.2; Demographic characteristics of respondents at live bird markets in Katsina Metropolis, Nigeria. (n=50)

Variables	Frequency at market	Percentage (%)
Age group(year)	Mean=21.52 SD=24.76	
<20	6	12
21-30	21	42
31-40	11	22
>41	12	24
Sex		
Male	50	100
Female	0	0
Level of education		
None	3	6
Primary	11	22
Secondary	33	66
Tertiary	3	6
Specific duty		
Marketer	19	38
Poultry processor	31	62
Experience on job		
<1year	4	8
1-5years	7	14
>5years	39	78

Table 4.3: Mean score rank of attitudes and practices with respect to demographic data of respondents at live birds Market, Katsina Metropolis, Nigeria.

Variable	Attitude mean rank	P value	Practice mean rank	P value
Level of Education				
Secondary	17.50	0.04*	17.18	0.002*
Tertiary	29.50		33.00	
Specific duty				
Seller	15.50	0.00*	20.50	
Dresser	31.63		28.56	0.006*
Experience on the job		0.016*		
1-5yrs	13.50		18.50	0.137
>5yrs	25.29		24.40	

Statistically significant at $p < 0.05$

4.4 Attitudes of Respondents at Live Bird Markets that Could Enhance the Transmission of *Toxoplasma gondii* Infection

Frequency of responses for specific questions on attitude is shown on Table 4.4. The mean score for attitude was 26.42±15.9. Majority 76% (38) did not use gloves when handling raw chicken meat. Few 13 (26%) of the respondents ate or drank while processing chicken. 64% (32) felt that raw chicken meat does not pose any threat to their health. Keeping cat at live bird market was considered safe by majority 92% (46). Feeding dogs and cats with raw poultry meat and offals was considered safe by 66% (33) while 76% (38) felt it was proper to use poultry feathers and offals as manure.

4.5 Practices of Respondents at Live Bird Markets that Could Enhance the Transmission of *Toxoplasma gondii* Infection

Mean score for practice was 21.74±9.21. Majority 60% (30) used well/borehole water. Raw chicken meat was handled with bare hands by 80% (40), while only 2% (1) used face mask and boots while processing chicken. Majority 96% (48) did not wash knife with soap and water after use on a particular chicken, while 84% (42) had been injured with knife while processing chickens. Minority of respondents 16% (8) felt it was proper to dispose poultry waste and offals at refuse dump (Table 4.5).

Table 4.4; Attitudes of live bird market workers that may predispose them to *Toxoplasma gondii* infection in Katsina metropolis, Nigeria (n=50).

Questions	**Yes**	**No**	**No response**
Do you feel its safe using gloves when handling raw chicken meat?	12(24%)	38(76%)	
Do you think it's proper to wash your hands with soap and water after contact with raw chicken meat?	17(34%)	33(66%)	
Do you feel it is safe to eat or drink while processing chicken?	13(26%)	37(74%)	
Do you think raw meat of chicken can be a threat to your health?	9(18%)	32(64%)	9(18%)
Do you feel it is safe keeping cat at live bird market?	46(92%)	4(8%)	
Do you think it is safe to feed the market dog and cat with poultry meat and offals?	33(66%)	5(10%)	12(24%)
Is it proper to use poultry feathers and offals as manure?	38(76%)	7(14%)	5(10%)
If you were ill, will you admit working at chicken slaughter house to the health officer?	40(80%)	9(36%)	1(2%)

Table 4.5; Practices of live bird market workers that may predispose them to *Toxoplasma gondi* infection in Katsina metropolis, Nigeria (n=50)

Questions	**Yes**	**No**	**No response**
Is well or borehole water your source of water?	30(60%)	20(40%)	
Do you handle raw chicken meat with bare hands?	43(86%)	7(14%)	
Do you use face mask and boots when processing chicken?	1(2%)	49(98%)	
Do you wash knife with soap and water after use on a particular chicken?	2(4%)	48(96%)	
Have you been injured with knife while processing chicken?	42(84%)	8(16%)	
Is it proper to dispose poultry offal at refuse dump?	8(16%)	35(70%)	7(14%)

4.6 Assessment of Biosecurity practices at the Live Bird Markets in Katsina Metropolis

Biosecurity assessment of the LBM was in relation to fencing of the LBMs, infestation of the LBMs with rodents and presence of other animal species in the LBMs. The two LBMs were not fenced and rodents were seen in both of them. Other animal species were seen in both markets, notable among them was the presence of cats which were more in Gobarou 70% (7) than Central 30% (3) market. Most of the respondents (85%) and (87%) do not allow cats to have access into the bird cages at Central and Gobarau LBMs respectively. Most birds kept at Gobarau LBM were caged (87%) with only 13% of the birds not caged, while Central LBM had 75% of the birds caged and 25% not caged. There were more dogs seen in Central 62% (10) than in Gaborou 38% (6) market (Table 4.6).

4.7 Categorization and Correlation of Attitudes and Practices of Respondents

Frequencies from categorization of scores on attitude and practice by the respondents revealed that majority were categorized as poor 60% (30), 80% (40) for attitude and practice respectively. The respondents categorized to have good attitude were 28% (14), while 14% (7) had good practice at the slaughter house. Those categorized as fair were 12% (6) for attitude and 6% (3) for practice. The relationship between attitude and practice of the respondents revealed a positive pearsons correlation 0.717 which was statistically significant ($p < 0.05$) (Table 4.7).

Table 4.6; Biosecurity practices in Live Bird Markets in Katsina Metropolis, Nigeria (n=50)

Factors	**Central market (%)**		**Gobarau market (%)**	
	Yes	**No**	**Yes**	**No**
Is the market fenced?	-	100	-	100
Are birds caged	80	20	83	17
Presence of cats in the market	100	-	100	-
Presence of rodents in the market	100	-	100	-
Cats accessibility to birds in cages	15	85	86	14
Presence of other species of animals	100	-	100	-

Table 4.7: Categorization and correlation of attitude and practice score of respondents at live bird markets in Katsina Metropolis, Nigeria (n=50)

Score	**Category**	**Attitude**	**Practice**	**Rho**
0-39	Poor	30 (60%)	40 (80%)	0.717*
40-59	Fair	6 (12%)	3 (6%)	
60-100	Good	14 (28%)	7 (14%)	

*****Statistically significant at $p < 0.05$

CHAPTER FIVE

DISCUSSION

The 7.83% prevalence of *Toxoplasma gondii* infection in indigenous chickens at the two main slaughter houses in Katsina metropolis, indicates chicken meat may serve as a possible source of *Toxoplasma gondii* infection for consumers. The finding in the present study is similar to that reported by Velmurugan *et al*. (2008) who reported a 6.3% prevalence in Nigeria. The prevalence was however lower than that reported by Aganga *et al*. (1985) (44%) in Zaria, Ayinmode (2012) (33%) in Ibadan, Zia Ali *et al*. (2007) (57%) in Iran. This could be due to the fact that duration of infectivity decreases with increasing temperature, with infectivity maintained for at least 200 days at temperature range of 10-25°C, 1 month at 35°C and 1 day at 45°C (Dubey, 1998). Thus, considering that Katsina State normally records temperature of up to 38-43°C during hot season, this may shorten the exposure period of chickens to sporulated oocyts. All the above studies reported that chickens served as an intermediate host and source of infection to human especially when meat is consumed undercooked. The higher seroprevalence rate at Gobarau (9.06%) as compared to Central market (5.97%), may be due to the fact that Gobarau is a bigger live bird market with a larger collection of pooled indigenous chickens from weekly markets around the locality. Variation of seroprevalence of *Toxoplasma gondii* in domestic chickens between and within countries can be due to the testing method, number of samples examined, type and hygiene of animal breeding (Dubey, 2010a). The differences between the results of this study and other findings may also be connected to the test kit used, as the ELISA kit used in this study is still in the process of being used commercially. It has been tested for specificity on negative specific pathogen free (SPF) sera in Paris. It showed

specificity ranging from 0-30%, in contrast to IHAT which had specificity of 25% (Frenkel, 1981). On sensitivity, 10 positive samples of control group in Thailand showed a result of Specificity ranging from 50-133%, thus more sensitive in contrast to IHAT that showed 46% sensitivity (Frenkel, 1981). The cost of ELISA is lower as compared to MAT, but it is usually automated and very convenient for large scale surveys for avian toxoplasmosis. In similarity to ELISA kits for human toxoplasmosis, it is subjected to standardization (Shaapan *et al*., 2008). The epidemiological significance of *Toxplasma gondii* in chickens indicates that cats may have a role to play, because they disseminate the oocysts in the soil (Asgari *et al*., 2006). Most of these cats become infected by eating infected rodents, chickens and wild birds. Factors such as management practice, hygiene standard and density of cat may play a very important role in the infection of chickens with *Toxoplasma gondii* oocyst (Jones *et al*., 2007). Environmental conditions such as humidity, altitude and temperature also affect the survival of oocysts. The study area which is Katsina has a dry weather, Saharan-like environment which may also have contributed to the relatively low prevalence.

The demographic data of the slaughter houses showed most of the respondents are within the age group of 21-40 years, thus indicating they are mostly youths within the age group responsive to awareness and training. All the respondents were males mainly due to the culture and tradition of the region, in which such jobs are strictly for males. The live bird marketers were predominantly males, contrary to reports from southern part of the country where women were dominant (AICP, 2008). Significant proportion of the respondents had certain levels of education, hence enabling ease of communication and ability to create the

required awareness, considering the fact that level of literacy is strongly related to risk cognition and comprehension of public health campaigns (Abdullahi *et al.*, 2009).

Most of the respondents have been working at the live bird markets for over 5 years, which may be enough to establish some level of equilibrium to exposure to parasite in the markets. A number of factors are associated with the acquisition of *Toxoplasma gondii* oocysts by animals, such as hygienic standards in breeding, density of cats and environmental conditions (Kapperud *et al.* 1996; Tenter *et al.*, 2000).

The attitude and practice of the respondents was poor overall. The use of protective gears and hand washing with soap was not adopted by majority of the respondents. A common negative attitude was the perception of the respondents towards keeping cats in the market and disposal of poultry offals as manure. Poor disposal of carcass is not peculiar to Nigeria as it has been reported in other developing countries such as Thailand (Kilpatrick *et al*., 2006). Cats serve as reservoirs for animal and human toxoplasmosis, thus their presence is a very important risk factor (Dubey, 1995b). Re-infection of the cat may occur due to the attitude of feeding dogs and cats with poultry meat and offal. Cats can shed tens of millions of oocysts into the environment in several days, which can potentially infect birds before being sold and also pose huge risk to the human population in the market (Dubey, 2010a). Cats are known to be very influential in the epidemiology of toxoplasmosis due to their predatory habits and diets such as rodents, wild birds and viscera of chickens (Steven *et al*., 2000; Ayinmode, 2012). The only source of water at Central market was mainly well water (40%), which may pose a high chance of contamination of the meat. Well water has been reported to be contaminated with oocysts (Villena *et al*., 2004). The degree of contamination of well water may depend upon the degree of environmental hygiene and the

depth of the well. The slaughtering at the slaughter houses were not supervised, thus no examination of carcass and no strict observation of hygiene standards and practices. Slaughter and poor disposal of carcasses of birds in markets have particularly been associated with contamination of market environment (Indriani *et al*., 2010). Waste and viscera at the slaughter house are mostly bagged and sold to farmers as manure. Organic manure is usually used due to its relative availability, low cost and improvement of urban food production, thus leading to increased access to food by urban population (Baumgartner and Belevi, 2001).

Live bird markets (LBMs) are essential for marketing poultry in different countries including Nigeria. However it has been reported to serve both as key mixing points and sources of disease spread in animals and humans (Kung *et al*., 2003; Wan *et al*., 2011). Hence, it is essential to have some degree of biosecurity to curtail spread of diseases. Fencing was totally absent in the two LBMs, thus control of animal movement was absolutely unchecked. However, the World Health Organization (WHO, 2005) has recommended fencing of markets as a risk reduction measure. Majority of the birds were caged, while few displayed the birds on the floor for sale. The cages are mostly made of wood and wire mesh, which makes it difficult to clean and disinfect as against the recommended use of plastic or metallic material cages that are easily cleaned (FAO, 2010). The use of cages to keep poultry was also reported in other parts of Nigeria (Ajetomobi and Adepoju, 2010). The only rodent control programme is the presences of cats at the LBMs, some of the cats and rodents have easy access to the cages and stray animals in all the LBMs thus compromising biosecurity against *Toxoplasma gondii* transmission. Rodents are known carriers of at least 35 diseases, and constitute major carriers and reservoirs of

poultry pathogens, including toxoplasmosis (Adams, 2003; Maas *et al*., 2007). Several species of animals are kept in the LBMs, with Gobarou LBM having multi species such as cattle, camels, donkeys, small ruminants and water fowls. Literature shows that limiting the number of species of livestock sold in a market reduces the persistence of infection in LBMs (FAO, 2010). The present study did show that the slaughtering of birds in the open and indiscriminate disposal of carcasses and offals in LBMs easily compromise biosecurity, as infection can spread to the point of sale and waste disposal (Indriani *et al*., 2010). Birds are kept at the LBMs on an average of 3 to 4 days which is safe to some extent considering that tissue cysts arise 7 to 10 days post-infection. The possibility of having birds being kept in the market for more than 10 days cannot be ruled out, thus creating high chances of such birds developing tissue cyst before being sold out. Implementation of biosecurity measures and husbandry practices to prevent the introduction, replication and spread of infectious agents, has often been recommended to reduce the risk of infection or disease within LBMs (FAO, 2010; Fox, 2012). However, ineffective measures may create conditions that favour silent spread of disease agents within the LBMs through such operations (Yupiana *et al*., 2010). An important finding of the study was the correlation of attitude and practice of respondents with level of education, specific duty and duration on the job. A significant correlation was observed in the level of education with respondents having tertiary level showing the highest mean scores for attitude and practice against acquiring *Toxoplasma* infection. A previous study has shown that level of education affect attitude and practice of individuals towards the environment (Abdullahi *et al.*, 2009). The correlation of dresser with attitude and practice was higher than that of seller, which may be due to better exposure of the dresser to processing chicken meat. Respondents that were

over 5 years on the job had stronger correlation with attitude and practice than respondents within 1-5 years on the job.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the aforementioned findings, it can be concluded that *Toxoplasma gondii* infection was present in Local chickens (7.82%) slaughtered for human consumption in Katsina metropolis. The prevalence was higher in Gobarau LBM (9.06%) than in Central LBM (5.97%). The mean score of attitudes and practices of LBM workers was 26.42±15.9 and 21.74±9.21 respectively. Biosecurity practices were poor in both LBMs due to lack of fence (0%), rodent control (0%) and presence of other animal species (100%) such as cats which were more at Gobarau 7(70%) than at Central LBM 3(30%).

6.2 Recommendations

The following Recommendations were made:

i. There is the need to control cats in the LBMs by the workers, since cats play a key role in the transmission of *Toxoplasma gondii.*
ii. There should be adoption of good hygienic practices at LBMs and improvement in personal hygiene standards of individuals.
iii. There should be development of strategy of meat inspection at chicken slaughter houses and live bird markets as obtainable in abattoirs.
iv. Chicken meat should be properly cooked before consumption or properly frozen when stored.
v. There should be encouragement of farmers to adopt intensive system of managing birds.

vi. The State government should be committed to improving communication and education methods for veterinarians, physicians, cat owners, slaughter house workers and the general public about toxoplasmosis risk factors and mechanisms of prevention.

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APPENDICES

APPENDIX 1: QUESTIONNAIRE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,

FACULTY OF VETERINARY MEDICINE,

MACARTHUR FOUNDATION

AHMADU BELLO UNIVERSITY, ZARIA

SEROEPIDEMIOLOGY OF *TOXOPLASMA GONDII* INFECTION IN LOCAL CHICKENS SLAUGHTERED IN LIVE BIRD MARKETS IN KATSINA, NIGERIA

Questionnaire for members of the live bird market on attitude, practice and biosecurity toward *Toxoplasma* infection and transmission

Instruction: Please kindly answer the following questions by ticking and filling within the appropriate space provided for each question. The information obtained will be used for academic purpose solely and will remain absolutely confidential.

(I) PERSONAL DATA

1. Name ----------
2. Location ----------
3. Sex Male ( ) Female ( )
4. Age 10-20yrs( ) 21-30yrs( ) 31-40yrs( ) 41-50yrs( ) 50yrs and above( )
5. Education None( ) Primary( ) Secondary( ) Tertiary ( )
6. Your duty in poultry business Seller( ) Dresser( )
7. Your duration in poultry business <1year( ) 1-5years( ) >5years( )

(II) ATTITUDE

1. Do you feel its safe using gloves when handling raw chicken meat? Yes ( ) No ( ) No response( )
2. Do you think its proper to wash your hand with soap and water after contact with raw chicken meat? Yes ( ) No ( ) No response( )
3. Do you feel it is safe to eat or drink while processing chicken? Yes( ) No( ) No response( )

4. Do you think the tissues of chicken can be a threat to your health? Yes( ) No( ) No response( )
5. Do you feel it is safe keeping cat at live bird market? Yes( ) No( ) No response
6. Do you think it is safe to feed the market dog and cat with poultry meat and offals? Yes( ) No( ) No response( )
7. Is it proper to use poultry feathers and offals as manure? Yes( ) No( ) No response( )
8. If you were ill, will you admit working at chicken slaughter house to the health officer? Yes ( ) No ( ) No response( )

(III) PRACTICE

1. Is Tap or Borehole water your source of water? Yes( ) No( ) No response( )
2. Do you handle raw chicken meat with naked hands? Yes ( ) No ( ) No response( )
3. Do you use face mask and boot when processing chicken? Yes( ) No( ) No response( )
4. Do you wash knife with soap and water after use on a particular chicken?Yes ( ) No ( ) No response
5. Have you been injured with knife while processing chicken? Yes ( ) No ( ) No response( )
6. Is it proper to dispose Poultry offal at refuse dump? Yes ( ) No( ) No response( )

(IV) BIOSECURITY

1. Is the live bird market fenced? Yes ( ) No ( )

2a. Are there cats in the market? Yes ( ) No ( )

b. If yes, how many? …….

3a. Are birds kept in cages? Yes ( ) No ( )

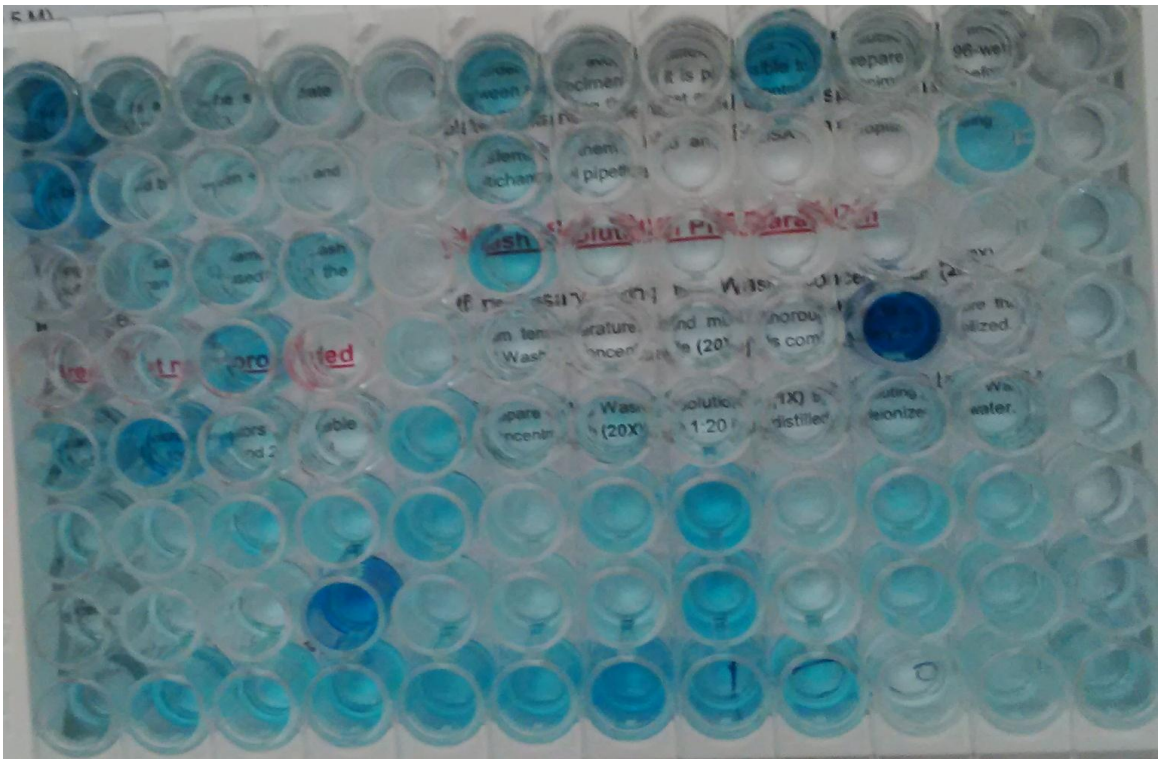
b. What is the average number of days birds stay before slaughter/sold? …….

4. Do cat have access into the cages? Yes ( ) No ( )
5. Are rodents present in the market? Yes ( ) No ( )

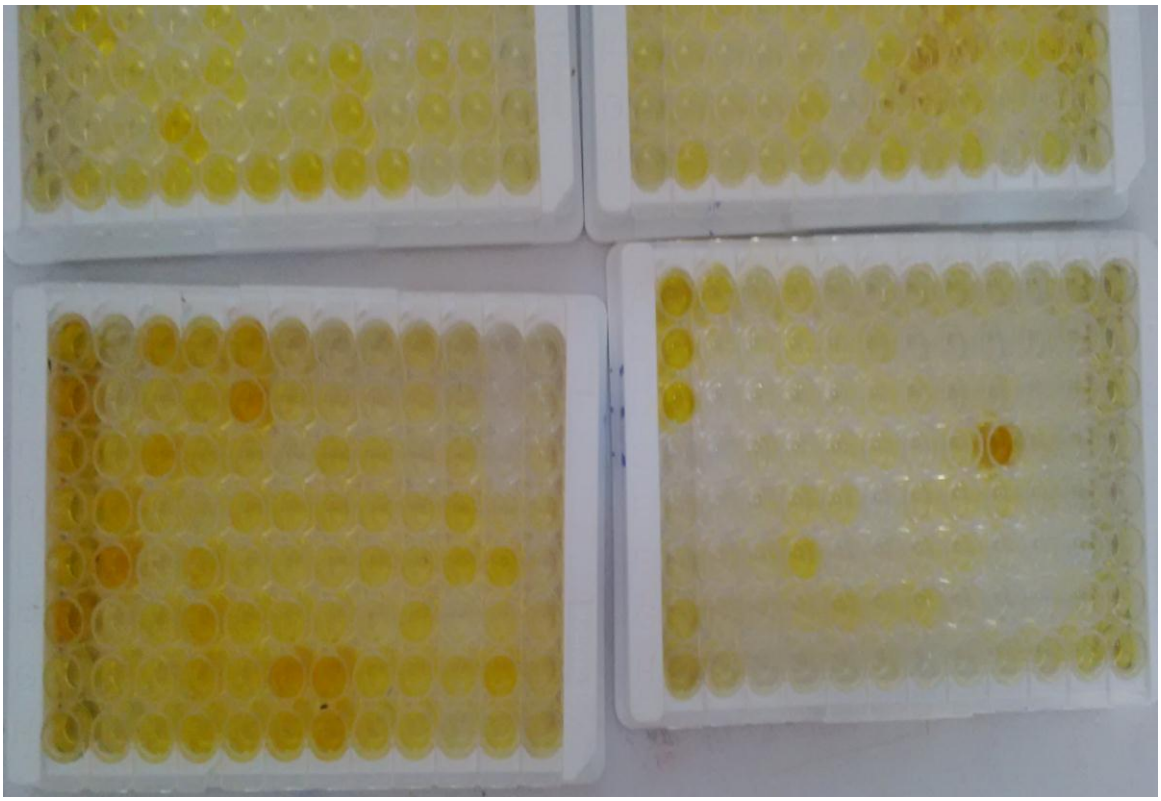
6a. Are there other animals sold in the market? Yes ( ) No ( )

b. If yes, indicate Cattle ( ) Sheep ( ) Goat ( ) Donkey

Appendix II: Blue coloration of microwells before the addition of stop solution.



Appendix III: Microwells showing yellow coloration after addition of stop solution.



Appendix IV: Microplate reader

