Congenital Transmission of *Toxoplasma gondii* and *Plasmodium falciparum* co-infections in pregnant women and their new born babies in the Kumasi Metropolis

A Thesis submitted to the Department of Theoretical and Applied Biology, College of Science, Kwame Nkrumah University of Science and Technology in partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY (Parasitology)

BY

Reginald Arthur- Mensah Jnr (BSc. Hons.)

July 2014

DECLARATION

Except for references which I have duly acknowledged, I hereby declare that this thesis is entirely a record of my own original research carried out under the supervision of Dr. John Larbi, Theoretical and Applied Biology Department (TAB) of Kwame Nkrumah University of Science and Technology (KNUST) Kumasi and Dr. Irene Ayi, Head of Department, Parasitology (NMIMR). I do further declare that this thesis has not been submitted in part or whole for any purpose elsewhere.

Reginald Arthur-Mensah Jnr

PG7998212

Student Name & ID	Signature	Date

Certified by:

Dr. John Larbi

Supervisor Name	Signature	Date

Dr. Irene Ayi

Supervisor Name	Signature	Date

Certified by:

Dr. I. K. Tetteh

Head of Dept.	Signature	Date

DEDICATION

I dedicate this research work to every pregnant woman. "*Lo, children are an heritage of the Lord: and the fruit of the womb is his reward"*. Psalm 127:3

ACKNOWLEDGEMENTS

First, I thank God for the enabling Grace from the start of this work to its perfect completion. To him be Praise and Glory now and evermore Amen.

To Dr. John Larbi, Department of TAB, KNUST, I am very grateful Sir for all your inputs into the fruitful output of this work. God richly bless you for your time, dedication and support throughout this work. Thank you.

To Dr. Irene Ayi, Head, Parasitology Department, NMIMR, I say a very big God bless you with heartfelt gratitude. You were more than a supervisor to me. Thank you so much for your time, financial inputs, expert technical advice and commitment throughout this work. You taught me a lot. I appreciate you immensely. Thank you.

My appreciation also to Mr. Emmanuel Awusah Blay, Parasitology Department, NMIMR. Your invaluable contributions to this work cannot go unmentioned. Thank you for the training sessions prior to laboratory procedures, paperwork and for your assistance in diverse ways. Your contribution to this work was invaluable. God bless you.

To the entire staff of the Parasitology Department of NMIMR who helped in one way or the other in this work. I appreciate your efforts and support towards this work. God bless you guys.

A warm hand of appreciation to maternity staff and midwives of Manhyia District Hospital, South Suntreso Government Hospital and the Aninwah Medical Center (AMC). This work would not have been possible without your consent and assistance during sample collection. I appreciate your efforts. Time spent with you is greatly treasured. Thank you.

Finally to my parents, Mr. Reginald Arthur-Mensah and Mrs. Esther Arthur-Mensah, Thank you for the support and encouragement throughout the period. You are the best!!! God bless you abundantly. Thank you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ACT	Artimisin-Based Combination Therapy
AMC	Aninwah Medical Center
Bp	Base pair
CDC	Center for Disease Control
CHRPE	Committee for Human Rights and Publication Ethics
CI	Confidence Interval
DOT	Direct Observed Treatment
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra Acetate acid
ELISA	Enzyme-Linked Immunosorbent Assay
GHS	Ghana Health Service
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPTp	Intermittent Preventive Treatment in pregnancy
IUGR	Intrauterine Growth Restriction
KATH	Komfo Anokye Teaching Hospital
KNUST	Kwame Nkrumah University of Science and Technology
LBW	Low Birth Weight
MDCK	Madin Darby Canine Kidney

NMIMR	Noguchi Memorial Institute for Medical Research
OD	Optical Density
PCR	Polymerase Chain Reaction
PV	Parasitophorous Vacuole
SP	Sulphadoxine-Pyrimethamine
TMN	Tubulovesicular Membranous Network
Rpm	Revolutions per minute
TAE	Tris-acetate Ethylene Diamine Tetra Acetic acid
UV	Ultraviolet
WHO	World Health Organisation

ABSTRACT

Toxoplasmosis is a disease caused by the protozoan parasite, *Toxoplasma gondii*. About 90% of the world's population is reported to be infected with the parasite. The disease is important for its serious implications in immunocompromised individuals including pregnant women and its severe consequences on fetuses in congenital transmission. Malaria caused by one of its most virulent species, *P. falciparum* continues to be a major health challenge in sub-Saharan Africa. This study investigated *T. gondii* and *P. falciparum* infections in pregnant women and their babies in the Kumasi metropolis. A total of 183 pregnant women and 186 babies were tested. Risk factors associated with toxoplasmosis and malaria infection were assessed by a questionnaire guide. Maternal and fetal blood was safely drawn from the appropriate blood vessels of the placenta after delivery. Blood blots were made on filter papers and whole blood was spun to obtain sera. Serum samples were tested for anti-*Toxoplasma* IgG and IgM using commercial ELISA kits and DNA was extracted from blotted blood samples for detection of *T. gondii* and *P. falciparum* infections by a nested PCR. An overall seroprevalence of 44.8% (82/183) anti-*T. gondii* IgG was recorded. Parasite prevalence as determined by PCR was 23% (42/183) for *T. gondii* only and 3.8% (7/183) for *P. falciparum* only. About 2% (4/183) pregnant women were co-infected with both parasites. This is the first report of *Toxoplasma* infection in the Kumasi metropolis. Results reveal an appreciable prevalence in pregnant women thus calling for the need to create awareness and intensify public education in women of child bearing age and implement measures for screening of *Toxoplasma* infection during antenatal care.

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Toxoplasma gondii is a ubiquitous protozoan parasite of man and birds (Luft and Remington, 1988). It causes the disease toxoplasmosis. It belongs to the phylum Apicomplexa. The Apicomplexa are defined by the presence of a complex of apical secretory organelles such as the micronemes, rhoptries and dense granules. This phylum includes other important disease causing pathogens such as *Plasmodium*, *Eimeria*, *Cyclospora*, *Babesia* and *Crypstosporodium* (Mcleod *et al*., 1991; Wong and Remington, 1993).

Toxoplasmosis is distributed worldwide. Approximately one-third of the world's population is predisposed to the parasite (Sensini, 2006). Data from serological studies estimates prevalence from 30% to 95% of populations of most industrialized countries to be chronically infected with the organism (Montoya and Remington, 2000; Asthana *et al*., 2006). In the USA, the seroprevalence of anti *T. gondii* antibodies stood at 24.6% in 2009 (Jones *et al*., 2009). Seroprevalence values from reported data in Brazil range from 50% to 80% (Spalding *et al*., 2005)

Studies in some parts of Africa have documented high prevalence of toxoplasmosis in people and animals. In Ethiopia, a survey using two different test kits; the Modified Direct Agglutination Test (MDAT) and ELISA on a total of 116 sheep and 58 goats revealed significant IgG seroprevalence of 52.6% and 24% in sheep and goats by the MDAT and 56% and 25.9% anti-*T. gondii* IgG in sheep and goats by ELISA respectively. Prevalence rates in humans ranged from 11.5% to 39% (Negash *et al*., 2004). *Toxoplasma*-specific seropositivity of 43.7% was recorded in Nigerian

women, with 25% observed in ages 15-18 years and 71.4% in ages 39-42 years (Olusi *et al*., 1996). In Ghana, an overall prevalence of 92.5% (147/159), serum anti-*Toxoplasma* IgG (73.6%), IgA (64.8%) and IgM (76.1%), as estimated by ELISA was found in pregnant women in the Greater Accra region (Ayi *et al*., 2009).

Toxoplasmosis is generally asymptomatic but can be symptomatic and cause life threatening conditions in congenital toxoplasmosis or in immunosuppressed patients such as HIV/AIDS patients, organ transplant recipients and cancer patients (Gavinet *et al*., 1997; Nissapatorn *et al*., 2004).

Toxoplasma gondii has three infectious forms. The tachyzoites (asexual stage), bradyzoites (in tissue cysts, also asexual) and the sporozoites (in oocysts and sexual reproduction).

Molecular genotyping has shown that approximately 90% of the *T. gondii* isolates that have been analysed can be classified into three clonal lineages namely; types I, II and III which have different pathogenicity. Type I is highly virulent in murine infections, whereas types II and III strains are relatively less virulent (Peyron *et al*., 2006).

Humans become infected by ingesting food, water and vegetables contaminated with oocysts from infected cat feaces, ingesting tissue cysts in under cooked or uncooked meat and vertical transmission from mothers to babies. Contamination of the environment by oocysts is widespread, as oocysts are shed by infected domestic cats and other members of the Felidae family. Domestic cats are the major source of contamination because oocyst formation is greatest in them. Cats may excrete millions of oocysts after ingesting one bradyzoite or one tissue cyst, and many tissue

cysts may be present in one infected mouse. Oocysts are shed for only a short period (1-2 weeks) in the life of the cat; however, the enormous numbers shed assure widespread contamination of the environment. Under experimental conditions, infected cats can shed oocysts after reinoculation with tissue cysts. Oocysts survive in moist soil for months to even years. Oocysts do not always stay in the soil; invertebrates like filth flies, cockroaches, dung beetles, and earthworms can mechanically spread these oocysts and even carry them onto food. The more oocysts there are in the environment, the more likely it is that prey animals will become infected, and this results in increased infection rates in cats and then to human populations (http://www.cdc.gov/parasites/toxoplasmosis/epi.html).

Congenital state of toxoplasmosis is due to vertical transmission of *T. gondii* from a seronegative pregnant woman, who becomes acutely infected and transmits to her foetus via the placenta. Multiple factors are associated with the occurrence of congenital *Toxoplasma* infection. These include the period of transmission, climate, cultural behaviour, eating habits and hygienic standards. This combination leads to marked differences of infection among countries. (Rorman *et al*., 2006). The rate of transmission to the foetus is 10–15% in the first trimester of gestation, which may increase to 68% in the third trimester (Thulliez *et al*., 1992). 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 (Holliman, 1995). Infection during the first trimester may lead to spontaneous abortion and stillbirths of the newborn while infection acquired later during pregnancy can result in chorioretinitis and mental retardation (McAuley *et al*.,

1994). Other clinical signs representative of the disease are low birth weights, hydrocephalus, cerebral calcifications and neurological injury (Couvreur, 2004).

Diagnosis of *T. gondii* infection rests upon conclusions drawn from clinical, radiological and laboratory investigations. In addition to confirming infection, such tests can aid in determining prognosis, influence management, and assist in monitoring response to treatment. The laboratory tests that are used most commonly for initial investigation are serological; targeting detection of IgG, IgM, and, in some circumstances, IgA specific for *T. gondii*. PCR tests have been developed using different gene targets. This technique has proven very useful in the diagnosis of clinical toxoplasmosis (Joynson and Guy, 2001).

Management of toxoplasmosis involves cooking food to safe temperatures. Beef, lamb, and veal and steaks should be cooked to at least 64ºC. Pork, ground meat, and wild game should be cooked to 72ºC before eating. Fruits and vegetables should be peeled or thoroughly washed before eating. Cutting boards, dishes, counters, utensils, and hands should always be washed with hot soapy water after they have contacted raw meat, poultry, seafood, or unwashed fruits or vegetables. Pregnant women should wear gloves when gardening and during any contact with soil or sand because cat waste might be in soil or sand. They are encouraged to keep their cats indoors and not adopt or handle stray cats. Cats should not be fed raw or undercooked meats (Lopez *et al*., 2000).

Malaria is one of the oldest diseases known to mankind which has had profound impact on our history for the past century. It's prevented economic development in vast regions of the earth and it continues to be a huge social, economic and health problem, particularly in the tropical regions.

Currently, an estimated 3.4 billion people are at risk of malaria. In 2012, WHO estimated that 207 million cases of malaria occurred globally (uncertainty range 135–287 million) and 627 000 deaths (uncertainty range 473 000–789 000) of which 80% of cases and 90% deaths occurred in Africa and 77% of deaths were in children under 5 years of age (WHO/WMR 2013).

In Ghana, malaria is hyper endemic and among pregnant women, it accounts for 38% of OPD attendance, 36% of admissions and 20% of maternal deaths. *P. falciparum* malaria is the most common species in Ghana which is very severe and turbulent during pregnancy (MOH/GHS, 2014).

Malaria is caused by protozoan parasite Plasmodia, also belonging to the parasitic phylum Apicomplexa. It is caused by five species of parasite that affect humans, and all of these species belong to the genus *Plasmodium*. They are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Of these, *P. falciparum* and *P. vivax* are the most important. Malaria due to *P. falciparum* is the most deadly form, and predominates mostly in Africa. More than 200 species of the genus *Plasmodium* have been identified that are parasitic to mammals including man, reptiles and birds (WHO/WMR 2013).

The principal mode of spread of malaria is by the bites of the female *Anopheles* mosquito. Of more than 480 species of *Anopheles*, only about 50 species transmit malaria, with every continent having its own species of these mosquitoes: *An. gambiae* complex in Africa, *An. freeborni* in North America, *An. culicifacies, An. fluviatilis, An. minimus, An. philippinensis, An. stephensi,* and *An. sundaicus* in the Indian subcontinent, *An. leucosphyrus, An. latens, An. cracens, An. hackeri, An.*

dirus, have been identified as the vectors for the transmission of *P. knowlesi* (Indra, 2008; Van den Eede, 2009)

When a female *Anopheles* mosquito bites an infected individual, it sucks gametocytes, the sexual forms of the parasite, along with blood. These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the salivary glands of the infested mosquito. When this female *Anopheles* mosquito bites another person for a blood meal, the sporozoites are inoculated into the blood stream of the fresh victim, thus spreading the infection (Barillas-Mury and Kumar, 2005).

Transfer of parasitized red blood cells due to *Plasmodium* from infected mother to the child transplacentally leads to congenital malaria (Neena *et al*., 2007). Congenital malaria has been reported due to all five *Plasmodium* species that commonly infect humans, although most cases are reported following *P. falciparum* or *P. vivax* malaria in the mother. In nonendemic countries, *P. malariae* causes a disproportionately higher number of congenital malaria cases due to its longer persistence in the host (Gitau *et al*., 2005).

Malaria in pregnancy occurs most often during the first and second pregnancies. Common morbidities in the mother include anaemia, fever, hypoglycemia, cerebral malaria, pulmonary edema, puerperal sepsis and mortality sometimes occurs from severe malaria and haemorrhage. Complications in the newborn include low birth weights, prematurity, intrauterine growth restrictions (IUGR) and deaths of the babies (Gitau *et al*., 2005).

The diagnosis of malaria is confirmed by blood tests and can be divided into microscopic and non-microscopic tests. Careful examination of a well-prepared and well-stained thick or thin blood film remains the gold standard for malaria diagnosis. Non-microscopic tests include rapid dipstick immunoassay, and Polymerase Chain Reaction assays. These tests involve identification of the parasitic antigen or the antiplasmodial antibodies or the parasitic metabolic products in maternal blood (Moody and Chiodini, 2000). The diagnosis of infant malaria can be confirmed by a smear for malaria parasite from cord blood or heel prick, anytime within a week after birth or even later (Lee *et al*., 2002).

The best available treatment, particularly for malaria is the artemisinin-based combination therapy (ACT). Antimalarial combinations increases efficacy, shorten duration of treatment and decrease the risk of resistant parasites arising through mutation during therapy (Bosman and Mendis, 2007). Vector control is the main way to reduce malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high levels to close to zero (Bosman and Mendis, 2007). For individuals, personal protection against mosquito bites represents the first line of defence for malaria prevention. Pregnant women are advised to sleep under insecticide treated mosquito nets (ITN) during the period of their pregnancy.

This study aims to assess *T. gondii* and *P. falciparum* infection status of mothers at delivery and their new born babies in the Kumasi Metropolis.

1.2 Problem Statement

Pregnancy and its related complications are significant on the list of conditions presented at health facilities in the country (MOH/GHS, 2013). Despite numerous

efforts been made to fight malaria in pregnancy, it still continues to be a major challenge in pregnant women and their babies. Pregnant women are at risk of developing complications of malaria in pregnancy such as maternal anaemia, intrauterine growth restriction (IUGR), intra-uterine death, stillbirth, premature delivery, low birth weights and neonatal deaths. Congenital toxoplasmosis is similarly known to be associated with such effects and clinical evidences of infection have been reported in the country. A previous study documented high titres of antibodies to *T. gondii* in both mothers and their babies (Anteson *et al*., 1980). In recent studies, high seroprevalence values (51.2% to 92.5%) of anti *T. gondii* antibodies were recorded amongst pregnant women attending antenatal clinics in the Greater Accra Region of Ghana (Ayi *et al*., 2009). Thus, the exposure to *T. gondii* infection is established in the country.

There could be possible infections with opportunistic parasites due to immunosuppression during the period of pregnancy especially in nulliparous women. The impact of possible negative effects due to aggravation of these parasites in mothers and unborn babies could cause associated consequences. The need therefore arises to assess the risk of infections with *T. gondii* and *P. falciparum* during pregnancy for appropriate management to monitor complications and improve maternal and child health.

1.3 Justification of Study

Screening of pregnant women for *Toxoplasma* infection is not practiced during antenatal clinics in Ghana. However, antenatal care policy for malaria prevention during the period of pregnancy with Sulphadoxine Pyrimethamine (SP) exists via the Intermittent Preventive Treatment of Malaria in pregnancy (IPTp-SP). This lack of

screening for toxoplasmosis may expose both mother and unborn child to the development of the disease which could have been managed by an early antenatal intervention. If screening for toxoplasmosis is to be added, then some empirical information on the detection, prevalence, associated effects and transmission rate from mother-to-child in the country is needful to serve as evidence. However, there is little reported data in this direction, hence the present study.

Further, the results of this study will provide preliminary data on the co-infection status of pregnant women and babies in the Kumasi metropolis. Results will contribute to knowledge and infection risk factors on both diseases in Ghana.

1.4 Objectives of Study

1.4.1 Main Objective

» To assess *T. gondii* and *P. falciparum* infection status of mothers at delivery and their new born babies in the Kumasi Metropolis.

1.4.2 Specific Objectives

» To estimate the prevalence of serum anti *T. gondii* IgG and IgM antibodies by ELISA in mothers and babies.

» To determine the prevalence of *T. gondii* infection in mothers and babies by detection of parasite DNA in blood samples using PCR.

» To determine the prevalence of *P. falciparum* infection in mothers and babies by detection of parasite DNA in blood samples using PCR.

» To determine *T. gondii* and *P. falciparum* co-infection status of mothers and their babies.

» To determine infection risk factors from responses to questionnaire interviews.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Toxoplasma gondii*

It is over a century since the discovery of the parasite, *Toxoplasma gondii*. The parasite was first detected by Nicolle and Manceaux in 1908. They found a protozoan in the tissues of a hamster-like rodent, the gundi, *Ctenodactylus gundii*, which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis. They initially thought the parasite to be leishmania but soon realized that they had discovered a new organism and named it *Toxoplasma gondii*, based on the morphology (*toxo* –arc or bow, *plasma* –life) and the host, the rodent (Nicolle and Manceaux, 1909).

Over the years, several groups of investigators have conducted research into the disease and the biology of the parasite; hence there is now knowledge about the parasite and its disease condition. In 1923, Janku reported the first case of chorioretinitis in a child. He found parasites in the eye and called them sporozoa. Later these sporozoa were identified to be *Toxoplasma gondii* in 1928 by Levaditi. Wolf and Cowen in 1938 identified *Toxoplasma gondii* as the cause of neonatal encephalitis being the first report of congenital transmission of *T. gondii*. Sabin described a triad of signs (retinochoroiditis, hydrocephalus and cerebral calcification) in an infant with congenital toxoplasmosis in 1941. Weinman and Chandler (1954) suggested that the transmission of *T. gondii* may be related to eating undercooked infected pork. From 1970 till date, there has been reports and increasing awareness of the risk to the foetus as a consequence of an acute and latent *T. gondii* infection in pregnant women (Dubey, 1993).

2.2 Taxonomy of *T. gondii*

The parasite is a member of the Kingdom: Protista, Sub Kingdom: Protozoa, Phylum: Apicomplexa, Class: Conoidasida, Subclass: Coccodiasini, Order: Eucoccidiorida, Suborder: Eimeria, Family: Sarcocystidae, Genus: *Toxoplasma,* Species: *Toxoplasma gondii* (Levine, 1977)

2. 3. *T. gondii* life forms

There are four invasive forms of *T. gondii*: tachyzoite, bradyzoite, merozoite, and sporozoite (Frenkel, 1973). Tachyzoites and bradyzoites are associated with the intermediate host, and merozoites and sporozoites with the definitive host. Tachyzoites and merozoites are responsible for the expansion of the population within a host, while the bradyzoites and sporozoites are capable of environmental transmission to new hosts (Frenkel, 1973). All of the infectious stages have the same basic morphology, with only minor variations.

2.3.1 Tachyzoites

Tachyzoites (*tachos* - fast) are the rapidly growing life stage of *T. gondii* that have also been called endozoites or trophozoites. It is the proliferative form and the feeding form. It can infect virtually any cell in the body. It divides by a specialized process called endodyogeny. These invasive stages are crescent-shaped cells (approximately 2 × 7 μm) with a slightly more pointed anterior end, the anterior being defined by the direction of motility (Dubey, 1993) [Figure 1].

They are comprised of a unique cytoskeleton (subpellicular microtubules, conoid), secretory organelles (rhoptries, micronemes, dense granules), endosymbiontic derived organelles (mitochondrion, apicoplast), eukaryotic universal organelles (nucleus, endoplasmic reticulum, Golgi apparatus, ribosomes), and specific

structures (acidocalcisomes), all enclosed by a complex membranous structure termed the pellicle (de Melo and de Souza, 1997). The nucleus is usually situated toward the central area of the cell and contains clumps of chromatin and a centrally-located nucleolus (Figure 2).

Tachyzoites enter host cells by actively penetrating through the host cell plasmalemma or by phagocytosis. After entering the host cell, the tachyzoite becomes ovoid and is surrounded by a parasitophorous vacuole (PV), which appears to be derived from both the parasite and the host cell. Soon after penetration, a tubulovesicular membranous network (TMN) develops within the PV. Some of the TMN membranes are connected to the parasitophorous vacuolar membrane. The TMN appears to be derived from the posterior end of the tachyzoite (Bonhomme *et al*., 1992; Dubremetz and Swartzman, 1993) [Figure 3]. However, convoluted tubules, structurally similar to the TMN, have been observed at the end of tachyzoites. The host cell ruptures when it can no longer support the growth of tachyzoites. The rates of invasion and growth vary depending on the strain of *T. gondii* and the type of host cells. After entry of tachyzoites into a host cell, there is a variable lag period before the parasite divides, and this lag phase is partly parasite dependent. Mouse virulent strains of *T. gondii* grow faster in cell culture than do avirulent strains, and some strains of *T. gondii* form more rosettes than others (Appleford and Smith, 1997).

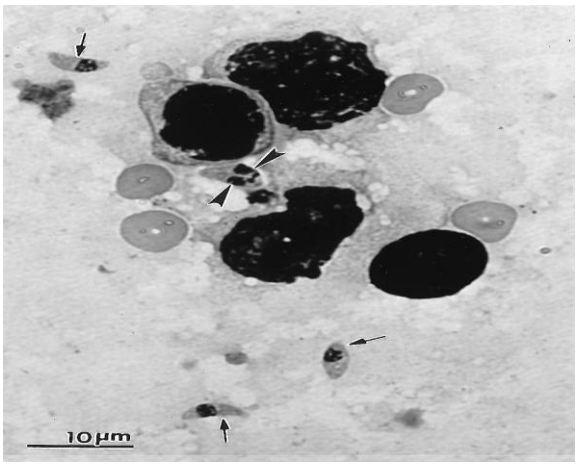

Figure 1: Tachyzoites of *T. gondii*. A dividing tachyzoite (arrowheads) and single tachyzoites (arrows). Impression smear feline lung, stained with Giemsa stain.

(Source: Weiss & Kim, 2006)

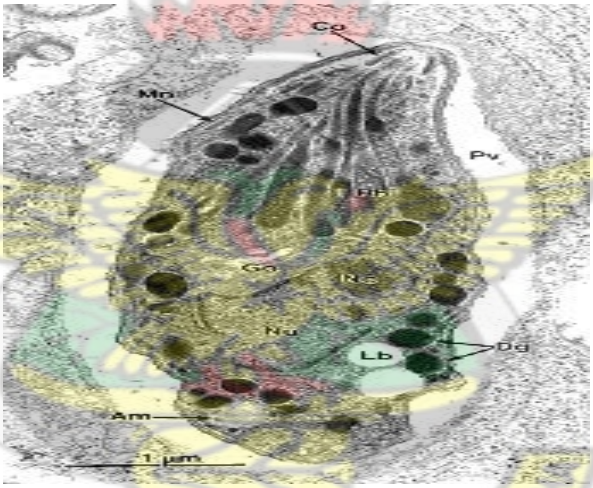

Figure 2: Transmission electron micrograph of a tachyzoite of the VEG strain of *T. gondii* in a mouse peritoneal exudate cell. Am, amylopectin granule; Co, conoid; Dg, electron-dense granule; Go, Golgi complex; Mn, microneme; No, nucleolus, Nu, nucleus; Pv, parasitorous vacuole

(Source: Weiss & Kim, 2006)

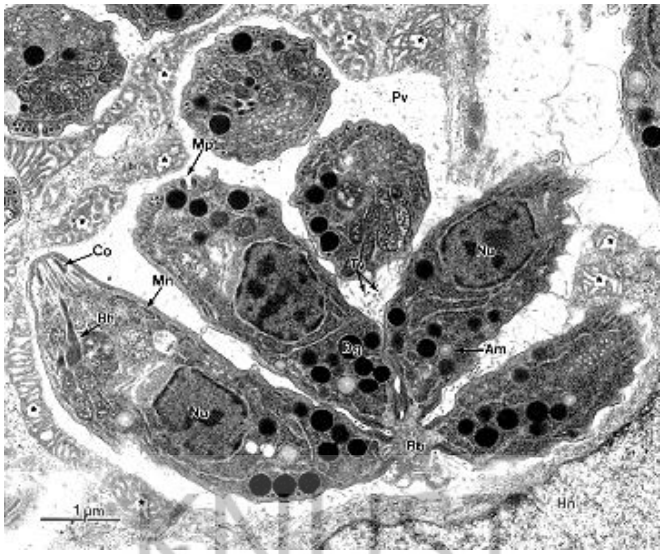

Figure 3: Transmission electron micrograph of four tachyzoites of the VEG strain of *T. gondii* in the final stages of endodyogeny that are still attached by their posterior ends to a common residual body (Rb).

(Source: Weiss & Kim, 2006)

2.3.2 Bradyzoites or tissue cyst

The term 'bradyzoite' (Gr. brady = slow) was proposed by Frenkel in 1973 to describe the stage encysted in tissues. Bradyzoites are also called cystozoites. They are approximately 7 by 1.5 µm in size. They possess similar organelles and inclusions as tachyzoites however, the nucleus of the bradyzoites is placed more to the posterior end (Figure 6). Movement is similar to that which occurs in the tachyzoites. The bradyzoites divide by endodyogeny but unlike tachyzoites this occurs in the tissue cyst. The contents of rhoptries in bradyzoites are usually electron dense, whereas those in tachyzoites are labyrinthine (Figure 4). However, the contents of rhoptries in bradyzoites vary with the age of the tissue cyst. Bradyzoites in younger tissue cysts may have labyrinthine rhoptries, whereas those in older tissue cysts are electron dense. Also, most bradyzoites have one to three rhoptries, which are looped back on themselves. Bradyzoites contain several amylopectin granules which stain red with PAS reagent; such material is either in discrete particles or

absent in tachyzoites (Figure 5). Bradyzoites are more slender than are tachyzoites. Bradyzoites are less susceptible to destruction by proteolytic enzymes than are tachyzoites (Jacobs *et al*., 1960), and the prepatent period in cats following feeding of bradyzoites is shorter than that following feeding of tachyzoites (Dubey, 1993 and Frenkel, 1976)

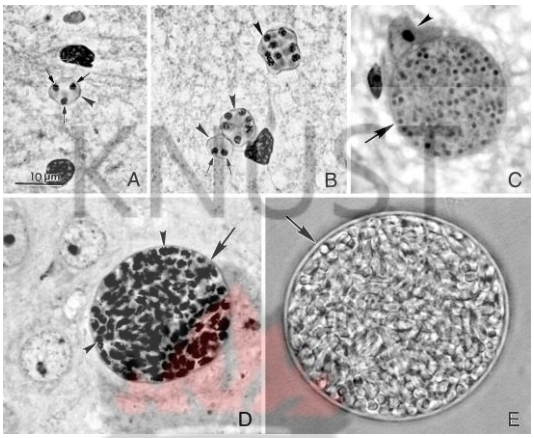

Figure 4: Tissue cysts of *T. gondii* in mouse brains. (A) Tissue cyst with three bradyzoites (B) Three tissue cysts with well-defined cyst walls (C) Intracellular tissue cyst in section. (D) Tissue cyst with numerous PAS-positive bradyzoites (E) Tissue cyst freed from mouse brain.

(Source: Weiss & Kim, 2006)

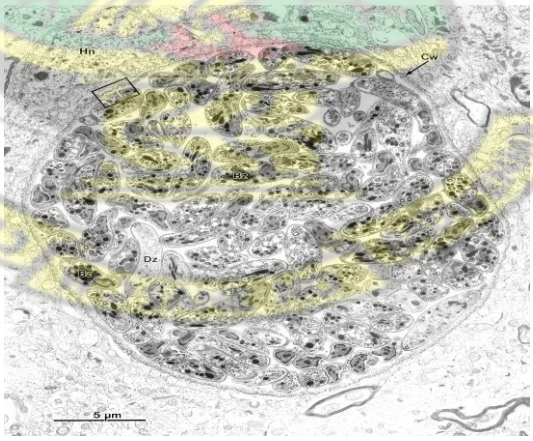

Figure 5: Tissue cyst in the brain of a mouse that was inoculated 8 months earlier with oocysts of the VEG strain of *T. gondii*. This ultrathin section of the cyst shows approximately 110 bradyzoites (Bz). The tissue cyst is surrounded by a relatively thin cyst wall (cw)

(Source: Weiss & Kim, 2006)

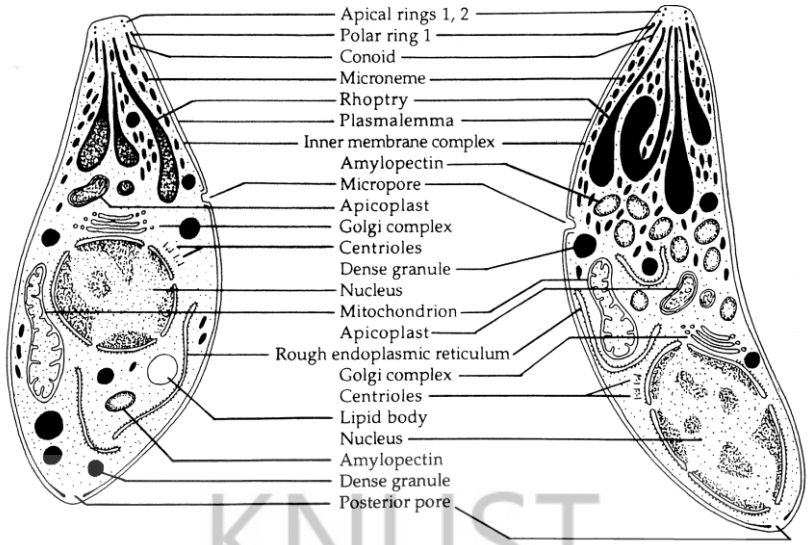

Figure 6: Schematic drawings of a tachyzoite (left) and a bradyzoite (right) of *T. gondii.* The drawings are composites of electron micrographs.

(Source: Weiss & Kim, 2006)

2.3.3 Oocysts and Sporozoites

Unsporulated oocysts are subspherical to spherical and are 10 by 12μm in diameter. Under light microscopy, the oocyst wall consists of two colorless layers. Polar granules are absent, and the sporont almost fills the oocyst. Sporulation occurs outside the cat within 1 to 5 days of excretion depending upon aeration and temperature. Sporulated oocysts are subspherical to ellipsoidal and are 11 by 13 μm in diameter. Each oocyst contains two ellipsoidal sporocysts without Stieda bodies. Sporocysts measure 6 by 8μm. A sporocyst residuum is present; there is no oocyst residuum. Each sporocyst contains four sporozoites (Ferguson *et al.*, 1978). Sporozoites are 2 by 6 to 8 μm in size with a subterminal nucleus (Ferguson *et al.*, 1979). Sporozoite formation begins when two dense plaques called anlagen appear at both ends of the sporocyst. Each nucleus divides into two and is incorporated into elongating sporozoite anlagen. Thus, four sporozoites are formed in each sporocyst (Ferguson *et al.*, 1978; 1979) [Figure 7]. A prominent residual body is left after sporozoite are formed. The residual body is enclosed in a single-unit membrane.

Ultrastructurally, the sporozoite is similar to the tachyzoite, except that there is an abundance of micronemes, rhoptries, and amylopectin granules in tachyzoites (Figure 8).

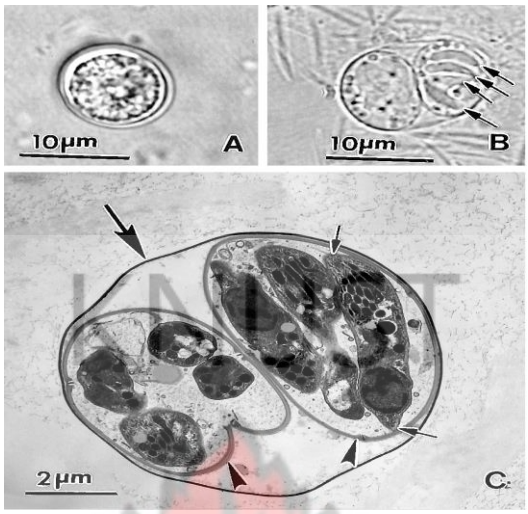

Figure 7: Oocysts of *T. gondii*. (A) Unsporulated oocyst. (B) Sporulated oocyst with two sporocysts. (C) Transmission electron micrograph of a sporulated oocyst. Note the thin oocyst wall (large arrow), two sporocysts (arrowheads), and sporozoites.

(Source: Weiss & Kim, 2006)

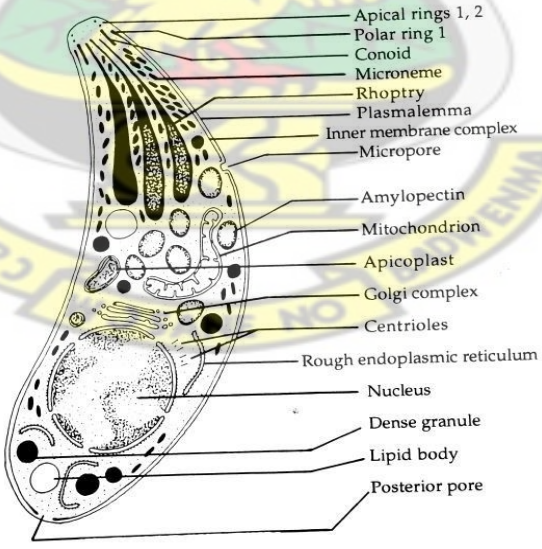

Figure 8: Schematic drawing of a *T. gondii* sporozoite.

(Source: Weiss & Kim, 2006)

2.4 Life and transmission cycle of *T. gondii*

Toxoplasma gondii is transmitted by three principal routes. Primarily, human can acquire *T. gondii* by eating raw or inadequately cooked infected meat, especially pork, mutton, and wild game, or uncooked foods that have come in contact with tissue cyst in infected meat. Secondly, humans can inadvertently ingest oocysts that cats have passed in their faeces, either from a litter box or from soil (e.g. soil from gardening, unwashed fruits or vegetables in unfiltered water). Lastly, women can transmit the infection transplacentally to their unborn foetus (Dubey, 1994). Transplacental infection occurs when an uninfected mother acquires infection during pregnancy. Parasitaemia is established in the mother when there is the invasion of the placenta, and finally *T gondii* spreads to foetal tissues. Transmission of *T. gondii* may also occur through blood transfusions and organ transplants (http://www.cdc.gov/parasites/toxoplasmosis/epi.html)

Toxoplasma gondii life cycle has three forms: tachyzoite, bradyzoite, and sporozoite. Asexual, sexual and oocyst stages of this organism develop in the small intestine of wild and domestic cats. Cats get infected by eating mice or birds or animal tissue containing infective oocysts. Sporozoites are found in environmentally resistant oocyts formed after the sexual stage of life cycle. In the intestine of the cat, the parasite develops through the typical coccidian life cycle. Unsporulated oocysts are shed in the faeces of infected cats. After a few days the oocysts sporulate and become infective in the environment for over a year. The oocysts are further ingested by the intermediate host (pig, sheep, cattle and humans). During the acute stages of *T.gondii* infection in intermediate host, tachyzoites invade and replicate within host cells. The tachyzoites invade all organs, including the heart, liver, spleen, lymph nodes, lungs, placenta, muscle, brain and central nervous system (CNS) [Figure 9].

During the latent infection, bradyzoites are present in tissue cyst. Tissue cyst may remain viable for the life span of the host. (Dubey and Beattie, 1988).

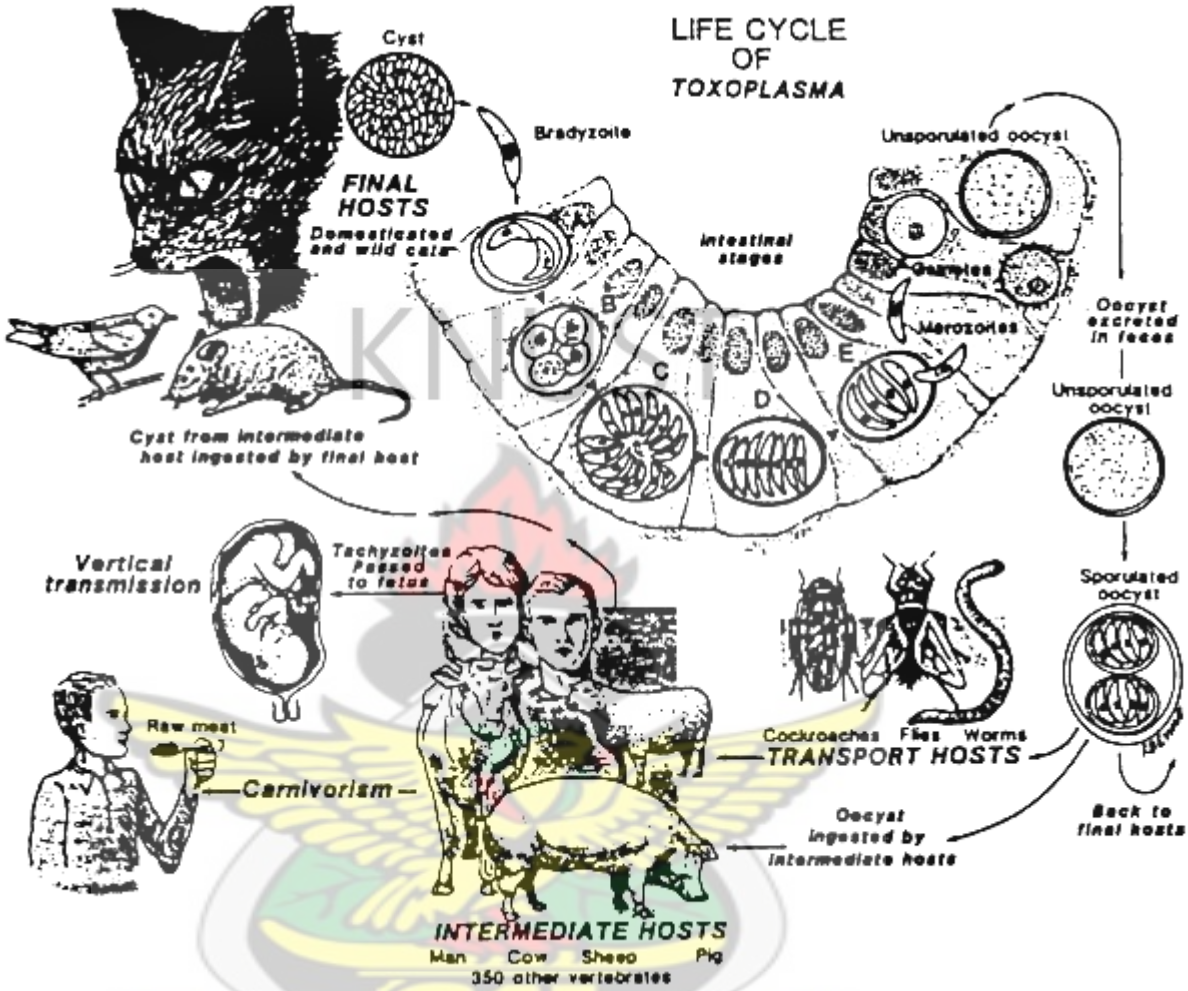

Figure 9: Life and transmission cycle of *T. gondii*

(Source: Dubey, 1994).

2.5 Congenital toxoplasmosis

Congenital toxoplasmosis is one source of *T. gondii* isolation in humans through amniotic fluid, placenta tissues, cord blood and tissues of aborted fetuses (Dunn *et al*., 1999). When infection occur for the first time during pregnancy, mother to child transmission of the parasite could cause congenital toxoplasmosis. The risk of

transmission depends on the gestation period at maternal infection, rising from approximately 6% for women infected at 10 weeks of gestation to 80% if the infection occurred at 38 weeks (Dunn *et al*., 1999). Transplacental passage is more common when maternal infection occurs in the latter half of pregnancy, but foetal injury is usually less severe. It may lead to miscarriage, stillbirth, or congenital defects depending on the stage of gestation when the infection occurs (Gagne, 2001). Roberts *et al*., (1999) reported that the frequency of foetal infection is higher when maternal infection occurs later in the pregnancy (usually in the third trimester). Most children with congenital toxoplasmosis appear developmentally normal but up to 3% have evidence of permanent neurological damage or bilateral visual impairment (Dunn *et al*., 1999). Placental contamination is a pre-requisite to congenital infection when there is maternal parasitaemia. The infected placenta then acts as a reservoir from which the parasite can spread to the foetus leading to multi-systemic disease (Thulliez *et al*., 1992). When the mother is chronically infected with *T. gondii*, the parasite is dormant in maternal tissues and there is no parasitaemic phase. Foetal transmission can occur immediately after maternal infection or be delayed by weeks (Martin, 2000). *T. gondii* infection in early pregnancy can lead to miscarriage or intra-uterine death. In foetuses that survive, lesions are predominantly cerebral due to cerebral vasculitis and necrosis (Martin, 2000). The earlier the infection occurs in pregnancy, the worse the outcome is for the foetus, both in term of survival and sequelae (Martin, 2000). Women who are seropositive before conception have the least risk of congenital toxoplasmosis while the greatest risk of congenital toxoplasmosis occurs during the first trimester of pregnancy (Remington *et al*., 1995; Evengard *et al*., 1999). However, the highest level of transmission of congenital toxoplasmosis occurs in the third trimester. This is thought to be related to the much

larger size of the uterus. If a woman's foetal loss is as a result of *T. gondii* infection; her subsequent pregnancies are safe as far as the parasitic infection is concerned until she becomes immunocompromised during subsequent pregnancies. Practically, only mothers with primary infection acquired during pregnancy are known to be at risk. If the infection was acquired before conception, there is no practical risk of congenital transmission (Martin, 2000). Although most congenitally infected children are asymptomatic at birth, they may develop some symptoms later in life. Hydrocephalus, chorioretinitis, intracerebral calcification, pancytopenia, and death may occur (Remington *et al*., 1995; Singh, 2003). Loss of vision was reported to be the most common sequela in congenitally infected children in a study done by Remington *et al* in 1995.

2.6 Population structure and Epidemiology of *T. gondii*

The population structure of *Toxoplasma gondii* has been analyzed with a variety of molecular probes. They reveal a clonal population structure in isolates with three dominant strain types (Howe and Sibley, 1995). These strains appear to have arisen from one or few crosses between ancestral isolates and then spread together with domesticated species. Large areas of the world, such as Africa or Asia, remain subtly explored. It is likely that *T. gondii*, which has multiple forms of transmission, can adapt its phenotype to different environments, and that either clonal or sexual reproduction might prevail in different habitats (Howe and Sibley, 1995).

Virulence in mice is the most recognized phenotypic marker. Type I strain led to a widespread parasite dissemination and death of mice in less than 10 days after inoculation of (< 10) tachyzoites. In contrast, mice survived to infection with the type II strain (50 percent lethal dose (LD50) > 103) and tachyzoite dissemination was

much less extensive. Type III is also generally considered as avirulent in mice, although progressive deterioration and death of mice, notably with neurological symptoms, can occur a few weeks or months after inoculation. The genetic differences between strains elicits a different immune response in the host that could in part explain the different patterns of virulence (Gavrilescu and Denkers, 2001; Fux *et al.,* 2003; Saeij *et al.,* 2005). The higher virulence of type I in mice compared to types II or III has been correlated with in vitro biological properties. Type I displays enhanced migration in vitro, as well as enhanced transmigration across polarized Madin Darby Canine Kidney (MDCK) or across extracellular matrix. It also shows a higher rate of ex vivo penetration of lamina propria and submucosa (Barragan and Sibley, 2002). This ability to cross epithelial barriers rapidly and reach the bloodstream within hours post-infection might be an important predeterminant measure of parasite dissemination in vivo in susceptible host species. In cell culture, type I grows faster than types II or III and has a lower rate of interconversion from tachyzoite to bradyzoite than type II strains. The higher growth rate of type I parasites has been suggested to be due to a higher reinvasion rate rather than a shorter doubling time (Saeij *et al.,* 2005).

Typing performed on all the isolates consecutively isolated in congenital cases in French laboratories revealed that they almost all belonged to type II. Amniotic fluids and placenta are readily available mainly in France where a systematic prenatal screening of congenital toxoplasmosis is performed (Ajzenberg *et al*., 2002).

Type II isolates are found in all the different aspects of congenital disease, including lethal infection, severe neuro-ocular involvement, isolated chorioretinitis, or latent toxoplasmosis. The main factor determining the severity of congenital infection remains the stage of pregnancy at which the disease is acquired. Thus type II strains,

which are non-virulent in mice, can sometimes prove highly pathogenic to the human fetus, but are also the only ones together with a very few type III strains in found in benign or latent congenital toxoplasmosis (Ajzenberg *et al*., 2002). Very few type I, atypical or recombinant strains have been isolated from congenital toxoplasmosis, but these are usually observed in severe cases. The higher growth rate of type I isolates in mice and their remarkable transepithelial migratory ability would imply a high risk of transplacental transmission and severe infection of the developing fetus (Barragan and Sibley, 2002). However, in some cases type I strains have been isolated from placenta without any development of congenital infection in children.

The conclusion that type II strains are most frequently responsible for congenital toxoplasmosis was drawn mainly from French data, but before it can be concluded that one genotype is more adapted than another to congenital transmission, data from different parts of the world have to be collated as the higher proportion of one type in human disease may simply reflect a disproportionately high infection with this genotype in other parts of the world.

2.7 Laboratory Diagnosis

2.7.1 Direct Methods

2.7.1.1 Microscopy

Tissue biopsies show tachyzoites or cysts which stain with haematoxylin and eosin in hispathological preparations (Dubey, 1994). The Romanovsky stains, such as Giemsa and Wright's also detect *T. gondii* which is most easily seen as clusters of slightly elongate to oblate nucleated bodies within a vacuole inside infected cells. The parasite can be found in various cell types including endothelial cells, fibroblast,

hepatocytes, myocytes, blood cells, macrophages and various cells of the central nervous system (Dubey, 1994).

2.7.1.2 Gene Amplification

The Polymerase chain reaction (PCR) diagnostic tests have proven useful in the diagnosis of infection in utero as well as in immunocompromised hosts. The detection of *T. gondii* tachyzoite DNA in body fluids and tissues by PCR amplification is effective to diagnose congenital, ocular (Montoya *et al*., 2000), and cerebral toxoplasmosis (Holliman 1995). PCR should be considered the gold standard for diagnosis of in utero infection. Sensitivity in initial reports was 100 percent, but subsequent studies have indicated this is very dependent on the gestational age of the infection (Montoya, 2002; Switaj *et al*., 2005). Sensitivity also varies with gene target for example, the B1 gene is present at 35 copies, and AF146527 is present at 300 copies. In a French study of 2000 consecutive amniotic fluid samples, it has been confirmed that a positive PCR correlates with disease and that the PCR is more sensitive than any other available test (Thulliez, 2002). Isolation of *T. gondii* from blood or body fluids (e.g. CSF, or amniotic fluid) establishes diagnosis of the acute infection.

2.8.1 Indirect Methods

2.8.1.1 Serology test

Detection of *T. gondii* specific antibodies is the primary diagnostic method in determining the parasite infection. Different serological tests have been used to detect antibodies to the parasite. *T.gondii* specific IgG, IgM, IgA or IgE antibodies can be detected with the serological methods. Newborn babies suspected of congenital toxoplasmosis are tested by both an IgM and IgA capture enzyme

immunoassay. In cases of congenital infection, however, antibody responses may be delayed in some infants, and the presence of transplacentally transferred maternal IgG may interfere further with the serologic confirmation of infection. For these reasons, all infants known to be at risk for congenital infection are examined further for clinical signs of infection (chorioretinal lesions, intracranial calcifications, or hydrocephalus) [Lynfield and Guerina, 1997].

Classically, serodiagnosis includes titration of specific immunoglobulin G showing past exposure and screening for specific IgM, which is suggestive of recent exposure or ongoing active infection. IgM detection could be due to naturally interfering IgM or persistence of IgM for long time after primary infection (Suzuki *et al*., 2001).

2.9 Therapy to Toxoplasmosis

Sulphadiazine and Pyrimethamine are the two drugs that are widely used for the treatment of toxoplasmosis (Guerina *et al*., 1994). These drugs have beneficial effect on toxoplasmosis when given in the acute stage but when there is active multiplication of the parasites, they will usually not eradicate infection (Guerina *et al*., 1994). The employment of combined drugs is known to be principally effective on the actively multiplying tachyzoites (Peterson *et al*., 2006b). When infection in uteri is documented using PCR on an amniotic fluid sample, the mother should be started on a combination of pyrimethamine and sulphadiazine with folinic acid supplementation. Good outcome has been reported with the use of high-dose pyrimethamine, clindamycin, co-trimoxazole and folic acid in patients after allogeneic haematopoietic stem cell transplantation (Rusiňáková *et al.*, 2009). Infected infants should be treated after birth up to one year of age with the same drugs whether the infection is over or latent and follow up is important up to adolescence (Martin, 2000).

The preferred treatment in immunocompromised patients is the standard combination of pyrimethamine and sulfadiazine. However, due to frequent serious side effects, alternative treatments are possible. In some patients, especially those undergoing immunosuppression due to stem cell transplantation, primary prophylaxis of cerebral toxoplasmosis is achieved by co-trimoxazole. Reduced doses of the standard regimen may be used as secondary prophylaxis during severe immunosuppression in these patients. However, due to an increased risk of myelotoxicity, other therapeutic measures are used (Prášil, 2009). In the last few years, there has been considerable progress towards the development of a vaccine for toxoplasmosis, and a vaccine based on the live-attenuated S48 strain was developed for veterinary uses (Kur *et al.*, 2009). However, this vaccine is expensive, causes side effects and has a short shelf life. Furthermore, the vaccine can revert to a pathogenic strain and, therefore, is not suitable for human use. However, various experimental studies have shown that it may be possible to develop a vaccine against human toxoplasmosis soon (Kur *et al.*, 2009).

2.10 Prevention of Toxoplasmosis

Proper washing of hands after handling meat, poultry or seafood with soap and water before doing other tasks will prevent *T. gondii* infection in humans (Dubey and Beattie, 1988). It is also important for pregnant women to wear gloves when they are gardening or touching soil or sand, because of possible presence of cat faeces and to wash their hands thoroughly after such events (Jones *et al*., 2003). Healthcare workers should educate women of childbearing age on information about *T. gondii* transmission. At the first antenatal visit, health care provider should educate pregnant

women about food hygiene and if possible, pregnant women should avoid exposure to cat faeces (Jones *et al*., 2003).

2.11 Malaria

Globally, an estimated 3.4 billion people are at risk of malaria. WHO estimates that 207 million cases of malaria occurred globally in 2012 (uncertainty range 135–287 million) and 627 000 deaths (uncertainty range 473 000–789 000). 80% and 90% of incident cases and deaths occurred in Africa respectively and about 77% of deaths were in children under 5 years of age (WHO/WMR, 2013).

Malaria is caused by five species of parasite that affect humans, and all of these species belong to the genus *Plasmodium*. They are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Of these, *P. falciparum* and *P. vivax* are the most important. Malaria due to *P. falciparum* is the most deadly form, and predominates in Africa (WHO/WMR, 2013).

In endemic countries, pregnant women constitute the main adult risk group for malaria and 80% of deaths due to malaria in Africa occur in pregnant women and children below 5 years. Every year, more than 30 million women in Africa become pregnant in malaria endemic areas and are at risk of *P. falciparum* malaria infection. *P. falciparum* infection during pregnancy is estimated to cause as many as 10,000 maternal deaths each year, 8% to 14% of all low birth weight babies, and 3% to 8% of all infant deaths (WHO/WMR, 2012).

2.12 Taxonomy of *Plasmodium*

The parasite belongs to the; Kingdom: Protista, Sub kingdom: Protozoa, Phylum: Apicomplexa, Class: Conoidasida, Sub class: Coccidiasina, Order: Eucoccidiorida,

Family: Plasmodiidae, Genus: *Plasmodium,* Species: *falciparum*, *malariae*, *ovale*, *vivax, knowlesi* (WHO/WMR 2012).

2.13 Life and transmission cycle of *Plasmodium* parasites

The malaria parasite has a complex, multistage life cycle occurring within two living beings, the vector mosquitoes and the vertebrate hosts. The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by a toolkit of more than 5,000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell types to evade host immune responses (Floren *et al*., 2002; Greenwood *et al*., 2008;). The parasite passes through several stages of development such as the sporozoites (Gr. *Sporos* = seeds; the infectious form injected by the mosquito), merozoites (Gr. *Meros* = piece; the stage invading the erythrocytes), trophozoites (Gr. *Trophes* = nourishment; the form multiplying in erythrocytes), and gametocytes (sexual stages). All these stages have their own unique shapes, structures and protein complements. The surface proteins and metabolic pathways keep changing during these different stages and this helps the parasite to evade the immune system, while also creating problems for the development of drugs and vaccines (Floren *et al*., 2002).

2.13.1 Sexual stage

Female *Anopheles* mosquitoes are the definitive hosts for malaria parasites. The sexual phase is called sporogony and results in the development of innumerable infecting forms of the parasite within a mosquito that induce disease in the human host following injection with a mosquito bite.

When the female *Anopheles* draws a blood meal from an infected individual, the male and female gametocytes of the parasite find their way into the gut of the mosquito. The molecular and cellular changes in the gametocytes help the parasite to quickly adjust to the insect host from the warm-blooded human host and then to initiate the sporogonic cycle. The male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinetes that burrow into the mosquito midgut wall to develop into oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of between 8 and 15 days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host (Figure 10). It has been found that the infected mosquito and the parasite mutually benefit each other and thereby promote transmission of the infection. The *Plasmodium*-infected mosquitoes have a better survival and show an increased rate of blood-feeding, particularly from an infected host (Ferguson and Read, 2004, Barillas-Mury and Kumar, 2005).

2.13.2 Asexual stage

Man is an intermediate host for malaria. The sporozoites inoculated by the infested mosquito initiate this phase of the cycle from the liver, and continues within the red blood cells, which results in the various clinical manifestations of the disease.

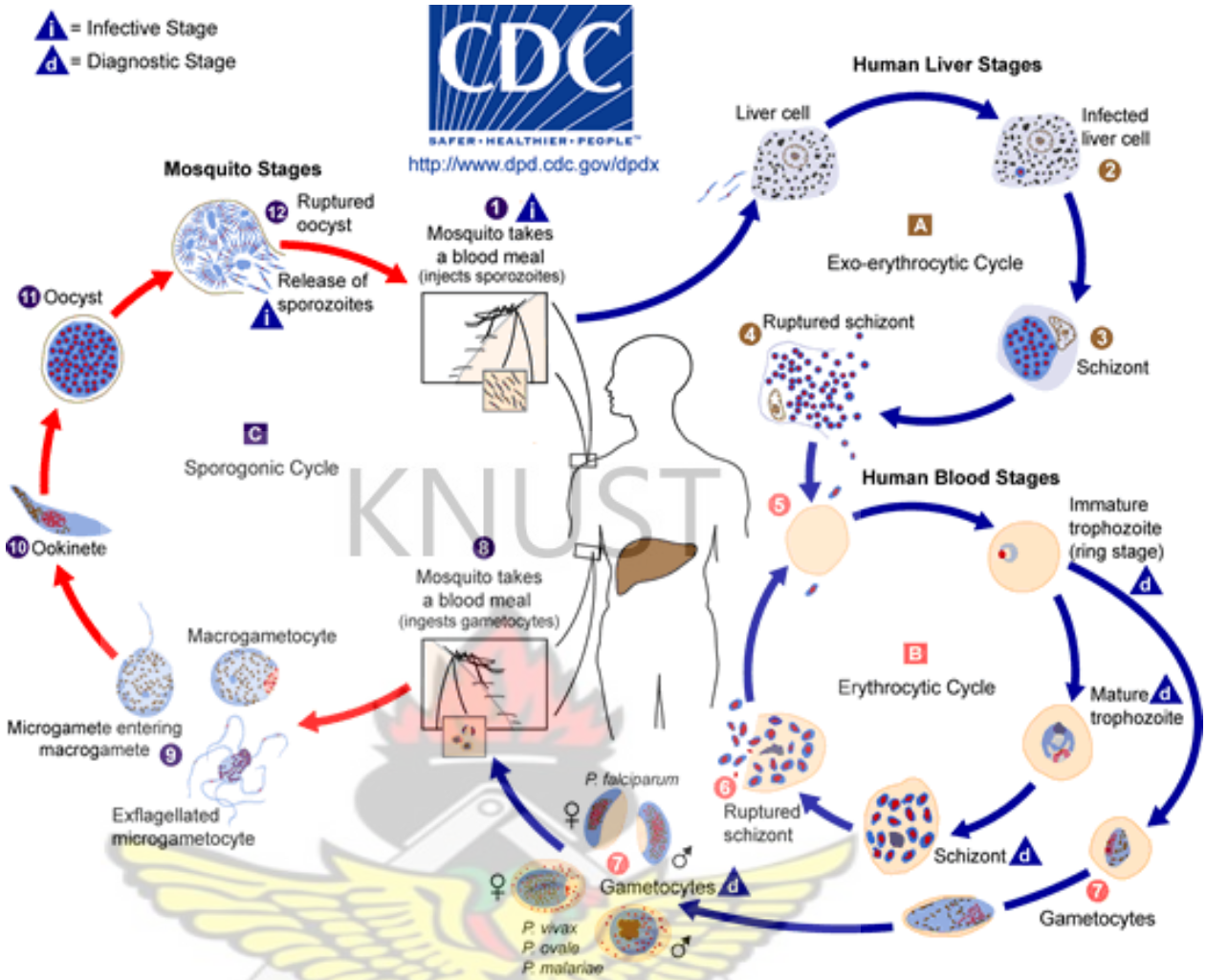

Figure 10: Life and transmission cycle of malaria parasites.

(Source: http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html)

2.13.2.1 Pre-erythrocytic Phase

With a mosquito bite, tens to hundred invasive sporozoites are introduced into the skin. Following the intradermal deposition, some sporozoites are destroyed by the local macrophages, some enter the lymphatics, and some others find a blood vessel (Vaughan *et al*., 2008; Olivier *et al*., 2008). The sporozoites that enter a lymphatic vessel reach the draining lymph node where some of the sporozoites partially develop into exoerythrocytic stages and may also prime the T cells to mount a protective immune response (Good and Doolan, 2007).

The sporozoites that find a blood vessel reach the liver within a few hours. It has recently been shown that the sporozoites travel by a continuous sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein family and an actin–myosin motor (Baum *et al*., 2006; Münter *et al*., 2009). The sporozoites then negotiate through the liver sinusoids, and migrate into a few hepatocytes, multiply and grow within parasitophorous vacuoles. Each sporozoite develop into a schizont containing 10,000–30,000 merozoites or more in case of *P. falciparum* (Jones and Good, 2006; Kebaier *et al*., 2009). The growth and development of the parasite in the liver cells is facilitated by favourable environment created by the circumsporozoite protein of the parasite (Prudêncio, 2006). The entire pre-eryhrocytic phase lasts about 5–16 days depending on the parasite species (Figure 10). On average, *P. falciparum* takes 5-6 days, 8 days for *P. vivax*, 9 days for *P. ovale*, 13 days for *P. malariae* and 8-9 days for *P. knowlesi* (Vaughan *et al*., 2008). The pre-erythrocytic phase remains a "silent" phase, with little pathology and no symptoms, as only a few hepatocytes are affected. This phase is also a single cycle, unlike the next erythrocytic stage which occurs repeatedly (Vanghan *et al*., 2008).

In *P. vivax* and *P. ovale* malaria, some of the sporozoites may remain dormant for months within the liver. Termed as hypnozoites, these forms develop into schizonts after some latent period, usually of a few weeks to months. It has been suggested that these late developing hypnozoites are genotypically different from the sporozoites that cause acute infection soon after the inoculation by a mosquito bite and in many patients cause relapses of the clinical infection after weeks to months (Collins, 2007).

2.13.2.2 Erythrocytic Schizogony

Red blood cells (RBCs) are the centre stage for the asexual development of the malaria parasite. Within the red cells, repeated cycles of parasitic development occur with precise periodicity, and at the end of each cycle, hundreds of fresh daughter parasites are released that invade more red blood cells. The merozoites released from the liver recognize, attach, and enter the red blood cells by multiple receptor–ligand interactions in as little as 60 seconds. This quick disappearance from the circulation into the red cells minimises the exposure of the antigens on the surface of the parasite, thereby protecting these parasite forms from the host immune response (Alan *et al*., 2006; Olivier *et al*., 2008). The invasion of the merozoites into the red cells is facilitated by molecular interactions between distinct ligands on the merozoite and host receptors on the erythrocyte membrane. *P. vivax* invades only Duffy blood group-positive red cells, using the Duffy-binding protein and the reticulocyte homology protein found mostly on the reticulocytes. *P. falciparum* uses several different receptor families and alternate invasion pathways that are highly redundant. Varieties of Duffy binding-like homologous proteins and the reticulocyte binding-like homologous proteins of *P. falciparum* recognize different RBC receptors other than the Duffy blood group or the reticulocyte receptors. Such redundancy is helped by the fact that *P. falciparum* has four Duffy binding-like erythrocyte-binding protein genes, in comparison to only one gene in the DBL-EBP family as in the case of *P. vivax*, allowing *P. falciparum* to invade any RBC (Ghislaine *et al*., 2009) [Figure 10].

The process of attachment, invasion, and establishment of the merozoite into the red cell is made possible by the specialized apical secretory organelles of the merozoite,

called the micronemes, rhoptries, and dense granules. The initial interaction between the parasite and the red cell stimulates a rapid wave of deformation across the red cell membrane, leading to the formation of a stable parasite–host cell junction. Following this, the parasite pushes its way through the erythrocyte bilayer with the help of the actin–myosin motor, proteins of the thrombospondin-related anonymous protein family and aldolase, and creates a parasitophorous vacuole to seal itself from the host-cell cytoplasm, thus creating a hospitable environment for its development within the red cell (Kasturi and Narla, 2007).

The principal nutrient for the growing parasite, Hemoglobin, is ingested into a food vacuole and degraded. The amino acids thus made available are utilized for protein biosynthesis and the remaining toxic heme is detoxified by heme polymerase and sequestrated as hemozoin (malaria pigment). The parasite depends on anaerobic glycolysis for energy, utilizing enzymes such as plasmodium aldolase etc. As the parasite grows and multiplies within the red cell, the membrane permeability and cytosolic composition of the host cell is modified (Kirk, 2001; Virgilio *et al*., 2003). These new permeation pathways induced by the parasite in the host cell membrane help not only in the uptake of solutes from the extracellular medium but also in the disposal of metabolic wastes, and in the origin and maintenance of electrochemical ion gradients.

The erythrocytic cycle occurs every 24 hours in case of *P. knowlesi,* 48hrs in cases of *P. falciparum, P. vivax* and *P. ovale* and 72hrs in case of *P. malariae.* During each cycle, each merozoite grows and divides within the vacuole into 8–32 fresh merozoites, through the stages of ring, trophozoite, and schizont. At the end of the

cycle, the infected red cells rupture, releasing the new merozoites that in turn infect more RBCs (Figure 10).

A small proportion of asexual parasites do not undergo schizogony but differentiate into the sexual stage gametocytes. These male or female gametocytes are extracellular and nonpathogenic and help in transmission of the infection to others through the female anopheline mosquitoes, where they continue the sexual phase of the parasite's life cycle (Louis *et al*., 2002) [Figure 10].

2.14 Congenital malaria

Congenital malaria was first described in 1876 (Romand *et al*., 1994). It has been documented for many years, but it was previously thought to be uncommon especially in indigenous populations. More recent studies, however, show that incidence has increased, and values between 0.30 to 33.00% have been observed from both endemic and nonendemic areas (Sotimehin *et al*., 2008). It is caused by transmission of infected maternal erythrocytes across the placenta into the fetal circulation. This phenomenon is known to occur during normal pregnancy. Infected erythrocytes may not be found until a high level of parasitaemia is established. Since transmission of infected maternal erythrocytes can occur before or during delivery, it is difficult to distinguish between autual congenital or perinatal acquisition of malaria. Malaria and pregnancy are generally believed to be mutually aggravating conditions. The pathological changes due to malaria and the physiological changes associated with pregnancy have a synergistic effect on the course of each other (Kakkilaya, 2009). Pregnancy exacerbates malaria through a nonspecific hormone-dependant depression of the immune system; protective antiplasmodial activity is suppressed at pregnancy (Okwa, 2003).

The following hypotheses to explain the altered immunity associated with pregnancy are offered; Reduced lymphoproliferative response sustained by elevated levels of serum cortisol, loss of cell-mediated immunity in the mother, the presence of placenta, a new organ in the primigravidae, allows the parasite to bypass the existing host immunity, or allows placenta-specific phenotypes of *P. falciparum* to multiply; pregnant women display a bias towards type-2 cytokines and are therefore susceptible to diseases requiring type-1 responses for protection like Tuberculosis and malaria, and *P. falciparum* has the unique ability of cytoadhesion due to chondroitin sulfate A and hyaluronic acid for parasite attachment to placental cells (Kakkilaya, 2009).

In pregnancies complicated by malaria, both fetal growth retardation and preterm birth contribute to low birth weight (Chedraui *et al*., 2009). Clinical malaria is rare in newborns from endemic areas with high incidence of malaria in pregnancy. This rare occurrence has been attributed to the transplacental transfer of maternally derived antibodies to malaria, and the inhibitory effect of foetal haemoglobin on malaria parasite development (Gitau and Eldred, 2005).

The prevalence of congenital malaria in a community based prospective study in infants in southern Ghana in 1998 was 13.6% by Polymerase Chain Reaction (PCR) and 2.5% by microscopy (Wagner *et al*., 1998). In the study, the risk of infection was three times higher during the rainy season (March to November) as compared to the dry season. Also, Blood samples of untreated newborns less than 1 week of age at the time of referral to Korle Bu Teaching hospital in Accra, during the peak malaria seasons (April to July) of 2008 and 2010 were examined for malaria parasites. In the 2008 study, nine cases of *P. falciparum* parasitaemia were diagnosed by microscopy in 405 (2.2%) newborns. All nine newborns had low parasite densities (≤50 per

microlitre). In 2010, there was no case of parasitaemia by either microscopy or rapid diagnosis tests in 522 newborns; however, 56/467 (12%) cases of *P. falciparum* were detected by Polymerase Chain Reaction (Enweronu-Laryea *et al*., 2013).

Congenital malaria in the newborn usually manifests after the first week of life with symptoms such as fever, poor suckling, lethargy, anaemia, hepatosplenomegaly, jaundice, irritability, and drowsiness. These clinical features are similar to the manifestations of neonatal sepsis and other neonatal conditions that more commonly cause morbidity and mortality in the newborn period (Mazzi *et al*., 2010; Opiyo and English, 2011).

2.15 Laboratory diagnosis

Peripheral smear examination for malarial parasite is the gold-standard in confirming the diagnosis of malaria. Thick and thin smears prepared from the peripheral blood are used for the purpose. The peripheral blood smear provides comprehensive information on the species, the stages, and the density of parasitemia with a sensitivity of 5 to 10 parasites/μL of blood for an experienced laboratory professional. The efficiency of the test depends on the quality of the equipment and reagents, the type and quality of the smear, skill of the technician, the parasite density, and the time spent on reading the smear. Thick smears allows the red blood cells to be hemolyzed and leukocytes and any malaria parasites present will be the only detectable elements. Thick smears are therefore used to detect infection, and to estimate parasite concentration. Thin films preserve the parasite and erythrocyte morphology and are used to differentiate parasite species as well as to quantify the percentage of infected erythrocytes (WHO/WMR, 2013) [Figures 11, 12, 13, 14, 15]

Detections based on PCR have been found to be highly sensitive and specific for detecting all 5 species of malaria, particularly in cases of low level parasitemia and mixed infections. The PCR test is reportedly 10-fold more sensitive than microscopy, with one study reporting a sensitivity to detect 1.35 to 0.38 parasites/µl for *P. falciparum* and 0.12 parasites/µl for *P. vivax.* The PCR test has also been found useful in unraveling the diagnosis of malaria in cases of undiagnosed fever (Castelli and Carosi, 1997).

2.15.1 *Plasmodium* species life forms

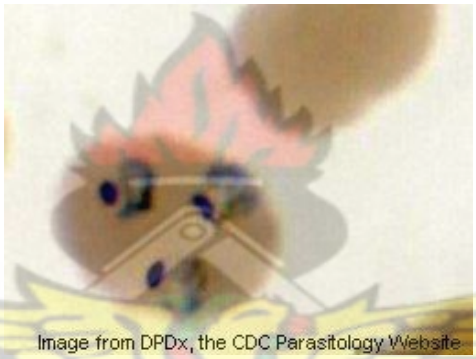

Figure 11: *Plasmodium falciparum*: Early trophozoites have the characteristic signet ring shape. Also, unique to *P. falciparum* is the presence of multiple trophozoites in one cell.

(Source: http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html)

Figure 12: Trophozoites: *Plasmodium vivax*. Red blood cells infected by *P. vivax* are often larger than uninfected red blood cells. They approximately 1.5 times the size of a normal cell.

(Source: http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html)

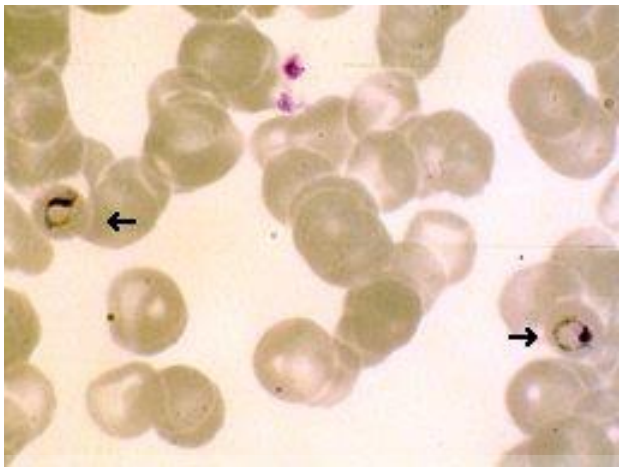

Figure 13: Trophozoites: *Plasmodium malariae*: Characteristic trophozoites of *P. malariae* showing the ring shape and the tendency of infected cells to be of normal or smaller size (arrows).

(Source: http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html)

Figure 14: Gametocyte*: Plasmodium falciparum*: The gametocytes of *P. falciparum* have a crescent or banana shape.

(Source: http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html)

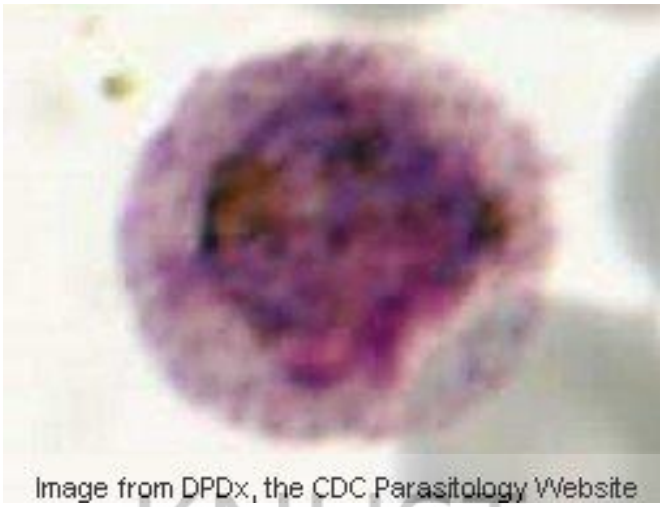

Figure 15: Gametocyte*: Plasmodium ovale*: A round gametocyte that is larger than normal red blood cells. It has a granular appearance as well as Schuffner's dots.

(Source: http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html)

2.16 Therapy and management of malaria

The need for effective control continues to threaten populations in many parts of the world especially Africa. Increasing drug resistance among the malaria parasites and increasing insecticide resistance among mosquitoes have made malaria control more difficult and heightened the need for effective vaccines or improved control.

Currently recommended interventions include vector control through the use of insecticide treated nets (ITNs), IPTp-SP, indoor residual spraying (IRS) and chemoprevention for the most vulnerable populations' particularly pregnant women and infants (WHO/WMR, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted at health facilities within the Kumasi Metropolis. The Kumasi Metropolis has an approximate area of 254 square kilometers and it is located between latitudes 6º35" and 6º40" N and longitudes 1º30" and 1º35" E. The current population is estimated at about 1,517,000, with a growth rate of 2.5% per year. Average temperature ranges from 21.5ºC to 30.7ºC. Annual rainfall is 625 m with peaks in the months of June to September. The rainfall pattern is generally good and evenly distributed (KMA, TCP Kumasi).

3.2 Study sites

The Manhyia District Hospital (MDH) at Manhyia, the South Suntreso Government Hospital (SSH) at Suntreso and the Aninwah Medical Center (AMC) at Emena were the targeted study sites for sample collection.

The Manhyia District Hospital at Manhyia, is centrally located in the metropolis. It is one of the largest hospitals in the city and serves patients from the Oforikrom Township, Dichemso, Aboabo, Sawaba, Krofrom, Asawase, Mooshi Zongo, Manhyia, Adum and kejetia. It has intensive care units that cater for medical and trauma emergencies. The maternity ward of the hospital has a total bed capacity of 20 and provides good health care for pregnant women who report for antenatal health care and near term pregnant women about to deliver.

The South Suntreso Government Hospital at Suntreso is located towards the northern part of the city. It attends to patients from communities such as Abuakwa, Tanoso,

Sofoline, Abrepo, Santasi, Patasi, Kotwi, Bantama, Ridge, North and South Suntreso and other surrounding townships. It is a big facility and meets the various health needs of its clients. It has the general Out Patient Department (OPD) and other specialized clinics such as the Optometry, Ear, Nose and throat (ENT), Obstetrics and Gynaecology amongst others. The maternity center houses the labour wards, lying-in wards and the emergency Mother and Baby Unit (MBU). It has a total bed capacity of 35 with dedicated nurses and midwives who provide the best maternal care to pregnant women who partronize their services.

The Aninwah Medical center (AMC) at Emena is one of the main referral private health facilities located towards the southern part of the city. The hospital serves patients from the Emena Township, Ayigya, Ayeduase, Kotei, Deduako, New site, Appeadu, Awomaso, Oduom, Ejisu and Atonsu. The facility has a well-structured maternity unit which houses staff well equipped to providing best health care for women attending antenatal and postnatal clinics. Total bed capacity is 25.

These hospitals were selected based on their location, environmental contribution to study objectives and the patronage of their maternity services.

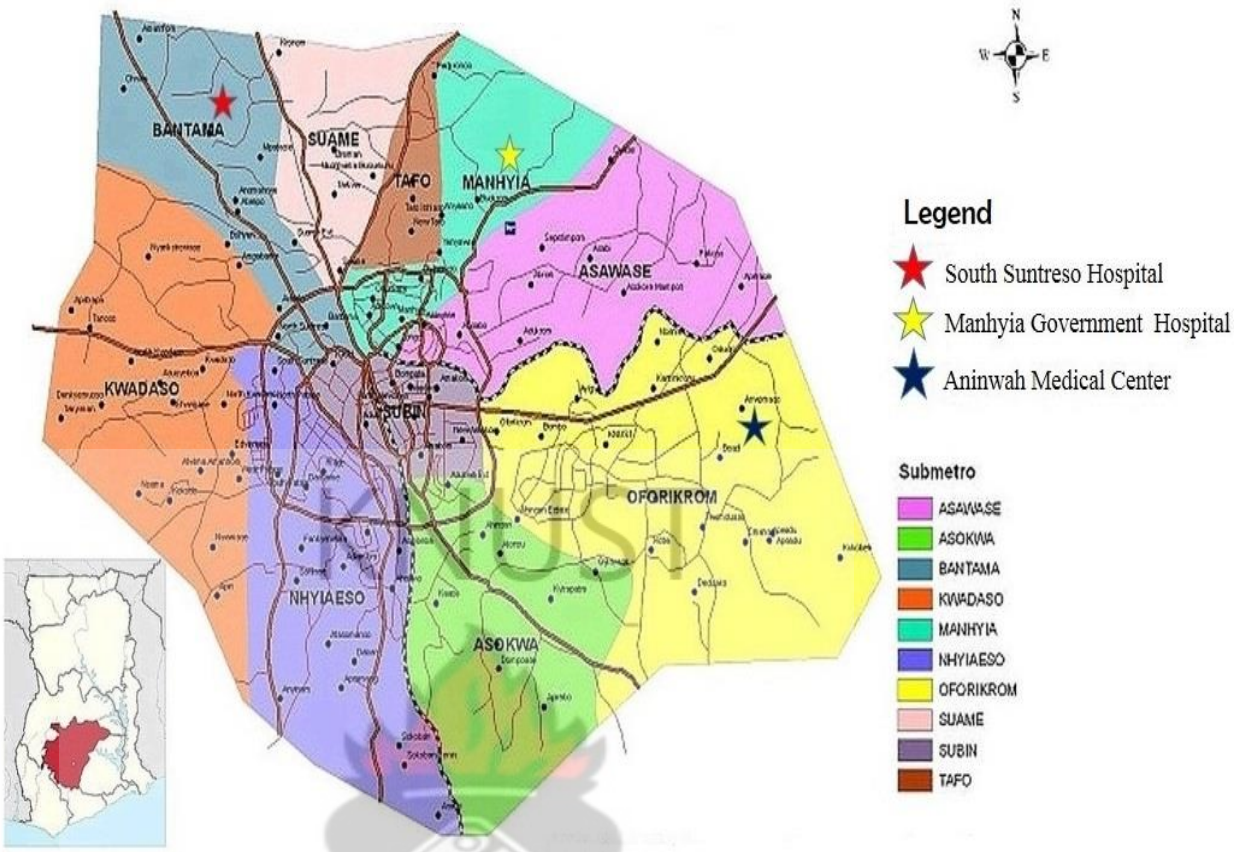

Figure 16: Map showing study sites and sub-metro areas of Kumasi

(Source: KMA, TCP)

3.3 Study design

This was a hospital based cross-sectional study that involved women who delivered at the selected health facilities. Study participants were recruited based on written consent after educating them on the study details. Sample collection was done between the months of September 2013 and January 2014.

After delivery, the placenta or afterbirth of each participant were collected soon after their expulsion in a kidney dish. The umbilical vein of the fetoplacental region of the placenta was located within the umbilical cord and with a sterile hypodermic syringe, 5ml blood was drawn to represent foetal blood. Foetal blood was dispensed into

labeled EDTA tubes. A new hypodermic syringe was used to draw 5ml of blood from the basal plate endometrial arteries from the uteroplacental region of the placenta representing maternal blood. Blood was dispensed into labeled EDTA tubes bearing mother's codes. Blood blots were also prepared for each blood sample that was collected.

ELISA for anti *T. gondii* IgG and IgM antibody detection was performed on maternal and foetal serum samples. DNA extraction and PCR procedures to detect *T. gondii* and *P. falciparum* DNA was performed on maternal and foetal blood from the blotted blood samples.

Questionnaires were administered to obtain personal and socio-demographic information from mothers such as their names, ages, residence, educational background, place of work. Questions were also asked on toxoplasmosis and malaria infection knowledge and exposure to infection risk factors. Some questions on exposures to infection risk factors included ownership of a cat, type of livestock meat consumed and preferred state of cooking before consumption, eating of vegetables and preferred state of consumption and malaria prophylaxis during pregnancy. A data collection book was also designed to obtain other relevant data from mothers and to record post birth outcomes. This was obtained from their maternal health record book. Data recorded included last BP reading, last HB reading, gravidity and parity, history of spontaneous abortions and still births, sex and weight of babies.

All laboratory procedures were undertaken at the Research laboratory of the Department of TAB, KNUST and Parasitology laboratory (NMIMR) in accordance with biosafety rules and regulations. Results were computerized and analyzed with statistical software according to the study objectives.

3.4 Sample size estimation

The minimum sample size estimation for the study was obtained using the Kish lisle formula for a cross-sectional study;

$N= [Z^2 (P) (1-P)] / (Error)^2$, Kish. (1965).

Where N is the Sample size, Z is the co-efficient of significance (1.96) for significance level (α) of 5 %, E represents the allowable error margin (0.05), P is the prevalence rate of disease. Using the previous prevalence of toxoplasmosis estimated at 92.5% (Ayi *et al*., 2009), a minimum sample size of 138 was obtained at a 95 % confidence interval of width ± 5 %.

$N= [1.96^2 (92.5)*(1-92.5)] / (0.05)^2$

N= 138.

A 31 % allowance was thus added to compensate for sample loss or any unforeseen eventualities.

3.5 Sample collection

3.5.1 Foetal blood and maternal blood

Samples were collected from 1st September 2013 to 15th January 2014. Sampling followed the working shift schedules of the maternity clinics of the hospitals. They were the morning shift from 08:00 to 14:00 hours, afternoon shift from 14:00 to 20:00 hours and the night shift from 20:00 to 08:00 hours. Convenient times were then chosen for sample collection.

After delivery, fresh placentas were collected in a kidney dish soon after its expulsion. The umbilical vein of the fetoplacental region of the placenta in the umbilical cord was located and 5ml of blood was drawn with a sterile disposable

hypodermic syringe to represent foetal blood. Foetal blood was dispensed into labelled ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. The placenta was lightly incised at the uteroplacental region with sterile surgical scissors and 5ml of blood was also drawn with a sterile disposable hypodermic syringe from the basal plate endometrial arteries to represent maternal blood. Maternal blood was dispensed into labelled EDTA vacutainer tubes bearing mothers' codes.

Blood blots were made on Whatman filter paper strips for each blood sample drawn. 0.5 ml to 1 ml of foetal and maternal blood was left in each syringe barrel during dispensing into EDTA tubes. Blood blots were carefully made on filter papers from the remaining blood in the syringe barrel by carefully pushing down the plunger to dispense blood unto the filter paper. Blood was allowed to soak well on filter papers, appropriately labelled and air dried thoroughly. They were then put into labelled zip-lock bags until ready for use. Zip-lock bags were kept at cool dry place at the Research laboratory of the Department of TAB, KNUST until transported to the Parasitology laboratory of NMIMR for testing.

Blood samples were kept in the sample refrigerators at the hospitals immediately they were dispensed into EDTA tubes. Then after each day's collection, samples were kept in a cool box containing ice packs and transported to the Research laboratory of the Department of TAB, KNUST for storage. After collections were ended at each study site, samples were transported in a cool box containing ice packs to the Parasitology laboratory of the NMIMR for laboratory procedures.

3.5.2 Questionnaire interviews and data collection

A structured questionnaire and a data collection book were designed to collate data from mothers. Responses to questionnaires were obtained by interview in

participants' language of understanding for some while others were self-administered under appropriate guidance. Questions sought personal and socio-demographic as well as *T. gondii* and *P. falciparum* infection risk related information from participants. (See appendix for copy of questionnaire).

3.6 Laboratory Procedures

3.6.1 Serum separation

Serum was obtained by centrifuging foetal and maternal blood samples at 14000rpm for 20 minutes. The supernatant (serum) was pipetted carefully with sterile pipette tips into sterile labelled 1.5 ml eppendorf tubes with mother and foetal codes making sure they was no disturbance of cell layers during transfer of cells. The serum samples were then stored at -40ºC until tested.

3.6.2 ELISA for the detection of anti-*T. gondii* antibodies

Serum samples were tested for the presence of anti-*Toxoplasma* IgG and IgM, using standard commercial 96-well ELISA Kit from (CTK Biotech, Inc., San Diego, USA) following manufacturer's instructions. ELISA results were recorded using a microplate reader (XFLUOR4 Version 4.51) as a measure of absorbance (Optical Densities) of the reaction intensity of *T. gondii* antigen and serum anti-*T. gondii* antibodies using a filter wavelength of 450 nm against the blank wells. Cut-off points and antibody index calculations were done according to manufacturers' recommendation to categorize seropositive and seronegative samples.

Antibody index calculations to categorize seropositives and seronegatives samples, was as a measure of the specimen optical density ratios. Specimen OD ratios (≥ 1.00) were interpreted as seropositive and Specimen OD ratios (< 1.00) were interpreted as seronegative.

3.6.2.1 Assay procedure for detection of IgG

Serum specimen for the samples was loaded into the microwell frame according to the designation of the sample on the ELISA working sheet. The wells designated as blank wells on the ELISA working sheet were left blank and no reagents were added. Wells designated as control wells (positive and negative controls) on the ELISA working sheet had 100 µl of the positive control reagents and negative control reagents respectively. Hundred micolitres of sample diluent was added to the test wells then 10 µl of each test specimen was added to each test well according to their designation on the ELISA working sheet. The microplate was then rocked gently for approximately 20 seconds and then covered with a sealant or a foil. The wells were then incubated at room temperature of about 37ºC for 30 minutes. After incubation, mixture was carefully removed and emptied from the wells carefully into a waste container. Each well was then loaded with 200 µl of diluted wash buffer and then shaken gently for about 20-30 sec. The wash buffer was then discarded completely and then tapped on absorbent paper. The wash procedure was repeated for four times to ensure that the wells were completely and thoroughly washed.

After washing, 100 µl of HRP (Horseradish Peroxidase) -anti-human IgG conjugates was added to each of the wells except the blank wells. The microplate was then covered with a sealant and incubated at 37ºC for 20 minutes. After the incubation period, the wells were washed with wash buffer for 5 times.

Fifty microlitres of Tetramethylbenzidine (TMB) substrate A and 50 µl of TMB substrate B was added to the test wells including the blank wells. The microplate was then covered with a sealant and incubated in the dark for 10 minutes at 37ºC. After

the incubation period, the reaction was stopped by adding 50 µl of stop buffer to each well and gently rocked for 30 seconds to ensure mixing.

3.6.2.2 Assay procedure for detection of IgM

Assay procedure for the detection of IgM anti *T. gondii* antibodies was the same as for IgG. However, 50 µl of test specimen was added to each well and 50 µl of positive and negative control reagents were added to the control wells. Incubation period after the addition of HRP-anti-human IgM conjugates and TMB substrate A and B was 37ºC for 30 minutes and 37ºC for 10 minutes respectively.

The microplate reader wavelength was set at 450 nm and the absorbance or Optical Density of each well was measured against the blank wells within 15 min after adding the stop buffer.

The results obtained from the microplate reader were interpreted according to the instructions of the manufacturers (CTK Biotech. Inc., San Diego, USA.). This was done by first setting up a cut –off point following manufacturer's instructions, calculating the specimen Optical Density (OD) ratio and validating the assay.

3.6.2.3 The cut-off value

The cutoff value was given as 0.15+N, where N was the mean OD of the negative control. However, if the mean OD value of the negative control was less than 0.05, then 0.05 was to be taken for the N value.

3.6.2.4 Calculation of specimen OD ratios

The OD of each specimen was calculated using the formula:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut –off value}}$$

3.6.2.5 Assay validation

The mean OD value of the *Toxoplasma* IgG positive controls was expected to be ≥ 1.0 and mean OD value of the *Toxoplasma* IgG negative control was to be ≤ 0.10. Mean OD value of the *Toxoplasma* IgM positive controls was expected to be ≥ 0.80 whiles the mean OD of the *Toxoplasma* IgM negative controls was to be ≤ 0.10.

3.6.2.6 Interpretation of specimen OD ratios

Test samples with an OD ratio <1.00 were seronegative. This indicates that there was no detectable anti- *T. gondii* IgM or IgG in the specimen. Test samples with specimen OD ratios ≥ 1.00 were interpreted as seropositive samples. This was indicative that there was detectable anti- *T. gondii* IgG and IgM in the samples.

3.6.3 Extraction of Genomic DNA from blotted blood samples

Genomic Deoxyribonucleic acid (DNA) was extracted from dried blotted blood samples using the TE buffer extraction method. Small pieces of filter paper blood blot were cut into sterile 1.5 ml Eppendorf tubes. 120 µl of absolute methanol was added to Eppendorf tubes containing the cut out filter paper blood blot to soak for 15 minutes. Filter papers were transferred to new sterile 1.5 eppendorf tubes to completely dry them for about 10 minutes after soaking in methanol. Afterwards, 120 µl of TE buffer was added to Eppendorf tubes containing the dried filter paper blood blot to soak. The tube and its contents were then incubated at 50ºC for 15 minutes to elute the blood from the filter papers. The buffer was to soften bonds between the filter paper so the blood will be in solution. Filter papers were kept at the bottom of the tube by pushing them gently with new pipette tips for each sample. DNA was extracted at 97ºC for 15 minutes. The tube and its contents was centrifuged briefly to settle any liquid condensing on the wall and lid and debris

pellets on the Eppendorf tubes. TE containing DNA was transferred into a new 1.5 ml Eppendorf tube without the filter papers. Eluted DNA was stored at -20ºC until analysis.

3.6.4 Polymerase Chain Reaction (PCR) for the detection of *T. gondii* DNA

Toxoplasma gondii parasite DNA was detected by amplification using a nested PCR method in a Takara Thermal Cycler (TP650 Version 3.00) with the SAG3 primers adapted from Su *et al*., (2006), and Prestrud *et al* (2008) with necessary optimizations. (Table 1). A nested PCR was necessary to increase the sensitivity and specificity of detecting the *T. gondii* DNA.

Table 1: Primer set sequence and product band size (SAG3)

Marker	Primer Sequence		Product size
SAG3	P43S1: CAACTCTCACCATTCCACCC	External forward	225bp
	P43AS1: GCGCGTTGTTAGACAAGACA	External Reverse	
	P43S2: TCTTGTCGGGTGTTCACTCA	Internal Forward	
	P43AS2: CACAAGGAGACCGAGAAGGA	Internal Reverse	

3.6.4.1 Nest one procedure

Reaction mixture containing SAG3 primers for detecting *T. gondii* parasite DNA was carried out in a 25 µl reaction volume containing 1× Gotaq, double distilled water (DDH_2O), 0.1 µM SAG3 forward primers and reverse primers and 5 µl of extracted DNA. (Table 2). Positive controls containing SAG3 DNA and negative controls having no DNA were included in every set of PCR amplification. The reaction conditions for the nest 1 PCR protocol was maintained at initial denaturation at 95°C for 4 minutes, followed by another denaturation at 94ºC for 30 seconds, annealing at

58°C for 1 minute, and extension at 72°C for 2 minute followed by another 5 minute at 72°C for a total of 25 cycles.

3.6.4.2 Nest two procedure

In the nested reaction, 1 µl of PCR products of each sample from the nest 1 reaction was amplified in a 25 µl reaction volume of reaction mix containing 1× Gotaq, double distilled water (DDH_2O), 0.3 µM SAG3 forward and reverse primers for nest 2. (Table 2). The reaction mixture was amplified at temperatures of initial denaturation at 95°C for 4 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 61°C for 1 minute, and extension at 72°C for 2 minute followed by another 5 minute at 72°C for a total of 35 cycles.

Table 2: PCR Protocol reaction mixture for *T. gondii*

Reagents	Nest 1		Nest 2	
	Final conc.	Volume (µl)	Final conc.	Volume (µl)
Go-Taq	1x	12.5	1x	12.5
DDH_20		7		10
SAG3 Foward Primer	0.1 µM	0.25	0.3 µM	0.75
SAG3 Reverse Primer	0.1 µM	0.25	0.3 µM	0.75
DNA Template		5		1 µl of Nest 1 product
Total		25 µl		25 µl

3.6.5 Gel electrophoresis for the detection of *T. gondii* DNA

Five microlitres of PCR products of each sample of nest 2 reaction was loaded into wells of 2% agarose gel in an electrophoresis chamber. The chamber was set at 100 volts and allowed to run for 30 minutes. The gel was stained in 0.3 mg/ml ethidium bromide solution for 15 minutes then visualized in a UV illuminator to identify corresponding bands to *T. gondii* (225 bp for SAG3 gene). A standard 100 base pair

DNA size marker was included in every gel that was run to aid reading of corresponding band sizes.

3.6.6 Polymerase Chain Reaction (PCR) for the detection of *P. falciparum* DNA

Gene amplification for the detection of *P. falciparum* DNA involved a nested PCR method adapted from Snounou *et al*., (1993). Two different primer sets were used in each amplification reaction using a Takara Thermal Cycler (TP650 Version 3.00). [Table 3].

Table 3: Primer set sequence and product band size (rFAL1, rFAL2)

Markers	Primer sequence		Product size
rPLU5	CCTGTTGTTGCCTTAACTTC	External forward	205bp
rPLU6	TTAAAATTGTTGCAGTTAAAACG	External Reverse	
rFAL1	TTAAACTGGTTTGGGGAAACAAATATATT	Internal Forward	
rFAL2	ACACAATGAACTCAATCATGACTACCCGTG	Internal Reverse	

3.6.6.1 Nest one procedure

In the first amplification, primer sets rPLU5 and rPLU6 were the forward and reverse primers used accordingly. The 20 µl final volume reaction mix contained 1×Gotaq, double distilled water (DDH_2O), 10 µM of forward and reverse primers and 1 µl of DNA template. (Table 4). The reaction conditions were performed for a total of 24 cycles of denaturation, annealing and extension. It involved an initial hot start at 95°C for 5 minutes, annealing at 58°C for 2 minutes and extension at 72°C for 2 minutes. Samples were maintained at 20°C for an infinite time for short-term storage in the thermal cycler prior to the nest 2 reaction.

3.6.6.2 Nest two procedure

In the second amplification, primer sets rFAL1 and rFAL2 were the forward and reverse primers respectively. Reaction mixture was amplified in a 20 ul final volume containing 1×Gotaq, double distilled water (DDH_2O), 0.2 µM of forward and reverse primers and 1 µl of nest one PCR product as DNA template. (Table 4). The reaction conditions were maintained as the same conditions for nest one but for a total of 30 cycles.

Table 4: PCR Protocol reaction mixture for *P. falciparum*

Reagents	**Nest 1**		**Nest 2**	
	Final conc.	**Volume (µl)**	**Final conc.**	**Volume (µl)**
Go-Taq	**1x**	**10.0**	**1x**	**10.0**
DDH_20		**8.2**		**8.2**
Foward Primer (rPLU5)	**10 µM**	**0.4**	**(rFAL1) 0.2 µM**	**0.4**
Reverse Primer (rPLU6)	**10 µM**	**0.4**	**(rFAL2) 0.2 µM**	**0.4**
DNA Template		**1**		**1 µl of Nest 1 product**
Total		**20 µl**		**20 µl**

3.6.7 Gel electrophoresis for the detection of *P. falciparum DNA*

Ten microlitres of nest two PCR products of each sample were resolved in a 2% agarose gel electrophoresis. The electrophoresis chamber was set at 100 volts and allowed to run for 30 minutes. The gel was stained in 0.3 mg/ml ethidium bromide solution for 15 minutes then visualized in a UV illuminator to identify corresponding bands for *P. falciparum* DNA. Expected band size was 205 bp. A standard 100 bp DNA size marker was included in every gel that was run to aim reading of corresponding band sizes.

3.7 Statistical Analysis

All data were entered into a computer using Microsoft Office Excel 2013 package according to the codes given and analysed using Statistical Package for Social Scientists (SPSS v 20). Categorical variables were summarized as percentages and analysed with the Pearson's Chi-square test to observe the differences among the various categories. Factors with p values < 0.05 were considered to have a statistically significant association with the infections.

3.8 Approval and Ethical Considerations

The study was reviewed and approved by the Ethical Committee on Human Research Publications and Ethics (CHRPE) of the Kwame Nkrumah University of Science and Technology (KNUST) and the Komfo Anokye Teaching Hospital (KATH), Kumasi. Permission and approval was also obtained from appropriate hospital authorities. All procedures were performed according to the guidelines for human experimentations in clinical research stated by the committee. Names and other information collected from participants were kept confidential and anonymous.

CHAPTER FOUR

RESULTS

4.1 Characteristics of study participants

A total of 183 pregnant women aged 18–50 years (mean age: 27.97 years ± 5.528 years) volunteered to participate in the study. The 18–25 years age group constituted the highest proportion of the study participants, 37.7% (69/183) and the 36–40 years age group recorded the least with 9.8% (18/183) [Table 5]. The educational background of the women varied from those who had never been to school or had any form of formal education to those who had attained up to tertiary level education. About 10% (19/183) had no formal education and 19.1% (35/183) had completed Universities and Polytechnics (Table 5). Majority of the women, 34.4% (63/183) were involved in apprenticeship such as sewing and hairdressing. Fifty-six (30.6%) were sellers of fruits and vegetables, fish, meat (beef, chevon, mutton, pork) and food in market places. Thirty-four of the women (18.6%) were civil servants and private sector workers and 1.6% (3/183) were students (Table 5).

Sixty-eight (37.2%) of the pregnant women were primigravida (had no previous pregnancy). Secundgravida and Multigravida women accounted for 22.4% (41/183) and 40.4% (74/183) respectively of the total number (Table 5). In all 82.5% (151/183) of the women had no history of spontaneous abortions or still births, 12% (22/183) had experienced it once, 2.7% (5/183) had experienced it twice and 2.7% (5/183) had three or more past experiences of spontaneous abortions or stillbirths. Of the 68 primigravida women, 3.8% (7/183) had past experiences of spontaneous abortions or still births.

The 183 women delivered 186 babies comprising 2 sets of twins. A total of 60.2% (112/186) male births and 39.8% (74/186) female births were recorded (Table 5).

Table 5: Characteristics of study participants

Participants	Factors	Categories	No. Involved	(%)
Mothers	Age	18-25	69	37.7
		26-30	55	30.1
		31-35	41	22.4
		36-40	18	9.8
		Total	**183**	**100.0**
	Educational background	No formal education	19	10.4
		Basic education JHS	64	35.0
		Secondary/vocational education SHS	65	35.5
		Tertiary education	35	19.1
		Total	**183**	**100.0**
	Occupation	Civil/Private workers	34	18.6
		Apprenticeship	63	34.4
		Shop attendants	6	3.3
		Students	3	1.6
		Hospitals	5	2.7
		Abattoir	0	0
		Farmers	3	1.6
		Market	56	30.6
		None	13	7.1
		Total	**183**	**100.0**
	Gravida Status	Primigravida	68	37.2
		Secundgravida	41	22.4
		Multigravida	74	40.4
		Total	**183**	**100.0**
Babies	Sex	Male	112	60.2
		Female	74	39.8
		Total	**186**	**100.0**

4.2 Questionnaire responses

All 183 pregnant women responded to all the question items in the questionnaire interview. Neither of the women had heard of toxoplasmosis nor knew of it as a disease. None had also ever been tested for it. On the contrary, all the women had heard about malaria and had knowledge of it as a disease.

4.3 Clinical Characteristics of study participants

Almost all the women, 96.4% (180/183) had normal BP readings (Systolic reading ≤140mmHg and Diastolic reading ≤ 90mmHg) [Table 6]. A total of 78.1% (142/183) of the women had normal HB levels (≥ 10.5 g/dl ≤ 14.5 g/dl), 2.2% (4/183) had high HB levels and 3.2% (6/183) were anaemic (Table 6). Spontaneous Vaginal delivery (SVD) accounted for 90.7% (166/183) of the women whilst 9.3% (17/183) went through a Caesarian Section (CS) to deliver. About 60% (111/183) of the babies were within the normal birth weight range (≥2.6kg ≤ 3.5 kg), 14% (26/183) were below the normal birth weight (≤ 2.5 kg) and 26.3% (49/183) were over weights (≥3.5kg) [Table 6].

Table 6: Clinical characteristics of study participants

Participants	Factors	Categories	No. Involved	(%)
Mothers	**Last BP reading**	Eminent Eclampsia	180	96.4
		Mild Eclampsia	3	1.6
		Eclampsia	0	0
		Severe Eclampsia	0	0
		Total	**183**	**100.0**
	Last HB reading	Severe Anaemia	6	3.2
		Eminent Anaemia	30	16.4
		Normal HB levels	142	78.1
		Above normal	4	2.2
		Total	**183**	**100.0**
	Blood Groups	A+	31	17
		A-	6	3.2
		B+	34	18.6
		B-	15	8.1
		AB+	4	2.2
		AB-	2	1.1
		O+	69	37.7
		O-	22	12.0
		Total	**183**	**100.0**
	Delivery method	SVD	166	90.7
		CS	17	9.3
		Total	**183**	**100.0**
Babies	**Birth weights**	Low birth weights	26	14.0
		Normal birth weights	111	59.7
		Over weights	49	26.3
		Total	**186**	**100.0**

4.4 Seroprevalence of anti *T. gondii* antibodies

An overall seroprevalence of 44.8% (82/183) serum anti-*T. gondii* IgG was estimated by ELISA among the women and 22.6% (42/186) serum anti-*T. gondii* IgG among the babies (Figure 17). Neither mothers nor their babies showed seropositivity to anti-*T. gondii* IgM. Overall seroprevalence of *Toxoplasma* infection in matched mothers and babies was 21.9% (40/183) [Figure 17].

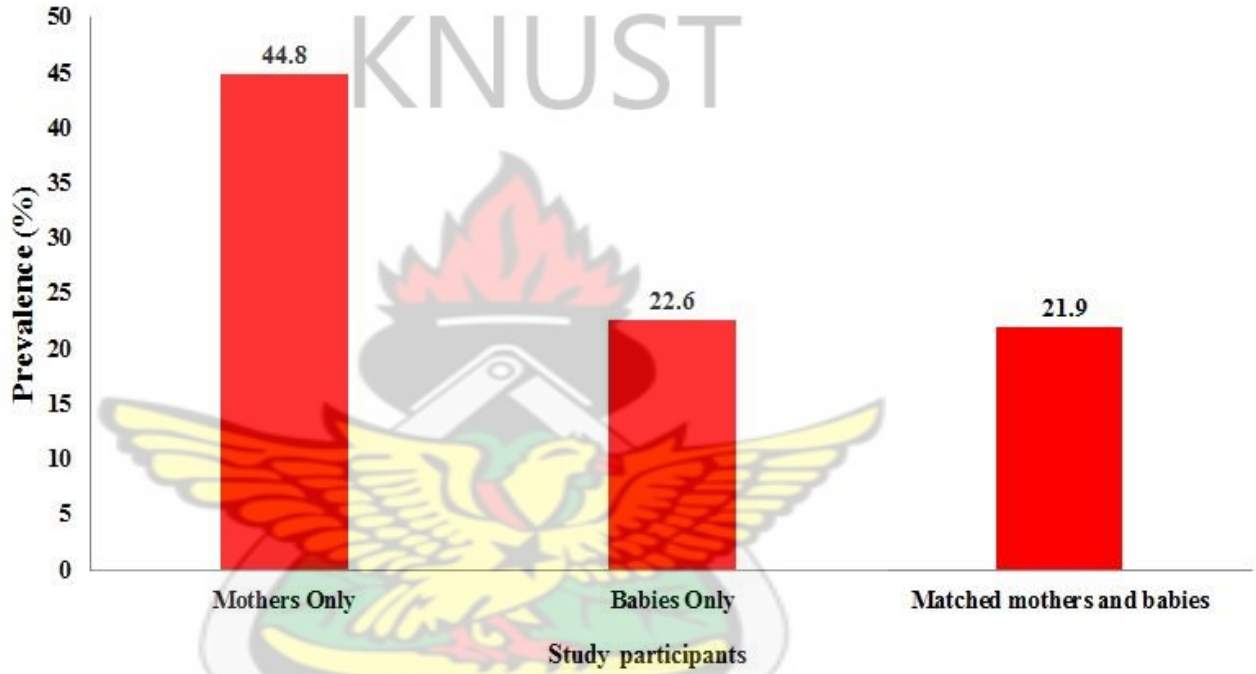

Figure 17: Seroprevalence of anti *T. gondii* IgG in the pregnant women and babies.

4.5 Detection of *T. gondii* DNA by PCR

Infection status of *T. gondii* among study participants was confirmed using the nested PCR method. Overall infection prevalence was 23% among the mothers. Forty-two mothers were positive for *T. gondii* infection (Table 7). The remaining 142 were negative and all babies also showed no infectivity to *T. gondii* infection. Figure 18

shows an electrophoregram of some positive samples of amplified *T. gondii* DNA (225 bp) by a nested PCR resolved in a 2% agarose gel.

Figure 18: Agarose gel electrophoregram of amplified *T. gondii* DNA by nested PCR. Lane M: 100 bp marker, Lanes 1-9: *T. gondii* positive samples, Lane P: SAG3 Positive control, Lane N: Negative control.

4.6 Detection of *P. falciparum* DNA by PCR

Prevalence of *P. falciparum* infection by the PCR method was detected in only maternal samples. Four percent (7/183) of the women were positive (Table 7). All cord blood samples were negative for *P. falciparum* infection. Figure 19 shows an electrophoregram of some positive *P. falciparum* amplified DNA (205 bp) by a nested PCR resolved in a 2% agarose gel.

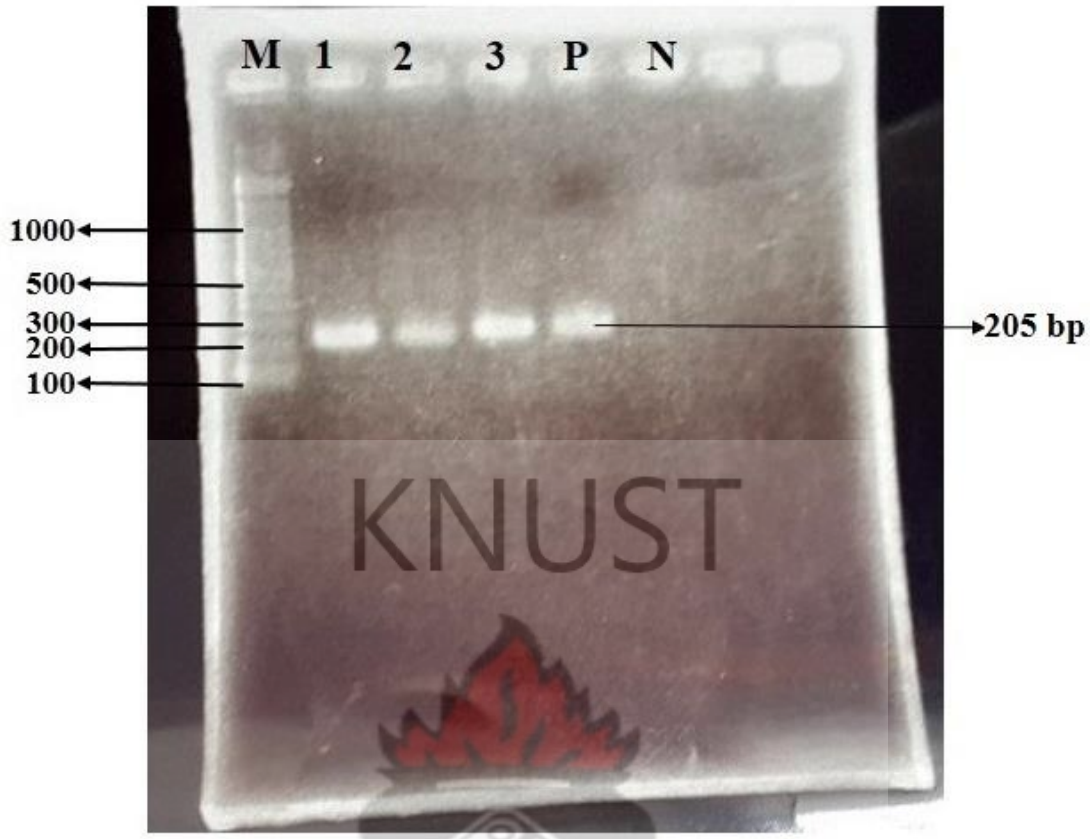

Figure 19: Agarose gel electrophoregram of amplified *P. falciparum* DNA by nested PCR. Lane M: 100 bp marker, Lanes 1-3: *P. falciparum* positive samples, Lane P: *P. falciparum* positive control, Lane N: Negative control.

Table 7: Overall *T. gondii* and *P. falciparum* infection prevalence among study participants

Test	*T. gondii* (Mothers)	*T. gondii* (Babies)	*P. falciparum* (Mothers)	*P. falciparum* (Babies)	*T .gondii* and *P. falciparum* (Mothers)	*T. gondii* and *P. falciparum* (Babies)
ELISA (IgG)	82/183 (44.8%)	42/186 (22.6%)	-	-	-	-
ELISA (IgM)	0	0	-	-	-	-
PCR	42/183 (23.0%)	0	7/183 (3.8%)	0	4/183 (2.2%)	0

4.7 Infection prevalence categorised by age group of mothers

Seroprevalence categorized by age group in years was: 17.2% for age group 18-25 years, 15.6% for age group 26-30 years, 10.7% for age group 31-35 years, and 4.1% for age group 36-40 years (Figure 20). Seroprevalence of IgG was high compared to actual detection of *T. gondii* parasite DNA in the age groups. *T. gondii* PCR prevalence decreased in older age groups (Figure 20). Age and pregnancy-related risk factors were not significantly associated with *T. gondii* infection (p=0.53). Infection prevalence of *P. falciparum* was within the 18-35 year age group (Figure 20). No statistical significance was observed with *P. falciparum* infection in relation to the age of the mothers (p=0.66).

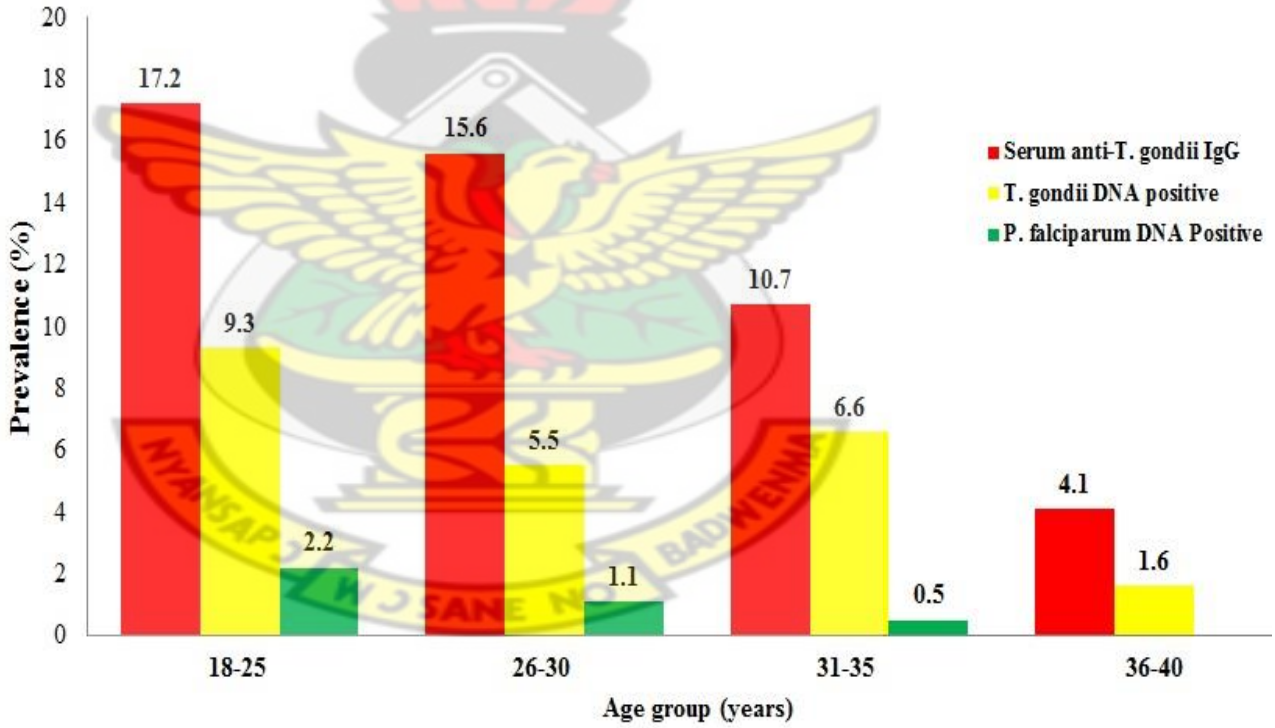

Figure 20: *T. gondii* and *P. falciparum* infection prevalence among mothers categorized by age groups

4.8 Infection prevalence and educational level of mothers

In general, the highest level of infection to both parasites was associated with mothers who had attained up to the basic level education (JHS) [Figure 21]. No significant association was observed with infection status and level of education attained ($p>0.05$). Thus, the level of education was independent on the infection status of the women.

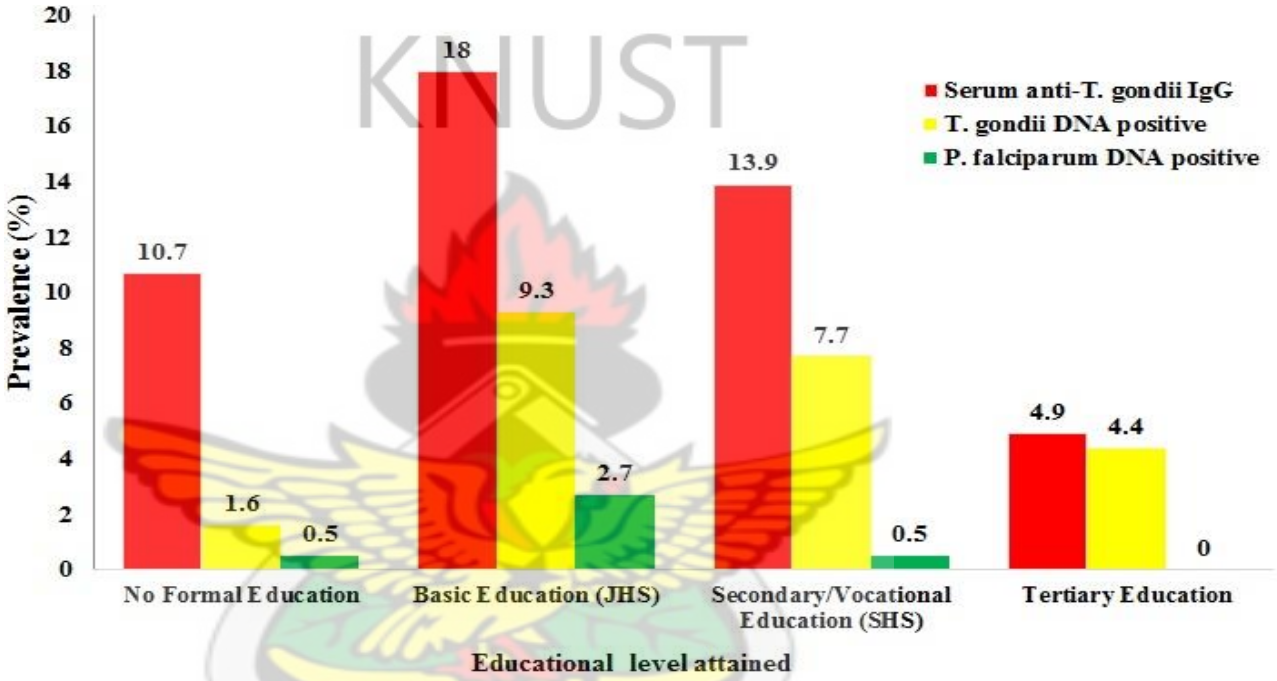

Figure 21: *T. gondii* and *P. falciparum* infection prevalence among mothers categorized by educational level attained

4.9 *T. gondii* infection prevalence and occupation of mothers

Within the high infection risk occupation group (workers at hospitals, abattoir, farmers, market vendors of fruits, vegetables and meat) prevalence was 28.1% (18/64) and 20% (21/105) within the low risk occupation group (civil/private sector workers, apprenticeship, shop attendants, students) [Figure 22]. However, no statstical significance was observed with *T. gondii* infection and occupation of the mothers (P=0.38).

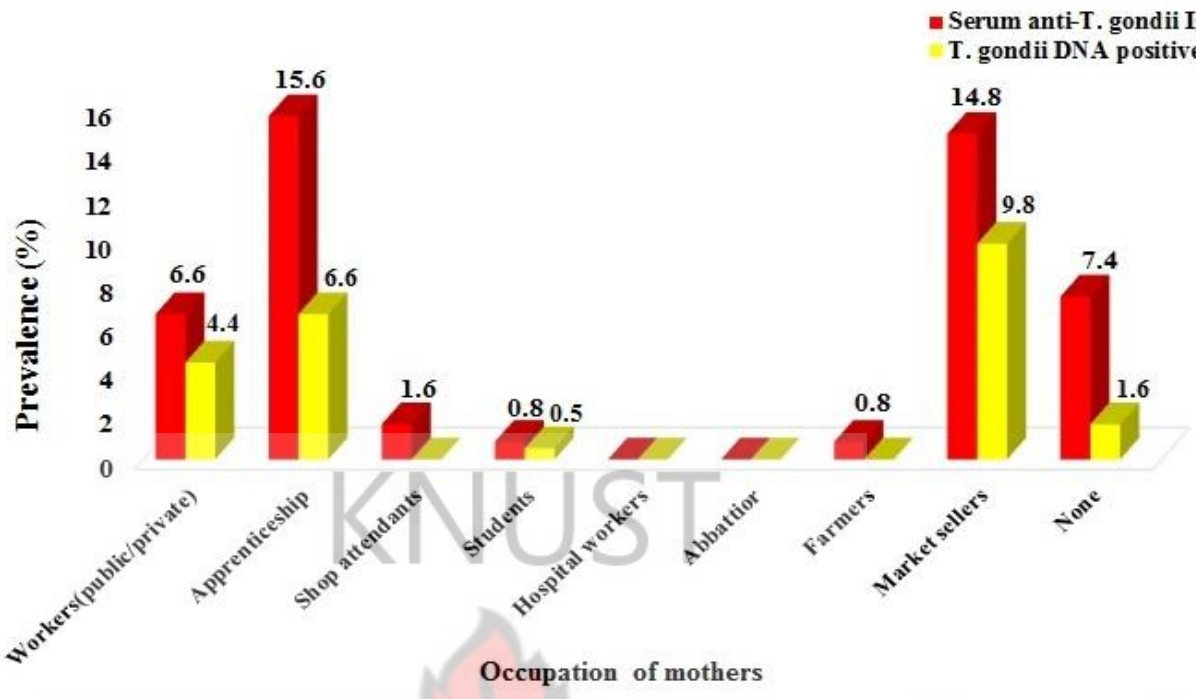

Figure 22: *T. gondii* infection prevalence and occupation of mothers

4.10 *T. gondii* infection status and risk factors considered

Infection status of the pregnant women and the related risk factors considered for *T. gondii* were all not significantly associated ($p>0.05$). Table 8 shows Chi-square (X^2) values and p-values of infection and the associated risk factors considered.

Table 8: *T. gondii* infection status and risk factors considered

Risk factors considered for *T. gondii*	X^2 value	p-value
Age of mothers	2.21	0.53
History of spontaneous abortions and/or still births	1.75	0.63
Contact with infected cat faeces	1.39	0.24
Consumption of infected meat from high transmission animals (chevon, pork, mutton)	2.96	0.94
Consumption of infected fruits and vegetables	0.30	0.86

4.11 *P. falciparum* infection status and treatment with SP-IPTp

A total of 97.3% (178/183) women took all three doses of SP at the weekly intervals under DOT during their antenatal visits until delivery (Table 9). The remaining women 2.7% (5/183) complained of mild allergic reactions to the drug so discontinued use (Table 9). However, they tested negative to *P. falciparum* infection.

Table 9: *P. falciparum* infection status and treatment with SP-IPTp

		Intake of SP-IPTp		
		Yes	**No**	**Total**
PCR Results (*P. falciparum*)	**Positive**	**7**	**0**	**7**
	Negative	**171**	**5**	**176**
	Total (%)	**178 (97.3)**	**5 (2.7)**	**183**

CHAPTER FIVE

DISCUSSION

Toxoplasma gondii and *Plasmodium falciparum* infections may be acquired or congenital and the diseases are important for their serious implications in immunosuppressed individuals including pregnant women as well as its severe consequences on foetuses in congenital transmission.

This is the first report from the Kumasi Metropolis and results reveal an appreciable prevalence of *T. gondii* infection in pregnant women and their babies.

Seroprevalence of anti-*T. gondii* IgG was lower (44.9%) in this study compared to report from the Greater Accra region. Ayi *et al*., 2009 recorded a serum anti-*T. gondii* IgG 73.6% among pregnant women from the Korle-Bu Teaching hospital and Achimota Hospital. This difference could partly be explained by geographical variation. There is reported variation of seroprevalence across regions within a given country (Jones, 2009). In the USA, the seroprevalence of toxoplasmosis was found to vary from 17.5% in the west to 20.5% and 29.2% in the south-midwest and north-east, respectively. This may be accounted for by the differences in climatic conditions where hotter areas are associated with higher seroprevalence values (Nijem, 2009) and this may be a probable reason for the high prevalence in Accra where the climatic conditions are generally hotter compared to Kumasi which falls within the forest belt. Hot weather has been found to favour the sporulation of *T. gondii* oocysts (Kistiah *et al*., 2011). The Presence of anti-*T. gondii* IgG identifies past exposures to infections of *T. gondii* in seropositive mothers. Mothers might have been exposed to the parasite in the past and presence of anti *T. gondii* antibodies

could be indicative of latent infection. An IgG avidity test could predict the time frame in which seropositive IgG mothers were infected. Detection of anti-*T. gondii* IgG in fetal serum indicate the transfer of maternal antibodies due to exposure to infection. Maternal antibodies are indicators of risk of infection (Charpak, 2004). IgG is secreted as a monomer that is small in size thus able to easily perfuse tissues. It is the only immunoglobulin that can pass through the human placenta thereby providing protection to the fetus in utero. It is also reported that predominant IgG antibodies found in newborns are IgG subclasses IgG_2 and IgG_3 against *T. gondii* antigens even though maternally transferred IgG_1 antibodies still persist in circulation (Buffolano *et al.,* 2006). The relative balance of these subclasses, in any immune complexes that form, helps determine the strength of the inflammatory processes that follow. Thus, subclass analysis of antibodies from mother to child against *T. gondii* antigens will further improve diagnosis of congenital *Toxoplasma* infection.

Anti- *T. gondii* IgM seropositivity were not detected in all maternal and fetal sera. The presence of IgM antibodies would be indicative of most recent, acute or on-going infections of circulating *T. gondii* antigens. IgM antibodies appear early in the course of an infection and is usually not expressed in acquired immunity and very rare in chronic infections (Charpak, 2004). Absence of anti. *T. gondii* IgM in maternal sera is suggestive of no on-going infections of *T. gondii*. IgM antibodies to *T. gondii* were also not detected in the serum of the newborns since they do not pass across the placenta and fetuses are unable to produce their own antibodies until they are about 6 to 12 months old (Charpak, 2004).

The frequency of *T. gondii* infections have been reported to increase in older age groups in both males and females in other studies (Bobic *et al*., 1998, Rosso, 2008,

Zemene *et al.*, 2012). However, in this study, there was no significant association between *Toxoplasma* infection and the age of the pregnant women. Results of this study agree with Ramsewak *et al.*, 2008 and Ayi *et al*., 2009, who also found that the age and pregnancy-related risk factors were not significantly associated with *T. gondii* infection.

Infection prevalence to both diseases was highest in mothers who had attained up to basic level education (JHS). This may be due to low level of knowledge to either or both diseases and much exposure to environmental contamination. This is consistent with WHO/WMR (2012) report on Child health which showed that children up to basic level education are most prone to diseases due to lack of knowledge and neglect and exposures to environmental contaminations to various diseases.

Pregnant women in the high risk occupation group (market vendors of fruits, vegetables, fresh meat and farmers) had high infection prevalence than pregnant women in the low risk occupation group (apprenticeship, civil/private workers, shop attendants and students). The market vendors of fruits, vegetables and fresh meat could have been infected directly from the foodstuff they sold. Foodstuff was most likely contaminated and these pregnant women might have handled and consumed them without any precaution. Contaminated fruits and vegetables have been reported as potential sources of *T. gondii* infection (Alvarado-Esquivel *et al.*, 2011). Fruits and vegetables are an important meal for the pregnant women during pregnancy. They provide needed vitamins and minerals for maternal and child development. 95.1% (173/183) women came into contact and ate fruits and vegetables when they were pregnant of which 62.4% (108/183) ate them fresh and raw. However, the consumption fruits and vegetables coupled with the preferred state of cooking vegetables before eating showed no significant association to *T. gondii* infection

(p=0.86). Lack of significant association could be attributed to thorough washing and treating of fruits and vegetables with saline solution or vinegar before consumption. Infections in the farmers could be attributed to contact via sporulated oocysts through broken skin in their farmlands from infected cat faeces, rodents and birds which are able to transport viable oocyst from one place to the other (http://www.cdc.gov/parasites/toxoplasmosis/epi.html). Farmers admitted to farming without protection on hands and feet which could have been possible routes of infection.

None of the practices also considered to be risk factors for infection status to *T. gondii* showed significant association among participating mothers (p>0.05). No statistical difference was found even with the most prevalent risk factor for *T. gondii* infection; exposure and possession of cats. Infection status of *T. gondii* from cats is established by contact with infected cat faeces (Dubey, 1994, Boothroyd, 2009). Infected cats excrete oocysts in their faeces which become infective after sporulation in the external environment. 41.2% (75/183) responded to having cats in their homes or immediate surroundings. In this study, no significant association between *T. gondii* infection and exposure to cats were found (p=0.24). Results from this study are consistent with studies done in Nigeria and Ethiopia (Ishaku *et al*., 2009, Zemene *et al.*, 2012) but contrary to results from Ayi *et al*., (2009) who recorded significant association between contact with cats and seroprevalence of *T. gondii.* However, non-association could be due to the fact that even though mothers had some sort of exposure to cats they were not in direct contact with infected cat faeces.

Twenty-two (12.1%) of the mothers had experienced spontaneous abortion once in previous pregnancies, 5 (2.7%) had experienced it twice and 5 (2.7%) had had it

thrice or more. In all 17.6% (32/183) had history of spontaneous abortions and/or still births. Spontaneous abortions and/or still births are important in the disease conditions presented by these parasites. Though history of spontaneous abortions did not show significant association with infection status (p=0.63), acute infections due to reactivation of latent infections of *T. gondii* due to immunosuppression during pregnancy could account for spontaneous abortions (Luft and Remington, 1992).

Infection from handling raw meat and consumption of high transmission risk farm animals (pork, mutton and chevon) as a route of infection is reported (Koskiniemi *et al*., 1989, Ghoneim *et al*., 2009). Serological evidence of *T. gondii* infection in farm animals in the country is also reported (Arko-Mensah *et al.*, 2000; Van der Puije *et al.*, 2000). *T. gondii* tachyzoites can penetrate unprotected and broken skin to cause infection. Though no statistical significance was found between handling of meat from these meat sources (p=0.94), all the women said they handled and prepared raw meat from these animals without any protection. They held blood contaminated meats with bare hands. About 67% (121/183) enjoyed the meat from these farm animals. These meats sources in the diet of the pregnant woman could increase the risk of infection if meats are contaminated and not properly cooked. The common practice in many Ghanaian homes of freezing at very low temperatures (<-4ºC) and thorough cooking of meat at very high temperatures (>70ºC) renders meat safe from *T. gondii* contamination and could have contributed to the lack of significant association with infection.

The low prevalence of malaria parasitaemia observed in this study is comparable to results from Stephens *et al*., 2014 who recorded *P. falciparum* parasitaemia of 5% among 320 pregnant women in Accra who visited antenatal clinics. Low levels of

malaria recorded in both studies might reflect improved education on malaria prevention during pregnancy and the introduction of IPTp-SP in Ghana. The IPTp is a public health intervention from WHO aimed at treating and preventing malaria episodes in pregnancy. It consists of the administration of doses of an efficacious anti-malarial drug (SP) at least twice during pregnancy regardless of the infection status of the pregnant woman. In Ghana, it has been instituted as part of the antenatal care for safe motherhood. All pregnant women who report to antenatal clinics are given at least 2 doses of SP in different gestational weeks under DOT. In the current study, 97.3% (178/183) of the pregnant women had at least 2 doses of SP during the period of their pregnancy. The remaining five complained of allergic reactions to the SP drug so discontinued intake. However, they tested negative to *P. falciparum* infection.

The standard therapeutic agent for the treatment of toxoplasmosis is a combination of sulphadoxine and pyrimethamine (SP) [Guerina *et al*., 1994]. This combination is active against the rapidly replicating tachyzoite stage of the parasite (Peterson *et al*., 2006). In this study, parasite prevalence of *T. gondii* as confirmed by PCR using SAG3 primers which are tachyzoite–specific was 23% (42/183). This warrants a high concern if SP is the same drug used in the treatment of toxoplasmosis. Patients diagnosed with toxoplasmosis are put on a daily dosage of sulphadoxine 3 g per day and pyrimethamine 50 mg per day for about 4-6 weeks till all circulating tachyzoites are cleared but for IPTp-SP, the doses are administered intermittently in different gestational weeks. It is administered as a single-dose comprising three tablets of 500 mg sulphadoxine and 25 mg pyrimethamine. The first dose is administered at 16 weeks gestation or more, second dose is given 4 weeks after the first dose and the

third dose is given 4 weeks after the second dose. This treatment regimen though effective at clearing *P. falciparum* parasitaemia might not be effective at clearing circulating *T. gondii* tachyzoites, hence might account for the prevalence recorded. Moreover, SP treatment to toxoplasmosis have limited efficacy against tissue cysts (Peterson *et al*., 2006b). Thus, *T. gondii* detection in placenta tissues of study participants might yield a high prevalence value. The detection of co-infections (2.2%) of both parasites could be due to opportunistic parasitism due to immunosuppression during the period of pregnancy. Possible negative effects due to aggravation of these parasites in the mothers could cause associated consequences in maternal health and in subsequent pregnancies. Appropriate management in monitoring pregnancies should therefore be developed to improve maternal and child health.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The data obtained from this study showed a notable prevalence of toxoplasmosis among pregnant women in the Kumasi Metropolis. There was the occurrence of co-infections detected in the mothers only. The risk of exposure to anti *T. gondii* IgG antibodies in some babies was due to maternal IgG transfer. None of the practices also considered as risk factors to *T. gondii* infection in this present study showed any significant association among the mothers ($p>0.05$). The low prevalence of malaria parasitaemia observed in the pregnant women is commendable as improved education on malaria prevention during pregnancy via the IPTp-SP in Ghana appears to make a positive impact.

6.2 Recommendations

The following recommendations are made:

- The consideration of routine screening of all pregnant women for *Toxoplasma* infection during antenatal clinics to minimize risk of infection towards improving maternal and child health.
- Intensive education on awareness and preventive measures to toxoplasmosis in the general populace especially among pregnant women as an opportunistic infection during pregnancy as have been done for malaria.
- A greater cross section of pregnant women should be sampled in further studies preferably in all regions of the country to obtain reliable epidemiological data on toxoplasmosis in Ghana.

» IPTp-SP prophylaxis should be continued strongly as it has proved efficient in the management of pregnancy malaria in Ghana.

REFERENCES

Ajzenberg D., Cogné N., Paris L. (2002). Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. *J. Infect. Dis*. 186**,** 684–689.

Alan F., Cowman J., Brendan S., Crabb A. (2006). Invasion of Red Blood Cells by Malaria Parasites.*Cell*. 124:755–766.

Alvarado-Esquivel C., Estrada-Martínez S., and Liesenfeld O. (2011). *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. *Parasit Vectors*, 16:4–235.

Anteson R. K., Sekimoto S., Furukawa S., Takao Y. and Nyanotor M. A. (1980). Studies on Toxoplasmosis in Ghana IV. Further Evidence of Congenital Diseases caused by *Toxoplasma gondii* Infections. *Ghana Med J*. 25, 146-148.

Appleford P. J., and Smith J. E. (1997). *Toxoplasma gondii*: the growth characteristics of three virulent strains. *Acta Trop*. 65:97–104.

Arko-Mensah J., Bosompem K. M., Canacoo J., Wastling M. and Akanmori B. D. (2000). The Seroprevalence of Toxoplasmosis in Pigs in Ghana, *Acta Tropica*, 76 (1), 27-31.

Asthana S. P., Macpherson C. N., Weiss S. H., and Stephens R. (2006). Seroprevalence of *Toxoplasma gondii* in pregnant women and cats in Grenada, West Indies. *J Parasitol*., 92: 644-645.

Ayi I., Edu, S. A. A., Apea-Kubi K. A., Boamah D., Bosompem K. M., Edoh, D. (2009). Sero-epidemiology of toxoplasmosis amongst pregnant women in the greater Accra region of Ghana. *Ghana Med J. 43 (3); 107 – 114.*

Barragan A., and Sibley L. D. (2002). Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. *J. Exp. Med*. 195**,** 1625–1633.

Barillas-Mury C. and Kumar S. (2005). *Plasmodium* –mosquito interactions: a tale of dangerous liaisons. *Cellular Microbiology*; 7(11):1539–1545.

Baum J., Richard D., Heale J. (2006). A Conserved Molecular Motor Drives Cell Invasion and Gliding Motility across Malaria Life Cycle Stages and Other Apicomplexan Parasites. *The Journal of Biological Chemistry*. February; 281:5197-5208.

Bobić B., Jevremović I., Marinković J., Šibalić D., Djurković-Djaković O. (1998). Risk factors for *Toxoplasma* infection in a reproductive age female population in the area of Belgrade, Yugoslavia. *European Journal of Epidemiology*; 14:605–610. [PubMed]

Bonhomme A., Pingret L and Pinon J. M. (1992). Review: *Toxoplasma gondii* cellular invasion. *Parasitologia* 54:31–43.

Boothroyd J. C. (2009). Toxoplasmosis. Encyclopedia of Microbiology. 3rd ed. Oxford: Elservier; pp.732-43.

Bosman A. and Mendis K. N. A Major Transition in Malaria Treatment **(2007).** The Adoption and Deployment of Artemisinin-Based Combination Therapies. *Am. J. Trop. Med. Hyg.*, 77,; 193–197.

Buffolano W., Beghetto E., Del Pezzo M. (2006). Use of recombinant antigens for early postnatal diagnosis of congenital toxoplasmosis. *J. Clin. Microbiol*. 43**,** 5916–5924.

Castelli F., and Carosi G. (1997). Diagnosis of malaria infection. A Handbook of malaria infection in the tropics. pp 114: Cambridge University Press, Cambridge UK

Centers for Disease Control and Prevention, CDC Malaria: AnophelesMosquitoes.http://www.cdc.gov/malaria/about/biology/mosquitoes/index.html (accessed 2014 February 10)

Centers for Disease Control and Prevention, CDC. Toxoplasmosis (EpidemiologyandRisk factors).http://www.cdc.gov/parasites/toxoplasmosis/epi.html (accessed 2013 November 6).

Charpak Y., Nicoulet I., and Blery C., (2004). Protective anti-donor IgM production after crossmatch positive liver-kidney transplantation. *Microbes infect* (2): 315-319 PMID 1476287

Chedraui P. A., Daily J, Wylie B, Weller P. F, Ramin S. M, and. Barss V. (2009) "Overview of malaria in pregnancy," http://www.uptodate.com (accessed 2014 February 10)

Collins W. (2007). Further Understanding the Nature of Relapse of *Plasmodium vivax* Infection. *The Journal of Infectious Diseases* 195:919–920.

Couvreur J. (2004). Infection in neonates and infants. *In:* Joynson DHM, Wreghitt TG. *Toxoplasmosis: A Comprehensive Clinical Guide* 2001; 254 – 276: Cambridge University Press, Cambridge UK

de Melo, E. J. T., and de Souza W. (1997). A cytochemistry study of the inner membrane complex of the pellicle of tachyzoites of *Toxoplasma gondii*. *Parasitol. Res*. 83:252–256

Dubey J. P. (1994). Toxoplasmosis. *J. Am. Vet. Med. Assoc.* 205: 1593-1598.

Dubey J. P. (1993). *Toxoplasma*, Neospora, Sarcocystis, and other tissue cyst-forming coccidia of humans and animals. 1 - 158. *In:* Kreier JP. *Parasitic Protozoa. Second Edition. Volume 6*1993. Academic Press, London.

Dubey J. P., and Frenkel J. K. (1976). Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *J. Protozool*. 23:537–546.

Dubey J. P. and Beattie C. P (1988). Toxoplasmosis of Animals and Man. *Boca Raton,* FL: CRC Press.

Dubremetz, J. F., and. Swartzman J. D. (1993). Subcellular organelles of *Toxoplasma gondii* and host cell invasion. *Res. Immunol.* 144:31–33.

Dunn D., Wallon M., Peyron F., Peterson E., Peckham C., and Gilbert R. (1999). Mother to child transmission of toxoplasmosis: risk estimates for clinical counseling. *Lancet*. 353: 1829-1833

Enweronu-Laryea C., Adjei O. G., Mensah B., Duah N. and Quashie N. B. (2013). Prevalence of congenital malaria in high-risk Ghanaian newborns: a cross-sectional study. *Malaria Journal*, 12:17

Evengard B., Lilja G., Capraru T., Malm G., Kussofsky E., Oman H., and Forsgren M. A. (1999). Retrospective study of seroconversion against *T. gondii* during 3,000 pregnancies in Stockholm. *Scand. J. Infect. Dis*. 31: 127-137.

Ferguson D. J. P., Birch-Andersen A, Siim J. C, and. Hutchison W. M. (1979). Ultrastructural studies on the sporulation of oocysts of *Toxoplasma gondii*. I. Development of the zygote and formation of sporoblasts. *Acta Pathol. Microbiol. Scand. Sect*. B 87:171–181.

Ferguson D. J. P., Birch-Andersen A., Siim J. C., and Hutchison W. M. (1978). Observations on the ultrastructure of the sporocyst and the initiation of sporozoite formation in *Toxoplasma gondii*. *Acta Pathol. Microbiol. Scand. Sect*. B 86:165–167.

Ferguson H. M and Read A. F. (2004). Mosquito appetite for blood is stimulated by *Plasmodium chabaudi* infections in themselves and their vertebrate hosts. *Malaria Journal*;3:12

Floren L., Michael P., Washburn J., Dale R. (2002). A proteomic view of the *Plasmodium falciparum* life cycle *Nature* October; 419:520-526.

Frenkel J. K. (1973). *Toxoplasma* in and around us. *BioScience* 23:343–352.

Fux B., Rodrigues C. V., Portela R. W. (2003). Role of cytokines and major histocompatibility complex restriction in mouse resistance to infection with a natural recombinant strain (type I–III) of *Toxoplasma gondii*. *Infect. Immun*. 71**,** 6392–6401.

Gagne S. S. (2001). Toxoplasmosis. Prim Care Update *Obstet. Gynecol*. 8: 122-126.

Gavinet M. F., Robert F., Firtion G. (1997) Congenital toxoplasmosis due to maternal reinfection during pregnancy. *J Clin Microbiol*; 35: 1276-7.

Gavrilescu L.C. and Denkers, E.Y. (2001). IFN-gamma overproduction and high level apoptosis are associated with high but not low virulence *Toxoplasma gondii* infection. *J. Immunol*. 167**,** 902–909.

Ghislaine M. D. C., Cofiea J. , Jiangb L., Hartlc D. L., Tracya E. , Kabatd J, Laurence H. M, Millera L. H. (2009). Glycophorin B is the erythrocyte receptor of *Plasmodium falciparum* erythrocyte-binding ligand, EBL-1. *PNAS* 31 March,;106(13):5348–5352

Ghoneim N. S. S., Hassanain N., Zeedan G., Soliman Y., Abdalhamed A. (2009). Detection of genomic *Toxoplasma gondii* DNA and anti-*Toxoplasma* antibodies in high risk women and contact animals. *Global Veterinaria,* 3:395–400.

Gitau G. M. Eldred J. M. (2005). Malaria in pregnancy: clinical, therapeutic and prophylactic considerations. *The Obstetrician & Gynaecologist.*; 7:5–11

Good M. F. and Doolan D. L. (2007). Malaria's journey through the lymph node. *Nature Medicine* ;13:1023-1024.

Greenwood B. M, Fidock D. A., Kyle D. E., Kappe S. H. I. Alonso P. L., Collins F. H,. Duffy P. E. (2008). Malaria: progress, perils, and prospects for eradication. *J. Clin. Invest.* 118:1266–1276.

Guerina N. G., Hsu H. W. Meissner H. C. Maguire J. H. Lynfield R. and Stenchenberg B. (1994). Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. The new England Regional *Toxoplasma* Working Group. *New Engl. J. Med*. 330: 1858-1863

Holliman R. E. (1995). Congenital toxoplasmosis: prevention, screening and treatment. *J Hosp Infect*; 30:179-190 [PubMed].

Howe D. K. and Sibley L. D. (1995). *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J. Infect. Dis*. **172,** 1561–1566

Indra V. (2008). *Plasmodium knowlesi* infections. in humans, macaques and In mosquitoes in peninsular Malaysia. *Parasit Vectors.*;1:26

Ishaku B. A. I, Umoh J., Lawal I., Randawa A. (2009). Seroprevalence and risk factors for *Toxoplasma gondii* infection among antenatal women in Zaria, Nigeria. *Res J Medicine & Med Sc,* 4:483–488.

Jacobs L., Remington J. S. and Melton M. L. (1960). The resistance of the encysted form of *Toxoplasma gondii*. *J. Parasitol*. 46:11–21.

Janku J. (1923). Pathogenesa a pathologická anatomie tak nazvaneho vrozeného kolombu zˇluté skvrany voku normálné velikem a microphthalmickém s nalezem parasitu v sítnici. *Casopis lékareu ceskyck* 62**,** 1021, 1052, 1081, 1111, 1138.

Jones B. L, Torrey E. F, and Yolken R. (2003). Drugs in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii*. Schizophr. *Res*. 62: 237-244.

Jones J. L., Dargelas V., Roberts J. (2009). Risk factors for *Toxoplasma gondii* infection in the United States. *Clin Infect Dis*; 49:878–884

Jones M. K and Good M. F. (2006). Malaria parasites up close. *Nature Medicine*; 12:170-171

Joynson D. H. M. and Guy E. C. (2001). Laboratory diagnosis of *Toxoplasma* infection. *In:* Joynson DHM, Wreghitt TG .Toxoplasmosis*: A Comprehensive Clinical Guide*; 296 – 318. Cambridge University Press, Cambridge UK

Kakkilaya B. S. (2009). "Malaria and pregnancy," http://www.malariasite.com (accessed 2013 March 8)

Kasturi H and Narla M. (2007). Erythrocyte remodeling by malaria parasites. *Curr Opin Hematol* 14:203–209

Kebaier C., Voza T., Vanderberg J. (2009). Kinetics of Mosquito-Injected *Plasmodium* Sporozoites in Mice: Fewer Sporozoites Are Injected into Sporozoite-Immunized Mice. *PLoS Pathog* 5(4)

Kish L. (1965). Survey Sampling. New York: John Wiley and Sons, Inc.

Kistiah K. B. A, Winiecka-Krusnell J., Karstaedt A., Frean J. (2011). Seroprevalence of *Toxoplasma gondii* infection in HIV-positive and HIV-negative subjects in Gauteng, South Africa. *South Afr J Epidemiol Infect*, 26(4):225–228

Kirk Kiaran. (2001). Membrane Transport in the Malaria-Infected Erythrocyte. *Physiological Reviews* April; 8 (2):495-537

Koskiniemi M., Lappalainen M., Hedman K. (1989). Toxoplasmosis needs evaluation. An overview and proposals. *Am J Dis Child*, 143:724–728

Kumasi Metropolitan Assembly-Town and Country Planning division-Ministry of Food and Agriculture.htm (accessed 2013 March 10)

Kur J., Holec-Gasior L., Hiszczyńska-Sawicka E. (2009). Current status of toxoplasmosis vaccine development. *Expert Rev. Vaccines*. 8: 791-808

Lee S. H, Kara U. A, Koay E., Lee M. A., Lam S., Teo D. (2002). New strategies for the diagnosis and screening of malaria. *Int J Hematol*; 76:291-293.

Levaditi C., Schoen R. and Sanchis B. V. (1928). L'encéphalo-myélite toxoplasmique chronique du lapin et de la souris. *C. R. Soc. Biol*. **99,** 37–40.

Levine, N. D. (1977). Taxonomy of *Toxoplasma*. *J Protozool*. Vol 24:36-41

Lopez A., Dietz V. J., Wilson M., Navin T. R., Jones J. L. (2000). Preventing congenital toxoplasmosis. *MMWR Recomm Rep*.; 49(RR-2): 59 - 68. [PubMed].

Louis H. M., Dror I. B., Marsh K., Ogobara K. D. (2002). The pathogenic basis of malaria. *Nature* February; 415(7):673-679.

Luft, B. J. and Remington, J. S. (1988). AIDS commentary: Toxoplasmic encephalitis. *J. Infect. Dis*. **157,** 1–6.

Luft, B. J. and Remington, J. S. (1992) Toxoplasmic encephalitis in AIDS. *Clin Infect Dis* 15: 211-22.

Lynfield R. and Guerina N. G. (1997). Toxoplasmosis. *Pediatr. Rev*. 18: 75

Martin F. (2000). Congenital toxoplasmosis: Value of antenatal screening and current prenatal treatment. *TSMJ*. 1:46-5

Mazzi E., Bartos A. E., Carlin J., Weber M. W., Darmstadt G. L. (2010). Bolivia Clinical Signs Study Group: Clinical signs predicting severe illness in young infants (<60 days) in Bolivia. *J Trop Pediatr* 56:307–316.

McAuley J., Boye K. M., Patel D., Beckman J., Schey W., Stein L., Wolters C., Johnson D., Meier P., Mack D. G., Mets M., Boyer K., Swisher C., Withers S., Holfels E., McLeod R. (1994). Early and longitudinal evaluation of treated infants and children and untreated historical patients with congenital toxoplasmosis: the Chicago collaborative treatment trial. *Clin Infect Dis*.; 38–72. [PubMed]

McLeod R., Mack D. and Brown C. (1991). *Toxoplasma gondii* – new advances in cellular and molecular biology. *Exp. Parasitol.* **72,** 109–121.

Ministry of Health, Ghana Health Service (2013). Guidelines for Malaria in Pregnancy. M. O.H, Accra, Ghana.

Montoya J. G., and. Remington J. S. (2000). *Toxoplasma gondii*, Principles and Practice of Infectious Diseases. Mandell G L., J E Bennett and R. Donlin (Eds) 5th Edn., Churchill Livingstone, Philadelphia, pp: 2858-2887.

Montoya J. G. (2002). Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J Infect Dis* 185**:**S73–82.

Moody A. H and Chiodini P. L. (2000). Methods for the detection of blood parasites. *Clin Lab Haematol*; 22:189-201.

Münter S., Sabass B., Selhuber-Unke C. (2009). *Plasmodium* Sporozoite Motility Is Modulated by the Turnover of Discrete Adhesion Sites *Cell Host & Microbe.*December;6 (17):551-562.

Neena V., Sadhna M., Sukla B., Aditya P. D. (2007). Congenital malaria with a typical presentation: A case report from low transmission area in India. *Malaria Journal*; 6:43.

Negash T., Tilahun G., Patton S.T., Prevot F., Dorchies P. H. (2004). Serological survey on toxoplasmosis in sheep and goats in Nazareth, Ethiopia. *Revue de Medicne Veterinaire. 155(10); 486–487*.

Nicolle C. and Manceaux L. (1909). Sur un protozoaire nouveau du gondi. *C. R. Seances Acad. Sci*.; 148**:** 369–372.

Nijem K. A-A. S. (2009). Seroprevalence and associated risk factors of toxoplasmosis in pregnant women in Hebron district, Palestine. *East Mediterr Health J,* 15:1279–1284.

Nissapatorn V, Lee C, Quek KF, Leong CL, Mahmud R, Abdullah KA (2004). Toxoplasmosis in HIV/AIDS patients: a current situation. *Jpn J Infect Dis*; 57: 160-5.

Okwa O. O. (2003) "The status of malaria among pregnant women: a study in Lagos, Nigeria," *African Journal of Reproductive Health*, 7, (3), : 77–83.

Olivier S., Mota M. M., Kai M., Miguel P. (2008). Interactions of the malaria parasite and its mammalian host *Current Opinion in Microbiology* ;11:352–359.

Olusi T., Grob U., Ajayi, J. (1996). High incidence of toxoplasmosis during pregnancy in Nigeria. *Scand J Infect Dis 28: 645-646*.

Opiyo N. and English M. (2011). What clinical signs best identify severe illness in young infants aged 0–59 days in developing countries? A systematic review. *Arch Dis Child*, 96:1052–1059.

Petersen E., Edvinsson B., Benfield T., Lundgren B. and Evengård B. (2006a). Diagnosis of *T. gondii* in bronchioalveolar lavage from HIV-infected patients. *Eur. J. Clin.Microbiol. Infect. Dis.* 25**,** 401–404.

Petersen E., Peyron F., Lobry J. R., Musset K., Ferrandiz J., Gomez-Marin J. E., Meroni V., Rausher B., Mercier C., Picot S., Cesbron-Delauw M. F. (2006b). Serotyping of *Toxoplasma gondii* in chronically infected pregnant women: predominance of type II in Europe and types I and III in Colombia (South America). *Microbes Infect*; 8(9-10): 2333 - 2340. [PubMed].

Prášil P. (2009). Current options for the diagnosis and therapy of toxoplasmosis in HIV-negative patients. *Klin. Mikrobiol. Infekc. Lek*. 15 : 83-90

Prestrud K.W., Asbakk K., Fuglei E., Mork T. and Tryland M. (2008). Direct high-resolution genotyping of *Toxoplasma gondii* in arctic foxes (Vulpes lagopus) in theremote arctic Svalbard archipelagoreveals widespread clonal Type II lineage. *Vet Parasitol* 158: 121-128

Prudêncio M., Rodriguez A., Mota M. M. (2006). The silent path to thousands of merozoites: the *Plasmodium* liver stage. *Nature Reviews Microbiology*;4:849–856

Ramsewak S., Gooding R., Ganta K., Seepersadsingh N., Adesiyun A. A. (2008). Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women in Trinidad and Tobago. Rev *Panam Salud Publica*.;23(3):164–70.

Remington J. S. Mcleod R. and Desmonts G. (1995). Infectious disease of the foetus and newborn. Infant Philadelphia: W. B. Saunders Company. Pp: 140-267.

Roberts F., Gavnet M. F., Anelle T., Raymond J., Tourte C. T., and Dupouy C. J. (1999). Value of prenatal diagnosis of congenital toxoplasmosis; retrospective study of 110 cases. *J. Clin. Microbiol*. 37: 2893-2898.

Romand S., Bouree P., Gelez J,. Bader-Meunier B., Bisaro F., and. Dommergues J. P. (1994) "Congenital malaria. Infected twins born to an asymptomatic mother," *Presse Medicale*, vol. 23, no. 17, pp. 797–800.

Rorman E., Zamir C. S., Rilkis I., Ben-David H. (2006). Congenital toxoplasmosis--prenatal aspects of *Toxoplasma gondii* infection. *Reprod Toxicol*; 21(4): 458 - 472. [PubMed]

Rosso F. (2008). Prevalence of infection with *Toxoplasma gondii* among pregnant women in Cali, Columbia, South America. *Am J Trop Med Hyg*, 78:504–508.

Rusiňáková Z., Raida L., Faber E., Tomková J., Bednaříková J., Indrák K., Novotný D. (2009). Toxoplasmosis after immunossuppressive therapy - our experience. *Klin. Mikrobiol. Infekc. Lek.* 15: 95-98.

Sabin A. B. (1941). Toxoplasmic encephalitis in children. *J Am Med Assoc*, 116:801–807.

Saeij J. P. J., Boyle J. P. and Boothroyd J.C. (2005). Differences among the three major strains of *Toxoplasma gondii* and their specific interactions with the infected host. *Trends Parasitol*. 21, 476–481

Sensini A., (2006). *Toxoplasma gondii* infection in pregnancy: Opportunities and pitfalls of serological diagnosis. *Clin. Microbiol. Infect.*, 12: 504-512. PMID: 16700697

Singh S. (2003). Mother to child transmission and diagnosis of *Toxoplasma gondii* infection during pregnancy. *Indian J. Med. Microbiol*. 21: 69-76.

Snounou G., Viriyakosol S., Jarra W., Thaithong S., Brown K. N. (1993). Identification of the four human malaria parasites species in field samples by the Poloymerase Chain Reaction and detection of a high prevalence of mixed infections. *Mol. Biochem Parasitol.* 58, 283-292.

Sotimehin S. A., Runsewe-Abiodun T. I., Oladapo O. T, Njokanma O. F, and Olanrewaju D. M. (2008) "Possible risk factors for congenital malaria at a tertiary care hospital in Sagamu, Ogun State, South-West Nigeria," *Journal of Tropical Pediatrics*, vol. 54, no. 5, pp. 313–320,.

Spalding S. M., Amendoeira M. R. R., Klein C. H and Ribeiro L. C. (2005). Serological screening and toxoplasmosis exposure factors among pregnant women in South of Brazil. *Rev Soc Bras Med Trop.,* 38: 173-177. PMID: 15821794

Stephens J. K., Ofori M. F., Quakyi I. A., Wilson M. L., Akanmori B. D. (2014). Prevalence of peripheral blood parasitaemia, anaemia and low birthweight among pregnant women in a suburban area in coastal Ghana. *Pan Afr Med J*. 17(Supp 1):3

Su C., Zhang X. and Dubey J. P. (2006) Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. *Int. J. Parasitol*. 36, 841–848.

Suzuki I.,A., Rosha R. J and Rossi C. L (2001). Evaluation of serological markers for the immunodiagnosis of acute acquire toxoplasmosis. *J. Med. Microbiol*. 50: 62-70.

Switaj K., Master A., Skrzypczak M., and Zaborowski1 P. (2005). Recent trends in molecular diagnostics for *Toxoplasma gondii* infections. *Clin Microbiol and Inf,* 11-3, Pp 171-176

Thulliez P., Stepick-Biek P., Edwards M. E. (2002). Fungal and protozoan infections. In AA Fanaroff, RJ Martin (eds) Neonatal-Perinatal Medicine, Diseases of Fetus and Infant, 7th ed., Mosby, St Louis, p. 745-755

Thulliez P., Daffos F., Forrestier F. (1992). Diagnosis of *Toxoplasma* infection in the pregnant woman and the unborn child: Current Problems. *Scand J Infect Dis.;* 84:22–28.

Van den Eede P. (2009) Human *Plasmodium knowlesi* infections in young children in central Vietnam. *Malaria Journal*; 8:249.

Van der Puije W. N. A., Bosompem K. M., Canacoo E. A., Wastling J.,M. and Akanmori B. D. (2000). The Prevalence of Anti-*Toxoplasma gondii* Antibodies in Ghanaian sheep and goats. *Acta Tropica,* 76(1), 15-20.

Vaughan A. M., Aly A. S. I., Kappe S. H. I. (2008). Malaria parasite pre-erythrocytic stage infection: Gliding and Hiding. *Cell Host Microbe.*11 September;4 (3):209–218.

Virgilio L. L, Tiffert T., Ginsburg H. (2003). Excess hemoglobin digestion and the osmotic stability of *Plasmodium falciparum*-infected red blood cells *Blood.* 15 May;101(10):4189-4194.

Wagner G., Koram K., Mcguinness D., Bennett S., Nkrumah F., Riley E. (1998). High incidence of asymptomatic malaria infections in a birth cohort of children less than one year of age in Ghana, detected by multicopy gene polymerase chain reaction. *Am J Trop Med Hyg*, 59:115–123.

Weinman, D. and Chandler, A. H. (1954). Toxoplasmosis in swine and rodents. Reciprocal oral infection and potential human hazard. *Proc. Soc. Exp. Biol.Med*. **87,** 211–216.

Weiss L. M. and Kim K. (2006). *Toxoplasma gondii*. The model Apicomplexan-Perspectives and Methods ; 200-220: Elsevier Science Ltd.

Wolf, A. and Cowen, D. (1938). Granulomatous encephalomyelitis due to a protozoan (*Toxoplasma* or *Encephalitozoon*). II. Identification of a case from the literature. *Bull. Neurol. Inst. NY* **7,** 266–290.

Wong, S. Y. and Remington, J. S. (1993). Biology of *Toxoplasma gondii*. *AIDS* **7,** 299–316

World Health Malaria. World Malaria Report 2013. http://www.who.int/malaria/publications/world_malaria_report_2013/report/en/ (accessed 2014 February 10)

World Health Organisation. World Malaria Report 2012 www.who.int/malaria/publications/world_malaria_report_2012/en (accessed 2013 March 8)

Zemene E., Yewhalaw D., Abera S., Belay T., Samuel A., Zeynudin A. (2012). Seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. *BMC Infect Dis*, 12:337.

APPENDICES

Appendix I: Questionnaire for Pregnant women

QUESTIONNAIRE

Kindly provide the needed information in the questionnaire below.

You may tick the appropriate box or boxes as indicated.

Thank you for your participation.

Part A: SOCIO-DEMOGRAPHIC DETAILS

ID Number: …… Date :……/……/…… (dd/mm/year)

Name: …….

Age: ...……..

Telephone Number: ……..

Area of residence: ……

Duration at Residence: ……..

Hometown: ……

1. Educational background
   a. No formal education ☐
   b. Elementary school/JHS ☐
   c. Secondary school/Vocational school ☐
   d. Tertiary (Polytechnic, University etc.) ☐
   e. Others (Specify)……..

2. Where do you work?
   a. Office (Bank, school etc.) ☐
   b. Hospital (Labour ward, Theatre, Accident/Emergency Centre, Blood Bank etc.) ☐
   c. Hospital (Records, OPD, Nurse/Midwife, wards etc.) ☐
   d. Slaughter House ☐
   e. Farm/Garden ☐
   f. Market (Sells vegetables, raw meat, fish etc.) ☐

g. Vocational Center (Sewing, Hairdresser etc.) ☐
h. Shop Attendant /Owner (Sells general home goods, provisions, stationary etc.) ☐
i. Other (Specify)……..

Part B: TOXOPLASMOSIS-RELATED INFORMATION

1. Have you ever heard of *Toxoplasma gondii* or *Toxoplasmosis*? Yes ☐ No ☐
2. Have you ever been tested for *Toxoplasma* infection? Yes ☐ No ☐

If answer to Question 2 is "No", please skip Questions 3 and 4. Go to Question 5.

If "Yes", please answer Questions 3 and 4 before continuing with Question 5.

3. When was the test conducted?
   a. 3 to 6 months ago ☐
   b. Up to a year ago ☐
   c. More than a year ago ☐
   d. Other (Specify)……
4. What was the result? Positive ☐ Negative ☐ No idea ☐
   b. If Positive, did you receive any treatment? Yes ☐ No ☐
5. Would you like to be tested for *Toxoplasma* infection during this pregnancy?
   Yes ☐ No ☐
6. How many pregnancies have you had before the current pregnancy?
   a) None (this is my first) ☐
   b) One ☐
   c) Two ☐
   d) Three ☐
   e) More than three ☐
7. Have you ever had a stillbirth, spontaneous abortion(s) [miscarriage(s)]?
   a) Once ☐
   b) Twice ☐
   c) Three times or more ☐

d) Never ☐

8. How old is your current pregnancy?

a) 1 to 3 months ☐

b) 4 to 6 months ☐

c) 7 to 9 months ☐

9. Do you live with a cat? Yes ☐ No ☐

10. Do you have a sand box for your cat? Yes ☐ No ☐

11. Do you eat meat? Yes ☐ No, I'm a vegetarian ☐

12. Have you ever handled raw meat from pig, sheep goat or cow? Yes ☐ No ☐

If answer to Question 11 is "No" Please skip the following questions and go to Question 15.

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

a. Cooked but tough ☐

b. Cooked till soft ☐

c. Cooked tough or soft ☐

14. Which meat do you eat? (*Please choose as many as are applicable*)

a. Pork ☐ d. Chevon/sheep meat ☐

b. Mutton/Goat meat ☐

c. Beef ☐

e. Others(specify)……

15. Do you eat vegetables? Yes ☐ No ☐

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

a) Fresh and raw ☐

b) Steamed ☐

c) Cooked ☐

Part C: MALARIA -RELATED INFORMATION

17. Have you ever heard of Malaria? Yes ☐ No ☐

18. Were you infected with Malaria during your pregnancy? Yes ☐ No ☐

19. Were you given Malaria prophylaxis drugs during the Antenatal clinics? Yes ☐ No ☐

20. Did you adhere to all the precautions that were taught during your Antenatal sessions about Malaria Prevention? Yes ☐ No ☐

Thank you very much for your participation.

Appendix II: Consent form

CONSENT FORM

Statement of person obtaining informed consent:
I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____
NAME: _____

Statement of person giving consent:
I have read the information on this study/research or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide that I want to take part in it.

I understand that I may freely stop being part of this study at any time without having to explain myself.

I have received a copy of this information leaflet and consent form to keep for myself.

NAME:_____
DATE: _____
SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to
_____ (Name of Participant), in the local language, is a true reflection of what l have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

MOTHER'S SIGNATURE (maintain if participant is under 18 years): _____

MOTHER'SNAME:
_____

FATHER'S SIGNATURE (maintain if participant is under 18 years): _____

FATHER'SNAME:
_____