

SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN KANO METROPOLIS, KANO STATE.

BY

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ZARIA**

NOVEMBER, 2016

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CARE IN KANO METROPOLIS, KANO STATE.**

BY

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P13SCMC8053

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE

STUDIES

AHMADU BELLO UNIVERSITY ZARIA

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF SCIENCE DEGREE IN MICROBIOLOGY**

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCE,

AHMADU BELLO UNIVERSITY,

ZARIA

NOVEMBER, 2016

DECLARATION

The research presented in this dissertation entitled “**Seroprevalence and Risk Factors Associated with *Toxoplasma gondii* Infection among Pregnant Women Attending Antenatal Care in Kano Metropolis, Kano State**” has been performed by me at the Department of Microbiology under the supervision of Prof. O. S. Olonitola and Dr. A. B. Suleiman. 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 at this university or diploma at any other institutions.

Umar Haruna ADAMU

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Signature

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Date

CERTIFICATION PAGE

This Dissertation entitled **“SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN KANO METROPOLIS, KANO STATE”** by **UMAR HARUNA ADAM**, meets the regulations governing the award of the degree of Masters of Science in the Department of Microbiology, Ahmadu Bello University, Zaria and it has been approved for its contributions to knowledge.

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DEDICATION

This work is dedicated to God the Almighty, all knowing and ever sufficient, the Alpha and the Omega, the Beneficent and the Merciful.

ACKNOWLEDGEMENTS

My sincere gratitude goes to Almighty God, the ruler of the universe, the custodian of wisdom and the giver of knowledge for His divine protection and provision throughout the academic journey and who has made this dissertation a reality.

I also appreciate my parents for standing solidly by me both in the rain and sunshine, also my brothers and sisters; I thank you all for your wonderful support and contributions. Remain blessed forever.

I hereby register my profound gratitude and appreciation to my wonderful supervisors, Prof. O. S. Olonitola and Dr. A. B. Suleiman for their encouragement, advice, suggestions and corrections throughout the project period. I pray Almighty God rewards you accordingly.

I am also greatly indebted to my able Prof. M.B. Shitu for his fatherly role, financial and moral supports. Sir, I pray God Almighty who sees and rewards in secret to reward you more abundantly.

Also worthy of mention are the contributions of my able and pragmatic Lecturers of this great Department. My sincere thanks go to my colleagues and all my friends in this reputable institution for your supports and contributions.

More so, i thank Dr. Eka Ella who did not only play an academic role but went the extra mile to act as a father to me. Sir, it is my sincere prayer that none of your generations will ever lack help. No amount of thanks would be sufficient enough to reciprocate your good gestures toward me.

To Dr. Mikail Kashimbila of Bayero University Kano, I express my profound gratitude to you for all your inputs, contributions morally, financially and academically. I will never miss your rewards from God Almighty.

I will ever remain grateful to Prof. Garba Kawu, Dr. Abdullahi Kawo, Dr. N. T. Dabo and Dr. Aminu Bukar . I thank you all for your immense contributions towards my academic pursuit.

The support of Mr. Essein Unyime Cosmas can never be forgotten in my life. Sir, you are wonderful. May God in His infinite mercy reward you abundantly.

To all the staff of Hospital Management Board Kano State, Murtala Muhammed and Abdullahi Wase Specialist Hospital, Kano State, I say thank you for your co-operation and support.

ABSTRACT

Toxoplasma gondii infection among women of reproductive age especially pregnant women can lead to embryonic death, foetal reabsorption, foetal death, mummification fetus, still-birth and neonatal death. This study establishes the seroprevalence of *Toxoplasma gondii* infection and its associated risk and demographic factors among pregnant women attending antenatal care in the selected hospital in Kano Metropolis, Kano State. Enzyme linked Immunosorbent Assay (ELISA) was used to assess the presence of *Toxoplasma gondii* IgG, IgM and IgG IgM specific antibodies and IgM positive isolate were further screened by Polymerase Chain Reaction (PCR) to detect the presence of *BI gene*. Data collected was subjected to Chi-square test using statistical package for social science version 20 to determine the association between risk factors and *Toxoplasma gondii* infection. This study revealed the prevalence of 34.23%, 13.08% and 3.85% of IgG, IgM and IgG IgM antibodies respectively. Correlating the ELISA results obtained from the serum samples analyzed in this study with the demographic and risk factor associated with the infection as follows: age, reproductive history, stages of pregnancy, occupation, level of education, presence of cat and sources of drinking water, there was no significant association in the occurrence of *Toxoplasma gondii* infection ($P > 0.05$), only presence of cat and sources of drinking water ($P < 0.05$) shows a significant association. Seven positive IgM isolates with higher titer value were screened using PCR, only one isolate confirmed positive for *Toxoplasma gondii BI gene* in the serum samples of pregnant women. This study established the presence of *Toxoplasma gondii* infection in Kano metropolis with overall prevalence of 43.4%. Hence campaign to intensify hygiene standard should be sustained in Kano metropolis and environs.

TABLE OF CONTENTS

Cover Page	i
Title Page	ii
DECLARATION	ii
CERTIFICATION PAGE	iv
ACKNOWLEDGEMENTS	vi
ABSTRACT.....	viii
TABLE OF CONTENTS.....	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF PLATES	xiv
ABBREVIATION.....	xv
CHAPTER ONE	1
1.0 INTRODUCTION.....	1
1.1 Statement of the Research Problem	3
1.2Justification of the Study	3
1.3Aim of the Study.....	4
1.4Objectives of the Study	4
CHAPTER TWO	6
LITERATURE REVIEW	6
2.0 The parasite- <i>Toxoplasma gondii</i>	6
2.1 History and taxonomy	6
2.2Morphology of <i>Toxoplasma gondii</i>.....	10
2.2.1Oocysts 10	
2.2.2Tachyzoites	10
2.2.3Bradyzoites	11
2.3.Cell Biology of <i>T. gondii</i>.....	11
2.4 Life Cycle of <i>Toxoplasma gondii</i>	16
2.4.1 Feaco-oral route	17
2.4.2Vertical transmission	17
2.5Nutrition in <i>Toxoplasma gondii</i>	18
2.6Toxoplasmosis	19

2.7	Clinical manifestations of Toxoplasmosis	21
2.8	Human Toxoplasmosis	22
2.9	Toxoplasmosis and pregnancy	25
2.10	Ocular Toxoplasmosis	26
2.11	Congenital Toxoplasmosis	26
2.12	Epidemiology and global distribution of Toxoplasmosis	30
2.13	Transmission of Toxoplasmosis	34
2.14	Economic importance of Toxoplasmosis	35
2.15	Behavioral changes associated with <i>Toxoplasma</i> Infection	35
2.16	Diagnosis of Toxoplasmosis	36
2.17	Prevention and control of Toxoplasmosis	38
2.18	Treatment of Toxoplasmosis	39
CHAPTER THREE		40
MATERIALS AND METHODS.....		40
3.0	Study area	40
3.1.	Study population	40
3.1.1.	Inclusion criteria	40
3.1.2.	Exclusion criteria	40
3.2	Ethical approval	40
3.3	Sample size determination	42
3.3.1	Data Collection	43
3.3.2	Sample collection.....	43
3.4	Laboratory Diagnosis.....	45
3.4.1	Assay procedure.....	45
3.4.1	Interpretation of ELISA results	46
3.5	Nested PCR Assay	47
3.5.1	DNA extraction method:.....	48
3.5.2	Assay procedure.....	49
3.5.4	PCR condition.....	49
3.5.5	Procedure for Agarose gel electrophoresis	49
3.6	Data analysis	50
CHAPTER FOUR.....		51

4.0	RESULT	51
	CHAPTER FIVE	63
	5.0DISCUSSION	63
	CHAPTER SIX	66
	CONCLUSION AND RECOMMENDATION	66
	6.0CONCLUSION	66
	6.1 RECOMMENDATION	66
	REFERENCE	68
	APPENDIX I	78
	APPENDIX II	78
	APPENDIX III	80
	APPENDIX IV	82

LIST OF TABLES

4.1: The seroprevalence of <i>T. gondii</i> based on age group of pregnant women examined.....	55
4.2: The seroprevalence of <i>Toxoplasma gondii</i> infection among pregnant women in relation to history of miscarriage.....	56
4.3: Seroprevalence of <i>T.gondii</i> antibodies in relation to the stages of pregnancy.....	57
4.4: The seroprevalence of <i>T.gondii</i> antibodies with respect to occupation of pregnant women examined.....	58
4.5: Seroprevalence of <i>T. gondii</i> antibodies with the level of education of pregnant women examined	59
4.6: The prevalence of <i>T.gondii</i> antibodies based on contact with cats.....	60
4.7: Seroprevalence of <i>Toxoplasma gondii</i> infection among pregnant women in relation to sources of drinking water.....	61

LIST OF FIGURES

2.1. Taxonomical classification of apicomplexan parasites.....	9
2.2: Schematic drawing of a tachyzoite cell showing intracellular organelles.....	15
2.3: Life Cycle of <i>T.gondii</i>	20
3.1: Map of Kano State Metropolis.....	41
3.2: Distribution of blood samples collected from consented pregnant women attending antenatal at selected hospitals.....	44
4;1: A ven diagram of <i>Toxoplasma gondii</i> antibodies IgG,IgM andIgG IgM among pregnant examined	54

LIST OF PLATES

4.1; Amplicon of B1 gene of <i>T.gondii</i>	62
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ABBREVIATION

AIDS: Acquired Immune Deficiency Syndrome

ELISA: Enzyme Link Immunosorben Assay

IgG: Immunoglobulin G.

IgM: Immunoglobulin M.

NPC: Nationalpopulation Census.

PCR: Polymerase Chain Reaction

CHAPTER ONE

1.0 INTRODUCTION

Toxoplasma gondii is a protozoan parasite which infects almost one-third of the world's population usually causing mild, non-specific clinical features (Dubey and Beattie, 1988; Tenter *et al.*, 2000). It was first discovered in 1908 by Nicolle and Manceaux in an African rodent (*Ctenodactylus gondii*) (Siel *et al.*, 2003), the name *Toxoplasma gondii* ("Toxon"-means arc, "Plasma"-form in blood) was derived from its crescent shape and form in the animal from which it was first isolated (Frenkel *et al.*, 1970). Toxoplasmosis is among the global major zoonotic diseases and the third leading cause of food-related deaths in the United States of America in 2004 (Torgerson and Macpherson, 2011).

The organism infects herbivorous, omnivorous and carnivorous animals, including birds (Arko-Mensah *et al.*, 2000; Bisson *et al.*, 2000). It is caused by *Toxoplasma gondii*, an Apicomplexa protozoan parasite (Tenter *et al.*, 2000), with cats as the definitive host, and warm-blooded animals as intermediate hosts (Dubey, 2010). There are two main routes of transmission which have been described in humans namely oral- ingestion of the parasite and through placental transmission to the fetus if infection occurs in recently pregnant women.

The oral routes may involve the ingestion of raw or undercooked meat that contains cysts, the ingestion of water or food contaminated with oocysts (Pelloux *et al.*, 1997; Dubey, 2004; Dawson; 2005). After ingesting raw or undercooked meat, ingesting cat-shed oocysts via contaminated soil, food, water or it can be acquired through congenitally by transplacental transmission of tachyzoites (Montoya and Rosso, 2005). Infection with *T. gondii* during pregnancy can result in fetal death, neonatal death or various congenital defects, such as hydrocephalus, central nervous system abnormalities and chorioretinitis (Dubey, 2004).

Acute primary maternal toxoplasmosis if acquired during first trimester of pregnancy can cause significant morbidity and mortality in developing fetus, induce abortion and loss of vision (Montoya and Liesenfeld, 2004; Pleyer *et al.*, 2007; Singh, 2003; Thiebaut, *et al.*, 2007). In general, human being can become infected horizontally with *T. gondii* by ingesting raw or undercooked meat or insufficiently treated meat containing viable tissue cyst (Cook *et al.*, 2000; Garcia *et al.*, 2006), by ingesting food or water contaminated with sporulated oocysts (Dubey, 2004). It can also occur by accidentally ingesting oocysts from the contaminated environment with infected cat feces (Clementino *et al.*, 2007) or vertically via trans-placental transmission from pregnant mother to the fetus through tachyzoites in the circulation (Dubey, 2004; Dubey, 2009a; Dubey, 2009b; Kravetz, and Federman, 2005).

Toxoplasmosis is also a serious problem in immune-compromised patients (Jones *et al.*, 2009). Recently, highly virulent genetically atypical strains of *T. gondii* have been incriminated with pneumonia, even in immune-competent individuals (Leal *et al.*, 2007). In a study in Lagos State *Toxoplasma* IgM was common in the first trimester (16.7%) while *Toxoplasma* IgG was seen mostly in the third trimester (46.7%). IgG and IgM were seen to be significantly associated with parity (Deji -Agboola *et al.*, 2011). Recent studies have shown that polymerase chain reaction (PCR) testing of amniotic fluid is useful for identification or exclusion of fetal *T. gondii* infection (Romand *et al.*, 2001). Ultrasound can be used for the detection of fetal lesions due to congenital toxoplasmosis (Gagne, 2001). A PCR was also used to detect the parasite in blood samples from patients who presented with acute toxoplasmic lymphadenopathy or to complement culture and serologic testing for the diagnosis of active toxoplasmosis (Burg, 1995).

1.1 Statement of the Research Problem

Most infections in humans are asymptomatic, but the parasite can produce a devastating disease (Jin *et al.*, 2005). Only about 10% of infected individuals develop signs and symptoms. However, in pregnant women and immunosuppressed individuals the clinical disease can be severe (Torda, 2001). Infection during pregnancy can cause spontaneous abortion and neurological disorders such as blindness and mental retardation in congenitally infected newborn babies (Dubey and Beattie, 1988; Hayed and Pollak, 2000).

Infection of human and livestock with *T. gondii* may cause embryonic death, foetal reabsorption, foetal death, mummification fetus, still-birth and neonatal death (Wang *et al.*, 2011).

Consumption of undercooked meat infected with the parasite can facilitate zoonotic transmission (Bisson *et al.*, 2009; Wang *et al.*, 2011). *Toxoplasma gondii* infection can be life threatening in congenitally infected immunosuppressed patients (Chinta *et al.*, 1988) and those undergoing cancer chemotherapy (Ghazei, 2006). Its greatest impact is in the late clinical stage of acquired Immunodeficiency Syndrome where up to 25 percent of patients developed Toxoplasmic encephalitis (Luft *et al.*, 1993; Lucas *et al.*, 1993). This causes heavy economic loss. The death and abortion in pregnant women as a result of this infection could lead to the reduction of population. Insufficient awareness of the disease condition and its risk factors has resulted in toxoplasmosis in pregnant women. There is paucity of information on the prevalence of *T. gondii* in women attending antenatal care clinics in Kano metropolis.

1.2 Justification of the Study

Scenario of animal rearing in Kano state, Nigeria poses danger for the transmission of zoonotic pathogens such as *Toxoplasma gondii*. This ubiquitous parasite can be transmitted directly by

human-animals, contact with contaminated soil, faeces or herbage (Jittapalapong *et al.*, 2008), thus there is a high risk of exposure in homes especially where domestic animals are kept such as cat, sheep, goat and cattle are kept. The presence of cats and dogs around abattoirs tend to facilitate the transmission cycle and hence meat could also be contaminated with oocysts from soil particularly where sanitary measures are lacking such as homes and abattoirs.

Serological screening of toxoplasmosis in pregnant women attending antenatal care is not a routine procedure in health centers in Kano metropolis. Therefore, a cross-sectional study will be performed to determine the current status of infection among pregnant women and the possible risk factors associated with infection.

There is a gap in knowledge on the current status and seroprevalence of *Toxoplasma gondii* in females attending antenatal care in Kano metropolis. This study is premised upon the non-availability of information on the current status of *Toxoplasma gondii* infection. This demand for the investigation of the current status of infection among pregnant women attending antenatal care in Kano and the possible risk factors associated with infection. There by reducing the rate of abortion, neonatal death and purchase of drugs by government and individuals.

1.3 Aim of the Study

To establish the Seroprevalence and risk factors associated with *Toxoplasma gondii* infection among pregnant women accessing antenatal care in Kano metropolis.

1.4 Objectives of the Study

1. To determine the seroprevalence of *Toxoplasma gondii* IgG IgM and IgG/IgM antibodies among pregnant women attending antenatal clinics in Kano metropolis using ELISA kits.

2. To confirm the presence of *Toxoplasma gondii* B1 gene in the serum of antenatal women using PCR.
3. To determine the demographic and associated risk factors responsible for contracting and transmission of *Toxoplasma gondii* infection.

CHAPTER TWO

LITERATURE REVIEW

2.0 The parasite-*Toxoplasma gondii*

Toxoplasma gondii bradyzoites invade the intestinal epithelium. Besides systemic dissemination after conversion to the invasive tachyzoite stage, some organisms inside the epithelium go through five different developmental stages that reproduce asexually by endodyogeny, where two daughter cells are created inside one through schizogony, involving the formation of multiple merozoite cells around a previously divided nucleus (Dubey *et al.*, 1988). Probably stemming from the merozoite forms, the sexual stages (gamonts) are formed three to fifteen days after tissue cyst ingestion, consisting of a female gamont and several bi-flagellated microgametes formed by division of the mature male gamont, the sexual stages are found just under the host cell microvilli in the small intestine, specially the ileum aided by its flagella, the male microgamete fertilizes the female gamont starting the formation of a zygote contained within a thick walled oocyst (Dubey, 1993). Oocysts are discharged from the ruptured epithelial cells into the intestinal lumen of the cat and excreted unsporulated to the environment via cat feces (Dubey, 1993).

After one to twenty days, oocyst sporulation results in the formation of two internal sporocysts containing four sporozoites each. *Toxoplasma gondii* is a promiscuous protozoan parasite with an extremely wide geographic and host range (Innes, 1997).

2.1 History and taxonomy

Toxoplasma gondii is an apicomplexan protozoan parasite. It was first described in 1908 in the tissues of a small North African rodent, the *gondii* (*Ctenodactylus gondii*) by Nicolle and Manceaux and independently in Brazil by Splendore, in an infected rabbit (Dubey, 1993). Identification of *Toxoplasma* as a human pathogen started with the description of *Toxoplasma-*

like organisms in human retinal tissue by the ophthalmologist Josef Jankú in 1923, and was followed by additional reports of clinical cases in humans (Dubey, 1993). Determination of its pathogenicity in animals and humans caused an increased interest in this organism and led to the discovery and description of the parasite's complete life cycle in 1970 (Dubey *et al.*, 1970). Classical taxonomy places *Toxoplasma gondii* in the phylum Apicomplexa (that is possessing an apical complex), in a class where all organisms share a conoid structure as a special characteristic. The important human pathogens *Plasmodium spp.* and *Cryptosporidium spp.* are related protozoans that also belong to the Apicomplexa (Roberts and Janoy, 2005). *Toxoplasma* belongs to the family Sarcocystidae along with at least three other closely related genera that are known as animal parasites: *Neospora spp.*, *Hammondia spp.* and *Sarcocystis spp.* (Dubey *et al.*, 2005; Kopecká *et al.*, 2006). Only one species has been assigned to the genus *Toxoplasma*. The first human case of toxoplasmosis was described by Jankú in 1923 (Cox, 2002; Sukthana, 2006).

This parasite has been successfully isolated from tissue in neonate with encephalitis by animal inoculation (Wolf *et al.*, 1939; Cox, 2002). This served to be the first example of an organism causing disease in utero (Cox, 2002). Adult infection with the parasite was first described by Pinkerton and Henderson in 1941 and childhood infection by Sabin in 1942 (Frenkel, 1973).

Toxoplasmic retinochoroiditis was first identified by Wilder in 1952 in the retinas of 50 human cases (Jacobs *et al.*, 1954). Reactivation of a latent infection in man was described by Frenkel in 1956 (Frenkel, 1973).

In 1954, Weinman and Chandler first investigated the possible transmission of the parasite through the consumption of undercooked meat (Jacob *et al.*, 1960). They found that *T. gondii* cysts from cat, pigs, rodent 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 *T. gondii* was completely elucidated by the discovery of a sexual stage in the small intestine of cats (Tenter *et al.*, 2000). *T. gondii* has three infective stages: the sporozoite stage in oocysts in faeces, the rapidly dividing tachyzoites found during an acute infection and the slowly dividing bradyzoite stage in cysts during latent infection (Peterson and Dubey, 2001; Jones *et al.*, 2001b)

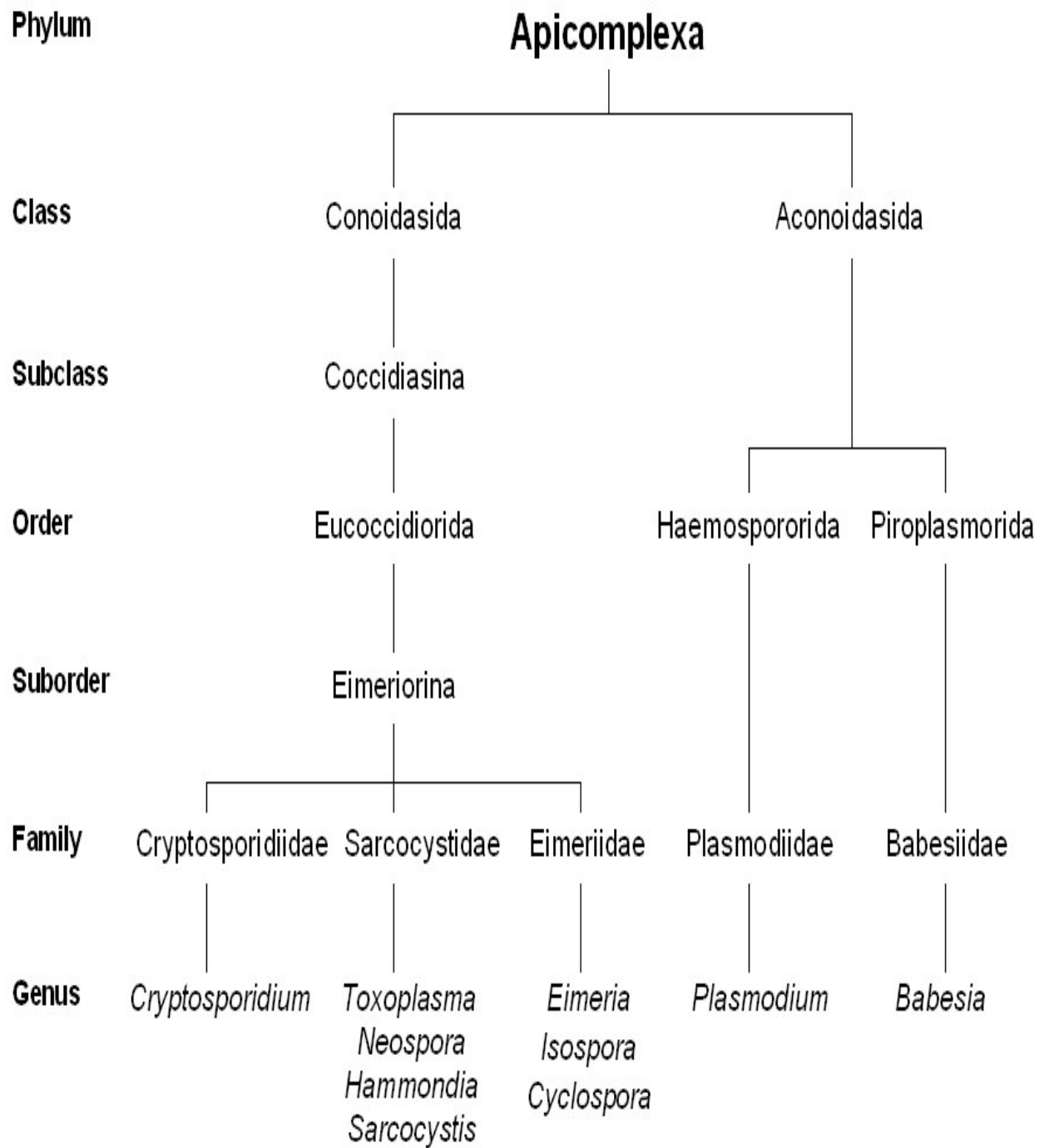


Figure 2.1 Taxonomical classification of apicomplexan parasites; some animal parasites have been omitted from this for simplicity. Classification according to (Stubb *et al.*, 1997)

2.2 Morphology of *Toxoplasma gondii*

T. gondii has three infective stages: the sporozoite stage in oocysts in feces, the rapidly dividing tachyzoites found during an acute infection and the slowly dividing bradyzoite stage in cysts during latent infection (Peterson and Dubey, 2001; Jones *et al.*, 2001b).

2.2.1 Oocysts

Oocysts are 10µm to 12µm in size and are produced within the intestine of the definitive host. Sporozoites are found within these oocysts, which are shed in the feces of the definitive host (Peterson and Dubey, 2001).

2.2.2 Tachyzoites

Tachyzoites are crescentic in shape and approximately 6µm long and 2 µm wide (Bhopale, 2003). They are coated by a three-unit membrane, namely a plasmalemma inner membrane consisting of two closely situated membranes, all of which formed the pellicle (Dubey *et al.*, 1998; Bhopale, 2003). The various organelles making up the tachyzoite (Figure 1.1) include apical and polar rings, rhoptries, micronemes, conoid, subpellicular microtubules, mitochondria, micropores, smooth and rough endoplasmic reticula, Golgi complexes, ribosomes and a nucleus consisting of an achromatin mass and a nucleolus (Dubey *et al.*, 1998).

Tachyzoites spread via the blood system in lymphocytes, macrophages and free in 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 then 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). *T. 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 (Carruthers, 2002). The pressure of the host's immune system on the parasite stimulates 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).

2.2.3 Bradyzoites

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). 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). Tissue cysts are able to persist for the duration of the host's life time and bradyzoites can be released from these cysts to form tachyzoites again causing a reactivated infection in immunocompromised host (Montoya and Liesenfeld, 2004).

2.3. Cell Biology of *T. gondii*

Toxoplasma gondii is an intracellular parasite that is able to successfully infect its host and replicate in a large variety of nucleated animal cells (Dobrowolski *et al.*, 1997). An intracellular location is needed by the parasite for reproduction and most of its metabolic requirements and to escape from the host's immune system (Rabenau *et al.*, 2001).

Infection of cells by *Toxoplasma gondii* is an active event that is powered by the parasitocytoskeleton and mediated by secretion of different parasite molecules that participate in cellbinding, cell invasion and the formation of the parasitophorous vacuole (PV)(Carruthers *et al.*,1997; Dobrowolski *et al.*, 1997). Infection of a cell begins with recognition and binding to matrix and cell surface receptors and requires apicalorientation of the zoite on the host cell membrane (Rabenau *et al.*,2001). Different GPI-anchored surface antigens, SAG and SAG-related proteins that are present on the parasite membrane act as initial ligands for these receptors (Dzierszinski *et al.*,1999;lekutis *et al.*, 2001; Kamani *et al.*, 2010).

Initial anchoring of the parasite to cell surfaces and matrices mediated by SAG proteins is followed by apical contact and sequential protein secretion onto the intended cell surface from two types of tubular organelles of the apical complex, micronemes and rhoptries(Carruthers *et al.*,1997). The proteins secreted by the micronemes, MIC proteins, possess a variety of conserved adhesive domains that function as ligands for different host proteins and carbohydrates. MIC proteins are processed and secreted as various complexes of adhesive and accessory proteins (Dowse and Soldati, 2004). Although secreted into the extracellular environment, receptor bound MIC proteins remain attached to the parasite plasma membrane. During cell penetration, host surface-anchored MIC proteins are capped along the parasite membrane towards the posterior end of the zoite and are eventually shed by proteolysis (Kim, 2004; Dowse *et al.*, 2006).

Thus attached to the substrate, *Toxoplasma* penetrates the cell without disrupting the membrane by pushing itself along with the host cell membrane through a constriction called the moving junction while simultaneously secreting the contents from the rhoptries (Carruthers *et al.*,1997). The creation of the moving junction requires a tight molecular interaction between the host

cell membrane, microneme and rhoptry (ROP) proteins as well as incorporation of rhoptry material into the membrane of the nascent vacuole (Carruthes *et al.*, 2006; Hakanson *et al.*, 2001; Sibley, 2001). Progressive host cell membrane invagination ends with closure of the moving junction behind the parasite and creation of the parasitophorous vacuole. During parasitophorous vacuole formation, host cell membrane proteins are selectively excluded from the vacuole membrane forming a host cell protein-denuded intracellular compartment (Charron *et al.*, 2004). Establishment of the parasite within the parasitophorous vacuole is dependent on the secretion of proteins from a third type of organelles, the dense granules. The function of dense granule (GRA) proteins and ROP proteins is to modify the parasitophorous vacuole in such a way that the parasite is able to subvert host cell metabolic functions (Sibley *et al.*, 1995; Coppers *et al.*, 2000; Sinai *et al.*, 2001; Coppers *et al.*, 2006). The parasitophorous vacuole membrane does not fuse with the host cell endolysosomal pathway, which might endanger the parasite, although import of lysosome-contained host molecules to the parasitophorous vacuole does occur (Coppers *et al.*, 2006).

Parasitophorous vacuole is closely associated to host-cell mitochondria, endoplasmic reticulum and microtubules and allows the diffusion of low-molecular weight nutrients through membrane pores (Sinai *et al.*, 1997; Sinai *et al.*, 2001; De Souza, 2006; Melo *et al.*, 2006). Intracellular organisms need host cell nutrients for their own biosynthetic pathways (Roos, 1999; Darling *et al.*, 1999; Coppers *et al.*, 2000; Chaudhary *et al.*, 2004; Roos, 2004). After a lag phase of about 4 to 6 hours, the tachyzoite starts a series of successive mitotic divisions with production of multiple daughter cells until the host cell eventually lyses. Parasites have also the ability to actively egress the host cell before lysis. Released organisms propagate infection by rapidly invading adjacent cells and starting new intracellular division cycles. The pathological effect of *Toxoplasma*

infection is caused by cell destruction due to parasite loads as well as by the inflammatory response recruited at the site. Activation of a specific immune response directed by activated macrophages and T-lymphocytes, with interferon- γ acting as the main effector cytokine of a Th1 type immune response induces tachyzoites to stay inside the host cell (Alibert, 2005). Conversion to the bradyzoite stage by turning-on of bradyzoite-specific protein expressions swiftly follows (Lyson *et al.*, 2002; Roberts, 2002). This stage is slowly replicative, non-lytic and creates a host-cell derived tissue cyst possessing low immunogenicity. Encysted bradyzoites can remain for years in tissues of the infected host, notably in skeletal and cardiac muscles, retina and brain. With the onset of cellular immunosuppression, bradyzoites once again revert to the tachyzoite stage and start rapid replication, cell lysis and dissemination into other parts of the human body eventually causing death of the host if left untreated.

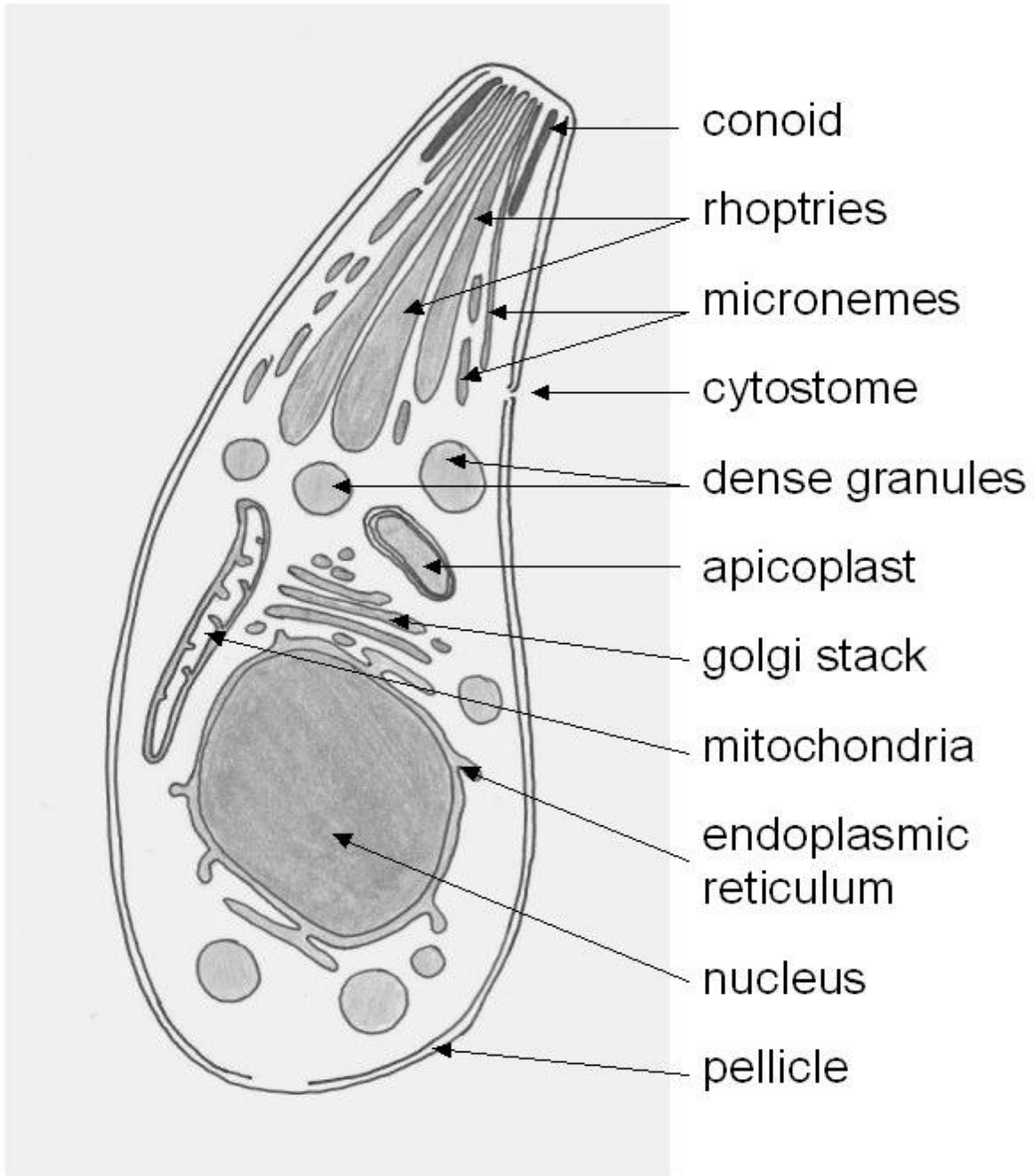


Fig 2.2: Schematic drawing of a tachyzoite cell showing intracellular organelles(Dubey,2004).

2.4 Life Cycle of *Toxoplasma gondii*

Toxoplasma is a parasite that possesses a heterogeneous life cycle requiring the participation of different animal hosts. The definitive hosts of this organism belong to the family of Felidae, either domestic or wild cats or other felids such as lynxes (Dubey 1972; Miller *et al.*, 1972). Sexual reproduction of *Toxoplasma gondii* takes place in the intestinal epithelium of felines, starting with ingestion by the definitive cat, host of tissue cysts containing the latent stage of the parasite, the bradyzoite. After digestion of the cyst wall by gastric acid, bile and lytic enzymes of the upper digestive tract, the released bradyzoites invade the intestinal epithelium besides systemic dissemination after conversion to the invasive tachyzoite stage, some organisms inside the epithelium go through five different developmental stages that reproduce asexually by endodyogeny, where two daughter cells are created inside one and by schizogeny, involving the formation of multiple merozoite cells around a previously divided nucleus (Dubey *et al.*, 1998). Probably stemming from the merozoite forms, the sexual stages (gamonts) are formed three to fifteen days after tissue cyst ingestion consisting of a female gamont and several bi-flagellated microgametes formed by division of the mature male gamont, the sexual stages are found just under the host cell microvilli in the small intestine, especially the ileum aided by its flagella, the male microgamete fertilizes the female gamont starting the formation of a zygote contained within a thick walled oocyst (Roberts *et al.*, 2005). Oocysts are discharged from the ruptured epithelial cells into the intestinal lumen of the cat and excreted unsporulated to the environment via cat feces (Dubey, 2004).

After one to twenty days, oocyst sporulation results in the formation of two internal sporocysts containing four sporozoites, oocysts are very resistant to environmental conditions; they can preserve their infectivity for several months or years until ingestion by a new host (Dubey, 2004). Ingestion of oocysts by other felines can enhance systemic infection as well as the

formation of sexual stages, while infection in non-feline (intermediate) hosts including humans will result only in systemic infection. After primary invasion of the intestinal epithelial cells of the new host, the sporozoites undergo stage conversion to the actively disseminating form, the tachyzoite reproduction by asexual division (endodyogeny) which occurs in the intestinal epithelium and after crossing the lamina propria (Dubey, 2004). The tachyzoites rapidly travel from the intestinal tract to other parts of the body including muscles and internal organs, specially the brain, establishing a latent infection by conversion to the slowly replicating form (Dubey, 2004).

The bradyzoite enclosed in tissue cysts, *Toxoplasma* bradyzoites are maintained viable in the infected organ for many years, if not for the entire duration of the host's life (Dubey, 2004).

Persistent latent infection in tissues helps to ensure that some of the parasite will be passed on to a new host through consumption of meat from infected animals (Dubey, 2004). Human beings are thus susceptible to infection by *Toxoplasma* either through:

2.4.1 Feaco-oral route

The ingestion of environmental oocysts containing sporozoites or tissue cysts in inadequately cooked meat from other intermediate host such as pigs, sheep or other warm blooded animals (Dubey, 2004). As most warm-blooded animals (including birds) can be infected, they act as potential sources for transmission of *Toxoplasma* infection through meat cysts. Additionally, the widespread prevalence of feline species ensures dissemination of this parasite through oocyst contamination of the environment (Dubey, 2004).

2.4.2 Vertical transmission

This is acquired from the infected mother through the placenta to the fetus.

2.5 Nutrition in *Toxoplasma gondii*

Acquisition of nutrients by a eukaryotic cell occurs through three different ways: passive diffusion of small uncharged or lipophilic molecules through the plasma membrane; active transport by protein transporters of hydrophobic or charged molecules such as ions, and internalization of macromolecules through cell membrane endocytosis including phagocytosis. Although endocytosis of macromolecules is a relatively well-known process in extracellular and intracellular kinetoplastid protozoan parasites, knowledge on the endocytic pathway of apicomplexan parasites is still incomplete (Morgan *et al.*, 2002; Robibaro *et al.*, 2002). In intracellular plasmodia, macromolecules from the environment around the infected erythrocyte are internalized by the parasite by fluid-phase (FP) endocytosis, bypassing the erythrocyte cytoplasm through a system of ducts or tubulovesicular structures (Pouvelle *et al.*, 1991; Goodyer *et al.*, 1996; Lauer *et al.*, 1997; Haldar *et al.*, 2001).

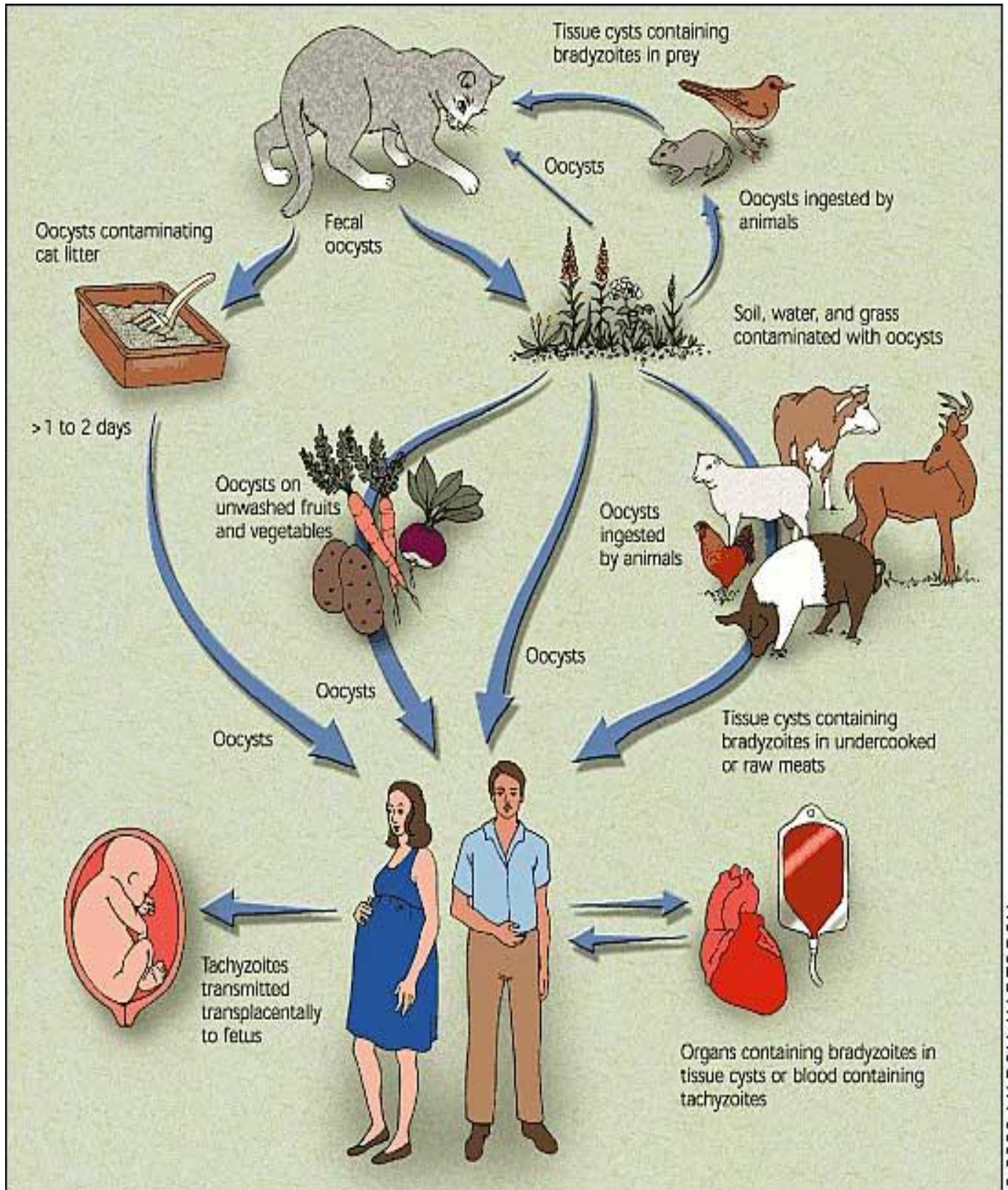
Internalization of morsels of host cell cytoplasm by invagination of the parasitophorous vacuole membrane into the cytostome or micropore of the parasite occurs in order to obtain nutrients from hemoglobin (Senaudet *et al.*, 1976; Olliaro *et al.*, 1995; Hoppe *et al.*, 2004; Weber, 2004). *Toxoplasma gondii* is an auxotroph for some amino acids and nucleobases and requires them from the host cell in order to survive and multiply (Bzik, 2004; Chaudhary *et al.*, 2004; Fox *et al.*, 2004; Roos, 2004). Although it is not known if active nutrient transport mechanisms are needed by extracellular parasites during their relatively brief stay in the extracellular environment, free forms of the parasite are capable of internalizing molecules through putative transporters located in the tachyzoite membrane (Coppens, 2005; Massimine *et al.*, 2005; Sehgal *et al.*, 2005) or through endocytosis at the parasite cytosome (Nichol *et al.*, 1994; Pavesio, 1994). Resembling malaria parasites, intracellular *Toxoplasma* bradyzoites appear to internalize amorphous bits of cyst matrix and vesicles through their cytostome. Additionally, fetuin, a fetal

calf serum protein, has been found inside tachyzoites propagated in vitro, and intracellular tachyzoites acquire cholesterol intercepted from the host cell LDL-degradative pathway, although how these substances enter the parasite is not elucidated (Gross *et al.*, 1993, Coppens *et al.*, 2000).

Recent data shows delivery of membrane-protected host cell lysosomes into the parasitophorous vacuole, indicating that the intracellular parasite can have access to degradation products of already digested macromolecules by subverting the host cell endo-lysosomal system (Coppens *et al.*, 2006). *T. gondii* internalizes different substances through trans membrane passage of macromolecules and also by endocytosis at the cytostome. Electron microscopy of *T. gondii* tachyzoites has shown that the cytostome can be sometimes coated with a bristle clathrin-like coat (Nichols *et al.*, 1994; Pavesio, 1994), which is a classical structure, formed under coated pits for capping and internalization of trans membrane receptor-bound ligands in eukaryotic cells. *T. gondii* possesses a Rab5 homologue that localizes to tubulovesicular structures adjacent to the Golgi, indicating the possibility of an endosomal compartment (Joiner, 2002; Robibaro *et al.*, 2002). 2.3.

2.6 Toxoplasmosis

Toxoplasmosis is a widespread disease and has been classified as the third most common cause of food-borne deaths in the USA. Over the last twenty years, it has also emerged as one of the most common opportunistic infections associated with HIV and AIDS, is a major cause of mortality in AIDS patients in developing countries (Carruthers, 2002).



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Fig 2.3 Life circle of *Toxoplasma gondii* parasite (Pediatric Review, 1997)

2.7 Clinical manifestations of Toxoplasmosis

The clinical manifestations of toxoplasmosis vary, depending on parasite characteristics such as virulence of the strain and inoculum size, as well as host factors such as genetic background and immune status (Montoya and Liesenfeld, 2004). The infection presents with a wide range of clinical manifestations in man, land and sea mammals, and various bird species (Akyar, 2011). When symptoms develop, they are nonspecific and include malaise, fever, sore throat, and myalgia. Clinical manifestations of toxoplasmosis are caused by cell destruction due to multiplying tachyzoites, which most commonly affect the brain, liver, lungs, skeletal muscles and eyes. Oocyst-induced infection may be more severe than that induced by ingestion of tissue cysts. Signs may persist for one to twelve weeks but more severe disease is very rare in immunocompetent individuals (Tenter *et al.*, 2000). Clinical cases, quite few may develop ocular toxoplasmosis (retinitis), but this is more commonly associated with congenital infection (Perkins, 1990).

Approximately 10% of congenital toxoplasmosis results in abortion or neonatal death. A clinical sign of congenital toxoplasmosis is not apparent at first in most cases but infection acquired after birth is usually asymptomatic. Intra-uterine meningoencephalitis could lead to the development of the following: cerebrospinal fluid (CSF) abnormalities, hydrocephalus, microcephaly, chorioretinitis, seizures, and deafness. Some of the severely affected infants die in utero or within a few days of birth. (Foulon *et al.*, 1988). Other signs include maculopapular rash, generalized lymphadenopathy, hepatomegaly, splenomegaly, jaundice, and thrombocytopenia. The clinical course is usually benign and self-limited myocarditis, pericarditis, and pneumonitis which are rare complications. Infants with congenital infection are asymptomatic at birth in (70%) to (90%) of cases, although visual impairment, learning disabilities, or mental retardation will become apparent in a large proportion of children several months to years later.

Infection may be associated with other diseases such as HIV/AIDS in humans or immunosuppressive therapy in any species (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, and liver.

Rarely do infants who are born to mothers living with AIDS or mothers who are immunocompromised for other reasons, have chronic infection with *T gondii* that was acquired congenitally in utero as a result of reactivated maternal parasitemia.

2.8 Human Toxoplasmosis

T. gondii is a ubiquitous parasite that can be found throughout the world. In humans, infection by this organism confers life-long detectable antibody titers and this characteristic has been used as a marker of infection for sero-epidemiological studies. Antibodies against *Toxoplasma* have been detected in most populations that have been surveyed, with infection rates that vary according to the habits of the population and seem to be lower in colder areas of the world (Sukthana, 2006). The probable source of infection, either tissue cysts or oocysts, is determined by the meat-eating habits and presence of infected cats in the near environment. Most cases of human toxoplasmosis are asymptomatic and *Toxoplasma gondii* usually behaves as a benign parasite, eliciting moderate inflammatory reaction and causing little damage to the host. Symptomatic primary infection is unusual (about 10% of infections) and its manifestations in healthy persons are unspecific: enlarged cervical lymph nodes, sometimes accompanied by muscle pain, fever and malaise and in fewer cases hepatosplenomegaly. Symptoms are usually self-limited and there is

no need for treatment except in those rare cases where more severe manifestations do occur, such as eye inflammation (Montoya and Liesendfeld, 2004).

Nevertheless, *Toxoplasma*'s continuous presence in the body presents a threat if the immune system ever becomes compromised such as in patients with untreated HIV infection or those who receive immunosuppressive therapy for cancer treatment or organ transplantation (Montoya and Liesendfeld, 2004). Additionally, *Toxoplasma*-seronegative immunosuppressed patients receiving a transplanted organ (mostly hearts) from an infected donor are also at risk for disseminated acute toxoplasmosis. Reactivation of latent parasites in chronically infected persons at the onset of cellular immunosuppression is influenced by the degree of immune deficit and genetic characteristics of the infecting parasite (Suet *et al.*, 2003; Boothroyd, 2005; Saeij *et al.*, 2005). *Toxoplasma* preferentially encysts in muscles and tissues of the central nervous system including retinal cells, active intracellular multiplication, cell lysis and dissemination of tachyzoites with accompanying inflammatory response in the surrounding tissue produces extensive damage and potential death if left uncontrolled. The reactivation of *Toxoplasma* due to immunosuppression exhibits a varied clinical presentation, most frequently producing focal neurological symptoms; it can also cause mental alterations, encephalitis, chorioretinitis or pneumonitis (Montoya *et al.*, 2002). In another type of clinical setting, acute *Toxoplasma* infection can be potentially damaging to the developing embryo/fetus of women who acquire primary toxoplasmosis during their pregnancy. The rate of transplacental transmission of *Toxoplasma* is correlated to the stage of pregnancy in which the mother becomes infected: the earlier the stage, the lesser the risk of fetal infection. However, the earlier parasite transmission occurs, the higher the degree of damage to the developing child (Dunnet *et al.*, 1999).

The fetal lesions caused by congenital toxoplasmosis can vary from sub-clinical manifestations such as non-inflammatory retinal scars in an otherwise healthy looking baby to chorioretinitis, hydrocephalus and mental retardation in those babies infected during the first trimester of pregnancy (Rorman *et al.*, 2006). The ocular manifestations of *Toxoplasma gondii* infection can thus be a manifestation of primary acquired infection. Congenital infection or reactivation can be acquired during immunosuppression (Holland *et al.*, 2004). The diagnosis and treatment of toxoplasmosis are dependent on the type of clinical presentation of the disease. Diagnosis in immunocompetent patients with suspicion of primary infection including pregnant women is made mostly through detection of *Toxoplasma*-specific IgG, IgM and IgA antibodies, as well as evaluation of differences in antibody titers (Montoya *et al.*, 2004).

While sero-diagnosis is possible in newborns with suspected congenital infection, Trans placental passage of maternal IgG antibodies precludes the use of IgG detection as marker for fetal infection until the first year of life, when the maternal IgG antibodies have disappeared from the baby's circulation. Additionally, not all of the infected babies have detectable IgM or IgA antibodies at birth (Candolfi, 2001; Pinon *et al.*, 2001). In immunosuppressed patients, the absence of anti-*Toxoplasma* antibodies mostly eliminates the diagnosis of a previous infection except in the case of bone-marrow transplants. However, when latent chronic infection precedes the immunosuppressive state, there often is no clear change in the antibody titers that will signal a reactivation of the parasite. This also applies to reactivation in an immunocompetent patient of a previous eye lesion (Holland *et al.*, 2003; Holland *et al.*, 2004). Alternative diagnostic method such as PCR has to be used for direct detection of the parasite in biological samples. The treatment of toxoplasmosis is based on the use of folic acid synthesis inhibitors such as pyrimethamine, sulfadiazine or trimethoprim-sulfamethoxazole and antibiotics that target protein

synthesis such as clindamycin. As treatment with folic acid synthesis inhibitors is not recommended during the first trimester of gestation, a macrolide antibiotic, spiramycin, is used instead. None of the available treatment options are effective on encysted parasites, which are why continuously immunosuppressed patients may need to be maintained on prophylactic treatment to avoid reactivation (Soave, 2001; Miro *et al.*, 2004).

2.9 Toxoplasmosis and pregnancy

Toxoplasmosis infection in pregnancy may lead to fetal infection in the uterus (Buxton *et al.*, 2007; Petersen, 2007). Early in pregnancy, this can cause multiple problems including brain damage, damage to the eyes, fever, enlargement of the liver and spleen, jaundice, rash and even death of the fetus (Ayi *et al.*, 2009). Later in the pregnancy, toxoplasmosis can cause mild disease and delayed reactions such as eye problems (Heyman, 2004). The rate of transmission to the fetus is 10-15% in the first trimester of gestation, which may increase to 68% in the third trimester. Thus maternal infections early in pregnancy are less likely to be transmitted to the foetus than infections later in pregnancy but early foetal infections are likely to have more severe consequences than late infections (Ayi *et al.*, 2009). In humans, most infected fetuses (approximately 75%) do not have obvious clinical signs at birth, many (approximately 80-85%) are likely to have manifestations such as chorioretinitis and mental retardation later in life (Guerina *et al.*, 1994).

Toxoplasma gondii is capable of causing severe disease in animals other than humans (Beattie *et al.*, 1988; Dubey, 1998). *T. gondii* parasite can cause lethal infection in the developing foetus (Buxton, 1990). The ability of the foetal immune system to respond to *T. gondii* developed progressively after 70 days of gestation, so that infection before this age result in rapid foetal

death with reabsorption, mummification, maceration or abortion of the fetus (Buxton and Finlayson, 1986).

2.10 Ocular Toxoplasmosis

T. gondii is the most common cause of retinochoroiditis in humans worldwide, accounting for (28%) to (55%) of all cases of posterior uveitis (Pavesio and Lightman, 1996; Lappalainen and Hedman, 2004; Bonfioli and Orefice, 2005; Vallochi *et al.*, 2005). Retinochoroiditis commonly occurs as a result of congenital infection but can also be due to an acquired or reactivated infection (Bonfioli and Orefice, 2005; Dubey and Jones, 2008). It is usually a self-limiting disease with lesions healing within six weeks (Rothova, 1993). Active lesions usually present with a white focus of necrotizing retinochoroiditis close to old pigmented scars. These lesions are usually circular or oval in shape and they vary in size. In the congenital forms, the lesions are usually bilateral and central and in the acquired forms, the lesions are usually unilateral and solitary (Pavesio and Lightman, 1996). Parasites reach the eye as free tachyzoites or as cysts which rupture, releasing tachyzoites. Once infected cells lyse, tachyzoites invade the retina and multiply in the surrounding cells causing an inflammatory response (Rothova, 1993; Roberts and McLeod, 1999). Clinical manifestations of acute retinochoroiditis include pain, photophobia and progressive loss of vision over time, especially when there is macular or optic nerve involvement (Pavesio and Lightman, 1996; Dubey and Jones, 2008).

2.11 Congenital Toxoplasmosis

Toxoplasmosis is a significant cause of congenital disease. Congenital toxoplasmosis occurs in between 1 and 10 per 10 000 live births in Europe (Cook *et al.*, 2000; Tenter *et al.*, 2000; Montoya and Liesenfeld, 2004). Transplacental transmission occurs when an immunocompetent woman acquires a primary infection during pregnancy (Hegab and Al-Mutawa, 2003), or may

also be due to a reactivated infection in immunocompromised women (Dubey and Jones, 2008). Primary infections acquired four to six months before conception usually result in no transplacental transmission to the foetus (Tenter *et al.*, 2000; Jones *et al.*, 2001b). Primary infection during pregnancy may result in severe damage or death of the foetus and long-term sequelae in the child. The risk of congenital infection increases from the first trimester (10-25%) to the third trimester (60-90%) with the development of a good blood flow (Jones *et al.*, 2001b; Hegab and Al-Mutawa, 2003; Dubey and Jones, 2008). The severity of the disease, however, is the highest in the first trimester and lowest in the third trimester (Jones *et al.*, 2001b; Hegab and Al-Mutawa, 2003). Infection within the first two trimesters may result in death of the foetus in utero or spontaneous abortion. Infection in the last trimester usually results in newborns that are asymptomatic at birth, but may develop symptoms later in life (Montoya and Liesenfeld, 2004). Most children born with congenital toxoplasmosis are asymptomatic at birth; however, approximately 80% of them will develop neurological or ocular sequelae later in life (Rothova, 1993; Roberts and McLeod, 1999; Boyer, 2000; Jones *et al.*, 2003; Dubey and Jones, 2008). Approximately 10% of prenatal infections result in abortion or neonatal death, and 10–23% of infected newborns show clinical signs of toxoplasmosis at birth (Luft and Remington, 1992). There may be mild disease such as reduced vision, or it may cause severe abnormalities such as blindness, mental retardation and epilepsy (Tenter *et al.*, 2000). The classic triad of signs is hydrocephalus, retinochoroiditis and intracranial calcifications, and occurs in approximately 10% of all infected newborns (Tenter *et al.*, 2000).

Toxoplasmosis in immunocompromised hosts reactivated latent infections of toxoplasmosis in immunocompromised individuals, such as those with HIV/AIDS, Hodgkin's disease or those undergoing immunosuppressive therapy, can be life threatening (Tenter *et al.*, 2000). It has

become an increasing problem worldwide due to the AIDS epidemic. Studies prior to widespread use of antiretroviral treatment show that approximately 10% of AIDS patients in the USA and 30% in Europe die from toxoplasmosis (Luft and Remington, 1992). The brain is the most common site of infection in immunocompromised individuals. Toxoplasmic encephalitis (TE) occurs in approximately 40% of AIDS patients worldwide (Tenter *et al.*, 2000; Aoun *et al.*, 2006). Highly active antiretroviral therapy (HAART), which helps to decrease the viral load and improve CD4+T-cell counts, and prophylactic treatment against reactivation of latent *T. gondii* infections, have helped to decrease the incidence of TE (Tenter *et al.*, 2000; Sukthana, 2006).

Early symptoms of TE include a persistent bilateral headache. Disease progression leads to severe manifestations such as confusion, lethargy, mental state changes, seizures, ataxia and coma, and the outcome may be fatal (Hill and Dubey, 2002; Montoya and Liesenfeld, 2004). Approximately two-thirds of all people living with HIV are in sub-Saharan Africa. According to the UNAIDS 2008 report on the global AIDS epidemic, approximately 5.7 million South Africans were living with HIV at the end of 2007. In areas such as this, where it is estimated that almost 1000 AIDS related deaths occur every day, toxoplasmosis could theoretically pose more of a threat than almost anywhere else in the world. Diagnosis Toxoplasmosis is frequently asymptomatic and clinical manifestations, when present, are usually non-specific and mimic other infections, making definitive clinical diagnosis very difficult (Hill and Dubey, 2002; Kompalic-Cristo *et al.*, 2004).

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 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). 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). Serological tests are used to detect increased antibody levels such as IgG, IgM, IgA and IgE (Jones *et al.*, 2003). In a primary *T. gondii* infection, IgM appears a few weeks after infection, followed by IgA and IgE. These acute phase immunoglobulins peak after about two months, and are usually undetectable by serological tests by six to nine months but can persist for longer periods of time (Montoya and Rosso, 2005; Sukthana, 2006). IgG, which appears after IgM, peaks after four months and persists at low levels throughout the duration of the host's life (Sukthana, 2006).

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). A further problem is that IgM may persist for longer than expected periods and discrimination between recent and older infections may therefore be a problem (Ho-Yen *et al.*, 1992; Remington *et al.*, 2004). This is an important factor when diagnosing toxoplasmosis in immunocompromised individuals as the presence of IgG indicates a risk for the reactivation of a latent infection, and IgM indicates the possibility of an acute infection. In pregnant women, positive IgM results indicate the likely acquisition of infection during gestation and a positive IgG and negative IgM result indicates a previous infection (Montoya and Rosso, 2005).

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).

Some of these problems can be overcome with the use of PCR. This method has both advantages and disadvantages. Advantages are that the detection of nucleic acid is not affected by the condition of the immune system, 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 non-viable *T. gondii* remnants that do not cause disease, and may yield false negative results due to inhibition (Johnson *et al.*, 1993).

These problems with PCR can, however, be overcome and more rapid and sensitive methods are regularly being developed. These advances in PCR techniques are helping to make it an invaluable diagnostic tool.

2.12 Epidemiology and global distribution of Toxoplasmosis

Toxoplasmosis is widespread and capable of infecting many mammalian species. 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). It is estimated that between 30% and 65% of all persons worldwide are

infected with *Toxoplasma* (Tenter *et al.*, 2000). The interest in toxoplasmosis has been stimulated over the last few years by the finding that this infection is widespread biologically as well as geographically. It is widespread perhaps, 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).

Transmission of tachyzoites to the fetus can occur via the placenta following primary maternal infection. The incidence of prenatal *T. gondii* infections within the same or similar populations have been estimated to range from about 1 to 120 per 10,000 births (Patton, 1993). Rarely, does infection by tachyzoites occur from ingestion of unpasteurized milk or by direct entry into the bloodstream through a blood transfusion or laboratory accident; but it does occur through transplantation of an organ that contain tissue cysts. *T. gondii* has been recovered from locations throughout the world, except Antarctica. Seroprevalence among adults could be as high as 90% in many countries (Akyar, 2011). Some studies have reported of incidence of primary maternal infection during pregnancy to range from about 1 to 310 per 10,000 pregnancies in different populations in Europe, Asia, Australia and the Americas (Opsteegh *et al.*, 2011). In Brazil, a recent report has it that 53.03% of pregnant women were positive for IgG and 3.26% were positive for IgM (Vaz *et al.*, 2010). *T. gondii* seropositivity among pregnant women, their fetuses, neonates, and AIDS patients have been investigated in Qatar (Vaz *et al.*, 2010). Widespread occurrence is confirmed in East Mediterranean (Akyar, 2011). In many developing countries, the exact prevalence of toxoplasmosis is not well articulated unlike in the developed world (Lindstrom *et al.*, 2006), but there is large variation between countries. In France, for

example, around 88% of the population are carriers, probably due to a high consumption of raw and lightly cooked meat (Ancha and Szyfres, 2003). In Germany, the Netherlands and Brazil there are high prevalence rates of 68%, 80% and 67% respectively (Henriquez *et al*, 2009). In Britain about 22% of pregnant women are carriers, while in South Korea the rate is 4.3% (Tenter *et al.*, 2000). The *T. gondii* seroprevalence for the Dutch human population has decreased from 40.5% in 1995/1996 to 26.0% in 2006/2007 (Hofhuis *et al.*, 2010). This is thought to be an effect of the decreased prevalence in consumption animals, especially in pigs, due to increased intensive indoor farming. A stable infection pressure from the environment is suggested by the unchanged seroprevalence in sheep when compared to studies in the eighties (Opsteegh *et al.*, 2011). However, differences may have been missed due to methodological differences between studies (Opsteeghet *et al.*, 2011).

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 *T. 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 coinfecting with HIV and *T. gondii* are expected to ultimately develop toxoplasmosis, the high seroprevalence combined with the HIV-pandemic indicate that 2.5–10 million people in this region may be at risk dying from toxoplasmosis (Lindstrom *et al*, 2006). Similarly, high incidences have been found in the Central Africa region (Dubey *et al.*, 2005). There is paucity of published work on toxoplasmosis among countries in East Africa. However, a work carried out among three tribes: Baganda, Masai, and Bondei, using serological test showed widespread distribution of *T. gondii* seroprevalence (Tenter *et al.*, 2000).

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 a study in Lagos State, IgM was common in the first trimester (16.7%) while *Toxoplasma* IgG was seen mostly in the third trimester (46.7%). IgG and IgM were seen to be significantly associated with parity (Deji-Agboola *et al.*, 2011). Reporting further on toxoplasmosis in Nigeria (Onadeko *et al.*, 1996) observed that polydactylism, a common congenital abnormality, was traced to reinfection or recrudescence of toxoplasmosis which accounted for high antibody levels. He also observed an association between high prevalence of toxoplasmosis and overcrowding with poor environmental sanitation challenges, including considerable contamination with cat faeces. 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 brackets, 71.4% presented with serological evidence of toxoplasmosis (Olusi *et al.*, 1996).

In Northern Nigeria, more work has been reported on toxoplasmosis. In a study conducted in 2009 by (Ishiaku *et al.*, 2009) among pregnant women attending antenatal care in Zaria, Kaduna State, the study showed that overall prevalence of *T. gondii* IgG and IgM antibodies in pregnant women in Zaria was 29.1% and 0.8% respectively. Studies conducted by (Aganga *et al.*, 1999) two decades ago in Zaria showed a prevalence of (39.5%) and (0.4%) of chronic and acute infection respectively. High seroprevalence of toxoplasmosis have been reported among some animals of economic importance, such as sheep (Okoh *et al.*, 1984), chicken in Zaria (Aganga, 1985), pet dogs in Zaria (Aganga and Ortese, 1984), and dogs in Maiduguri (Kamani *et al.*,

2010). Studies in other parts of the world also showed a decline in infection. *T. gondii* infection has continued to decline since, these studies reveal the high preponderance and spread of human and 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 (Clementino *et al.*, 2010).

2.13 Transmission of Toxoplasmosis

Carnivorous animals are often infected with *Toxoplasma* through ingestion of bradyzoites from tissue cysts in infected prey, as are persons who eat undercooked meat, particularly that of pigs, sheep and goats. Infection can also be through the milk of sheep, goats and cattle, and sometimes through chicken eggs (Eyles, 2001). *Toxoplasma* cysts are less commonly found in poultry and rarely found in beef. Its prevalence in commercial farm animals has decreased significantly with the advent of intensive management practices (Clementino *et al.*, 2009). Free range poultry, swine, small ruminants, marsupials and some wild game are more likely to harbour cysts. Tachyzoites are killed relatively easily by pasteurization, and uncommonly survive gastric digestion but any kind of cooking will definitely kill tachyzoites in an egg.

Oocysts are only shed by cats but unsporulated oocyst in fresh feces is not uninfected. Appropriate oxygen, humidity, and temperature are necessary for sporulation to occur. Sporulated oocysts are the most environmentally resistant life stage of the parasite (Halland, 2004). Ingestion of even as few as ten oocysts may infect an intermediate host, while ingestion of 100 or more oocysts can cause a patent infection in a cat, which may shed tons of hundreds of millions of oocysts (Patton, 1993).

In utero transmission of *Toxoplasma* occurs only if primary infection of the mother occurs during pregnancy. Parasitemia then results in placentitis and infection of the fetus. This is more likely to

occur in man, sheep and goats, and sometimes in mice, cats and dogs. Under normal circumstances, a female that has been exposed to *Toxoplasma* 4-6 months prior to pregnancy will develop sufficient immunity to protect herself and the fetus for the rest of her life (Vaz *et al.*, 2010). However, if the immune response is suppressed by drug therapy or disease such as AIDS in man, both the mother and the fetus may become susceptible to infection again (Tenter *et al.*, 2000). The risk of vertical transmission to the fetus increases from the first trimester (10-24%) to the third trimester (60-90%), and the potential of congenital defect is more severe with earlier infections (Patton, 1993).

2.14 Economic importance of Toxoplasmosis

Toxoplasmosis leads to a myriad of diseases. The risk-prone group of individuals including fetuses, new-born babies and immunologically impaired patients develops chorioretinitis, lymphadenitis, or rarely, myocarditis and polymyositis (Jones *et al.*, 2003). It can cause more serious progression and complications such as abortion, when accompanied with some other infection such as human immunodeficiency virus (HIV), and catalyzes: birth defects (Ouermi *et al.*, 2009), reproductive disorders (Montoya and Liesenfeld, 2004), and transmission of Hepatitis B virus (HBV). 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 *T. gondii* is widespread and constitutes a public health concern (Zhao *et al.*, 2011), resulting in significant reproductive losses (Dubey, 2009; Walsh *et al.*, 1999).

2.15 Behavioral changes associated with *Toxoplasma* Infection

One of the dramatic characteristics of *T. gondii* is its ability to change the behaviour of its host. It has been reported that infected rats and mice are less fearful of cats, and some of the infected rats

seek out cat-urine-marked areas. This effect is advantageous to the parasite, as the setting catalyzes the proliferation of the parasites as the infected rat is eaten by the cat (Beyer *et al.*, 1986). The mechanism for this change is not completely understood, but It could also be a result of subtle effects on the nervous system (Obendorf *et al.*, 1996; Berdoy *et al.*, 2000). However, there is evidence that toxoplasmosis infection raises dopamine levels and concentrates in the amygdala in infected mice. (Henriquez *et al.*, 2009). Perhaps, this could be as a result of low-grade encephalitis marked by presence of cysts in the brain, which induces the production of neurotransmitter (dopamine), which acts similarly to dopamine reuptake inhibitor type antidepressants and stimulants (Laing *et al.*, 1996). This observation of behavioral change among rats and mice has led to speculation that *Toxoplasma* may have similar effects in man, even in the latent phase that had previously been considered asymptomatic (Tenter *et al.*, 2000). Correlations have been found between latent *Toxoplasma* infections and various characteristics such as: decreased novelty seeking behavior, slower reactions, as well as lower rule-consciousness and greater jealousy (in men) greater warmth, conscientiousness and moralistic behavior (in women).

2.16 Diagnosis of Toxoplasmosis

Serologic tests are the primary means of diagnosis. Immunoglobulin (Ig) G-specific antibodies achieve a peak concentration during about one to two months after infection and remain positive indefinitely. For patients with sero-conversion or a fourfold increase in IgG antibody titer, specific IgM antibody determinations should be performed as the presence of *T gondii*-specific IgM antibodies may indicate acute or recent infection. Enzyme immunoassay tests are the more sensitive assays for IgM, detection, and this can be achieved 2 weeks after infection. Peak concentrations of IgM antibody is achieved in one month, but decreases thereafter to an

undetectable level within 6 to 9 months. This undetectable level can persist for as long as 2 years, and during this period, it confounds the differentiation of acute and remote infections.

Tests to detect IgA and IgE antibodies, which decrease to undetectable concentrations sooner than IgM antibodies are useful for the diagnosis of congenital infection and infections in other patients, such as pregnant women, for whom more precise information about the duration of infection is needed.

Detection of *T. gondii* DNA in amniotic fluid by polymerase chain reaction assay has been shown to be a safe and accurate method of diagnosis. Serial fetal ultrasonography examinations should be performed in cases of suspected congenital infection to detect any increase in size of the lateral ventricles of the central nervous system or other sign of fetal infection. Quantitative screening for IgG antibodies to *T. gondii* is used to determine the immune status of pregnant women and newborns. Anti-Toxo IgG antibodies may persist throughout life. Consequently, a steady anti-*Toxoplasma* IgG titer shows earlier exposure, whereas a fourfold or greater rise shows active infection. Furthermore, among infants, serial determination of the anti-Toxo, IgG level will assist in determining between *T. gondii* infection that occurred congenitally (Plateau level) or neonatal (increase in titer) (Akyar, 2011). If the diagnosis for an infant is unclear at the time of delivery, evaluation of the infant should include ophthalmologic, auditory, and neurologic examinations; lumbar puncture; and computed tomography of the head. Attempt should be made to isolate *T. gondii* from the placenta, umbilical cord, or blood specimen from the infant by mouse inoculation.

Patients with HIV infection who are infected latently with *T. gondii* have variable titers of IgG antibody to *T. gondii* but rarely have IgM antibody. Although sero-conversion and fourfold increases in IgG antibody titers may occur, the ability to diagnose active disease in patients with

AIDS is impaired by immunosuppression. In HIV-infected patients who are seropositive for *T. gondii* IgG, *T. gondii* encephalitis is diagnosed presumptively on the basis of the presence of characteristic clinical and radiographic findings. If the infection does not respond to an empirical trial of anti-*T. gondii* therapy, demonstration of *T. gondii* organisms, antigen, or DNA in biopsied tissue, blood, or cerebrospinal fluid may be necessary to confirm the diagnosis.

2.17 Prevention and control of Toxoplasmosis

Improper handling of cat litter and not necessarily ownership of cat is accepted as a risk factor of toxoplasmosis (Walker *et al.*, 2008). This definitely determines what measures would be effective in preventing or controlling the spread of toxoplasmosis. There are general sanitation and food safety steps needed to be taken to prevent one from becoming infected with *Toxoplasma gondii* infection and these include: (i) Cats found to be shedding oocysts should be removed from the premises temporarily and treated to eliminate shedding. Since cats are usually meticulous groomers, it is unlikely that oocysts will be found on their fur. This means that regular handling will not be a significant risk. (ii) Microwave cooking, salting and smoking do not consistently kill all infective *Toxoplasma* stages. So meat should be frozen to -12°C for at least 24 hours to kill *Toxoplasma* tissue cysts, but it must be noted that sporulated oocysts can survive at -20°C for up to 28 days. (iii) Kitchen utensils and surfaces that have come in contact with raw meat should be washed with soap and scalding hot water to kill any bradyzoites or tachyzoites present. (iv) Individuals should always wash their hands thoroughly after contact with cat stool, litter or litter box. (v) Cat feces should be disposed of daily to reduce the risk of transmission. Feces and dirty litter can be disposed of in a septic system if the litter is biodegradable, sealed tightly in a plastic bag and placed in the garbage, or incinerated. Backyard compost units do not produce sufficient heat to destroy oocysts and other pathogens potentially

present in fecal material. (vi) Keep cats out of sandboxes and other areas where children play to prevent the cats defecating there (Tenter *et al.*, 2000). (vii) Unwashed fruits or vegetables as well as unpasteurized milk should not be eaten. (viii) One must ensure that all avenues that could bring one in contact with cat faeces either directly or indirectly are blocked, while the intermediate host population must be properly checked.

2.18 Treatment 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).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study area

The study was carried out in Kano metropolis. Kano State is a city located in northwest geopolitical zone in Nigeria. Kano urban area has estimated land mass of 137 km². It has eight Local Government Areas with an estimated population of 2, 828, 8361(NPC, 2006). Kano is known for its commercial activities of buying and selling goods and services. It is the most populous state in Nigeria.

3.1. Study population

A Convenient sampling method was used in the selection of the study population. Murtala Mohammed Specialist Hospital and Abdullahi Wase Specialist Hospital were selected. The bloodsamples were collected from child bearing women attending antenatal care clinic.

3.1.1. Inclusion criteria

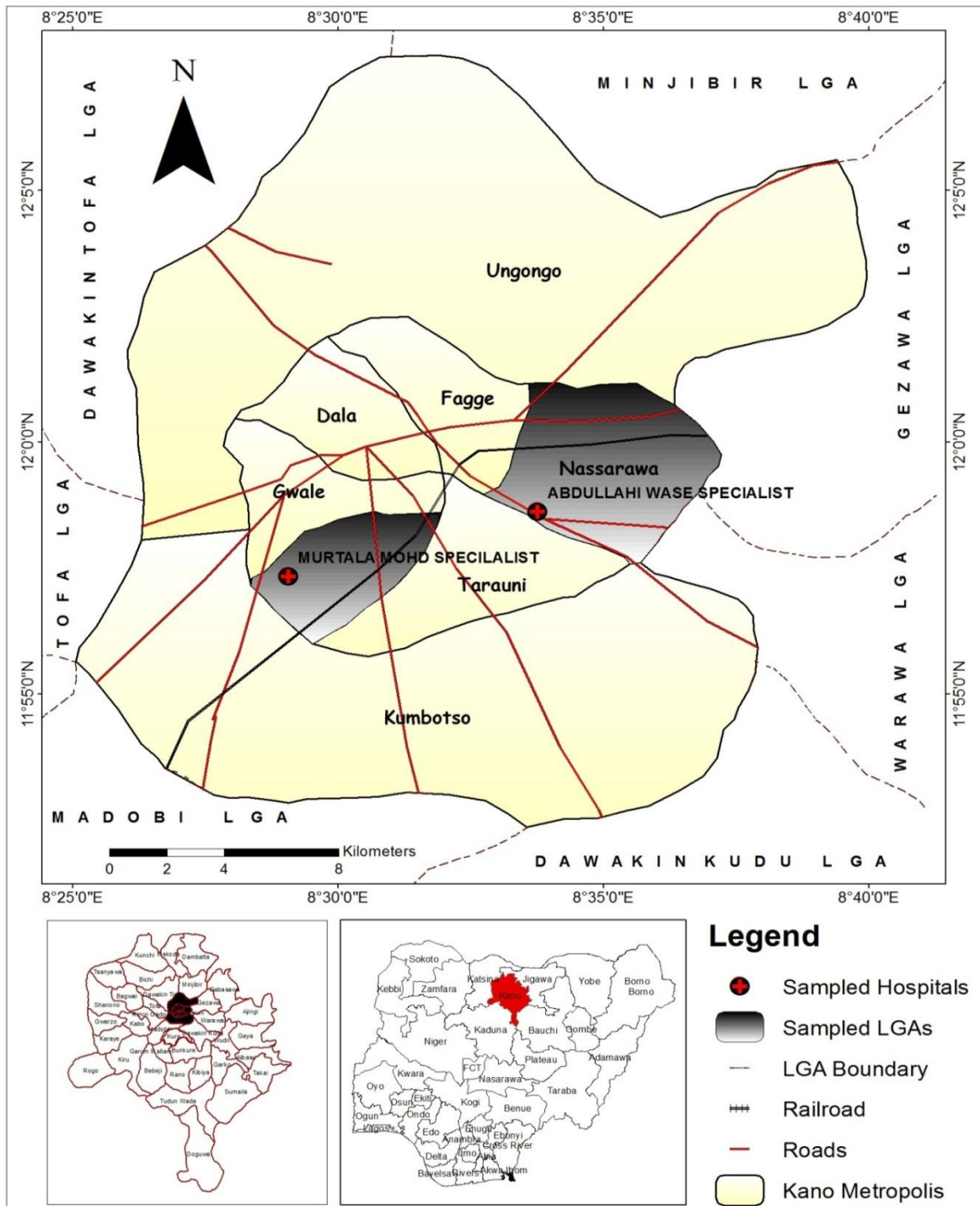
Consenting pregnant women attending antenatal clinics in Kano metropolis were the case study.

3.1.2. Exclusion criteria

Pregnant Women who did not consent or did not register with the clinics were excluded during theresearch.

3.2 Ethical approval

Ethical clearance was obtained from Hospital Management Board, Kano State (Appendix i).



**Fig 3.1:Map of Kano State Metropolis
Adapted from Administrative Map of Kano state showing study areas(2003)**

3.3 Sample size determination

The following formula (Daniel, 1999) was used.

$$N = Z^2 P (1 - P) / d^2$$

Where n = sample size,

Z = Z statistic for a level of confidence,

P = expected prevalence or proportion (Ishiaku *et al.*, 2009) (21.6%)

d = precision (in proportion of one; if 5%, d = 0.05).

Z statistic (Z): For the level of confidence of

95%, which is conventional, Z value is 1.96. In these studies, the results with 95% confidence intervals (CI).

$$N = (1.96)^2 \times 0.216(1-0.216) / (0.05)^2$$

$$= 0.8297856 \times 0.784 / 0.0025$$

$$= 260.220764$$

$$= 260$$

Sample size = 260 samples.

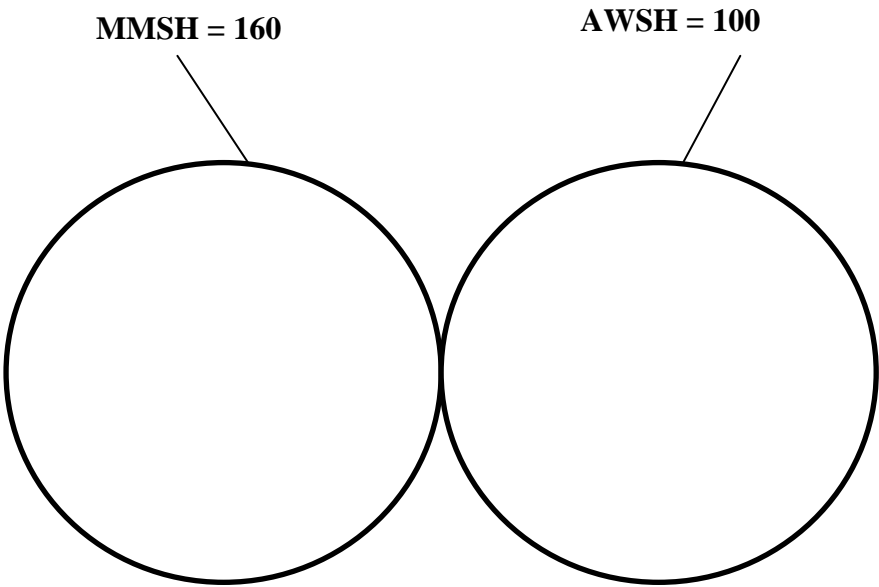
3.3.1 Data Collection

Data were collected by the use of questionnaire to obtain information on the socio-demographic and risk factors such as Age, History of Miscarriage, Trimester, Level of Education Presence of Cat and source of drinking water which may be associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care which was captured using questionnaire in (appendix iii).

3.3.2 Sample collection

A total of two hundred and sixty (260) blood samples were collected from consented pregnant women registered with the antenatal at Muhammad Abdullahi Wase specialist Hospital Kano State and Murtala Mohammed Specialist Hospital Kano State using convenient sampling method. The samples container were labeled first, 5 mL of blood samples were collected with the help of Lab. Technician. The blood samples were collected aseptically by disinfecting the area by swabbing using cotton wool with 70% alcohol, using 5ml syringe from the patient and dispense into anti coagulated sample bottles, once the blood sample was dropped into the anticoagulant sample bottle, the containers was then rotated gently to mixed well to avoid coagulation before it was kept on arack. The samples collected for each clinic day were preserved at a temperature of 4°C in a freezer at Microbiology department of that hospital until the next day it was then separated by picking the plasma at the uppermost layer using sterile and dried Pasteur pipette into a non-ant coagulated bottle. Both the whole blood and the plasma samples bottles were kept in the freezer at a temperature of -20°C at hospital Laboratory.

After the required number of samples was obtained, they were then conveyed in an ice pack for onward transportation to Microbiology Laboratory, Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria for processing.



MMSH = Murtala Muhammad Specialist Hospital

AWSH = Abdullahi Wase Specialist Hospital

Fig 3.2: Distribution of blood samples collected from consented pregnant women attending antenatal care at selected hospital.

3.4 Laboratory Diagnosis

Plasma samples were analysed using Enzyme Linked Immunosorbent Assay(ELISA)using the Diagnostic Automation ELISA of *Toxoplasma gondii*IgG and IgM antibodies(Diagnostic Automation,inc.,Calabar USA)and Polymerase Chain Reaction(PCR),primers sequence were obtained from (Inqaba Biotech South Africa).

3.4.1 Assay procedure

All samples and reagent under refrigeration were brought to room temperature on a bench that was initially made clean and disinfected using detergent. A sterile micro titer plate with 96 wells,1-12 in rows and A-H column wise was used. First five in rows was labeled reagent blank, positive, negative control,calibrator one and calibrator two were labeled according to the labeled on the wells of the micro titer plate. Fromwell number six that is A6 to H12 which was up to ninety one wells were used for specific patient sample. This was done to each plate of IgG and IgM.The unused strips were returned to the scalable bag with desiccant, sealed and immediately refrigerated. Test sera was diluted, Calibrator and Control sera 1:21 For I gG T(10 μ L + 200 μ L), while IgM was 1:81(10uL + 80uL) in Serum Diluent except reagent blank on the wells of micro titer plate provided. The wells were mixed very well. To individual wells, 100 μ L of the appropriate diluted Calibrator, Controls and patient sera was added to the wells.100microliter was also added to the reagent blank well, it was then incubated at room temperature (21 to 25⁰C) for 25 minutes +/- 5 minutes. Liquid from all wells were aspirated out.The prepared 250-300 μ L of diluted Wash Buffer was added to each well. All liquid were aspirated out of the wells. The washing of the coated plates were repeated for a total of three (3) washes. After the final washed, the plate was blotted on paper toweling to remove all liquid from the wells.One hundred μ L Conjugate was added to each well, including reagent blank well, bubbles were avoided upon

addition as they may yield erroneous results. The wells were incubated at room temperature (21 to 25 °C) for 25 minutes +/- 5 minutes. The wash was repeated as described in Step 5. 100µL chromogen/substrate solution (TMB) was added to each well, including the reagent blank well, a constant rate of addition across the plate was maintained. Wells were incubated at room temperature (21 to 25 °C) for 10-15 minutes. Reaction was stopped by addition of 100 µL of Stop Solution (IN H₂SO₄) following the same order of Chromogen/Substrate addition, including the reagent blank well. The plate was tapped gently along the sides, to mix contents of the wells. The plate was read immediately. The developed color was read on an ELISA plate reader equipped with a 450 nm filter.

3.4.1 Interpretation of ELISA results

Calculated mean of the two calibrator values was obtained. A Correction Factor was determined by Diagnostic Automation/Cortez Diagnostics, Inc. each lot of kits. The Correction Factor was printed on the Calibrator vial. Cutoff Calibrator Value - The Cutoff Calibrator Value for each assay is determined by multiplying the Correction Factor by the mean Calibrator O.D. determined in Step 1. Calculate an Immune Status Ratio (ISR) for each specimen by dividing the specimen O.D. Value by the Cutoff Calibrator Value determined in Step 3. E.g. O.D.s obtained for Calibrator = 0.38, 0.42

Mean O.D. for Calibrator = 0.40 for IgG but IgM was 0.55

Correction Factor = 0.50

The patients' ISR (Immune Status Ratio) values are interpreted as follows: Table 3.1 ISR interpretation of results

ISR Reading Interpretation

≤ 0.90	No detectable antibody to <i>Toxoplasma gondii</i> by the ELISA test.
> 1.10	Indicates presence of detectable antibody to <i>Toxoplasma gondii</i> by the ELISA test. Indicative of current or previous infection. The individual may be at risk of transmitting <i>Toxoplasma gondii</i> infection, but is not necessarily currently contagious.

3.5 Nested PCR Assay

The nested PCR were performed. Seven IgM positive samples were selected at random considering those with high titervalue. The DNA was extracted from serum samples using Phenol Extraction Method. Samples were amplified using Gel Electrophoresis Machine to detect a fragment from the *T.gondii* B1 gene, which is present in 35 copies and is conserved in the *T. gondii* genome, as described by Burg. *et al.*, (1989). The primers that were used in the first round of the PCR were inner primers and presented below :

<i>T. gondii</i> primer sequence	Position	Target gene	Amplified PCR product size(bp)
F ₁ -5'GGAAGTGCATCCGTTTCATGAG-3' R ₁ -5'TCT-TTAAAGCGTTCGTGGTC-3'	694-714	<i>Bl gene</i>	100
F ₂ -5'GCCATAGCTTGCAGTCAC TG-3' R ₂ -5'GGCGACCAATGC-GAATAGACC-3'	700-776.	<i>Bl gene</i>	100

Key: F₁= First Round Forward Primer F₂= Second Forward Primer
R₁=First Reverse Primer R₂= Second Reverse Primer

3.5.1 DNA extraction method:

Two hundred microliter of the clients blood samples were pipetted and dispensed into a micro-tube, 400 microliter of lysis buffer was added to the mixture and then vortexed and incubated for 1 hour then 400 microliter of phenol chloroform was added to the mixture it was centrifuged at 18000rpm for 10 minutes. The upper layer was picked and then dispensed into a new tube using a sterile pipette subsequently 400 microliter of chloroform was added to the new tube containing the upper layer it was then vortexed and then centrifuged for at 13000rpm for 10 minutes. The upper layer was pipetted into a new micro tube then 40 microliter of 70% absolute ethanol was added to the mixture with addition of 1000 microliter of 3 moles of sodium acetate, it was then incubated at -20°C overnight. It was then centrifuged at a temperature of 4°C for 20 minutes, the ethanol was then discarded. Then 400 microliter of 70% ethanol was added and then it was centrifuged at 13000rpm for 10 minutes, the ethanol was discarded. The sediment was then allowed to air dry. Fifty microliter of ultra-pure water was added to the sediment that was air dried and stored at a temperature of -20°C for future use.

3.5.2 Assay procedure

Only serum samples that were found to be IgM seropositive were used for PCR for the detection of *T.gondii* DNA. A total of seven IgM seropositive samples were used for Polymerase Chain Reaction for the detection of *T.gondii B1 gene*

3.5.3 The PCR master mix preparation

One microliter of each primer making 2 microliters were pipetted and then dispensed into micro tubes labeled A-H where A is the negative control while B-H is the client sample. Two microliters of DNA extracted was dispensed into the micro tubes except A labeled that is the negative control to make up 4 microliters of the client samples and 2 microliters of negative control. Sixteen of deionized water was added to the client samples to make up of 20 microliters while 18 microliters of deionized water will be added to the negative control that made up 20 microliters of the negative control. This mixture is then vortex for seconds and then subject to PCR machine.

3.5.4 PCR condition

The following temperature was set. The cycling conditions for both PCR are 90°C for five minutes; followed by 30 cycles at 94°C for 30 seconds, 55°C for 90 seconds, and 72°C for one minute, and a final extension of 72°C for 10 minutes.

3.5.5 Procedure for Agarose gel electrophoresis

About 1.5g of Agarose powder was dissolved in 100mls of TAE buffer using micro wave oven. It was allowed to cool for 45°C, five microliters of ethidium bromide was added and gently mixed. The mixture was poured into a casting tray already assembled with combs positioned. It was then allowed to solidify, thereafter combs were removed. The casting tray was removed and immerse

inside the electrophoresis tank containing 10 times TAE running buffer, 10mls of the DNA was mixed with 5 microliter of loading dye and then loaded into individual wells. The ladder one hundred base pair was loaded into the first well. The tank was then covered and connected to the power pack and the voltage was set at 75v and run for 40 minutes, the casting tray was then removed and observed under gel documentation system connected to a computer. The PCR was run in DNA Laboratory Kaduna, Kaduna State.

3.6 Data analysis

The data obtained from the questionnaire and the result of the laboratory analysis were entered into Microsoft Excel and analysed using (Statistical Package for Social Science version 20). The Pearson Chi-square test at 95% confidence interval and 0.05 level of significance was used to determine the relationship between demographic data and prevalence rate.

CHAPTER FOUR

4.0 RESULT

Fig 4.1: The prevalence of *Toxoplasma gondii* antibodies among pregnant women attending antenatal care clinic days at Murtala Muhammad Specialist Hospital and Abdullahi Wase Specialist Hospital in Kano Metropolis, Kano State are presented in fig 4.1. All samples collected were tested for the presence of *Toxoplasma gondii* IgG, IgM and IgG IgM were as follows: anti-*T. gondii* IgG 89 (34.23%), followed by anti-*T. gondii* IgM 34 (13.08%) while individual with both IgG IgM had 10 (3.85%).

Table 4.1: Indicates the seroprevalence of *T. gondii* based on age group of pregnant women examined. Individuals within the age group of 31-40 had the highest latent IgG and acute IgM infection of (39.82%) and (13.95%) respectively, followed by age group 15-30 with acute infection of (13.13%) and 41-50 years with the least acute infection of (10.53%). There was no statistical significant association between prevalence of antibodies and age group of pregnant women ($P > 0.05$).

Table 4.2: Shows seroprevalence of *Toxoplasma gondii* infection among pregnant women in relation to history of miscarriage. We observed that women who had experience (miscarriage) had the highest prevalence of latent (IgG) infection with 27 (41.54%) compared to those that did not experience any miscarriage with a prevalence of 62 (31.79%). Furthermore, those that did not experience any miscarriage had the highest acute infection (IgM) with a prevalence of 26 (13.33%) compared to those with miscarriage 8 (12.31%).

Table 4.3: Shows seroprevalence of *T. gondii* in relation to the stages of pregnancy, women within the second trimester recorded the highest prevalence 7 (10.66%) of both IgG+ IgM

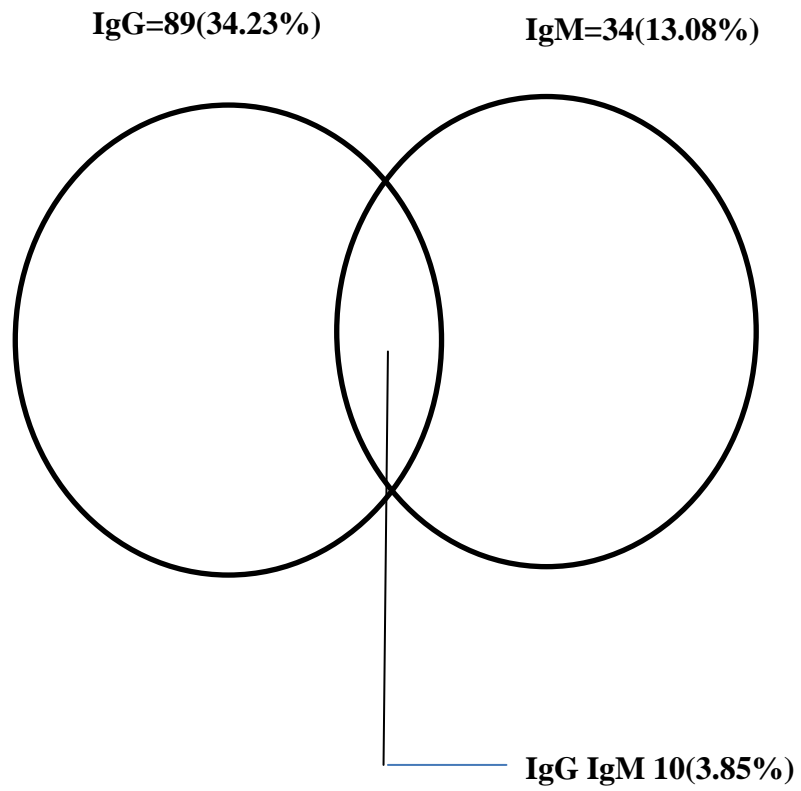
antibodies, followed by those in third trimester 2 (1.55%), whereas the least prevalence was noted among those in first trimester 1 (1.54%). Furthermore, Individuals within first trimester recorded the highest percentage of IgG antibodies 29 (44.62%) and IgM antibodies 20 (30.76%). However, there was no significant relationship between the presence of *T. gondii* antibodies and gestation age ($P>0.05$). It is also worth noting that the seroprevalence of acute infection decreased as the gestation period progresses.

Table 4.4: Shows the seroprevalence of *T. gondii* antibodies with respect to occupation of pregnant women examined. The highest seroprevalence of latent (IgG) and acute (IgM) infection of *T.gondii* antibodies was observed among the house wife with 74(60.16%) and 28(22.76%) respectively, respectively while the least was observed among student with seroprevalence of (4.49%) and (4.49%) for IgG and IgM.

Table 4.5: Shows the seroprevalence of *T.gondii* antibodies as it concern with the level of education of pregnant women. The highest prevalence was recorded among individuals with non-formal education with a seroprevalence of latent infection to be (35.96%), while the least seroprevalence of acute infection was among those in Tertiary Level of Education (11.76%). In addition, there was no significant association between *T. gondii* antibodies and level of education ($P>0.05$).

Table 4.6: Presents prevalence of *T. gondii* antibodies based on contact with cats. A higher seroprevalence of both latent and acute infection of *T.gondii* antibodies are (39.62%) and (22.64%) respectively was recorded among those who had regular contacts with cats compared to those who had no contact with cats. However, there was a statistical significant association between seroprevalence of *T. gondii* antibodies and contacts with cats ($P<0.05$).

Table 4.7: Present seroprevalence of *Toxoplasma gondii* infection among pregnant women in relation to sources of drinking water. Individuals who drink well water recorded the highest *T.gondii* antibody seroprevalence of acute infection (29.1%), while those who used Sachet water had the highest latent infection with a seroprevalence of (37.75%).



Key:
IgG = Immunoglobulin G
IgM = Immunoglobulin M
IgG IgM = Immunoglobulin G and M

Figure 4.1: Percentage distribution of T.gondii antibodies in pregnant women examined.

Table 4.1: Seroprevalence of *T.gondii* base on age group of pregnant women examine

Age group	No. examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
15-30	198	65 (32.82)	26 (13.13)	9 (4.55)
31-40	43	17 (39.82)	6 (13.95)	1 (2.33)
41-50	19	7 (36.84)	2 (10.53)	0 (0.00)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=2.130$; $p=0.91$; $df=4$

Key

IgG=Immunoglobulin G,**IgM**=ImmunoglobulinM,**IgGIgM**=both Immunoglobulin G an
Immunoglobulin M.+**VE**=Positive

χ^2 =Chi-square,p=Probability,df=degree of freedom.

Table 4.2: Seroprevalence of *Toxoplasma gondii* infection among pregnant women in relation to history miscarriage.

Abortion (miscarriage)	No. examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
Yes	65	27 (41.54)	8 (12.31)	2 (3.08)
No	195	62 (31.79)	26 (13.33)	8 (4.10)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=2.115$; $p=2.082$; $df=2$

Key

IgG=Immunoglobulin G, **IgM**=Immunoglobulin M, **IgGIgM**=both Immunoglobulin G and Immunoglobulin M. +**VE**=Positive.

χ^2 =Chi-square, p =probability, df =Degree of freedom.

Table4. 3:The seroprevalence of *T.gondii* in relation to stages of pregnancy,

Stages of pregnancy	No.examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
First trimester	65	29 (44.62)	20 (30.76)	1 (1.54)
Second trimester	66	7 (10.66)	13 (19.69)	7 (10.66)
Third trimester	129	53 (41.09)	1 (7.75)	2 (1.55)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=10.08$; $P=0.121$; $df=4$

Key

IgG=ImmunoglobulinG, **IgM**=ImmunogloblinM,**IgGIgM**=both Immunoglobulin G an
 Immunogloblin M. +**VE**=Positive.

χ^2 =Chi-square,p=probability,DF=degree of freedom.

Table 4.4: Seroprevalence of *Toxoplasma gondii* infection among pregnant women in relation to occupation

Occupation	No. examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
Student	89	4 (4.49)	4 (4.49)	2 (2.25)
Civil servant	34	11 (32.35)	2 (5.88)	1 (2.94)
Housewife	123	74 (60.16)	28 (22.76)	7 (5.69)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=5.41$; $p=0.49$; $df=4$

Key

IgG=Immunoglobline G, **IgM**=Immunogloblin M ,**IgGIgM**=both Immunoglobline G and M.

+VE=Positive.

χ^2 =Chi-square,**p**=probability,**df**=degree of freedom.

Table 4.5: Seroprevalence of *T. gondii* antibodies in relation to the level of education of pregnant women.

Level of education	No. examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
Primary	46	15 (32.61)	7 (15.22)	3 (6.52)
Secondary	91	32 (35.16)	10 (10.99)	6 (6.59)
Tertiary 34	10 (29.41)	4 (11.76)	0 (0.00)	
Non formal	89	32 (35.96)	13 (14.16)	1 (1.12)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=10.04$; $p=0.36$; $df=6$

Key

IgG=Immunoglobulin G, **IgM**=Immunoglobulin M, **IgGIgM**=both Immunoglobulin G and M.

+VE=Positive.

χ^2 =Chi-square, p =probability, df =degree of freedom.

Table 4.6: Seroprevalence of *T. gondii* antibodies based on contact with cats among the pregnant women examine.

Domestic animals	No. examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
Cat	53	21 (39.62)	12 (22.64)	3 (5.66)
Others	207	68 (32.85)	22 (10.63)	7 (3.38)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=9.62$; $P=0.02$; $df=2$

Key

IgG=Immunoglobulin G, **IgM**=Immunoglobulin M, **IgGIgM**=both Immunoglobulin G and M.
+VE=Positive.

χ^2 =Chi-square, **p**=Probability, **df**=degree of freedom.

Table 4.7: Seroprevalence of *Toxoplasma gondii* infection among pregnant women in relation to sources of drinking water

Source of	No. examined	IgG+ve(%)	IgM+ve(%)	IgGIgM+ve(%)
Drinking water				
Well water	34	8 (23.53)	10 (29.41)	2 (5.88)
Sachet water	151	57 (37.75)	14 (9.27)	5 (3.31)
Pipe borne water	71	23 (32.39)	10 (14.08)	2 (2.82)
River	4	2 (25.00)	0 (0.00)	0 (0.00)
Total	260	89 (34.23)	34 (13.08)	10 (3.85)

$\chi^2=16.85$; $P=0.05$; $df=6$

Key

IgG=Immunoglobulin G, **IgM**=Immunoglobulin M, **IgGIgM**=both Immunoglobulin G and M.

+VE=Positive.

χ^2 =Chi-square, **p**=probability, **df**=degree of freedom.

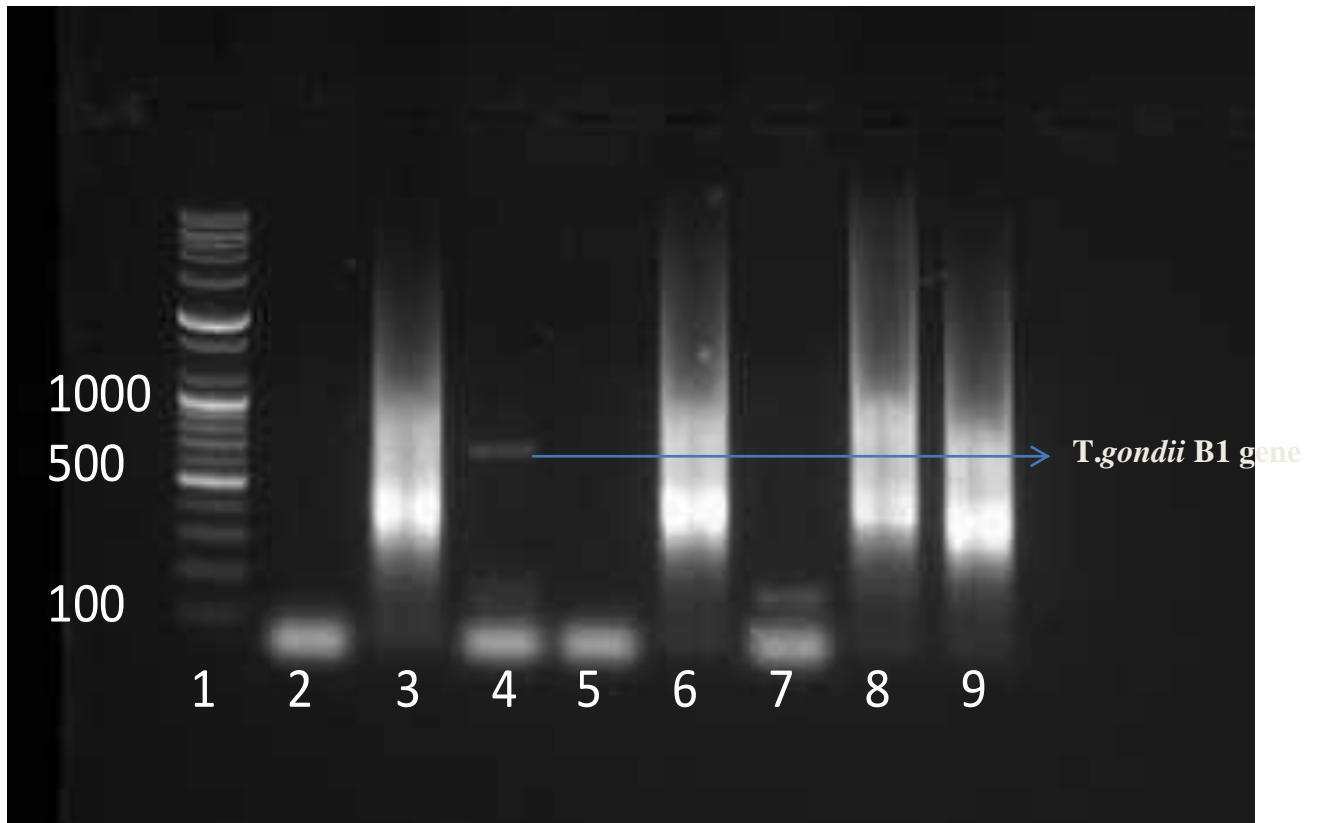


Plate 4.1:

Amplicon of B1 gene of *T.gondii* with size of 700bp. Lane 2 (negative control), Lane 3 to Lane 9 (patient sample) where lane 4 shows the presence of amplicon size.

Molecular marker (100bp as size).

CHAPTER FIVE

5.0 DISCUSSION

This study recorded an overall prevalence of 43.4% that comprises of pregnant women who were positive for anti-*T gondii* IgG and IgM with 34.23%, and 13.08% respectively. The high prevalence recorded in this study is comparable with the findings reported by Akingbami *et al.*, (2010) and Dezi-Agboola *et al.*, (2011) with prevalences of (40.8%) and (33.8%) respectively in Lagos. However, our prevalence was higher than the finding reported by Ishiaku *et al.*,(2009) in Zaria with a prevalence of (29.1%) and 0.8% for IgG and IgM antibodies respectively. The reason for the high prevalence observed in this study could be due to the fact that Kano metropolis have a higher temperature which favour the sporulation of oocyst.

In relation to age, age group 31-40 had the highest seroprevalence for both latent and acute infection (IgG and IgM respectively). Younger pregnant women (15-30) had higher (13.13%) seroprevalence of acute infection compare to older pregnant women (41-50) with acute seroprevalence of (10.53%). This may be attributed to poor hygiene, low standard in living and regular contact with cat, this study disagreed with the finding reported by Al-ani *et al.*,(2004).

All factors mentioned above may increase the chance of exposure to *Toxoplasma gondii* infection especially possession of cat, so also the fact that eating of contaminated food containing *T.gondii* oocyst.

In relation Miscarriage, a higher seropositivity (41.54%) of *T.gondii* latent infection was observed among pregnant women who have suffered miscarriage in the past compare to those that have not suffered any miscarriage (31.79%). These suggest that Toxoplasmosis is possible risk for miscarriage.

In respect to occupation, the least prevalence of *T. gondii* infection among students was noted as follows: IgG and IgM 4 (4.49%), Ig IgM was 2 (2.25%), while the highest prevalence was recorded among house wives in this order IgG 74 (60.16%), IgM 28(22.76%) and IgG+IgM 7 (5.67%). The reason for low prevalence among student may be due to the fact that students are restricted to a particular place and their food and environment are monitored to a considerable extent. Higher prevalence was observed among the house wife may be as a result of poor sanitation and contact with cat excrement.

Results from this study also indicates a higher prevalence of *T.gondii* antibodies among those with no formal education as follows: IgG 32(35.96%), IgM 13 (14.16%), those who attend tertiary education recorded the following; IgG 10 (29.41%) IgM 4 (11.76). This shows that the prevalence of *T. gondii* antibodies decreases with increase in level of education. However, educated pregnant women are more likely to maintain personal hygiene by washing cutting boards, knives, with soap and detergents before and after use compared to non-educated women.

In relation to domestic animals, the present study shows a higher prevalence among women who had cats as pet animals; this may be as a result of regular contact with cat litters. This agrees with the finding reported by Al-omar *et al.*, (1993).

Based on sources of drinking water, this study found a higher latent infection IgG 57 (37.75%) in those drinking sachet than those who drink well water IgG 8 (23.53%) but well water had the highest prevalence of IgM antibodies 10 (29.41%). However, the highest acute infection associated with well water consumption could be as a result of contamination of well water with oocysts from the surrounding soil. Furthermore, individuals who drink from the river had the least prevalence of *T gondii* antibodies. The reason for this could be due to the fact that only four

pregnant women were examined in that category. Also, it could be suggested that these four individuals do not reside Kano metropolis as there is no river water in the Metropolis.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0 CONCLUSION

This study has established 43.4% seroprevalence of *T.gondii* in Kano metropolis. There are no significance associations of *T gondii* infection with all demographic and riskfactors measured in this research ($P>0.05$), except for cat and sources of drinking waterthat show significant association with *T. gondii* antibodies ($P<0.05$).Polymerase chain reaction was used to confirmed the presence of *Toxoplasma gondiiB1 gene*.Therefore cerebrospinal fluid should also be included in further research for the confirmation *Toxoplasma gondii*, but its require greater technical know-how and experience.

6.1 RECOMMENDATION.

1.Since contact with cats cannot be avoided either directly or indirectly therefore they should be dewormed regularly.

2.For those that have no option rather than to drink well water, the water should be boiled at high temperature before used as drinking water or it should be preserve in a freezer before used.

3.This gives room for public awareness, to continue to educate the public through sensitization work- shop.

4.There should be a public campaign on *T.gondii* awareness among pregnant women attending antennal care clinic

Proper hygienic practice should be encouraged in all our daily activities.

5. Eating not properly processed food should be discouraged more especially eating food from unregistered vendors.
6. Those that sell close to gutters, water drainage were there will be highly expectation of contamination should be standardize and it should be enforce as a law.
7. Hands should be thoroughly washed with soap and water regularly to avoid contamination.
8. More research should be carrying out and the outcome of the research should be confirm and extended to the authority responsible for an appropriate action.
9. Therefore cerebrospinal fluid should also be included in further research for the confirmation, but its requiring greater technical know-how and experience.
10. Nigerian government should implement a ministry were kits of different organism should be synthesis rather than shipping from abroad.

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APPENDIX I



KANO STATE

HOSPITALS MANAGEMENT BOARD

BOARD HEADQUARTERS
P.M.B 2546, Post Office Road, Kano

HMB/GEN/488/VOL. I

06/11/1435AH = 21/08/2015

Umar Adam Haruna
Department of Microbiology,
Ahmadu Bello University Zaria,
Kaduna.

PROVISIONAL ETHICAL CLEARANCE

Sequel to your request to conduct a research titled "SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH TOXOPLASMA GONDII INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN KANO METROPOLIS". In the light of the above, I am mandated to convey provisional clearance to proceed on your study based on the following conditions.

- i. That the consent of all participants must be obtained by filling in consent form.
- ii. That you should liaise with the Management of the facility for appropriate guidance.
- iii. That any publication related to the study should be brought to the knowledge of the Ethical Committee for approval.
- iv. That a copy of your finding should be submitted for documentation, record and final approval, please.


Best regards.

✓
MAIMUNAH KHALID ABDUL'AZIZ
Asst. Secretary II
FOR: EXECUTIVE SECRETARY

CC:

The Chief Medical Directors,
MMSH, MAWSH,
Hospitals Management Board,
Kano.

Also is for your information and noting, please.


MAIMUNAH KHALID ABDUL'AZIZ
Asst. Secretary II
FOR: EXECUTIVE SECRETARY



APPENDIX II

APPENDIX II

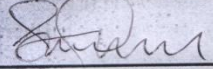
INFORMED CONSENT FORM

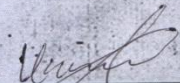
My name is Umar Haruna, a postgraduate student in the department of Microbiology Ahmadu Bello University, Zaria. I am conducting research on "Seroprevalence and Associated Risk factors of Toxoplasma Gondii infection in Females attending Antenatal Clinics, Kano State.

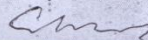
I would be grateful if you will participate in this research.

Please note that this participation is a voluntary venture and you have the right to refuse or withdraw from this research without any ill -feelings towards you from me, any group persons or organization.

I understood the aim of the research and have agreed to participate in the research.


Signature of Subject


Signature of Investigator


Signature of Witness

APPENDIX III

Department of Microbiology

Faculty of Science

Ahmadu Bello University, Zaria

Topic:- SEROPREVALENCE AND RISK FACTORS ASSOCIATED WITH *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN KANO METROPOLIS, KANO STATE.

Research questionnaire

Dear Respondent,

I am a postgraduate student of the above named university carrying out a research work on the seroprevalence and associated risk factors of *Toxoplasma gondii* infection in females attending antenatal clinics in Kano metropolis, Kano State.

I wish to therefore solicit for your humble co-operation to kindly offer to me your honest opinions and accurate responses to the under listed questions to the best of your knowledge. I will be grateful if you contribute to the fulfillment of these project by answering the below questions. Thank you for your anticipated co-operation. Yours faithfully,

Umar Haruna Adamu

Please tick the appropriate options given, all information given is necessary.

Bio-Data

1. Age:

a. 15-30 [] b. 31-40 [] c. 41-50 [] d. 51-60 []

2. Any Abortion before:

a. Yes [] b. No []

3. Stages of Pregnancy:

a. First trimester [] b. Second trimester [] c. Third trimester []

4. Occupation:

a. Student [] b. Civil Servant [] c. House wife []

5. Educational Qualification

a. certificate [] b. Secondary school certificate [] c. Tertiary [] d. non
formal education-----

6. Type of domestic Animal?

a. Others [] b. Pig [] c. Cat []

7. Sources of drinking water

a. Well water [] b. Sachet water [] c. pipe borne water [] d. River water []

APPENDIX IV

IgG and IgM ELISA Results

IgM plate 1 0.142														
					0.753521	0.697183	0.795775	1.014085	1.232394	0.774648	1.119718			
0	0	0	0	0	0	0	0	0	0	0	0.859155	Negative	Negative	
1	0	0.5	0.880282	0.873239	1.056338	0.34507	1.056338	0.78169	0.739437	1.457746	0.922535	equivocal	Negative	
1.943662	0.697183	0.556338	0.43662	0.507042	0.478873	0.732394	0.661972	1.352113	1.028169	1.267606	3.204225	positive	equivocal	
1.732394	0.633803	0.690141	0.15493	0.746479	0.852113	0.774648	0.43662	0.880282	1.140845	1.549296	0.859155	positive	equivocal	
0.112676	0.478873	4.084507	0.197183	0.253521	0.232394	0.661972	0.422535	0.443662	1.309859	1.450704	0.669014	equivocal	equivocal	
0.197183	0.422535	0.28169	0.119718	0.338028	0.28169	0.739437	1.661972	0.690141	1.873239	1.014085	0.880282	equivocal	equivocal	
4.197183	0.429577	0.211268	0.626761	0.147887	0.133803	0.380282	0.577465	0.661972	1	0.795775	1.147887	positive	equivocal	
												positive	3	0
												Negative	1	2
												Equivocal	3	5
												Total	7	7
					0.606452	0.477419	0.551613	1.293548	0.548387	0.667742	1.090323			
2.683871	0	0	0.290323	0.53871	0.196774	0	2.167742	1.051613	0	0	0	positive	Negative	
0.329032	0.103226	0.258065	0.76129	0.987097	0.670968	0.493548	0.425806	0.590323	0.335484	0.445161	0.212903	equivocal	equivocal	
0.522581	0.729032	1.322581	0.893548	0.480645	0.683871	0.387097	0.274194	0.458065	0.312903	0.287097	0.780645	equivocal	equivocal	
0.441935	0.941935	2.029032	0.254839	2.903226	0.693548	0.703226	1.158065	0.66129	2.051613	0.56129	0.396774	equivocal	equivocal	
0.206452	0.203226	0.009677	0.283871	0.351613	0.03871	0.716129	0.419355	4.070968	1.03871	0.151613	0.490323	equivocal	equivocal	
0.46129	0.912903	0.425806	0.906452	4.409677	0.122581	0.416129	0.448387	0.241935	0.887097	0.190323	0.332258	equivocal	equivocal	
0.364516	0.219355	0.867742	0.567742	0.303226	0.33871	0.43871	0.535484	0.254839	0.203226	0.387097	0.403226	equivocal	equivocal	
												positive	1	0
												Negative	0	1
												Equivocal	6	6
												Total	7	7
					0.51	1.36	0.53	0.69	0.55	0.84	0.54			
0.49	-	-	-	-	0.92	-	0.15	-	0.32	-	-	equivocal	Negative	

	0.36	0.97	0.62	1.19	0.18	0.28	0.39	0.78	0.75	0.27	0.13	0.38	equivocal	equivocal	
	1.91	0.58	0.46	0.66	0.62	0.19	1.22	1.77	0.43	2.72	1.19	0.26	positive	equivocal	
	1.47	0.39	0.47	0.55	0.23	0.62	0.22	0.59	0.42	0.29	0.65	0.57	positive	equivocal	
	0.34	0.69	0.50	0.53	0.24	0.83	0.27	0.22	0.53	0.57	0.26	0.32	equivocal	equivocal	
	0.07	0.07	0.09	0.16	0.08	0.08	0.17	0.20	0.18	0.22	0.20	-	Negative	Negative	
IgG Plate 1 1.131													positive	2	0
													Negative	1	2
													Equivocal	3	4
													Total	6	6
					1.992927	1573.828	0.947834	0.825818	0.786914	0.745358	0.79664				
0	0.238727	1.157383	0.3855	0.454465	0.761273	0.197171	0.351017	0.3687	0.351017	0.207781	0.353669		Negative	equivocal	
1.422635	1.595049	0.494253	1.078691	0.76481	1.746242	0.291777	0.629531	0.774536	1.241379	1.038904	0.320071		positive	positive	
908.9302	0.91954	1.526083	1712.644	0.564103	0.709991	0.892131	0.50221	1.132626	0.458002	0.366932	1.928382		positive	equivocal	
1373.121	1.809903	0.937224	0.305924	1.127321	1.689655	1.429708	1.785146	1.627763	1.215738	0.43855	0.660477		positive	positive	
0.787798	1.642794	0.717065	0.882405	1.694076	0.525199	1.635721	1.210433	0.348364	0.609195	0.320071	0.637489		equivocal	positive	
1.356322	1.480106	0.298851	1.361627	1.50221	0.586207	0.458002	0.528736	1.371353	0.237843	0.478338	0.527851		positive	positive	
0.870911	1.335102	0.301503	0.793103	0.482759	1.527851	0.465959	0.737401	0.6313	0.431477	0.400531	0.433245		equivocal	positive	
IgG Plate 2 0.733													positive	4	5
													Negative	1	0
													Equivocal	2	2
													Total	7	7
					0.991814	0.570259	0.755798	0.819918	0.428377	#VALUE!	0.834925				
2.751705	0.177353	0.27558	1.094134	3.68895	4.199181	0.315143	1.451569	4.199181	0.747613	0.261937	5.388813		positive	equivocal	
0.150068	0.234652	0.043656	0.120055	0.780355	0.398363	0.364256	0.478854	0.517053	0.815825	3.804911	0.440655		equivocal	equivocal	
0.514325	0.508868	0.304229	0.559345	0.567531	0.55116	0.328786	0.260573	0.414734	4.267394	0.19236	0.190996		equivocal	equivocal	
0.270123	0.907231	2305.593	0.099591	0.418827	0.219645	0.136426	0.783083	0.169168	0.244202	0.697135	0.624829		equivocal	equivocal	
0.383356	0.79809	0.46794	0.424284	0.527967	0.691678	0.824011	0.953615	1.12824	0.323329	0.914052	1594.816		equivocal	equivocal	
0.34925	0.287858	0.676671	0.245566	0.278308	0.99045	0.351978	0.583902	0.3206	2.830832	0.968622	0.612551		equivocal	equivocal	
0.282401	0.418827	0.305593	0.356071	0.748977	0.859482	4.380628	0.563438	0.583902	0.590723	3.289222	1452.933		equivocal	equivocal	
IgG plate 3													positive	1	0
													Negative	0	0

0.275												Equivocal	6	7	
												Total	7	7	
					0.872727	1.836364	1.232727	10.59273	1.661818	1.952727	3.454545				
3.541818	0.763636	1.261818	0.785455	6.429091	1.207273	2.334545	1.992727	6.901818	0.883636	0.476364	1.603636	positive	equivocal		
10.13455	3.305455	0.76	1.898182	1.363636	0.8	1.938182	1.174545	3.389091	1.138182	0.363636	0.741818	positive	positive		
0.905455	0.538182	4970.909	3.236364	0.418182	0.949091	0.912727	1.785455	1.832727	1.603636	6.610909	1.352727	equivocal	equivocal		
1.076364	0.421818	2.505455	0.418182	2.745455	0.690909	2.269091	1.410909	1.974545	1.029091	1.687273	0.967273	equivocal	equivocal		
0.72	0.305455	0.338182	0.189091	0.981818	1.581818	1.218182	0.676364	2.825455	5.996364	5.661818	2.2	equivocal	equivocal		
0.378182	0.527273	0.349091	0.465455	0.734545	0.298182	0.28	0.403636	0.410909	0.341818	0.425455	0.538182	equivocal	equivocal		
0.24	0.232727	0.134545	0.247273	0.192727	0.12	0.305455	0.163636	0.305455	0.309091	0.414545	0.392727	equivocal	equivocal		
												positive	2	1	
												Negative	0	0	
												Equivocal	5	6	
												Total	7	7	
															Total
IgM	positive	6	0	3	1	2	0	2	4	3	6	5	3	35	
	Negative	2	5	5	2	3	3	3	1	2	2	3	3	34	
	Equivocal	12	15	12	17	15	20	18	18	18	15	15	16	191	
	Total	20	20	20	20	20	23	23	23	23	23	23	22	260	
IgG	positive	7	6	6	4	7	7	9	8	11	8	6	8	87	
	Negative	1	0	1	0	0	0	0	0	0	0	0	0	2	
	Equivocal	13	15	14	16	14	17	15	16	13	16	17	16	182	
	Total	21	21	21	20	21	24	24	24	24	24	23	24	271	
IgM IgG	positive	13	6	9	5	9	7	11	12	14	14	11	11	122	
	Negative	3	5	6	2	3	3	3	1	2	2	3	3	36	
	Equivocal	25	30	26	33	29	37	33	34	31	31	32	32	373	
	Total	41	41	41	40	41	47	47	47	47	47	46	46	531	

