SERO-PREVALENCE AND FACTORS ASSOCIATED WITH TOXOPLASMOSIS AMONG PREGNANT WOMEN RECEIVING ANTENATAL CARE IN PLATEAU STATE

BY

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ATTESTATION

I hereby declare that this project was written solely by me as a record of my research work carried out under the supervision of Professor Adebola Olayinka. It was never presented previously in any application for the award of a degree either within or outside the country. The words of other researchers have been duly acknowledged.

_____ _____

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CERTIFICATION

This thesis entitled “Sero-prevalence and factors associated with toxoplasmosis among pregnant women receiving antenatal care in Plateau State” by Mariam Florence Ogo meets the regulations governing the award of the degree of Master in Public Health of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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This project is dedicated to my beloved father Late Major. (rtd.) Joseph L. Obenefiro for his loving memory.

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SUMMARY

Toxoplasmosis, one of the TORCH's infections in pregnancy is caused by *Toxoplasma gondii* an obligate intracellular protozoan parasite which can cause severe complications for an infected mother if the primary infection was acquired during pregnancy. This includes spontaneous abortions, low birth weight, congenital deformities and intrauterine deaths. In many developed countries routine screening for *Toxoplasma gondii* is offered to pregnant mothers as part of the routine screening services offered during antenatal care visits for early detection and prompt treatment. This service in Plateau State and elsewhere in Nigeria is largely limited as it is not part of the routine screening program.

This study was therefore undertaken to determine the Sero-prevalence of *T. gondii* infection in pregnant women attending antenatal and factors associated with it. The type of immune response in the infected women as well as their awareness of zoonotic infections was also investigated.

The study was a cross-sectional study involving 356 pregnant women in the reproductive age group who were attending antenatal care in Plateau State Specialist Hospital and Vom Christian Hospital, Plateau State, Nigeria between February and April 2015. Consenting pregnant women were recruited based on systematic sampling. Enzyme-Linked Immunosorbent Assay (ELISA) method was used to determine the Sero-prevalence of Toxoplasma IgG and IgM antibodies. A semi- structured questionnaire was used to collect information socio-demographic characteristics and factors associated with the Toxoplasma infection. Sero-prevalence was determined through frequency distribution of

seropositivity to *T. gondii*. Multivariate logistic regression was used to identify factors associated with toxoplasmosis.

The overall sero-prevalence of antibodies against *T. gondii* among the study participants was 12.1%. Forty-three (12.1%) of them were sero-positive for IgG and 1 (0.8%) was positive for IgM. The sero-prevalence of *T. gondii* infection was higher in pregnant women with no formal education (OR=4.27; 95% CI=1.47 -12.59), among the Hausa/Fulani ethnic group (OR=2.99; 95% CI=1.35 - 6.61). And in those who drank untreated water (OR=3.05; 95% CI=1.36 - 6.86). The study also demonstrated that tasting meat while cooking was protective against s *T. gondii* infection (OR=0.47; 95% CI=0.24 - 0.94). Similarly, Other factors such as HIV status, owning a cat, cleaning cat litter, type of meat preference, living in urban areas, being married and eating raw vegetables were not significantly associated.

At multivariate analysis Educational level, tasting of meat while cooking, drinking untreated water and ethnicity were all found to be associated with *T. gondii* infection in the study participants. The awareness of zoonotic infection among the pregnant women was based on myths.

We recommend health education on preventive measures against *T. gondii* infection and other zoonotic diseases by avoiding factors that could predispose the pregnant women to the infection during antenatal care. Policy makers and caregivers should consider introducing routine screening of toxoplasmosis on the high risk group.

LIST OF ACRONYMS

%	Percent
ANC	Antenatal care
CI	Confidence interval
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
EIA	Enzyme Immuno Assay
ELISA	Enyme-Linked Immunsorbent Assay
FCT	Federal Capital Territory
HIV	Human Immunodeficiency syndrome
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFA	Indirect Fluorescent Assay
LD_{50}	Lethal dose
LGA	Local Government Area
OD	Optical density
OR	Odds ratio
PCR	Polymerase Chain Reaction
µl	Microlitre

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CHAPTER ONE

INTRODUCTION

1.1 Background

Toxoplasmosis is a parasitic zoonotic disease caused by *Toxoplasma gondii* (*T. gondii*). The organism was first described in 1908 by Charles Nicolle and Louis Manceaux in a North African rodent *Ctenodactylus gundi.*[1] *T. gondii* is a member of the TORCH group of infections which include (Toxoplasmosis, Other Agents (Syphilis, Varicella-Zoester, human parvovirus B19), Rubella, Cytomegalovirus (CMV) and Herpes viruses which can cause congenital abnormalities and even death in foetuses. It is one of the most common parasitic zooonoses globally where it has been estimated that up to one-third of the world human populations has been exposed to this parasite.[2] It is an obligate intracellular protozoan parasite capable of infecting all warm-blooded mammals and birds including humans which are secondary hosts.[1] The primary host are cats (felids) where sexual reproduction occurs in the digestive epithelium.[2]

Toxoplasmosis can be transmitted horizontally (acquired) or vertically (congenitally). The acquired infection involves ingestion of oocyst excreted by cats via ingestion of raw or uncooked infected meat containing encysted bradyzoites of the parasite, consumption of fruits, vegetables and water contaminated by oocyst of the parasite, consumption of unpasteurized milk due to poor domestic hygienic practices. Vertical transmission is across the placenta to the foetus.[3] There are other factors that influence the incidence of Toxoplasmosis, these include prevailing climatic conditions, cultural traditions and eating habits of a particular country and therefore its incidence and prevalence differ from one geographical region to another even within same country.[4]

Toxoplasmosis in humans presents various clinical manifestations. Immunocompetent adults have mild to transient symptoms such as fever, malaise and lymphadenopathy which goes unrecognized in approximately 90% of cases.[5] Toxoplasma encephalitis and disseminated toxoplasmosis have been reported in people with immunocompromised systems such as acquired immune deficiency syndrome (AIDS) patients and people on immunosuppressive therapy.[5] Women of child bearing age who get infected with *T. gondii* before pregnancy do not transmit it to the foetus however, if primary infection occurs during pregnancy it can be transmitted congenitally through the placenta with a risk of transmission ranging between 10-15% in the first trimester with higher risk in the third trimester of up to 68%.[6]

Toxoplasmosis acquired during pregnancy has been associated with severe complications such as spontaneous abortion or stillbirth, low birth weight, intracranial calcification, neonatal death and congenital birth defects including hydrocephalus.[7] Neonates who acquired the infection congenitally may die within few days after birth. However, those that survive may develop severely impaired eyesight, hearing loss, jaundice. Neurological complication namely intellectual disability, cerebral palsy, seizures have been associated with it. Other problems like attention deficit hyperactivity disorder, obsessive-compulsive disorder and schizophrenia have also been linked with the infection[7,8] Latent toxoplasmosis have been implicated in suicidal behavior in humans and higher incidence of automobile accidents as a result of impaired psychomotor performance or elevated levels of risk- taking personality profiles.[9] It is estimated that the disease burden of toxoplasmosis as represented by disability-adjusted life years (DALY), is the highest among all food-borne pathogens.[10]

Studies on the sero-prevalence of toxoplasmosis in pregnant women receiving ante-natal care in Plateau State are largely limited. In this study we examined the sero-prevalence of toxoplasmosis among ante-natal care attendees in secondary health facilities in Plateau State, Nigeria and factors associated with the infection.

1.2 Statement of Problem

It is estimated that 6.5 million of under 5 children are affected by birth defect globally every year and of these 3.3 million of them die and 3.2 million that survive may be disabled for life.[8] Maternal infection such Toxoplasmosis have been implicated in 10-15% of these estimates. It has also been established that 80-90% of toxoplasmosis in pregnant women is largely asymptomatic and where symptoms exist they may be unspecific and as such mimic other infectious diseases.[5,11] However, toxoplasmosis in pregnant women has public health and socioeconomic implications for both the mother and unborn child. The sequelae of maternal infection with *T. gondii* could include spontaneous abortion or stillbirth which can cause psychological trauma.

Approximately 85% of infants with congenital toxoplasmosis show subclinical infection at birth.[5] This if left untreated has been associated with psychomotor and mental retardation, subsequent loss of vision or blindness due to one or more episodes of chorioretinitis. Hearing loss has been reported in 10%-30% and developmental delay in 20%-75% of this group of patients.[5] The socioeconomic impact associated with cost of treatment and cost of care for a pregnant woman with toxoplasmosis and an infant with congenital toxoplasmosis cannot be overemphasized.

Several epidemiological studies on the sero-prevalence of *T. gondii* infection in pregnant women has been well documented globally due to the long term complications it causes and as an important contributor to early and later childhood morbidity.[12] Global report on seroprevalence include 17.3 – 44% in Europe, [13,14] 9.1% in North America,[15] 51.7-92% in South America,[16] 0.8-25% in Asia [17,18] and up 92.5% in Africa..[19 In] Nigeria, previous studies reported ranges of 22.2% -44-4%.[20, 21 22 23]

1.3 Justification for the Study

Previous studies carried in the Benue River Basin which included Plateau State was based on women of child bearing age which did established a high sero-prevalence of 43.7%. Data on the sero-prevalence of toxoplasmosis in pregnant women in the State is limited. This study will therefore provide data on the sero-prevalence of toxoplasmosis of pregnant women accessing antenatal care services in the State and factors associated with *T. gondii* infection. Serological screening of pregnant women for Toxoplasmosis in Plateau State, North Central Nigeria is not routinely carried out as part of the antenatal care services. This is despite the risk of transmission from an infected mother to her unborn child during pregnancy and the socio-economic and psychological antecedent attached to it. Several reports have shown that countries such as France and Austria where systematic screening of toxoplasmosis in pregnant women is routinely done has greatly reduced the occurrence congenital transmission of *T. gondii* infection in newborns through intervention by treatment of the pregnant women and proper post-natal monitoring of affected children who were infected in utero.

The information derived from this investigation will add to the existing knowledge on toxoplasmosis in pregnant women globally and give better understanding of factors associated with toxoplasmosis in pregnant women in the State. This may help policy makers in the State and region on the need to introduce intervention for early detection, prompt treatment and monitoring of *T. gondii*. The feedback could provide essential information that can assist pregnant women, their primary care givers and obstetricians on informed choices and information needed on lowering the risk of acquiring the infection and effective prevention strategies.

1.4 Aim and Objectives

1.4.1 General objective

The main aim of the study is to determine the sero-prevalence of *Toxoplasma gondii* among pregnant women receiving antenatal care in Plateau State Specialist and Vom Christian Hospitals and the potential factors associated with the infection.

1.4.2 Specific objectives

1. To determine sero-prevalence of *T. gondii* in pregnant women among antenatal care attendees.
2. To characterize the immune response in the pregnant women.
3. To identify factors associated with Toxoplasmosis in pregnant women in Plateau State.
4. To determine the level of awareness of zoonotic infection among these pregnant women.

CHAPTER TWO

LITERATURE REVIEW

2.1 Toxoplasmosis and Toxoplasma gondii

2.1.1 Historical Overview

Toxoplasmosis has been described as the most widespread zoonotic parasitic infection caused by an intracellular protozoan called *Toxoplasma gondii* and infects most warm-blooded animals and humans, [24] but the primary host is felidae (cat) family. The parasite *T. gondii* and the disease it caused was first described in 1908 by Nicolle and Mancaeux in the rodent *Ctenodactylus gundi* in Tunsia and in the domestic rabbit *Oryctolagus cuniculus in Brazil by Splendore.*[25,26] The name *Toxoplasma gondii* was suggested by Nicolle and Mancaeux based on the shape of the infectious stage (mod. L *toxon*: arc or bow, *plasma*: life;).[27] Gondii may have resulted from the misspelling of the original host gundi (*Ctenodactylus gundi).*[28]

Toxoplasmosis was first recognized clinically as a disease in a domestic dog by Mello.[29] The first conclusively identified congenital toxoplasmosis was carried out by three pathologists (Wolf, Cowen and Paige) from New York, USA in an infant girl in the delivered full-term by C-section on May 23rd 1938 at Babies hospital.[30] The infant had convulsive seizures and chorioretinitis in both eyes at three days old which later progressed to encephalomyelitis and died eventually at a month of age. At post mortem, brain, spinal cord, and right eye were examined. Free and intracellular T. gondii were found in lesions of encephalomyelitis and retinitis of the girl and viable T. gondii was isolated in animals inoculated with tissues from the girl.[31] A review of additional cases showed that these represented examples of congenital transmission. Although, the

mothers were asymptomatic, they did have antibodies.[32] This showed *Toxoplasma* to be a human pathogen capable of and associated with congenital transmission. Sabin summarized all that was known of congenital toxoplasmosis in 1942. He proposed typical clinical signs of congenital toxoplasmosis as hydrocephalus or microcephalus, intracerebral calcification, and chorioretinitis. These signs helped in the clinical recognition of congenital toxoplasmosis.[31]

2.1.2 Taxonomic classification of. *T gondii*

Toxoplasma gondii belongs to the phylum apicomplexa which consists of more than 5000 species, including important human and veterinary pathogens. Other apicomplexans include Plasmodium species, the causative agent of malaria, Eimeria species are important agents in the poultry industry due to coccidiosis, Cryptospodium and Neospora caninum species.[33] The class Conoidasida consist of *T. gondii* and they are basically intracellular parasites which requires the host's intestinal tract to complete the sexual stage of their life cycle.[34, 35] It belong to the order Eucoccidiorida and the family Sarcocystidae where life cycle requires more than one obligatory host and the only species in the genus is Toxoplama.[36]

2.1.3 Genetic variability of *T. gondii*

Genetically *T. gondii* was considered to be clonal in structure with low genetic diversity and grouped into three distinct clonal lineages namely I, II, III[37,38] derived from the recombination of two discrete ancestral gene pools although recombination phenomena here is an occasional event.[39,40] The lineages differ genetically from each other by 1% or less.[41] The lineage II strain based on LD_{50} are relatively avirulent in mice but capable of readily establishing chronic infections with characteristic tissue cysts which are highly

infectious orally.[41,42] but notably, have been implicated as the cause in more than 70% of cases of human toxoplasmosis in USA and France.[39,43,44] Several studies have demonstrated lineage I to be more virulent and have shown greater capacity of crossing biological barriers such as gut epithelia, the blood-brain barrier or placenta and such have been associated with severe congenital toxoplasmosis in Europe.[45,46]

2.1.4 Morphology of *T. gondii*

There are three different morphological and infectious forms of *T. gondii* associated with the developmental stages of the life cycle of the parasite. These include tachyzoites normally in groups or clones (aggregates of numerous tachyzoites) and are the actively dividing form, bradyzoites in tissue cysts, the slow dividing form and the sporozoites in oocysts.[47] The various forms are linked together in the life cycle.

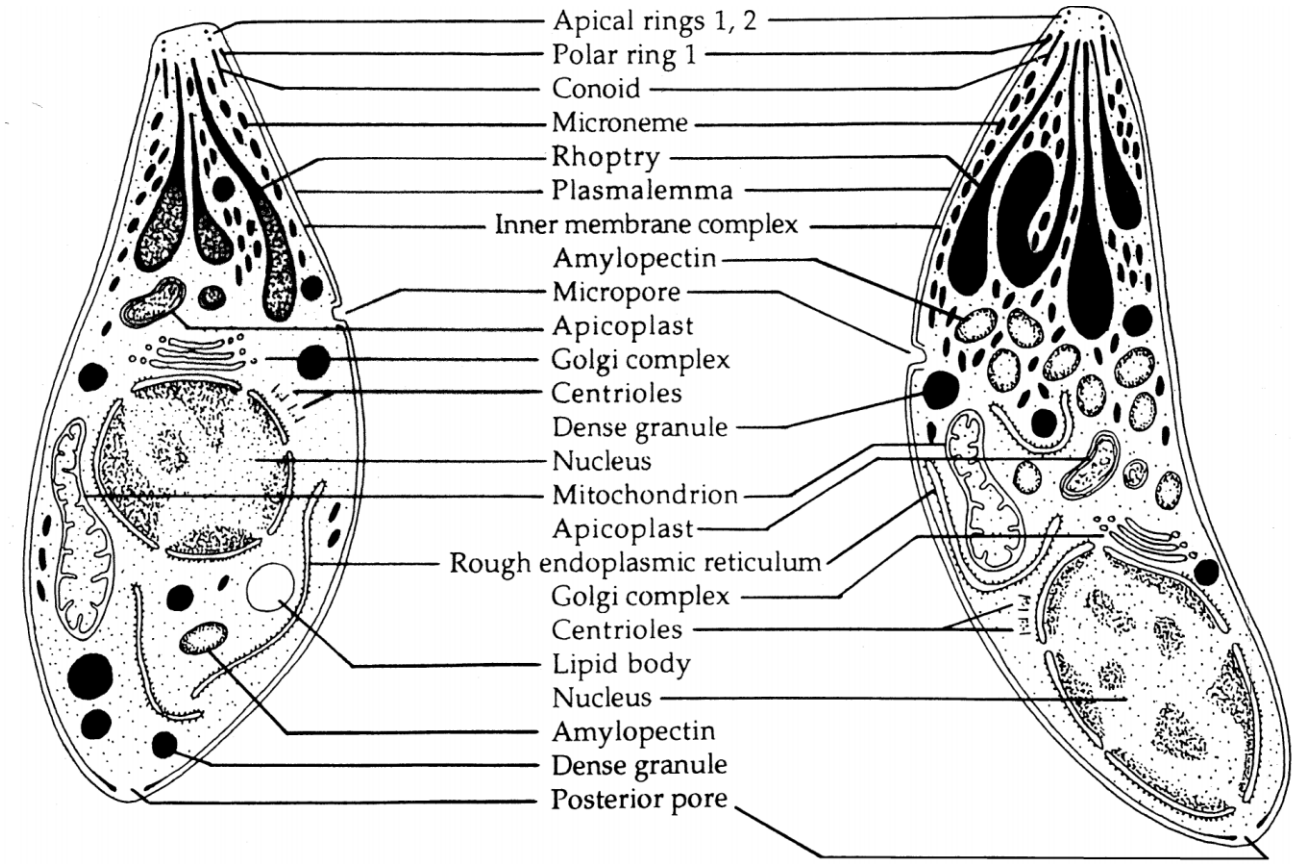


Figure 1: Schematic drawings of a tachyzoite (left) and a bradyzoite (right) of *T. gondii*.[47]

2.1.4.1 Tachyzoites

The term“tachyzoite” (meaning speed in Greek) was coined by Frenkel[48] to describe the stage that rapidly multiplied in any cell of the intermediate host and in non-intestinal epithelial cell of the definitive host. The tachyzoite is often crescent shaped, approximately 2 by 6 mm (Fig. 1), with a pointed anterior (conoidal) end and a rounded posterior end. Ultrastructurally, the tachyzoite consists of various organelles and inclusion bodies including a pellicle (outer covering), apical rings, polar rings, conoid, rhoptries, micronemes, micropore, mitochondrion, subpellicular microtubules, endoplasmic reticulum, Golgi complex,ribosomes, rough and smooth endoplasmic reticula, micropore, nucleus, dense granules, amylopectin granules (which may be absent), and a multiple-membrane-bound plastid like organelle which has also been called a Golgi adjunct or apicoplast.[47]

2.1.4.2 Bradyzoites

The term "bradyzoite" (brady; slow in Greek) was also coined by Frenkel[48] to describe the stage of the parasite multiplying slowly within a tissue cyst. The tissue cyst wall is elastic and thin (<0.5 μm thick) and it encloses hundreds of crescent shaped bradyzoites each approximately 7 by 1.5 μm in size.[49] The tissue cyst develops within the host cell cytoplasm. Tissue cysts grow remain intracellular as the bradyzoites divide by endodyogeny.[50] Tissue cysts vary in size; young tissue cysts may be as small as 5μm in diameter and contain only two bradyzoites, while older ones may contain hundreds of organisms. Bradyzoites differ structurally only slightly from tachyzoites. They have a nucleus situated toward the posterior end whereas; the nucleus in tachyzoites is more centrally located. Bradyzoites are more slender than are tachyzoites. Bradyzoites are less

susceptible to destruction by proteolytic enzymes than are tachyzoites and the prepatent period in cats following feeding of bradyzoites is shorter than that following feeding of tachyzoites. Cysts are usually formed in neural tissues such as the eye and the brain, and the muscular tissues. However, visceral organs including the lungs, kidneys, and liver can also be infected. Intact tissue cysts probably do not cause any harm and can persist for the life of the host without causing a host inflammatory response.[47]

2.1.4.3 Oocyst

Unsporulated oocysts of *T. gondii* are almost spherical and are 10 by 12 mm in diameter. The oocyst wall consists of two colorless layers under light microscopy. It lacks polar granules but the sporont almost fills the oocyst. Adverse weather conditions such as heating, cool climates and arid conditions can destroy the oocyst.[50] Sporulation occurs outside the cat within 1 to 5 days of excretion depending on climatic conditions of aeration and temperature. Sporulated oocysts are 11 by 13 um in diameter (Fig. 2B and C). Each oocyst contains two ellipsoidal sporocysts without Stieda bodies. Sporocysts measure 6 by 8 mm. A sporocyst residuum is present; there is no oocyst residuum. Each sporocyst contains four sporozoites (Fig.2 C). The sporulated oocyst is infectious and can remain so for up to one year under favorable conditions such as warm moist soil.[46]

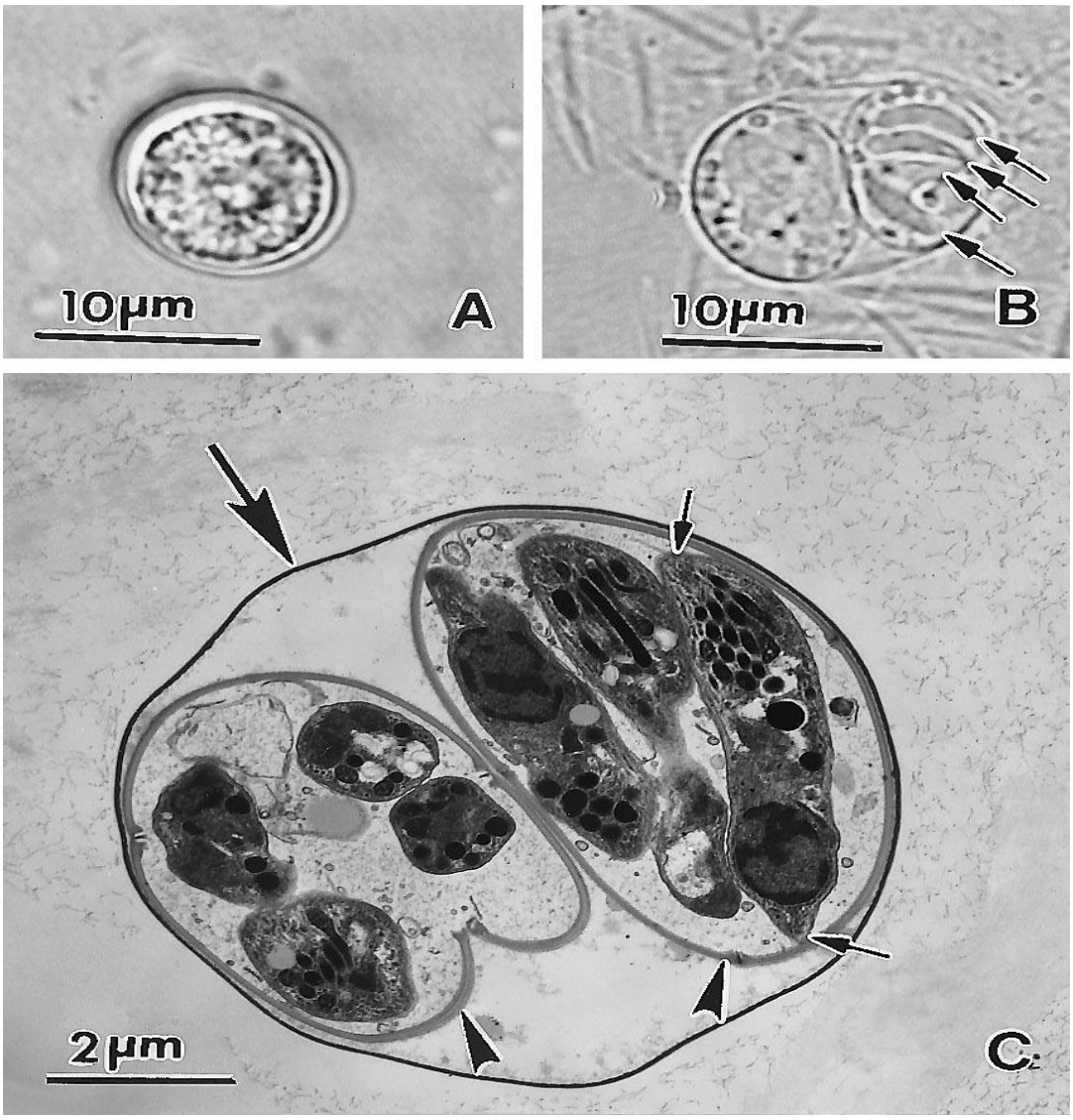


Figure 2: Oocysts of *T. gondii*

Oocysts of *T. gondii*. (A) Unsporulated oocyst. Note the central mass (sporont) occupying most of the oocyst. (B) Sporulated oocyst with two sporocysts. Four sporozoites (arrows) are visible in one of the sporocysts. (C) Transmission electron micrograph of a sporulated oocyst. Note the thin oocyst wall (large arrow), two sporocysts (arrowheads), and sporozoites, one of which is cut longitudinally (small arrows).[47]

2.1.5 Life cycle of *T. gondii*

The life cycle of T. gondii can broadly be grouped into two components. A sexual component that occurs only within cats (Felids, wild or domestic) and therefore, referred to as the definitive host. Secondly, an asexual component that can occur virtually in all warm-blooded animals and this includes humans, cats, mice and birds.[28] These are referred to as the intermediate host as only asexual reproduction occurs in them.

2.1.5.1 Sexual reproduction in the Feline definitive host

A definitive host of *T. gondii* becomes infected by ingesting sporulated oocyst through infected animals containing parasite tissue cysts, tachyzoites or bradyzoites. Enzymatic action in the digestive tract causes the release of the bradyzoites in the small intestine. Inside the epithelial cell of the small intestine the parasites undergo sexual development and reproduction by a process called endodyogeny which is a budding process whereby two daughter cells are formed inside the original or mother cells which are then consumed by the developing daughter cells. The sexual reproduction process produces millions of thick – walled zygote containing cysts known as oocysts.[47]

2.1.5.2 Shedding of the Oocyst

Matured infected epithelial cells eventually rupture and oocysts are release into intestinal lumen where they shed in the cat's faeces.[47] Oocysts become infectious after sporulation which can then spread to soil, water, food and anything contaminated with the faeces. The oocysts can survive and remain infective for many months in cold and dry climates.[51]

2.1.5.3 Asexual reproduction in the intermediate host

The asexual life cycle occurs in all intermediate hosts including humans and cats. Ingestion of oocysts by humans and other warm-blooded animals is one of the route of infection through consumption of unwashed vegetables or contaminated water or handling of faeces (litter) of an infected cat. On ingestion of an oocyst or tissue cyst by an intermediate host, the resilient cyst wall is dissolved by proteolytic enzymes in the small intestine discharging sporozoites from within the oocyst. The parasite then invades cells in and surrounding the intestinal epithelium and inside these cells through the process of endodyogeny they differentiate into tachyzoites. Inside the host cells the tachyzoites replicate inside specialized vacuoles referred to as parasitophorous vacuoles created in the process of parasite entry into the cell.[52] Tachyzoites replicate inside the vacuole until the host cell ruptures releasing other tachyzoites through the bloodstream to infect other tissues and cells including the brain. The response from the host immune system following infection causes the tachyzoites to convert into bradyzoites, the semi-dormant slow dividing cellular stage of the parasite.[52] Clusters of these bradyzoites inside the host cell are called tissue cysts and predominately found in muscle tissue, brain, eyes and heart and they could persist for life.

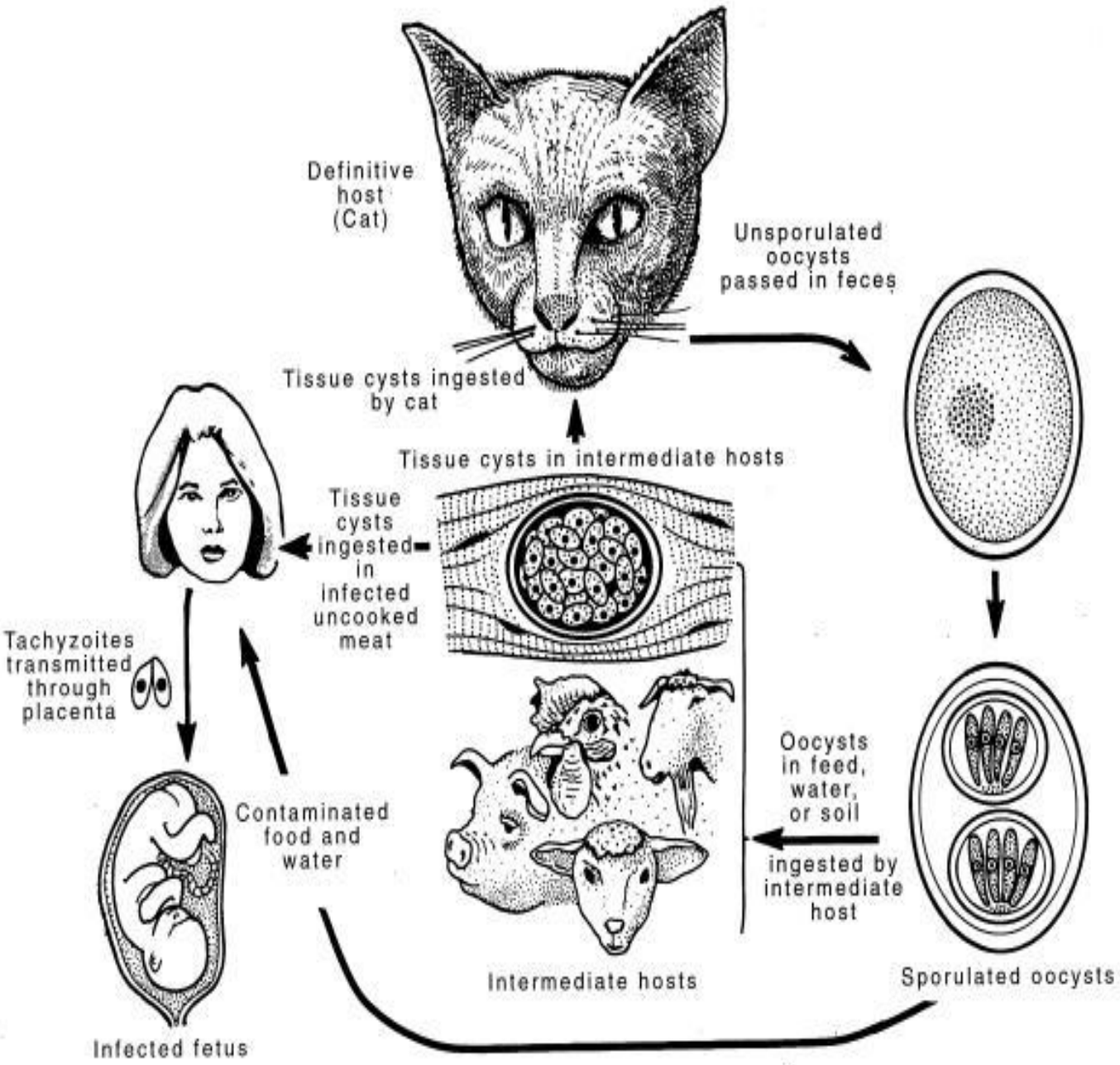


Figure 3: **Life cycle of *Toxoplasma gondii*** [47]

2.1.6 Transmission and pathogenesis of *T. gondii*

The transmission of *T gondii* occurs primarily by three main routes where two involve horizontal transmission through tissue cyst and oocyst whereas, the third one has to do with vertical (congenital transmission). In humans the horizontal transmission occurs through the ingestion of undercooked or raw meat containing tissue cysts or of water or foodstuffs contaminated by oocysts that have been excreted in the feces of infected cats. After ingestion, the outer walls of cysts and oocysts are disrupted by enzymatic degradation, and the infective stages (bradyzoites and sporozoites, respectively) are liberated into the intestinal lumen.[53] Subsequently, they invade and multiply within their surrounding cells and become tachyzoites inside the cells. Following that, the tachyzoites circulate via blood or the lymphatic system with the possibility of infecting all cells and tissues (acute phase).[54]

During the acute phase, parasites can be excreted through different biological fluids (faeces, urine,), but these tachyzoites are very unstable and are easily destroyed. In sub-acute infection, the replication of tachyzoites in the intestine is inhibited, but not those localized in the nervous system. Then, under the force of the systemic immune response and/or chemotherapy, conversion of rapidly dividing tachyzoites to quiescent bradyzoites occurs. This conversion represents the chronic phase of infection, which is associated with persistence of bradyzoites within tissue cysts.[55]

Further spread of the parasite follows its release from disrupted cells, with subsequent invasion of contiguous cells and the blood. Because *T. gondii* can infect virtually all cells and tissues, its dissemination is wide-spread. The vertical (congenital) transmission occurs when a pregnant woman acquires Toxoplasma infection acutely during gestation

and infection is passed to the fetus through the placenta which may result in fatal outcome for the unborn child. Several factors appear to influence the transmission of the parasite such as the climate and the rate of exposure to the factors that enhances transmission of the parasite.

2.1.7 Clinical manifestation of *T. gondii* infection in Pregnant Women

Acute Toxoplasma infection in immunocompetent pregnant women goes un-recognized in approximately 90% of cases. Signs and symptoms of acute infection are often so minor that they escape the memory of women who give birth to infants with congenital toxoplasmosis.[53] The most commonly recognized clinical manifestation of recent infection is non-specific flu-like symptoms and lymphadenopathy, usually involving a few nodes or even a single node. Diffuse lymphadenopathy may develop. The nodes most commonly involved are in the cervical, suboccipital, supraclavicular, axillary, and inguinal regions. The enlarged nodes are usually discrete, of variable firmness, nontender, and nonsuppurative. The lymphadenopathy may be associated with fever, headache, and fatigue. In some instances lymphadenopathy persists or recurs over a period of >6 months.[53]

2.1.8 Congenital toxoplasmosis in Pregnancy

Congenital toxoplasmosis is a public health problem globally. The parasite may cause inflammatory lesions in the fetal brain, retina and choroid, leading to severe morbidity and even death of the fetus Mother- to - child transmission of toxoplasmosis occurs predominantly in women who acquire their primary infection during pregnancy.[56] Congenital transmission, in certain rare cases, has been detected in chronically infected pregnant women whose infection was reactivated because of their immunocompromised

condition.[57,58] Maternal-fetal transmission occurs between 1 and 4 months following placental colonization by tachyzoites. The placenta remains infected for the duration of the pregnancy, and therefore may act as a reservoir supplying viable organisms to the fetus throughout pregnancy.[59] The risk of vertical (congenital) transmission without intervention or treatment, studies have shown that it increases with gestational age, with the highest rates (60% to 81%) in the third trimester compared with 6% in the first trimester.[60,61] Disease severity, however, decreases with gestational age, with first trimester infection resulting in fetal loss or major sequelae.[60,61,62]

The overall risk of congenital infection from acute *T. gondii* infection during pregnancy ranges from 20% to 50% without treatment.[61,63,64]

Classic congenital toxoplasmosis is characterized by the tetrad described by Sabin in 1942: chorioretinitis, hydrocephalus, intracranial calcification and convulsion.[63] Signs such as intracranial calcification, microcephaly, hydrocephalus, and severe intrauterine growth restriction strongly suggest in utero infection in the presence of documented maternal infection.[64]

The incidence of Toxoplasma infection is known to depend on the prevailing climatic conditions, cultural traditions and eating habits of a particular country and it therefore varies from one geographical area to another, even within same country. Congenital toxoplasmosis can be prevented by informing women how to avoid infection during pregnancy (primary prevention), by routine serological screening of pregnant women for a recent Toxoplasma infection (secondary prevention) which allows for correct prenatal diagnosis and timely treatment or by serological testing and treatment of infected

neonates. In countries with a high risk of toxoplasmosis, such as France and Austria, screening for Toxoplasma infection has been practiced widely.

2.1.9 Epidemiology and risk factors of toxoplasmosis

The sero-prevalence of *T. gondii* infection in pregnant women have been examined in different parts of the world. Available data revealed a sero-prevalence of 17.32% to *T. gondii* infection was reported in a study conducted in Central London in ethnically diverse antenatal attendees. The study found ethnicity, eating undercooked meat and drinking unpasteurised milk to be significantly associated with toxoplasmosis. Owning cats or as pets was not associated with *T. gondii* infection in these pregnant women the study suggested.[13] In South America, a study carried out in Brazil had an overall sero-prevalence of 51.7% of toxoplasmosis in pregnant women. They reported residency in rural area, low per capital income, low educational level and multigravid to be significantly associated with the infection. Presence of cats, water consumption from public system and raw or poorly cooked meat were not associated *T gondii* infection in this study.[16] In Asia, report from Thailand indicated a *T. gondii* sero-prevalence of 25% and drinking unclean water, occupation, and age group demonstrated significant association with toxoplasmosis. Here pregnant women who ate undercooked meat, had cats revealed no significant association.[18] In Africa, a study from Ghana reported a T. gondii sero-prevalence of 92.5% and contact with cat faeces was the major risk factor in that study.[19]

In Nigeria, previous studies reported sero-prevalence of 22.2% from Maiduguri in Borno State among antenatal attendees. This study further indicated level of education, rearing of cats and consumption of suya were associated with *T. gondii* infection.[20] Reports from

Zaria showed sero-prevalence of 29.1% in pregnant women in antenatal care and factors such as level education, drinking untrated water and tasting of meat while cooking were significantly associated with toxoplasmosis. A seroprevalenve of 40.8% was reported from a study in Lagos and they found high prevalence among the pregnant women who kept pets.[22]

2.1.10 Diagnosis of *T.gondii* infection

The diagnosis of *T. gondii* infection is mainly established by direct evidences showing the presence of the parasite (immunoperoxidase stain) or its DNA (polymerase chain reaction [PCR]) in body tissues/fluid or indirect evidence showing the presence of antibodies against the parasite also by isolation of the parasite from body tissues/fluids by inoculation in laboratory mice or on tissue culture cells.[65,66]

2.110.1 Serological diagnostic methods

Several serological tests are used for the detection of antibody types (IgG, IgM, IgA and IgE) since toxoplasmosis is associated with no or non- specific symptoms especially in pregnant women with efficient immune systems. The serological test relies on the detection of different antibody types in body fluids mainly serum.[67] Examples include Sabin-Feldman Dye Test which detects virtually antibody types against *T. gondii* (IgG, IgM, IgA and IgE). Indirect fluorescent assay (IFA) mainly for IgG and IgM, Agglutination test and Enzyme immunoassay (EIA) such Enzyme Linked Immunosorbent Assay (ELISA) and Enzyme-linked fluorescent immunoassays (ELFA) are the most common laboratory Toxoplasma diagnostic test and EIA which also detects the presence of all antibody types. IgG avidity test is used to discriminate recently acquired infections from those that occurred in the more distant past. The test is based on

the measurement of the functional affinity (avidity) of Toxoplasma-specific IgG antibodies.[68]

The antibodies produced at the beginning of the infection usually have a low average affinity which usually increases progressively over weeks or months.[67] IgM antibody levels rise from 5 days to weeks following acute infection, and peaking after 1 to 2 months and decline more rapidly than IgG.[63] However, IgM antibodies can decrease to low or undetectable levels in many they may persist for years following acute infection.[58] IgG antibodies appear later than IgM and are usually detectable within 1 to 2 weeks after the infection, peaking within 12 weeks to 6 months after acute infection. They will be detectable for years after acquired infection and are usually present throughout life.[58] If IgG and IgM are both negative, this indicates the absence of infection or extremely recent acute infection.[69] If testing reveals a positive IgG and negative IgM, this indicates an old infection (infection greater than 1 year ago). If both IgG and IgM are positive, this indicates either a recent infection or a false-positive test result.[58]

2.1.10.2 Molecular diagnostic methods

The commonly utilized technique for obtaining direct evidence for toxoplasmosis is PCR[70] especially in immuno-compromised patients, congenital infection and ocular toxoplasmosis. However, the most common use of PCR is the prenatal diagnosis of congenital infection using amniotic fluid in pregnant women with serological evidence of a primary infection.[71] The specificity and positive predictive value of PCR tests on amniotic fluid samples are to 100% whereas the sensitivity varies and estimates based on many studies carried out showed it to be 70-95%.[67,72]

Monoplex and multiplex PCR techniques are both useful in identifying T. gondii in biological samples with the utilization of different target genes. These targets include B1 gene,[73] 529 bp,[74,75] 18S ribosomal DNA and P30.[76,77] These PCR targets are mainly used in PCR or real-time PCR assays. Several other single-copy sequences, including the SAG2, SAG3, SAG4, and GRA4 genes which provide high resolution for detection and genotyping of *T. gondii* have also been used as PCR targets in research studies.[38] However, the sensitivity of the PCR is enhanced if the target sequence exists in multiple copies that are specie specific. The most widely-used PCR target gene is the 35-fold repetitive B1 gene.[78]

2.1.11 Treatment

Treatment of toxoplasmosis in pregnant women is usually carried out with two main goals depending on whether or not fetal infection has occurred. If maternal infection has occurred but fetus is not infected spiramycin is used for fetal prophylaxis that is to prevent spread of organism across the placenta from mother to fetus.[56] Spiramycin is a macrolide antibiotic that is concentrated in but does not readily cross the placenta, and therefore is not reliable for treatment of fetal infection**.**[56] Use is aimed at preventing vertical transmission of the parasite to the fetus, and it is indicated only before fetal infection. If fetal infection is confirmed by a positive result of PCR of amniotic fluid at 18 weeks of pregnancy or later, treatment with pyrimethamine, sulfadiazine and folinic acid is recommended[56] as this combination kills parasites in tissues and prevent organ lesions in foetus. However, if the infected pregnant woman is already receiving spiramycin, the recommendation is to switch to this combination because of the high transmission rates observed after 18 weeks of gestation. Treatment with pyrimethamine, sulfadiazine, and

folinic acid is also used for patients who have acquired the infection after 18 weeks of gestation, in an attempt to prevent fetal infection from occurring. If transmission has occurred, to provide treatment for the foetus Pyrimethamine is not used earlier because it is potentially teratogenic.

2.1.12 Prevention and control

The control of human toxoplasmosis is dependent on the reduction or elimination of the transmission of the parasite. The consumption of undercooked meat containing viable tissue cysts is the major route of infection in most parts of the world as well poor water hygiene, ingestion of oocyst-contaminated soil and water, or contact with infective oocysts, are other important sources of infection.[67] The prevention of toxoplasmosis is primarily directed towards health education related to avoiding personal exposure to the parasite through improved environmental and personal hygienic practices. Many countries have introduced education programmes aimed at reducing the incidence of congenital toxoplasmosis.[79] Detection of antibodies is very important for pregnant women especially through routine screening. This is an effective way to find the infection, and then to provide treatment. It is also an efficient way to stop congenital toxoplasmosis in newborns. Good animal husbandry practice and animal welfare should be set up and popularized for food-producing animals, which may also decrease the risk of human infection. If such strategies and measures can be implemented, it should be possible to effectively control and reduce the prevalence of *T. gondii* in pregnant women. Development of an effective vaccine against T. gondii appears to be an achievable goal, as primary infection results in a life-long protection against the parasite.[80] The most

effective approach for vaccine development has been the use of non-virulent mutated strains of the parasite.[81]

CHAPTER THREE

METHODOLOGY

3.1 Study Area

The study was conducted from February – April 2015 in Plateau State, Nigeria. It is located in the North Central geopolitical zone of Nigeria lying 9.1667°North, 9.7500°East with an area of 26,899 square kilometers with an estimated population of 3, 206,531 and of these 1, 607,533 are females based on the 2006 census. The adjacent States are Bauchi to North East, Kaduna to North West, Nasarawa to South West and Taraba to South East. The study sites were Plateau State Specialist Hospital (PLSSH) and Vom Christian Hospital (VCH) situated in Jos North and Jos South Local Government Areas (LGAs) respectively. PLSSH is the major State owned secondary health facility centrally located in an urban setting whose catchment areas for antenatal clinic (ANC) include Jos metropolis and environs, referrals from other LGAs and adjacent States. Routine screening tests and PMTCT services are available for pregnant women. VCH is also a secondary health facility established by missionaries located in Vom in a rural setting with antenatal attendees from Vom and surrounding villages and offers routine services as well. Agriculture is the major occupation of the people and the areas are generally characterized by cold climate.

3.2 Study design

A cross-sectional study was conducted involving pregnant women attending ANC at PLSSH and VCH. Pregnant women registered for their first visits were enrolled based on informed consent and invited to participate. Confidentiality was maintained by interviewing each study participant privately with their identifying number on the data

collection tool. Venous blood was drawn from each respondent and all the samples were processed at Plateau State Human Virology Laboratory (PLASVIREC).

3.3 Study population

The study populations were 356 pregnant women in the reproductive age group (15- 49 years) receiving antenatal care at PLSSH in Jos North L.G.A and VCH in Jos South L.G.A. in Plateau State during the study period.

3.3.1 Inclusion criteria

Pregnant women attending antenatal care on their first booking in the two health facilities and who gave informed consent were included in the study.

3.3.2 Exclusion criteria

Sick pregnant women on admission in both health facilities were excluded from the study.

3.4 Sample Size Determination

Sample size was calculated based on the formula for cross-sectional study[82]

$$n = \frac{Z\alpha^2 pq}{d^2}$$

Where

n = Minimum sample size

Z = Desired confidence interval expressed as a t value from normal standard table at (95% CI) =1.96

p = Expected prevalence of toxoplasmosis among pregnant women on antenatal care (29.1%[21]

q = 1-p = Expected proportion of pregnant women without toxoplasmosis (0.709)

d = Margin of error was set at 5% (0.05)

Substituting the formula

$$n = \frac{1.96^2(0.291)(0.709)}{0.05^2}$$

$$= 317$$

Addition of 10% was done for the anticipated non-response rate which gave a minimum sample size of 349

3.5 Sampling Technique

A systematic random sampling was used to select the final study participants. The calculated sample size was allocated to the two health facilities proportional to the number of weekly attendees in these facilities and time allotted for sample collection. PLSSH receives approximately sixty pregnant women for ANC and was allocated 249 from the calculated sample size whereas, the weekly attendees for VCH were twenty and it received 100.Using the formula shown below sampling interval of three (kth) was used to select the respondents from the list of the bookings at the health facilities weekly after a random selection of one at the beginning.

Calculation of sampling interval at PLSSH and VCH

$$K = \frac{N}{n}^{83}$$

$$K = \frac{60}{20}(3.0)$$

VCH

$$K = \frac{20}{8}(3.0)$$

Where N is population size (list of booked pregnant women) and n is the number of weekly desired samples

3.6 Data Collection Instruments

3.6.1 Semi-structured Questionnaire

A semi-structured Open-ended questionnaire with 3 sections (A, B and C) was used to collect data on the respondents. Section A: captured Socio-demographic characteristics of the respondents, section B: Biological plausible factors and section C: clinical history and information of the respondents.

3.7 Sample collection and methods

Venous blood (2 ml) was collected aseptically using plain vacutainers tubes and needles. All samples were transported at 2-8°C using ice packs to Plateau State Human Virology Laboratory (PLASVIREC). Serum was obtained by Centrifugation at 14000 rpm for 10 minutes and was stored at -20°C until use.

3.7.1 Laboratory analysis

3.7.1.1 Enzyme Linked Immunosorbent Assay

Commercial human *T. gondii* Enzyme Linked Immunosorbent Assay (ELISA) detection kits (Prestige Diagnostics U.K Ltd) were used to detect IgG and/or IgM antibodies qualitatively from the serum samples

3.7.1.2 Toxoplasma IgG Elisa Test

All reagents including the ninety-six well *T. gondii* coated microplate from the manufacturer were brought to room temperature before the test procedure. Pre-coated microplate was labeled appropriately (Blank, positive, negative controls and samples). Sample diluent (100µl) was added into appropriate wells except the blank well. Ten (10µl) of the serum samples were then added to the sample wells and thoroughly mixed

by repeated pippetting until the mixture turned blue. Fifty (50µl) of the positive and negative control was dispensed into their respective wells while nothing was added to the blank well. The microplate was swirled gently for 20-30 seconds to mix and then covered with plate cover provided in the kit and incubated for 20 minutes at 37°C for antigen – antibody reaction if present in the patient's serum. At the end of the incubation period the plate cover was discarded and well as the contents of the microwell. Each well was washed five times with 350µl of wash buffer with a concentration of 1: 40 to remove any unbound antibody. The plate was blot dried at the end of the fifth washing cycle. Fifty 50µl of HRP conjugate was added to each well except the blank well. The contents was mixed gently and covered with plate cover and incubated at 37°C for 20 minutes in the dark. A second round of washing was carried following the steps earlier described. A drop of the substrate A and B was added into the wells except the blank well. The microplate was incubated at 37°C for 10 minutes and the mixture was gently mixed, it was incubated further at 37°C for additional 10 minutes. The reaction was stopped by adding 50 µl of stop solution to each well. The plates were then read for absorbance at a wavelength of 450 nm against the blank well within 15 minutes after adding the stop solution using an ELISA reader (Spectrophotometer).

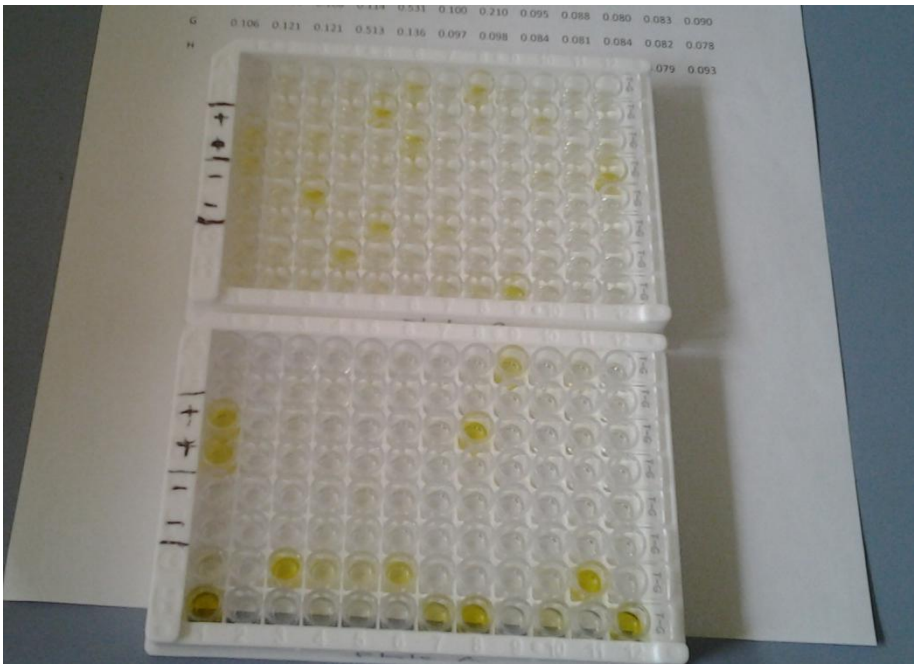


Plate 1: ELISA microtitre Plates showing the results of some samples for *T. gondii* IgG test

3.7.1.3 Toxoplasma IgM test

The Procedure was performed according to manufacturer's protocol. Pre-coated microplate was labeled appropriately (Blank, positive, negative controls and samples). Sample diluent (100µl) was added into appropriate wells except the blank well. Ten (10µl) of the serum samples were then added to the sample wells and thoroughly mixed by repeated pippetting until the mixture turned blue. Fifty (50µl) of the positive and negative control was dispensed into their respective wells while nothing was added to the blank well. The microplate was swirled gently for 20-30 seconds to mix and then covered with plate cover provided in the kit and incubated for 20 minutes at 37°C for antigen – antibody reaction if present in the patient's serum. At the end of the incubation period the plate cover was discarded and well as the contents of the microwell. Each well was washed five times with 350µl of wash buffer with a concentration of 1: 40 to remove any unbound antibody. The plate was blot dried at the end of the fifth washing cycle. Fifty 50µl of HRP conjugate was added to each well except the blank well. The contents was mixed gently and covered with plate cover and incubated at 37°C for 20 minutes in the dark. A second round of washing was carried following the steps earlier described. A drop of the substrate A and B was added into the wells except the blank well. The microplate was incubated at 37°C for 10 minutes and the mixture was gently mixed, it was incubated further at 37°C for additional 10 minutes. The reaction was stopped by adding 50 µl of stop solution to each well. The plates were then read for absorbance at a wavelength of 450 nm against the blank well within 15 minutes after adding the stop solution using an ELISA reader (Spectrophotometer).

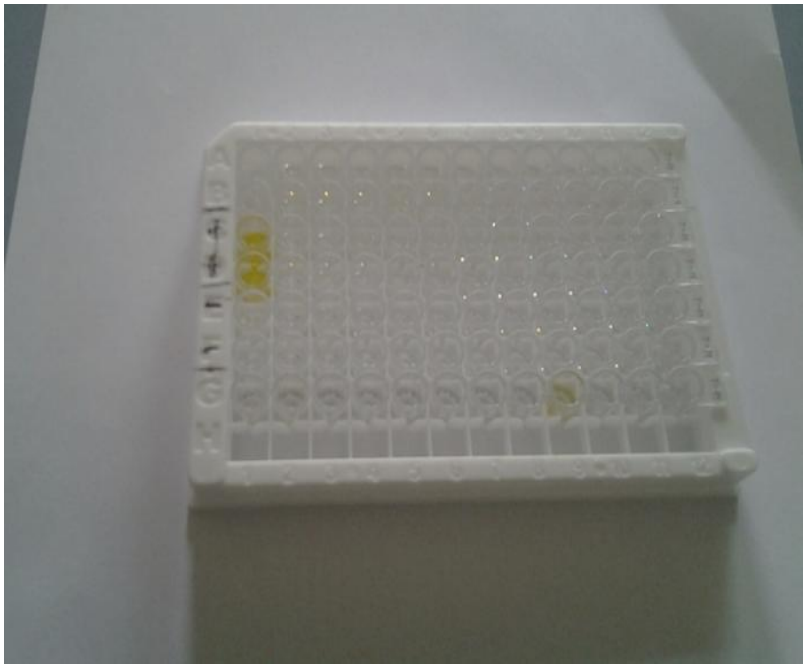


Plate 2: ELISA microtitre Plates showing the results of some samples for *T. gondii* IgM test

3.7.1.4 Cut-off Value for IgG test

The cut-off value was set using the formula 2.1 x negative control optical density (OD) , as instructed by the manufacturer if the OD value of the negative control was lower than 0.09 the cut-off was calculated using 0.09 absorbance but if it was greater than 0.09 the actual absorbance of the negative control was used.

3.7.1.5 Cut-off Value for IgM test

The cut-off value was set using the formula 2.1 x negative control optical density (OD) , as instructed by the manufacturer if the OD value of the negative control was lower than 0.05 the cut-off was calculated using 0.05 absorbance but if it was greater than 0.05 the actual absorbance of the negative control was used

3.7.1.6 Interpretation of results

Positive for Toxoplasma IgG: sample OD was ≥ cut-off OD

Negative for Toxoplasma IgG: sample OD was < cut-off OD

For IgM:

Positive for Toxoplasma IgM sample OD was ≥cut-off OD Negative for Toxoplasma IgM: sample OD was < cut-off OD

3.8 Data Analyses

Data collected were extracted and entered into an Excel spreadsheet and exported to EPi-Info 7.0 Centers for Disease Control and Prevention Atlanta, GA where further cleaning of data and analyses was carried out,

3.8.1 Univariate analyses

The univariate analyses carried out involved frequency distributions for categorical variables and descriptive statistics (means, medians, standard deviations) for continuous and discrete

variables. Categorical variables were presented using frequency distribution tables. Univariate analyses were used to summarize the characteristics of the respondents in the study as well as description of the response variables from the participants to *T. gondii* infection.

3.8.2 Bivariate analysis

Bivariate analysis was used to investigate any association between the response variable (*T. gondii* infection) with socio demographic and other variables of interest. The $\chi 2$ test was used to test association between 2 variables and significant level was set at $p < 0.05$.

3.8.3 Multivariate analysis

Using unconditional logistic regression a model was constructed using variables that were significant in the bivariate analysis as well as variables that had p value of 0.25 or less to calculate adjusted odd ratios (OR) for the different determinants to *T. gondii* status. Variables at the final stage with $p < 0.05$ were considered statistically significant

3.9 Ethical Consideration

Ethical approval was obtained from the Scientific & Ethical Committee of the Plateau State Ministry of Health through the Plateau State Specialist Hospital and Vom Christian Hospital. Written informed consent was obtained from each study participant and a participant with positive result were referred to their caregivers for further evaluation and management. Confidentiality was maintained throughout.

3.10 Limitation of the Study

The awareness of Toxoplasmosis among the study participants was low hence some showed reluctance to participate but efforts were made to explain the benefits of participating and no harm in refusing consent as well.

CHAPTER FOUR

RESULTS

4.1 Study Population

A total of 356 pregnant were enrolled and participated in the study. Two hundred and eighty-nine (81.2%) pregnant women were from PLSSH and 67(18.8%) were from VCH.

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Table 1: Socio-demographic characteristics of the study population

Characteristics	Frequency (n=356)	Percent (%)
Age group (years)		
< 20	12	3.4
20 - 24	82	23.0
25 – 29	118	33.1
30 – 34	91	25.6
35 – 39	46	12.9
≥ 40	7	2.0
Marital status		
Married	349	98.0
Unmarried	7	2.0
Area of residence		
Urban	225	63.2
Rural	131	36.8
Ethnicity		
Hausa/Fulani	82	23.0
Igbo	13	3.7
Yoruba	14	3.9
Others	247	69.4
Educational level		
None	22	6.2
Primary	57	16.0
Secondary	172	48.3
Tertiary	105	29.5
Occupation		
Civil servant	58	16.3
Farming	31	8.7
General	95	26.7
Sell vegetables	23	6.5
Sell meat	21	5.9
Unemployed	128	35.9
Family income per month		
< 7, 500.00	45	12.7
7, 500.00 – 30, 000.00	214	60.1
> 30, 000.00	97	27.2
Gravidity		
Multigravid	264	74.2
Primigravid	92	25.8
Trimester		
First	40	11.2
Second	224	62.9
Third	92	25.8

The mean age of the respondents was 28 years with range from 17 to 45 years. Almost all the women were married 349 (98.0%), 228 (64.0%) had one form of employment and 264 (74.2%) were multigravid with 224 (62.9%) on second trimester of their pregnancy. The study participants who had post primary education were 172(48.3%) and 214 (60.1%) were medium income earners. The urban dwellers were 225 (63.2%).Table 1.

Table 2: Sero-prevalence of *T. gondii* in relation demographic characteristics

Characteristics	No tested (n %) n = 356	No of Positives (n) n = 43	Prevalence % [95% CI]
Age group(years)			
15 - 19	12 (3.4)	3	25.0 (5.48 - 57.19)
20 - 24	82 (23.0)	11	13.4 (6.89 – 22.74)
25 - 29	118 (33.1)	10	8.5 (4.12 – 15.03)
30 – 34	91 (25.6)	11	12.1 (6.19 – 20.6)
35 - 39	46 (12.9)	6	13.0 (6. 24 – 25.2)
≥ 35	53 (14.9)	8	15.1 (6.74 – 27.51)
Marital status			
Married	349 (98.0)	41	11.7 (8.56 – 15-60)
Unmarried	7 (2.0)	2	28.6 (3.67 – 70.96)
Area of residence			
Urban	225 (63.2)	32	14.2 (9.93 – 19.48)
Rural	131 (36.8)	11	8.4 (4.27 – 14.53)
Ethnicity			
Hausa/Fulani	82 (23.0)	20	24.4 (15.60 – 35.12)
Igbo	13 (3.7)	0	0.0 (0.0)
Yoruba	14 (3.9)	1	7.14 (0.18 – 33.87)
Others	247 (69.4)	22	8.90 (5.67 – 13.17
Educational level			
None	22 (6.2)	6	27.2 (10.0 – 48.33
Primary	57 (16.0)	8	14.0 (6.26 – 25.79)
Secondary	172 (48.3)	18	10.5 (6.32 –16.03)
Tertiary	105 (29.4)	11	10.5 (5.35 – 17.97)
Occupation			
Civil servant	58 (16.3)	6	10.3 (3.89 – 21.17
Farming	31 (8.7)	2	6.5 (0.79 – 21 -75)
Vegetable seller	23 (6.5)	4	17.4 (4.95 – 38.78)
Meat seller	21 (5.9)	3	14.3 (3.05 – 36.34)
General	95 (26.7)	13	13.7 (7.49 – 22.26)
Unemployed	128 (36.0)	15	11.7 (6.71 – 18.59)
Family income			
<7, 500	45 (12.6)	6	13.3 (5.05 – 26.79)
7, 500 – 30, 000	214 (60.1)	33	15.4 (10.86 – 20.97)
>30, 000	97 (27.2)	4	4.1 (1.14 – 10.22)
Gravidity			
Multigravid	264 (74.2)	32	12.1 (8.44 – 16.68)
Primigravid	82 (23.0)	11	13.4 (6.89 – 22.74)
Trimester			
First	40 (11.2)	4	10.0 (2.79 – 23.66)
Second	224 (62.9)	29	12.9 (8.85 – 18.06)
Third	92 (25.8)	10	10.9 (5.34 – 19.08)

The overall sero-prevalence of *T. gondii* among the 356 study participants was 12.1%. Distribution of the *T. gondii* infection in our analysis revealed that of the 356 respondents, the age group < 20 years had the highest prevalence of 3(25%). The least prevalence of 10 (8.5%) was observed in the age group 25 – 29 which had 118 (33.1%) participants which was the largest age group in the study. Among the married participants, sero-prevalence for *T. gondii* was 11.8% while the unmarried had sero-prevalence of 28.6%.

The distribution according to inhabitation showed urban dwellers had sero-prevalence of 32(14.7%) as against 11(8.4%) for the rural dwellers. The Hausa/Fulani ethnic group recorded the highest sero-prevalence of 20 (24.1%) whereas; zero sero-prevalence was recorded among the Igbo ethnic group. We found 27.2% sero-prevalence in pregnant women with no formal education with a decreasing pattern as the educational level increases but no difference was observed in the sero-prevalence of participants with secondary and post secondary education.

Pregnant women whose occupation was selling vegetables and meat had sero-prevalence of 17.4% and 14.3% respectively. A low sero-prevalence of 4.1% was seen in the participants whose family income per month was greater than ₦7,500:00. Further analyses demonstrated that 23% of the pregnant women were primigravid with sero-prevalence of 13.4% and multigravid had sero-prevalence of 12.1% with 62.9% of them in their second trimester (Table 2).

Table 3: Distribution of the immune response to *T. gondii* infection among the respondents

Infection	Samples (n)	Positivity %)	95 %CI
Chronic Infection (IgG)	356	43 (12.1)	8.88 – 15.92
Acute infection (IgM)	356	1 (0.3)	0.0 – 0.8

The immune response in the pregnant women demonstrated that 43 (12.1%) were positive for anti *T. gondii* specific IgG only antibodies indicating previous infection and 1 (0.3) had IgM only specific antibodies indicating recent infection Table 3.

Table 4: Factors associated with *T. gondii* infection in relation to demographic characteristics of the pregnant women

Factor	*T. gondii* sero-prevalence		Bivariate	
	Positive (n)	Sero-prevalence %	OR [95% CI]	p-value
Level of education				
Formal education	37 (334)	11.1	0.33 (0.12 – 0.90)	0.03
No formal education	6 (22)	27.3		
Area of residence				
Urban	32 (225)	14.2	1.80 (0.88 – 3.72)	0.10
Rural	11 (131)	8.4		
Marital status				
Married	41 (349)	11.8	0.33 (0.06 – 1.71	0.17
Unmarried	2 (7)	28.6		
Duration at residence				
> I year	37 (294)	12.6	1.34 (0.54 – 3.34)	0.52
< 1 year	6 (62)	9.7		
Ethnicity/tribe				
Hausa/Fulani	20 (82)	24.4	3.52 (1.82 – 6.82)	< 0.001
Others	23 (274)	8.4		
Occupation				
Employed	28 (231)	12.0	0.99 (0.51 – 1.93)	1.000
Unemployed	15 (125)	12.0		
Family income/month				
< 7, 500.00	6 (44)	13.4		
≥ 7, 500.00	3 (312)	11.9	1.17 (0.46 – 2.80)	0.73
Gravidity				
Multigravida	32 (266)	12.0	1.02 (0.49 – 2.11)	1.000
Primigravida	11 (90)	12.2		
***T. gondii* knowledge**				
Yes	2 (8)	25.0	2.49 (0.49 –1 2.80)	0.24
No	41 (348)	11.8		

By bivariate analysis The factors associated with *T. gondii* infection the level of education which indicated having formal education was protective against *T. gondii* infection (OR 0.33, 95% CI 0.12 – 0.90, p 0.03). Pregnant women from the Hausa/Fulani ethnic group revealed significant association to *T. gondii* infection, (OR 3.52, 95% CI 1.82 – 6.85, ($p < 0.001$). Other factors such marital status, area of residence, HIV status, duration at residence, occupation, gravidity, family income and knowledge on *T. gondii* infection were not significantly associated. (Table 4).

Table 5: Factors associated with *T. gondii* infection in the respondents in relation to eating and drinking habits

Factor	*T. gondii* sero-prevalence		Bivariate	
	Positive (n)	Sero-prevalence %	OR [95% CI]	p value
Taste meat				
Yes	23 (253)	9.1		
No	20 (104)	19.2	0.42 (0.22 – 0.81)	0.007
Meat preference				
Food animals (Beef, Mutton, Chicken)	30 (180)	16.7	2.51 (1.26 – 4.98)	0.008
Others (Pork, Dog meat, Rat)	13 (176)	7.4		
Cooking method				
Boiling/stewing	18 (134	13.4	1.24 (0.65 – 2.37)	0.62
Frying/roasting	25 (222)	11.3		
Eating stick meat (suya)				
Yes	40 (331)	12.1	1.00 (0.29 – 3.52)	0.99
No	3 (25)	12.0		
Water type				
Untreated	34 (222)	15.3		
Treated	9 (134)	6.7	2.51 (1.16 -5.42)	0.01
Take vegetables				
Yes	42 (351)	12.0	1.84 (0.20 – 16.8)	0.47
No	1 (5)	20.0		
Form Vegetable is eaten				
Fresh/raw	21 (131)	16.0	1.81 (0.94 – 3.46)	0.07
Steamed/cooked	21 (220)	9.5		

The analysis of our data showed that 253 (71.1%) of the respondents in the study reported tasting their meat while cooking which was significant (OR 0.42, 95% CI = 0.22, 0.81, p

0.007) as against those who do not. The meat preference of the women indicated that those who eat meat from common food animals such as beef, mutton and chicken was significantly associated in comparison to those who reported eating uncommon meat such as dog meat, pork and rat meat (OR 2.51 95% CI=1.26- 4.98, p 0.008). The sero-prevalence among the study participants who reported drinking untreated water such well water was higher at 15.3% as against 6.7 % of those who drink treated water such as piped water. Other factors considered here such as cooking method, eating stick meat, vegetable consumption and the form vegetable is eaten were not significant (Table 5).

Table 6: Factors associated with *T. gondii* infection in relation to clinical information

Factor	*T. gondii* sero-prevalence		Bivariate	
	Positive (n)	Sero-prevalence %	OR [95% CI]	p-value
HIV status				
Positive	4 (16)	25.0	2.57 (0.79 – 8.37)	0.11
Negative	39 (340)	11.5		
Miscarriage/stillbirth				
Yes	14 (108)	13.0	1.12 (0.57 – 2.21)	0.74
No	29 (247)	11.9		
Blood transfusion < 1 year				
Yes	0 (8)	0.0	0.0 (0.00 – 0.00)	0.60
No	43 (348)	12.4		
Any symptoms				
Yes	13 (86)	15.1	1.42 (0.70 2.87)	0.32
No	30 (270)	11.1		

Factors considered in the study according the clinical information of the study participants such HIV status revealed 4.5% of the pregnant were positive to HIV with a sero-prevalence of 25% to *T. gondii* infection but it was not significantly associated with the *T. gondii* infection. Other factors included history of still birth or miscarriage, blood transfusion one year prior to the interview and any symptoms like fever, flu during the interview were all not significantly associated *T. gondii* infection (Table 6).

Table 7: Factors associated *T. gondii* infection in relation to presence of pets

Factor	*T. gondii* sero-prevalence		Bivariate	
	Positive (n)	Sero-prevalence %	OR [95% CI]	p-value
Own a cat				
Yes	20 (176)	11.4	1.14 (0.60 – 2.19	0.75
No	23 (180)	12.8		
Clean cat litter				
Yes	3 (42)	7.1	1.89 (0.56 – 6.43	0.44
No	40 (340)	12.7		
Handling raw dog/pig/rat meat				
Yes	17 (171)	9.9	0.68 (0.35 – 1.29)	0.26
No	26 (185)	14.1		

The seroprevalence of study participants

Of the 356 study participants 49.4% reported owning a cat or having cat in their neighbourhood and these participants had sero-prevalence of 11.4 % as against 12.7% of participants who reported not having but it was significantly not associated with toxoplasmosis. Handling uncommon meat such dog, pork or rat meat was also not significantly association with infection in these study participants Table 7.

Table 8: Logistic regression model for factors associated with the presence of *T. gondii* infection

Factor	OR	95% CI	p-value
Level of education (none)	4.29	1.47 – 12.59	0.007
Ethnicity (Hausa/Fulani)	2.99	1.35 – 6.61	0.006
Tasting meat while cooking	0.47	0.24 – 0.94	0.03
Drinking untreated water	3.05	1.36 – 6.86	0.007
Meat type (Beef, Mutton, chicken	1.49	0.65 – 3.40	0.34
Living in urban areas	1.21	0.54 – 2.68	0.64
Being married	0.23	0.04 – 1.36	0.10
HIV status	2.69	0.73 – 9.99	0.14
Eating raw vegetable	1.53	0.76 – 3.07	0.50
T. gondii knowledge	1.49	0.25 8.75	0.50

A multivariate model constructed using significant factors associated with *T. gondii* infection from the bivariate analysis showed the following factors being significantly associated with T. gondii infection in the multivariate logistic regression. The participants with no formal education (OR 4.29, 95% CI= 1. 47-12.59, p 0.007), pregnant women belonging to the Hausa/.Fulani ethnic group (OR2.99, 95% CI= 1.35 – 6.61 p 0.006), tasting of meat while cooking (OR 0.47, 95% CI 0.24 – 0.94, p 0.03), drinking untreated water (OR 3.05 95% CI= 1.36 – 6.86 p 0.007). Other factors included in the model such residence of the participants, marital status, HIV status, form vegetable is eaten and knowledge on *T. gondii* infection were not significantly associated with sero-positivity to *T. gondii* infection in the study participants (Table 8).

Table 9: Zoonotic diseases awareness among study participants

Disease	Aware (n %) N = 356	Unaware (n %)
Toxoplasmosis	2 (0.6)	354 (99.4)
Tuberculosis	56 (15.7)	300 (84.3)
Rabies	51 (14.3)	305 (85.7)
Lassa fever	27 (7.6)	329 (92.4)
Ebola	24 (6.7)	332 (93.3)

Our data analysis revealed that 160 (44.9%) had some prior awareness concerning zoonotic infections from cats, dogs and rats mostly based on myths than facts but only 2 (0.6%) had with regard to toxoplasmosis. Overall, 196 (55.1%) were not aware of any zoonotic infection or diseases (Table 9).

CHAPTER FIVE

DISCUSSION

Primary infection of *T. gondii* acquired during pregnancy can be transmitted to the foetus vertically which may cause serious complication including abortion, stillbirth, visual impairment and neurological disorders.[7] The overall sero-prevalence of *T. gondii* infection of 12.1% obtained in the study was statistically significant but lower when compared with other studies within Nigeria. For instances 29.1 % was reported from a study in pregnant women from the North West city of Zaria,[21] 22.2% from Maiduguri in North East[20] and 40.8% and Lagos in South West,[22] The most likely explanation to these differences in sero-prevalence among pregnant women of different regions can be explained by variation in geographical and climatic conditions, even within the same country of different regions as the oocyst sporulation is enhanced by hot and wet conditions.[4]

In this study, we found sero-prevalence of IgM indicating acute infection among only 1 (0.3%) participant which is in agreement with the study conducted in Zaria that found 3 (0.8%) IgM sero-prevalence[21] but this was slightly lower when compared with other part of Africa such as Gabon with 5.4 % IgM sero-prevalence.[84] Sero-prevalence to *T. gondii* infection have been shown to increase with age but this study found sero-prevalence to *T. gondii* declined with age and this is in accordance with reports from other parts of the world.[85] This could be due to behavioral differences and eating characteristics of the younger pregnant women compared to the older group and as such may get more exposed to some of the factors associated with toxoplasmosis in Pregnant women.[85] T. gondii sero-prevalence was found to be highest among the pregnant women with no formal education which was significantly associated with *T. gondii* seropositivity and this reduces as the

level of education increases which is in agreement with other studies which reported lower levels of education increased the likelihood of being infected with *T. gondii* infection.[21] Most of the study participants were married which is in agreement with the cultural practices of the people where the study was carried out. However, the few unmarried participants in this study had higher sero-prevalence to *T. gondii* infection probably caused by social factors such as adventurous outdoor eating associated with not being married which could predisposes them more to toxoplasmosis.

The area of residence was associated with high sero-prevalence to *T. gondii* intion though not significant. It was observed those pregnant women dwelling in urban areas had higher sero-prevalence compared to the participants from the rural areas. This can be due to lifestyles, eating habits and environmental conditions of those dwelling in urban areas as such more prone to factors that are associated with toxoplasmosis. The occupation of the study participants was not significantly associated with seropositivity to *T. gondii* but the pregnant women whose occupation involved selling vegetables and meat had higher prevalence compared to other groups or those that were unemployed. This could be explained that probably they were exposed to cat oocyst contaminated vegetables and meat and poultry products through handling without the necessary precaution.[85] We observed that pregnant women whose family income was high had the lowest sero-prevalence in the study may be having better socioeconomic status, they are able to reduce or avoid factors that will predispose them to *T. gondii* infection. There was no significant difference between the pregnant women were primigravid or multigravid but the pregnant women in their second trimester had higher sero-prevalence in this category

which is period where risk of foetal transmission is highest following infection without any intervention.[56]

There was significant association between the sero-prevalence and the different ethnic/tribal groups among our study participants. The Hausa/Fulani ethnic group had the highest sero-prevalence among the major ethnic groups as well as the other ethnic tribes and groups. The Igbo ethnic group had zero sero-prevalence. This is agreement with finding from a study in Asia which reported T. gondii infection to be more common among Malays than other ethnic groups.[86] This however is not in agreement with a study conducted in the Federal Capital Territory (FCT) which found no difference between the various ethnic groups in their study.[23] Differences of sero-prevalence between ethnic group could be due to or can be caused by cultural practices, eating habits and food preferences between the various ethnic groups.[4]

We found significant association between the types of water the study participants' drink and sero-prevalence to *T. gondii* infection. The pregnant women who reported drinking untreated water recorded significantly higher sero-prevalence compared to those drank treated water. This agrees with findings from previous studies both in Nigeria and elsewhere.[21, 85] This could be generally be explained that untreated water could contain *T. gondii* oocyst and as such they are continually exposed.

Cooking practices and habits including the type of meat preference by a pregnant woman are important factors for *T. gondii* transmission. The cooking of meat and meat products at high temperature rapidly destroys *T. gondii* cyst found in muscles of infected animals. However, this study found significant association and the sero-prevalence to *T. gondii*

infection in the pregnant women who taste their meat during cooking as compared to those who do not. We observed that those taste meat during cooking had lower sero-prevalence which was statistically significant in comparison to those who do not. Taking these factors of Toxoplasma infection into account, one would expect would expect an increase in *T. gondii* seropositivity among the pregnant women who taste their meat during cooking. Yet, this is not coherent with our findings. This may be due to the meat preference of those who said no to tasting of meat because it has been shown that food animals including poultry, beef and mutton are important source of *T. gondii* infection.[15] Our study also revealed that pregnant women who had meat preference for food animals recorded higher sero-prevalence of 16.7% as against 7.4% of those who had meat preference of other animals including dog, pork and rats and lower sero-prevalence was also recorded for pregnant women who history of handling raw dog, pig or rat meat.

Other factors assessed in this study that demonstrated high *T. gondii* seropositivity but there was no significant association include cooking method, form vegetable is eaten, HIV status and pregnant women who had flu-like symptoms during the interview. We found no difference in the seropositivity of those who eat stick meat (suya) and those who do not, owning a cat and cleaning of cat litter.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The Sero-prevalence *Toxoplasma gondii* infection among antenatal attendees in these health facilities in Plateau State is high with active infection which is significant in the transmission of toxoplasmosis. Educational level, tasting of meat while cooking, drinking untreated water and ethnicity was found to be associated with *T. gondii* infection in the study participants. The awareness of zoonotic infection among the pregnant women was mostly based on myths.

Therefore based on these findings, the following recommendations are offered

1. Health education on preventive measures against *T. gondii* infection and other zoonotic diseases by avoiding factors that could predispose the pregnant women to the infection during antenatal care by the care givers
2. The policy makers should consider introducing routine screening for toxoplasmosis on the high risk groups.
3. Pregnant women should cultivate proper hygienic practices so as to reduce exposure to infection such as:

a. washing of hands after handling raw meat or poultry products
b. Washing of fruits and vegetables before consumption
c. Proper cooking of meat and poultry products before eating

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APPEENDICES

Appendix 1: QUESTIONNAIRES FOR STUDY PARTICIPANTS

Date:…./…../…….. (dd/mm/year) **No:……**

QUESTIONNAIRE ON SERO-PREVALENCE AND FACTORS ASSOCIATED WITH TOXOPLASMOSIS AMONG PREGNANT WOMEN RECEIVING ANTENATAL CARE IN PLATEAU STATE

We would appreciate about ten minutes of your time to obtain some information from you. I am the principal investigator and a resident of the Nigeria Field Epidemiology and Laboratory Training Program Abuja, a Post-graduate student at the Department of Community Medicine Hamada Bello University, Zaria. The study is being conducted to find out how many pregnant women attending antenatal in Plateau State are infected with Toxoplasmosis and the associated factors. The information we get from you will assist us in this study and it will be treated as confidential and participation is voluntary. Thank you.

PART A: PERSONAL INFORMATION

1. ID/Hosp No: ……
2. Age at last birthday in (year)… 3. Area of residence: (Urban /Rural)……
4. Duration at residence: …… 5. Phone No: ……
6. Contact address: ……..
7. Marital Status
   a. Single ☐
   b. Married ☐
   c. Separated ☐
   d. Divorced ☐
   e. Widowed ☐

8. Educational background
   a. No formal education ☐
   b. Primary ☐
   c. Secondary/Vocational ☐
   d. Tertiary ☐
   e. Other (specify): ……

9. Occupation

- Employed
   a. Office (Civil Servant) ☐
   b. Garden/Farm/Abattoir ☐
   c. Market (sell vegetables, raw meat ☐
   d. Other (specify): ……..
- Unemployed ☐

10. What is your tribe /ethnic group?
   a. Hausa/Fulani ☐
   b. Igbo ☐
   c. Yoruba ☐
   d. Other (specify):……

11. What is your family income per month?
   a. < ₦7,500 ☐
   b. ₦7,500 – N30,000 ☐
   c. >₦30,000 ☐

PART B: TOXOPLASMOSIS-RELATED MEDICAL INFORMATION

12. Do you know any disease/s that could be got from cats, dogs, rats? Yes ☐ No ☐

13. If the yes what is it? (Specify).....

14. Have you ever heard of "*Toxoplasma or Toxoplasmosis"*? Yes ☐ No ☐

15. Have you ever been tested for *Toxoplasma* infection? Yes ☐ No ☐

If your answer to Question (Qu.) 14. is "No", please skip to Qu. 19

16. When was the test conducted?
    a. 3 to 6 months ago ☐
    b. Up to a year ago ☐
    c. More than a year ago ☐
    d. Other (specify)……..

17. What was the result? Positive ☐ Negative ☐ Don't know ☐

18. If positive, did you receive any treatment? Yes ☐ No ☐

19. How many pregnancies have you had before the current pregnancy?
    a. None (this is my first) ☐
    b. One ☐
    c. Two ☐
    d. Three ☐
    e. More than three ☐

20. Have you ever had a stillbirth, spontaneous abortion(s) [miscarriage(s)]?
    a. Once ☐
    b. Twice ☐
    c. Three times or more ☐
    d. Never ☐

21. Have you received whole blood or any blood component transfusion? Yes ☐ No ☐ during Last 12 months?

22. How old is your current pregnancy?
    a. 1 to 3 months ☐
    b. 4 to 6 months ☐
    c. 7 to 9 months ☐

23. Do you know your HIV status? Yes ☐ No ☐

24. If *Qu* 23 is yes what is it? Positive ☐ Negative ☐

PART C: FACTORS-RELATED TO TOXOPLASMOSIS

25. Do you own a cat or have cats in your compound or your neighbourhood?
    Yes ☐ No ☐

If "Yes" to Qu. 25 please answer Qu. 26. If "No" skip to Qu. 27

26. Do you have a special corner where the cat defecates in your house which you regularly clean? Yes ☐ No ☐

27. Have you ever handled raw pig, dog or rodent (rat) meat? Yes ☐ No ☐

28. Do you eat meat? Yes ☐ No ☐

If your answer to Qu. 28 is "No'' please skip and go to Qu. 35

29. In which form do you often eat your meat?

    a. Mildly cooked ☐
    b. Cooked till soft ☐
    c. Cooked but tough ☐

30. What form of cooking method do you prefer for your meat

    a. Frying ☐
    b. Roasting ☐
    c. Stewing ☐

31. Which types of meat do you eat? (Please c*hoose as many as applicable.*)

    a. Pork ☐
    b. Goat meat/mutton ☐
    c. Beef ☐
    d. Chicken ☐
    e. Dog meat ☐
    f. Rodent (rat) ☐
    g. Cat meat ☐
    h. Other (specify)……

32. Do you taste your meat while cooking it? Yes ☐ No ☐

33. Do you eat Stick meat (Suya)? Yes ☐ No ☐

34. If yes, what type do you enjoy most?

a. Pork ☐
b. Goat meat ☐
c. Beef ☐
d. Chicken ☐
e. Dog meat ☐
f. Rodent (rat) ☐
g. Sheep (Balangu) ☐
h. Others (specify)……

35. Do you like vegetables? Yes ☐ No ☐

36. In what state do you prefer your vegetables before eating them?

a. Fresh and raw ☐
b. Steamed ☐
c. Cooked ☐

37. What type of drinking water do you consume?

a. Piped water ☐
b. Well water ☐
c. Stream/River water ☐
d. Packaged water ☐

38. Do you have any of these symptoms?

a. Fever ☐
b. Flu-like symptoms ☐
c. Swollen lymph nodes ☐
d. None ☐
e. Other (Specify)……

Thank you very much for your time.

Interviewer's Name:_____ Signature:_____

***Appendix 2*: CONSENT FORM FOR STUDY PARTICIPANTS**

CONSENT FORM

Title: **Sero-prevalence and factors associated with Toxoplasmosis among pregnant women receiving antenatal care in Plateau State**

Principal Investigator: Dr Mariam Florence Ogo

Address: 1. Department of Community Medicine, Ahmadu Bello University Zaria, Kaduna State

2. Nigeria Field Epidemiology and Laboratory Training Program, 50 Haile Selassie Street Asokoro, Abuja

Supervisor: Prof. Adebola Olayinka, Department of Microbiology, Ahmadu Bello University Teaching Hospital, Shika, Zaria, Kaduna State

General Information about the study

Dear Madam,

You are kindly invited to take part in this study voluntarily and you are at liberty to opt out without any consequence.

Purpose of study:

To find out how many pregnant women attending antenatal care are infected with *Toxoplasma gondii* the causative agent of toxoplasmosis

Study Background:

Toxoplasmosis is a parasitic zoonotic disease that affects all warm blooded animals including humans. It is caused by *Toxoplasma gondii* and humans get infected through eating of improperly cooked meat, Fruits and vegetables and drinking of water contaminated by oocyst excreted by cats. If a pregnant woman gets the infection for the first time during pregnancy through these mentioned routes the disease can be passed to the unborn child through the placenta. This can cause various problems for both mother and child. For example, it can cause spontaneous abortion, miscarriage, the baby can die in the womb, or born alive with an abnormally big head, or with eye problems. Treatment is available for this disease when it is promptly detected.

How it will be done: Someone will ask you some questions related to Toxoplasmosis to fill the questionnaire and about a teaspoon full (2 ml) of blood will be taken from you by

an expert and this may cause little discomfort of needle prick. The blood will be tested for *Toxoplasma* infection and the test results will be communicated to you through your doctor.

Benefit

Results of the study will inform your doctor and care givers to give you the prompt care and it may also help elucidate factors associated with Toxoplasmosis in pregnant women.

Confidentiality

Your personal information including your name and all other details provided in the questionnaire will be kept confidential for reference purposes by investigators and not disclosed to anyone. All samples will be given identification codes.

Your rights as a Participant

This research has been reviewed and approved by the Institutional Review Board of Plateau State Specialist Hospital and Vom Christian Hospitals. If you have any question(s) about your rights as a research participant you can contact the IRB Office between the hours of 8 A.M - 4 P.M. You may also contact the chairmen

Agreement

The document describing the benefits and procedures for the research titled **Sero-prevalence and factors associated with toxoplasmosis among pregnant women receiving antenatal care in Plateau State** has been read and explained to me. I have been given an opportunity to ask questions about the research and answered to my satisfaction. I agree to participate.

_____ _____

Date Name and signature or mark of Participant

I certify that the nature and purpose, the benefit associated with participating in this research have been explained to the above individual.

_____ _____

Date Name /Signature of Person Who Obtained Consent

PLATEAU STATE SPECIALIST HOSPITAL JOS

Ref. No. PSSH/ADM/ETH. CO/2015/006
Old Bukuru Road,
P.M.B. 2113,
Jos, Nigeria
Tel. 073-462180
Fax: 073-464031

Email: psshjos@yahoo.com

Date: February 24, 2015

Reg.No: NHREC/05/01/2010b

Notice of Expedited Review and Approval

Re: Sero-prevalence and factors associated with toxoplasmosis among pregnant women receiving antenatal care in Plateau State

Name of Principal Investigator: Mariam Florence Ogo
Address of Principal Investigator: Dept of Community Medicine, A.B.U. Zaria, Kaduna State
Date of receipt of valid application: 11 February 2015
Date of meeting when final determination of research was made: February 24, 2015

This is to inform you that the research described in the submitted protocol, has been reviewed and *given expedited approval by the Health Research Ethics Committee.*

This approval dates from **24/02/2015** to **23/02/2016**. Note that no participant accrual or activity related to this research may be conducted outside of these dates.

All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study. In multiyear research, endeavor to submit annual report to the HREC early in order to obtain renewal of your approval and avoid disruption of research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit your research site without previous notification.

Dr. Bitrus Matawal, MBBS, FWACS
Chairman, HREC PSSH

VOM CHRISTIAN HOSPITAL
(COCIN)

P.M.B. 06 Vom, Plateau State, Nigeria. Tel: 08035860852
E-mail: vom.hospital@gmail.com

Our Ref. VCH/ADM/48/Vol. II/ Your Ref. Date 7th January 2015

APPROVAL OF RESEARCH PROPOSAL

This is to certify that the research proposal entitled:

"SERO-PREVALENCE AND FACTORS ASSOCIATED WITH TOXOPLASMOSIS IN PREGNANT WOMEN RECEIVING ANTENATAL CARE IN PLATEAU STATE".

By: MARIAM FLORENCE OGO.

Has been approved by the Committee on Research and Ethics.

Dr. Fredrick Dachung M.
(Chairman)

7.1.15
Date