SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION AMONG PREGNANT WOMEN IN THE ASHANTI REGION OF GHANA: EVIDENCE FROM THE MANHYIA DISTRICT HOSPITAL, KUMASI.

By

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DECLARATION

I hereby declare that this submission is my own work towards the award of the M.Sc Clinical Microbiology and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the Kwame Nkrumah University of Science and Technology except where due acknowledgement has been made in the text.

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DEDICATION

This work is dedicated to my mum Madam Owusu Georgina, late Uncle Mr. Osei-Wusu Aduomi, late dad Mr. Kwaku Addai, Madam Akua Afriyie Comfort and my entire family.

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Ebenezer! This is how far the most Merciful Lord has brought me. I am most grateful to God for the strength and knowledge imparted in me and availing His big shoulders to support me.

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ABSTRACT

Toxoplasma gondii infection is a zoonotic protozoan infection that affects warmblooded animals, including humans. Congenital toxoplasmosis occurs when unborn and developing foetus becomes infected with *T. gondii* through the placenta *in utero*. The study sought to determine the seroprevalence of *T. gondii* infection among pregnant women seeking antenatal care at the Manhyia District Hospital. Consent form, structured questionnaires, as well as, about 3mls of venous blood was taken from 110 pregnant women for the study. Analysis of blood sera using commercial ELISA kit (Teco Diagnostics, 1268 N, Lakeview Ave., Anaheim, CA 92807, USA) showed that, 71.8% (79/110) were seropositive for *T. gondii* IgG antibodies whiles 39.1% (43/110) were seropositive for *T. gondii* IgM antibodies. A total of 30 representing 27.3% of the pregnant women were seropositive for both *T. gondii* IgG and IgM antibodies. Again, 44.5% (49/110) was reported for pregnant women with only *T. gondii* IgG antibodies and 11.8% (13/110) for only *T. gondii* IgM antibodies. The overall seroprevalence of toxoplasma IgG and IgM antibodies for the study was 83.6% (92/110).

It is recommended that screening of pregnant women for toxoplasmosis be done during routine antenatal laboratory test. Again, it is recommended that, a study to determine the risk factors for *T. gondii* infection among pregnant women in the Ashanti region of Ghana be done.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Toxoplasma gondii infection, according to Ryan and Ray (2004), is a protozoan infection that affects warm-blooded animals, including humans. It is a zoonotic infection caused by *T. gondii*, an obligate intracellular protozoan of cats that infects humans and other mammals, including birds as intermediate hosts. *T. gondii* belongs to subphylum Apicomplexa, Class Sporozoa and exists in three main forms. These forms according to Török *et al.* (2013) include

i. The oocysts, which are the infective form and release sporozoites ii. The trophozoites/Tachyzoites, which are the asexual form responsible for cell invasion and are crescent shaped. They are seen during acute stage of infection and invades all types of mammalian cells, except non-nucleated red blood cells

(RBCs) iii. The tissue cyst/Bradyzoites, which are made up of intracellular trophozoites and develop within cytoplasm of host cell.

The tissue cyst and oocysts are the main forms of the parasite that are involved in its transmission.

Infection of *T. gondii* is high in areas with hot, low altitudes and humid climatic conditions according to a study by Jones *et al.* (2003). Human infection of toxoplasmosis occurs through

i. Ingestion of tissue cysts in poorly cooked meat ii. Ingestion of food or water contaminated with mature oocysts faeco-orally iii. Transplacental (vertical) transmission from mother to foetus *in utero* iv. Although rare,

needlestick injury, organ transplantation and blood transfusion from seropositive donors.

Toxoplasmosis, according to Ayeh-Kumi *et al.* (2010) and Montoya and Liesenfeld (2004), is estimated to affect nearly a third of the global population. It has been estimated by Ayeh-Kumi *et al.* (2010) that between 30% and 65% of the global population are infected with toxoplasmosis.

Congenital toxoplasmosis occurs when unborn and developing foetus becomes infected with *T. gondii* through the placenta *in utero*. Maternal-fetal transmission of *T. gondii* occurs between one and four months after placenta has been colonized by tachyzoites as reported by Dubey *et al.* (2009) and Stray-Pedersen (1993). Garweg *et al.* (2005) and Liesenfield *et al.* (1997) reported that, congenital toxoplasmosis has adverse health consequences on pregnancies and newborns. Jones *et al.* (2003), Dunn *et al.* (1999) and Stray-Pedersen (1993) also reported that, the risk of congenital infection from acute *T. gondii* infection in pregnancies ranges between 20% and 50% when strict treatment regimens are not adhered.

Török *et al.* (2013) reported that, *T. gondii* hardly cause infections except when acquired congenitally. Consequences such chorioretinitis, hydrocephalus, microcephalus, intracranial calcification, encephalitis and convulsions are commonly related to *T. gondii* infection in pregnancies. Studies by Dunn *et al.* (1999) and Stray-Pedersen (1993) suggested intrauterine growth restrictions and intrauterine foetal death as common signs of *in utero* infection of *T. gondii* in severe cases.

1.2 Problem Statement

T. gondii infection is a protozoan infection that affects warm-blooded animals, including humans. Toxoplasmosis is considered as the third leading infectious disease that causes food-borne deaths after traditional Listeriosis and Salmonellosis (Jones *et al.*, 2001 and Dubey and Jones, 2008). Toxoplasmosis, according to Ayeh-Kumi *et al.* (2010) and Montoya and Liesenfeld (2004), is estimated to affect nearly a third of the global population. Similarly, Ayeh-Kumi *et al.* (2010) reported that, between 30% and 65% of the global population are infected with toxoplasmosis.

Jones *et al.* (1996) and Jacquire (1995) reported that, seroprevalence studies of toxoplasmosis vary geographically. Garweg *et al.* (2005) and Liesenfield *et al.* (1997) reported that, congenital toxoplasmosis has adverse health consequences on pregnancies and newborns. Consequences such chorioretinitis, hydrocephalus, microcephalus, intracranial calcification, encephalitis and convulsions are commonly related to congenital toxoplasmosis. Dunn *et al.* (1999) and Stray-Pedersen (1993) reported intrauterine growth restrictions and intrauterine foetal death as common signs of *in utero* infection in severe cases.

Despite the consequences associated with congenital toxoplasmosis, pregnant women are not screened for toxoplasmosis in Ghana probably for reasons of cost and lack of knowledge about toxoplasmosis. This neglect in systemic education and screening of pregnant women during antenatal care raises issues of public health concerns and ought to be addressed. It is in the light of the above that the researcher sought to determine the seroprevalence of *T. gondii* infection among pregnant women. It is hoped that the study may prove useful to the Government of Ghana by formulating appropriate healthcare

policies and advance knowledge on seroprevalence of congenital toxoplasmosis in the academia.

1.3 Research Question

This study sought to determine the seroprevalence of *T. gondii* infection among pregnant women seeking antenatal care at the Manhyia District Hospital, Kumasi in the Ashanti region of Ghana.

1.4 Research Objective

1.4.1 Main objective

The main objective of the study was to determine the seroprevalence of *T. gondii* infection among pregnant women seeking antenatal care (ANC) at the Manhyia District Hospital, Kumasi in the Ashanti Region of Ghana.

1.4.2 Specific objectives

- To estimate the seroprevalence of *T. gondii* IgG antibodies among pregnant women seeking antenatal care at the Manhyia District Hospital, Kumasi.
- To estimate the seroprevalence of *T. gondii* IgM antibodies among pregnant women seeking antenatal care at the Manhyia District Hospital, Kumasi.

1.5 Research Scope

The study employed purposive random sampling of pregnant women attending ANC clinic at the Manhyia District Hospital in Kumasi in the Ashanti region of Ghana. About

3mls of venous blood was aseptically drawn and primary information obtained through administration of structured questionnaires. The study covered a period of six months from September, 2014 to March 2015.

1.6 Justification of the Study

Ayeh-Kumi *et al.* (2010) reported that, congenital toxoplasmosis is an issue of global public health concerns, particularly in pregnancies and newborns. Chorioretinitis, microcephalus, hydrocephalus, convulsions, encephalitis, intracranial calcification and in severe cases fetal deaths have been associated with congenital toxoplasmosis (Garweg *et al.,* 2005 and Liesenfield *et al.,* 1997).

A study by Ayi *et al.* (2009) estimated the overall seroprevalence of *T. gondii* antibodies IgG, IgA and IgM in the Greater Accra region as 92.5%. Global studies on seroprevalence of *T. gondii* infection, particularly in pregnant women, are still inconclusive. Ashanti region of Ghana has no published studies on the seroprevalence of *T. gondii* infection among pregnant women. It is for this reason that the researcher was motivated to carry out this study to determine the seroprevalence of *T. gondii* infection among pregnant women and also to advance knowledge on the seroprevalence of *T. gondii* infection among pregnant women in the Ashanti region and Northern part of Ghana. This study may prove useful to the Ministry of Health, Ghana in formulating national health policies.

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomic Classification of T. gondii

Toxoplasmosis is a zoonotic infection caused by *T. gondii*, an obligate intracellular protozoan of the cat family, as definitive host, and parasitizes humans and other mammals, including birds as intermediate host (Ryan and Ray, 2004). *T. gondii* belongs to Kingdom Protoctista or Protista, Subkingdom Protozoa, Phylum Apicomplexa, Class Sporozoasida, Order Fucoccidiorida, family Sarcocystidae, Genus *Toxoplasma* and Species *gondii*.

2.2 Forms of T. gondii

T. gondii exists in three main forms. These forms according to Török *et al.* (2013) include

i. The oocysts, which are the infective form and release sporozoites ii. The trophozoites/Tachyzoites, which are the asexual form responsible for cell invasion and are crescent shaped. They are seen during acute stage of infection and invades all types of mammalian cells, except non-nucleated red blood cells

(RBCs) iii. The tissue cyst/Bradyzoites, which are made up of intracellular trophozoites and develop within cytoplasm of host cell.

The tissue cyst and oocysts are the main forms of the parasite that are involved in its transmission.

2.3 Transmission of T. gondii

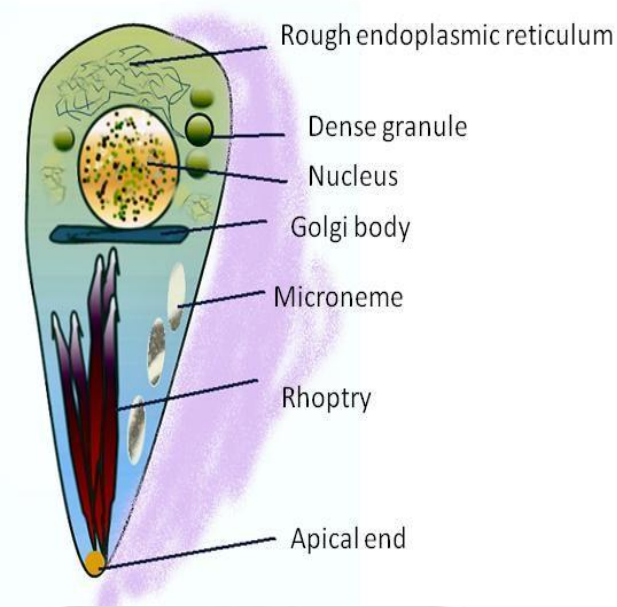
Cats, the definitive hosts of *T. gondii,* become infected by ingesting the sporulated oocysts or in some cases infected animals like rats or mice (Baron, 1996). The oocysts are highly contagious to most mammals, including humans and birds. Infection of *T. gondii* spread by one of the following four known routes

i. Ingestion of tissue cysts in raw or poorly cooked meat ii. Ingestion of food or water contaminated with mature oocysts faeco-orally iii. Transplacental (vertical) transmission from mother to foetus *in utero* iv. Although rare, needlestick injury, organ transplantation and blood transfusion from a seropositive donors.

2.4 Morphology of T. gondii

There are three main stages of *T. gondii* that are highly infectious to humans: the tachyzoites (in groups), the bradyzoites (in tissue cysts) and the sporozoites (in oocysts) (Dubey,1998). Sporozoites, bradyzoites and tachyzoites of *T. gondii* are ultrastructurally similar, with all three forms having similar number of rhoptries, although they differ in appearance, cell inclusions and organelles (Baron, 1996). According to studies by Dubey *et al*. (1998), bradyzoites differ structurally from tachyzoites. The nucleus of bradyzoites is situated towards the posterior end whilst the nucleus in tachyzoites is more centrally placed. Moreover the contents of rhoptries in tachyzoites are labyrinthine whereas that of the bradyzoites are usually electron dense (Kwofie, 2012).

Figure 2.4 Ultrastructure of *Toxoplasma gondii* as seen under the electron microscope



Source: Dubey *et al*., 1998

2.5 Life cycle of *T. gondii*

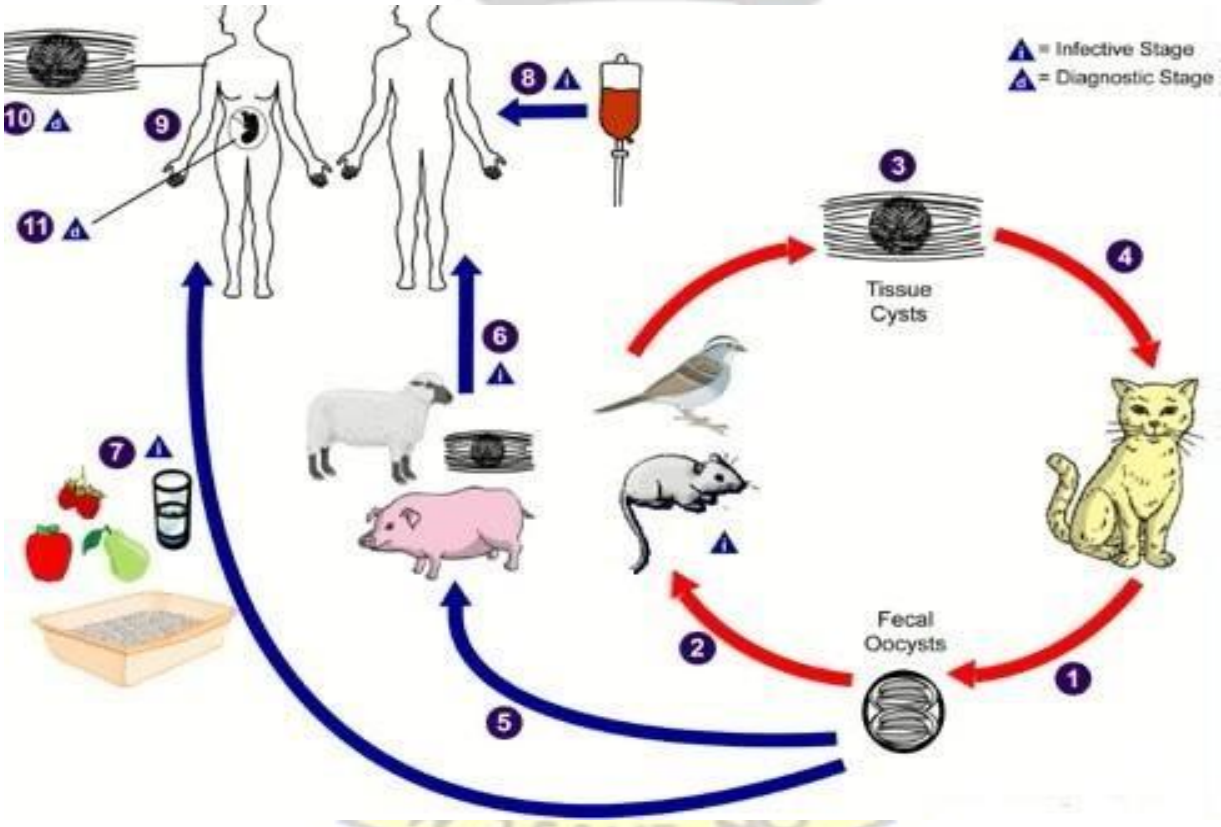
Following the discovery of the definitive host as members of the family Felidae, including domestic cats, the life cycle of *T. gondii* was promulgated in 1970 according to Baron (1996). Other warm blooded mammals including humans and birds serve as intermediate hosts.

Domestic cats become infected with *T. gondii* by ingestion of mouse contaminated with the *T. gondii* tissue cyst (Dubey, 1995). The parasites persist and passed to the stomach where they infect the epithelial cells of the small intestines of cats. The parasites then undergo sexual development and reproduce several zygotes, which contains oocysts.

Cat's epithelial cells infected burst and release oocysts, which are shed in cat's faeces (Dubey *et al.,* 2011). Intermediate hosts become infected by ingesting soil, water or plant materials contaminated with oocysts (Dubey, 2009 and Dubey *et al.,* 2011). Humans

become infected by ingesting unwashed vegetables or contaminated water or by handling litter of an infected cat (Baron, 1996 and Kapperud *et al.,* 1996). Oocysts transform into tachyzoites shortly after ingestion (Baron, 1996). These tachyzoites localize in neural and muscle tissue and develop into tissue cyst called bradyzoites, which contains the tissue cysts (Robert-Gangneux and Darde, 2012 and Miller *et al.,* 2009). Humans become infected by ingesting raw or poorly cooked meat containing the tissue cyst (Baron, 1996). Figure 2.4 below summarizes the activities involved in the life cycle of *T. gondii*.

Figure 2.5 **Life cycle of** ***Toxoplasma gondii***



Source: Centers for Disease Control and Prevention (CDC), 2013.

2.6 Pathogenesis of toxoplasmosis

Development of toxoplasmosis takes place after infection with *T. gondii* parasites congenitally or ingestion of contaminated tissue cysts in infected meat or sporulated oocysts in food or water contaminated with cat's faeces as reported by Baron (1996). During the incubation period of 5 to 18 days, the host cell becomes engaged and finally gets destroyed as a result of active multiplication by cell division of *T. gondii* as opined by Dubey *et al.* (1998).

Stray-Pedersen (1993) and Dunn *et al.* (1999) reported that, a more severe form of congenital toxoplasmosis involves infection of the retina and cause chorioretinitis which is characterized by vision impairment. When the brain is involved in congenital toxoplasmosis, mostly in immunosuppressed patients, necrotic abscess of the brain may occur as reported by Stray-Pedersen (1993) and Dunn *et al*. (1999).

2.7 Survival Mechanisms of *T. gondii*

T. gondii upon infection of humans and subsequent invasion undergo some processes to escape damage by the host's immune system. Among them include the use of plasmids which it uses to infect host cells (Henrik, 1999). This enables it to resist damage by the host's immune system.

The use of anti-apoptotic mechanism is also a mechanism employed by *T. gondii* to escape damage by host immune system. This according to Hippe *et al.* (2009) involves disruption of pro-apoptosis effector proteins like Bak and Bax. In disrupting these proteins, *T. gondii* changes shape and structure of the pro-apoptosis effector proteins.

This leads to the proteins' inability to be transported to the host cells and in the process apoptosis is initiated.

Autophagy of host's cell is another mechanism employed by *T. gondii* to escape damage. This is due to the ability of *T. gondii* to initiate autophagy. This reduces the volume of host cells, thereby reducing the host immune system's ability to destroy *T. gondii* according to Wang *et al.* (2009).

2.8 Epidemiology of *T. gondii*

Toxoplasmosis is considered as the third leading infectious disease that causes foodborne deaths after traditional Listeriosis and Salmonellosis according to Jones *et al.* (2001) and Dubey and Jones (2008). Studies into seroprevalence of toxoplasmosis vary considerably in different countries and continents as reported by Jacquire (1995). The variation, according to Furtado *et al.* (2013), is thought to be as a result of geographical, socio-economical and environmental factors such as host age, genetic and immune status of host. Among the other factors include parasite genotype, parasite load and stage of parasite development.

Maternal-fetal transmission of *T. gondii* occurs between one and four months after placenta has been colonized by tachyzoites as reported by Dubey *et al*. (2009) and Stray-Pedersen (1993). This by Garweg *et al.* (2005) and Liesenfield *et al.* (1997) has adverse health consequences on pregnancies and newborns. Studies by Furtado *et al*. (2013) reported that, the risk of mother-to-child transmission increases as pregnancy progresses. However, infection of *T. gondii* acquired in the early stages of pregnancy is more probable to result in clinical disease. Moreover, Jones *et al*. (2003), Stray-Pedersen (1993) and

Dunn *et al.* (1999) estimates that, the risk of congenital infection from acute *T. gondii* infection in pregnancies ranges between 20% and 50% when strict treatment regimens are not adhered. Dunn *et al.* (1999) estimated the risk of mother-to-child transmission in France between 1987 and 1995 to be 6% at 13 weeks gestational age, 40% at 26 weeks and 72% at 36 weeks gestations. Congenital toxoplasmosis transmission can result in severe to fatal sequelae in fetuses and newborns.

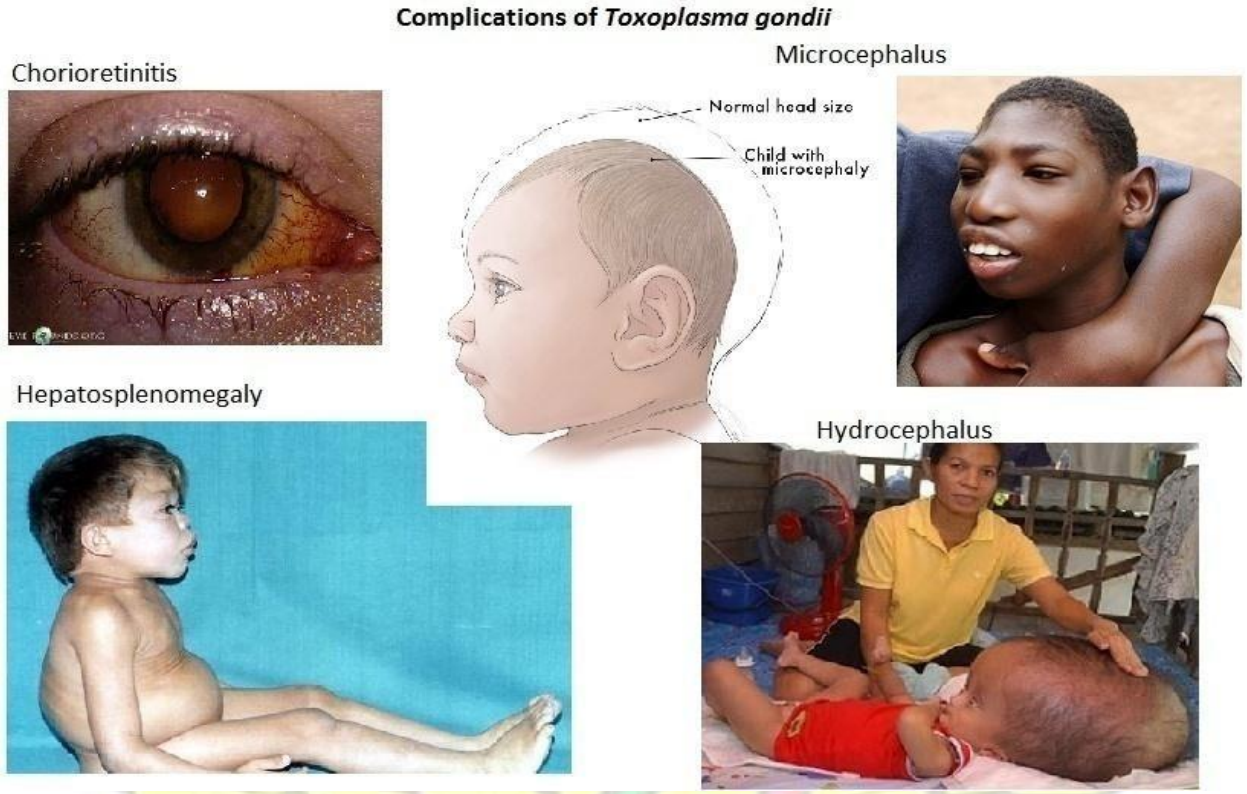
2.9 Clinical manifestations of toxoplasmosis

T. gondii infection during pregnancy poses threat on fetuses and newborns. Remington *et al*. (1995) and Mitchell *et al.* (1990) observed that, in pregnant women with AIDS and those on high dose immunosuppressive therapy like organ transplant, patients with malignancies and connective tissue disorders, there could be reactivation of *T. gondii* which may be transmitted to the foetus to cause infection. This may cause severe encephalitis, myocarditis, hepatitis or pneumonitis according to a study by Montoya and Remington (2008).

Other clinical manifestations of congenital toxoplasmosis include intracranial calcification, microcephalus, hydrocephalus, convulsion and severe restriction of intrauterine growth as observed by Dubey and Jones (2008), Jones *et al.* (2003), Dunn *et al.* (1999) and Stray-Pedersen (1993).

In neonates, extensive studies by Di Carlo *et al.* (2008) and Brown *et al.* (2009) revealed that, delay in treatment of toxoplasmosis could lead to development of severe sequelae, including neurological abnormalities and mental impairment.

Figure 2.9 Pictorial presentation of the Clinical complications of toxoplasmosis



Source: CDC, 2013

2.10 Toxoplasmosis in Meat animals

Toxoplasmosis in meat animals is often regarded as an issue of medical importance and public health concern because of the major economic importance of the disease to meat animals and humans as consumers. A study by Fayer (1981) suggested that although transmission of *T. gondii* by tissue cysts occurs among carnivores, transmission of tissue cysts of *T. gondii* by herbivorous and omnivorous meat animals is relatively higher. Studies by Arko-Mensah (1999) and Gilbert (2002) reported that, most adults acquire toxoplasmosis through ingestion of raw or poorly cooked meat contaminated with tissue cyst. Today, meat animals such as sheep, goats, cattle and pigs among others are consumed

on daily basis and at higher quantities and rates in Ghana. Vanderpuije *et al*. (2000) reported an overall prevalence of toxoplasma antibodies in Ghanaian sheep and goats at 30.5%. Arko-Mensah (1999) reported a prevalence of 39% toxoplasma antibodies in Ghanaian pigs. An average global seroepidemiological infection rate of 25%, 31% and 29% have been reported for cattle, sheep and goats respectively for *T. gondii* infection (Fayer, 1981 and Dubey, 1986a). These meat animals and/or their products such as unpasteurised milk, when contaminated with the oocysts of *T. gondii,* may pose health risks to Ghanaian consumers, particularly pregnant women. Tachyzoites of *T. gondii* can be transmitted in unpasteurized goat milk to human consumers, particularly pregnant women (Reimann *et al.*, 1975).

2.11 Global Seroprevalence of toxoplasmosis

Congenital toxoplasmosis has serious health consequences on pregnancies and newborns (Garweg *et al.*, 2005 and Liesenfield *et al.*, 1997). The risk of congenital infection from recent *T. gondii* infection in pregnancies ranges between 20% and 50% when strict treatment regimens are not adhered as studied by Jones *et al*. (2003), Dunn *et al.* (1999) and Stray-Pedersen (1993). Remington *et al.* (2001) and Dunn *et al.* (1999) reported that, the risk of congenital transmission of toxoplasmosis is low and ranges between 10% and 25% when acute maternal infection (IgM) is detected during the first trimester of gestation and high (65-90%) when acute maternal infection is detected during the third trimester of gestation.

Seroprevalence studies on toxoplasmosis vary geographically (Jacquire, 1995).

Toxoplasmosis, reported by Ayeh-Kumi *et al*. (2010) and Montoya and Liesenfeld (2004), is estimated to affect nearly a third of the global population. It has also been reported by

Ayeh-Kumi *et al.* (2010) that between 30% and 65% of the global population are infected with toxoplasmosis. The global seroprevalence is reportedly estimated as 46.1% (Jacquire, 1995). A study by Jones *et al.* (1996) indicated that toxoplasma encephalitis in HIV-infected individuals varied geographically. The seroprevalence of latent toxoplasma infection, according to Partisani (1991), in Europe, Africa and Latin America is estimated at 75-90%. Montoya and Liesenfeld (2004) estimated the seroprevalence of toxoplasma infection at 75% in El Salvador. A study also by Dupont *et al*. (2012) described the fatality of toxoplasma infection particularly in individuals with weakened immune system such as HIV/AIDS individuals and most importantly in pregnant women. Again, a seroprevalence of *T. gondii* IgG antibodies of

81.1% has been reported by Zemene *et al.* (2012) in the Southwestern Ethiopia and Gebremedhin *et al.* (2013) in the Central Ethiopia. Similarly, Akinbami *et al.* (2010) in Nigeria estimated the seroprevalence of toxoplasma IgG antibodies as 40.8%.

CDC between 1999 and 2004 estimated the seroprevalence of toxoplasmosis in the United States at 10.8% with seroprevalence among women of childbearing age (15-44 years) as 11.0% according to Jones *et al.* (2007). Nester *et al.* (2004) and Garcia *et al.* (1999) also reported that, the distribution of toxoplasmosis increases with age and women of child-bearing age.

2.12 Prevalence of toxoplasmosis in Ghana

In Ghana, prevalence studies on toxoplasmosis have been concentrated at the Greater Accra region. Anteson *et al.* (1978b) reported a seroprevalence of 76% of toxoplasma antibodies among pregnant women attending prenatal clinic at the Mamprobi Clinic, Accra. Ayi *et al.* (2009) reported the overall seroprevalence of *T. gondii* IgG, IgA and

IgM antibodies in the Greater Accra region as 92.5%. Ayi *et al.* (2009) in the Greater Accra region reported *T. gondii* IgG antibodies as 73.6% against 76.1% for *T. gondii* IgM antibodies. Studies by Ayeh-Kumi *et al.* (2010) reported 32.7% seroprevalence of *T. gondii* IgG antibodies against 29.7% for *T. gondii* IgM antibodies in the Greater Accra region. Ayeh-Kumi *et al.* (2010) reported an overall seroprevalence of *T. gondii* antibodies as 49.7% in the Greater Accra region. Kwofie (2012) in the Greater Accra region also reported the *T. gondii* IgG antibodies as 37.6% against 0.0% for *T. gondii* IgM antibodies.

2.13 Diagnosis of toxoplasmosis

Diagnosis of toxoplasmosis in humans is generally based on serology and on histologic examination of tissues, as well as, molecular methods using the Polymerase Chain Reaction (PCR).

2.13.1 Serological Detection of *T. gondii*

This technique involves the detection of *T. gondii* antibodies in the serum of infected patients. Key among the serological methods employed in diagnosing toxoplasmosis include the Sabin-Feldman Dye test, the Indirect Haemagglutination assay, the Indirect Fluorescent antibody assay (IFA), the direct agglutination test (DAT), the Latex agglutination test (LAT), the Enzyme-Linked Immunosorbent assay (ELISA) and the Immunosorbent agglutination assay test (IAAT).

According to Hill and Dubey (2002), the most effective and preferred method and hence the gold standard for diagnosing toxoplasmosis is the Sabin-Feldman Dye test. This method is extensively used due to its high sensitivity and specificity for toxoplasmosis.

However, it is costly and highly hazardous when used on live humans compared to ELISA as reported by Baron (1996).

2.13.2 Histologic Detection of *T. gondii*

This technique involves the detection of *T. gondii* in host tissue biopsy or necropsy. A study by Baron (1996) indicated that, histologic detection is most effective for immunosuppressed patients, who may have delayed synthesis of antibodies and have low volume of antibodies produced.

2.13.3 Molecular detection of *T. gondii*

This technique involves the detection of the genetic material (DNA) of biological samples. Molecular detection requires the use of Polymerase chain reaction (PCR) to isolate and amplify Deoxyribonucleic acid (DNA) from biologic samples as reported by Switaj *et al.* (2005). The use of PCR is most appropriate for patients with immunodeficiencies (Kwofie, 2012).

2.14 Treatment of toxoplasmosis

The principal drugs of choice used extensively to treat toxoplasmosis in humans are Spiramycin and Pyrimethamine. Spiramycin, a macrolide antibiotic, is used as fetal prophylaxis to prevent vertical transmission of *T. gondii* parasites to fetuses (Montoya and Remington, 2008). It is used after maternal infection of *T. gondii* has taken place whiles the fetus is uninfected.

Pyrimethamine and sulfadiazine are used after fetal infection has taken place. These drugs act as agents to produce a combined effect by blocking the pathway for cellular

metabolism that involves p-aminobenzoic acid (Sulphonamides) and folic-folinic acid cycle (Pyrimethamine) as reported by Caroline and Mark (2013).

However, Pyrimethamine and sulphonamide therapy must not be administered during the first trimester of pregnancy due to its potential teratogenicity (Montoya and Remington, 2008). Studies by Baron (1996) reported that, development of thrombocytopenia and/or leucopenia may occasionally occur as a result of Sulphonamide or Pyrimethamine therapy. However, a combination therapy with folic acid reduces thrombocytopenia.

2.15 Prevention of toxoplasmosis

In spite of the complexity in the mode of transmission of toxoplasmosis, prevention of it can be done by improved personal and group hygiene. Prevention of toxoplasmosis requires avoidance of tissue cysts and oocysts from the environment (Lappalainen and Hedman, 2004).

Institution of educational and public health programs, particularly during pre-natal care can reduce toxoplasma infection (Fabiana *et al.,* 2007). Among these programs include wearing of gloves when changing litter and ridding of cats faeces and thorough washing of hands with soap after handling raw meat and kitchenwares as reported Foulon (1992) and Hill and Dubey (2002).

Pregnant women and immunocompromised patients must avoid contact with soil, cats and consumption of raw meats and products like unpasteurised milk (Fabiana *et al.,*

2004). Fresh meat and other products should be cooked at 67ºC or frozen to -20ºC. Fruits and other raw vegetables must be washed thoroughly before consumption (Hill and Dubey, 2002).

2.16 Antibodies

Antibodies are group of glycoproteins, which are sensitized and secreted by plasma cells. They comprise light chain and heavy chain proteins that form a Y-shaped structure. Whiles the base of the Y-shaped structure is a conserved region and thus common with all antibodies, the tips of the forks of the Y-shaped structure are peculiar to each antibody (Selamawit, 2004). The tips react with antigens, while the conserved region acts on the immune system (Litman *et al.,* 1993). Antibodies are generally secreted in response to antigenic stimulation and thus constitute approximately 20% of plasma protein (Selamawit, 2004).

The principal goal of an antibody is to provide defense to the body by combining with antigens, thereby neutralizing bacteria, toxins, viruses and other pathogens and foreign bodies, including *T. gondii* (Maverakis *et al.,* 2015).

2.16.1 Types of antibodies/Immunoglobulins

There are basically five main types of antibodies formed in the body. These are

i. **IgG:** It is the most abundant immunoglobulin that are secreted in the body and forms approximately 80% of the total antibodies according to Selamawit (2004). It diffuses more easily relative to other immunoglobulins into extra vascular spaces, and neutralizes toxin while binding to microorganisms in the extra vascular spaces. It is therefore the only antibody that can cross the placenta in humans, where it provides immunity onto the foetus and newborn (Pier *et al.,* 2004). The conferred immunity produces protection for the baby for the first 6-

12 months of life, while the baby's own immune system matures. IgG has the ability to prevent the systemic spread of infections and promotes recovery from many infections.

ii. **IgM:** According to Selamawit (2004), IgM comprises approximately 10% of all immunoglobulins in the blood serum. It is synthesized by plasma cells early in primary infections to prevent the spread of pathogens during the early stages of infection (Pier *et al.,* 2004 and Geisberger *et al.,* 2006).

iii. **IgA:** It accounts for approximately 20% of all circulatory immunoglobulins (Selamawit, 2004). It is mostly present in serum, tears, sweat, milk, colostrums, saliva and other intestinal secretions (Pier *et al.,* 2004). IgA are synthesized as dimmers that fuse with a short J chain (polypeptide). This makes IgA more resistant to action by proteases. IgA in breast milk prevents colonization of the gastrointestinal tract by invading pathogens in the early stages of life of newborns (Selamawit, 2004). They are therefore unable to cross the placenta in humans.

iv. **IgD:** It is a monovalent immunoglobulin that accounts for less than 1% of all immunoglobulins (Selamawit, 2004). It is found on the surface of Blymphocytes where they join with monomeric IgM. This makes them serve as antigen receptors for activation of basophils and mast cells to produce antimicrobial factors as reported by (Geisberger *et al.,* 2006).

v. **IgE:** It is a monomer and accounts for 0.004% of all serum immunoglobulins (Selamawit, 2004). Pier *et al.,* 2004 reported that, IgE molecules bind to allergens and tissue cells, particularly mast cells and basophils. Reactions of antigens with IgE result in allergic reactions such as asthma, hives, and hay fever among others.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was undertaken at the Manhyia District Hospital, Kumasi in the Ashanti region of Ghana. The Ashanti region, the third largest region, is located in the Southern part of Ghana. The region lies between longitude 0.15W and 2.25W, and latitudes 5.50N and 7.46N. It occupies a landmass of 24,389km^2 and has 27 administrative Districts. There are over 40 hospitals, including the Manhyia District Hospital, serving some of the citizens

of the region. The Manhyia District Hospital is located at the Manhyia South District and serve as the third largest hospital in the Ashanti region. The hospital has high turnout of antenatal care (ANC) attendance and also has functional Obstetrics and Gynaecology (O&G) Clinic in the Ashanti region that is only second to Komfo Anokye Teaching Hospital (KATH).

3.2 Subjects

The study was undertaken at the Manhyia District Hospital because it constantly records high ANC attendance of at least 30 daily. The study involved recruiting pregnant women aged between 15 and 40 years and attending the ANC clinic. Study participants had to meet the inclusion criteria to be included in the study.

3.3 Nature of the Study

Research can be

a. Quantitative research: It is the type of research that aims at measuring or quantifying data and also comparing the data with past data and sometimes makes projections into the future.
b. Qualitative research: It is the type of research that involves gathering, analysing and interpreting data merely by observation. This type of research is somewhat open and exploratory. Tools for obtaining information or data include focus group discussion and interviews

c. Mixed-method research: This type of research combines both the qualitative and quantitative research designs in gathering, analysing and interpreting the data. It has become more common because it seeks to eliminate the weaknesses of both qualitative and quantitative research.

In order to fully satisfy the research question of the study, as well as, the research objectives, the mixed-method research design was the most preferred.

3.4 Data Consideration and Source

Sources of data for research could be either

i. Primary data: This is the type of data usually in its raw form and is mostly first-hand data or information. Data obtained is done by the researcher conducting the study

ii. Secondary data: It is the type of data that is not obtained by the researcher but someone else. Common sources include censuses, organizational records and through other qualitative methodologies.

In gathering data for the study, both primary and secondary data were obtained through administration of structured questionnaires and drawing blood samples from each enrolled pregnant woman. Secondary data was obtained from published journals and articles, as well as, information obtained from the Manhyia District Hospital.

3.5 Study Population

The study population involved pregnant women aged between 15 and 40 years who sought antenatal care at the Manhyia District Hospital, Kumasi and satisfied the inclusion criteria.

3.6 Inclusion/Exclusion Criteria

3.6.1 Inclusion Criteria

Pregnant women aged between 15 and 40 years and attending the ANC clinic at the Manhyia District Hospital, Kumasi were enrolled for the study. Again, pregnant women enrolled were certified to be medically fit by the Specialist Obstetrician and Gynaecologist, including having haemoglobin (Hb) concentration of at least 12.0 g/dl. Additionally, pregnant women were included in the study upon signing/thumb printing the consent form for the study.

3.6.2 Exclusion Criteria

i. Pregnant women who failed to sign/thumb print the consent form were excluded from the study ii. Pregnant women with Hb less than 12.0 g/dl were not considered for the study

3.7 Study Participants

Pregnant women within the study population who volunteered and satisfied the inclusion criteria were enrolled for the study**.**

3.8 Sample size

A total of 110 blood samples were drawn from pregnant women seeking antenatal care at the Manhyia District Hospital. This figure was arrived at using the relation

$$N = \underline{[\ Z^2\ (P)\ (1\text{-}P)\ ]}$$

$$(\text{Error})^2,$$

where N= Sample size, Constant set by convention, Z= 1.96, P= Previous study's prevalence as obtained from Ayi *et al.* (2009), P = 92.5% (0.925). Error was calculated at 5% (0.05).

$$N = \frac{[1.96^2 \text{ X } 0.925 \text{ X } (1-0.925)]}{0.05^2}$$

$$N = \frac{[3.8416\text{X } 0.925 \text{ X } 0.075]}{0.0025}$$

$$N = 106.6$$

In approximation, the Sample size, N was taken as 110 pregnant women.
Similarly, a total of 110 consent forms and questionnaires were administered, completed and returned for analysis.

3.9 Informed consent and Questionnaire administration

Each pregnant woman recruited for the study was made to sign or thumb print written consent form after the study had been explained to their full understanding. Questionnaires were also administered to obtain primary data from pregnant women recruited for the study.

3.10 Research Design

Research can be categorized into exploratory, descriptive and explanatory (causal). Explanatory (or causal) study is a valuable means of looking at cause and effect relationships between variables. It is mostly found in quantitative studies. Descriptive research seeks to describe the characteristics of population or phenomenon being studied.

The objective of descriptive research is to portray an accurate profile of person, event or situation.

Exploratory research on the other hand, according to a study by Saunders *et al*. (2007), is a valuable means of finding out what is happening; to seek new insights, to clarify an understanding of a problem.

Based on the above, this study combined both descriptive and exploratory research design to determine the seroprevalence of *T. gondii* infection among pregnant women attending ANC clinic at the Manhyia District Hospital, Kumasi.

3.11 Blood sample collection

About 3mls of venous whole blood was drawn aseptically from each enrolled pregnant woman into serum separator tubes. It was then centrifuged at 1000 rpm for 10 minutes and blood sera separated into labeled cryotubes and stored at -20ºC until use. Again, questionnaires and consent forms were administered to recruited pregnant women.

3.12 Analysis of Samples

3.12.1 ELISA Principle, Procedure and Precautions

Quantitative determination of the levels *T. gondii* IgG and IgM antibodies in patient's serum was determined using commercial ELISA kit (Teco Diagnostics, 1268 N, Lakeview Ave., Anaheim, CA 92807, USA) in accordance with manufacturer's procedure. See Appendix II for complete components of the ELISA kit for *T. gondii* IgG and IgM antibodies.

3.12.1.1 ELISA Principle

Purified *T. gondii* antigen is coated on the surface of microwells. Diluted patient serum is added to the microtiter wells, and *T. gondii* IgG and IgM-specific antibodies, if present, binds to the antigen. All unbound materials are washed away. Horse Radish Peroxidase (HRP) conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of Tetramethylbenzidine (TMB) reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of the IgG/IgMspecific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

3.12.1.2 Assay Procedure

All reagents contained in the ELISA kit (Catalog numbers: IGMT-96 and IGGT-96) were brought to room temperature (18-25ºC). Again, one volume of wash buffer was diluted with 19 equal volumes of distilled water and mixed before use. The following were the assay procedure used as directed by the commercial ELISA kit manufacturer (Teco Diagnostics, 1268 N, Lakeview Ave., Anaheim, CA 92807, USA).

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control and calibrators by adding 5µL of the sample to 200µL of sample diluents. Mix well
3. Dispense 100µL of diluted sera, calibrators, and controls into the appropriate wells. For the reagent blank, dispense 100µL sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well
4. Incubate at 37ºC for 30 minutes

5. At the end of the incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted wash Buffer (1X)
6. Dispense 100µL of enzyme conjugate to each well. Mix gently for 10 seconds
7. Incubate at 37ºC for 30 minutes
8. Remove enzyme conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted wash Buffer (1X)
9. Dispense 100µL of TMB Reagent into each well. Mix gently for 10 seconds
10. Incubate at 37ºC for 15 minutes
11. Add 100µL of stop solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds.
13. Read Optical Density (OD) at 450nm within 15 minutes with ELISA microwell reader.

3.12.1.3 Quality Control (QC) and Precautions

The following were the principal QC and precautions taken to ensure reliability and reproducibility in the result of the ELISA test as was required by the ELISA kit manufacturers

a. The O.D value of the reagent blank against air from a microwell reader was less than 0.250 as was required by manufacturer
b. The values for the cut-off calibrator *T. gondii* IgG and *T. gondii* IgM for both negative and positive controls were in the range on the certificate of analysis as provided by the manufacturer.
c. Blue (IgM) and green (IgG) colours were carefully made to change to yellow colour completely.

d. There were no air bubbles in each well before reading

e. Reading was strictly done within 15 minutes of stopping the reaction

3.12.1.4 Interpretation of Results

Optical Density (O.D) results obtained from the ELISA reader was interpreted as follows based on the manufacturers' instruction

i. **Negative –** *T. gondii* IgG and IgM O.D value less than 0.90 (< 32IU/ml) indicated negative for IgG or IgM antibody to *T. gondii*.

ii. **Equivocal –** O.D values between 0.91-0.99 is equivocal and hence, test should be repeated *iii.* **Positive –** *T. gondii* IgG and IgM O.D value of 1.00 or greater or 32 IU/ml or greater is positive for IgG or IgM antibody to *T. gondii.*

3.13 Data Analysis

Scientific Program for Social Sciences (SPSS, Version 16) software and Microsoft Excel were used to analyse the responses from the completed questionnaires. Pictorial presentation was employed to enhance the presentation of the study.

3.14 Ethical Consideration

Ethical clearance was sought from the Manhyia District Hospital. Informed written consent was sought from each pregnant woman before venous blood sample was drawn and questionnaire administered. Information obtained from respondents were strictly treated as confidential and used solely for academic purposes.

CHAPTER FOUR

RESULTS

4.1 General characteristics of study participants

Out of the 192 pregnant women approached for the study, 110 were enrolled based on the inclusion/exclusion criteria set out for the study. More so, from the 110 pregnant women enrolled for the study, 63.6% (70/110) were aged between 21 and 30 years with 30.0 % (33/110) between 31 and 40 years. Similarly, 33 and 44 representing 30.0% and 40.0% had attained basic and secondary/middle school educational levels respectively. A total of 20 representing 18.2% had no formal education. Again, 85.5% (94/110) and 13.6% (15/110) were within the third and second trimesters of pregnancy respectively. Married women were 96 representing 87.3% with 10.9% (12/110) and 1.8% (2/110) being single and widowed respectively. On occupation, 35.5% (39/110) and 27.3% (30/110) were traders and self employed respectively. Again, 28 representing 25.5% were unemployed.

From the questionnaire administered, none of the 110 (0.0 %) pregnant women enrolled for the study had ever been screened for toxoplasmosis.

4.2 Seroprevalence of ELISA Anti-*Toxoplasma gondii* IgG and IgM

ELISA conducted for *T. gondii* IgG antibodies indicated 79 were seropositive. This represented 71.8% with 28.2% (31/110) seronegative for *T. gondii* IgG antibodies. From the 110 pregnant women enrolled, 43 representing 39.1% were ELISA *T. gondii* IgM antibodies seropositive with 60.9% (67/110) seronegative for Anti-*T. gondii* IgM. Both *T. gondii* IgG and IgM antibodies were both present in 27.3% (30/110) of pregnant women enrolled. More so, 44.5% (49/110) and 11.8% (13/110) were reported for pregnant women with only *T. gondii* IgG and IgM antibodies respectively. The overall seroprevalence for the study was 83.6% (92/110).

Table 4.2 below summarizes the result of the seroprevalence of Anti-*Toxoplasma gondii* IgG and IgM.

Table 4.2: Seroprevalence of Anti-*Toxoplasma gondii* IgG and IgM

Result	**IgG (%)**	**IgM (%)**	**IgG and IgM (%)**
Positives	79 (71.8)	43 (39.1)	
	49 (44.5)*	13 (11.8)*	30 (27.3)
Negatives	31 (28.2)	67 (60.9)	
	61 (55.5)*	97 (88.2)*	
			80 (72.7)
Total	**110 (100)**	**110 (100)**	**110 (100)**

*only one *T. gondii* antibody

4.3 Age distribution among seropositives for ELISA Anti-*Toxoplasma gondii* IgG and IgM.

From the 79 seropositives for ELISA *T. gondii* IgG antibodies, 64.6% (51/79) were aged between 21 and 30 years. Additionally, 3.8% (3/79) were between 15 and 20 years with 31.6% (25/79) aged between 31 and 40 years. The highest seroprevalence of *T. gondii* IgG antibodies was between the ages of 21 and 30 years. It was also observed that 96.2% (76/79) were between the ages of 21 and 40 years.
The highest seroprevalence of *T. gondii* IgM antibodies of 55.8% (24/43) was between the ages of 21 and 30 with 95.3% (41/43) aged between 21 and 40 years. Table 4.3 below shows the age distribution among seropositives for ELISA Anti-*Toxoplasma gondii* IgG and IgM.

Table 4.3: Age distribution among seropositives for ELISA Anti-*Toxoplasma gondii* IgG and IgM

Result (Years)	№ of positives IgG (%)	№ of positive IgM (%)
15-20	3 (3.8)	2 (4.7)
21-30	51 (64.6)	24 (55.8)
31-40	25 (31.6)	17 (39.5)
Total	**79 (100)**	**43 (100)**

4.4 Duration of pregnancy among respondents seropositive for ELISA Anti-*Toxoplasma gondii* IgG and IgM

The study showed that, 87.3% (69/79) of the seropositives were in their third trimester of pregnancy with 11.4% (9/79) and 1.3% (1/79) in their second and first trimesters of gestation respectively. A total of 78 of the seropositives representing 98.7% were either in their second or third trimester of pregnancy.

Anti-*T. gondii* IgM were present in 79.1% (34/43) of the pregnant women in their third trimester gestation. Moreover, 20.9% (9/43) and 0.0% (0/43) of the seropositive *T. gondii* IgM antibodies pregnant women were in their second and first trimester gestation. All (100%) seropositive pregnant women were either in their second or third trimester of pregnancy.

Table 4.4 below provides a summary of the duration of pregnancy among pregnant women seropositive for ELISA Anti-*Toxoplasma gondii* IgG and IgM.

Table 4.4: Duration of pregnancy among respondents seropositive for ELISA Anti-*Toxoplasma gondii* IgG and IgM

Duration of Pregnancy	№ of positive IgG (%)	№ of positive IgM (%)
First trimester	1 (1.3)	0 (0.0)
Second trimester	9 (11.4)	9 (20.9)
Third trimester	69 (87.3)	34 (79.1)
Total	**79 (100)**	**43 (100)**

CHAPTER FIVE DISCUSSION

Congenital toxoplasmosis poses a severe risk to pregnancies and newborns and hence, remains an issue of public health concern as reported by Ayeh-Kumi *et al.* (2010) and Montoya and Liesenfeld (2004). In determining the seroprevalence of *T. gondii* infection among pregnant women attending ANC clinic at the Manhyia District hospital, it was observed that the seroprevalence of *T. gondii* IgG antibodies was 71.8% (79/110), which indicates a past exposure to *T. gondii*. This value of 71.8% is similar to a study by Ayi *et al.* (2009) in the Greater Accra region (Accra) which indicated a seroprevalence of anti-*T. gondii* IgG as 73.6%. However, the seroprevalence of *T. gondii* IgG antibodies for the current study is lower than 81.1% reported by Zemene *et al.* (2012) and Gebremedhin *et al*. (2013) in the Southwestern and Central Ethiopia respectively. Akinbami *et al.* (2010) in Nigeria reported a lower seroprevalence of *T. gondii* IgG antibodies as 40.8%. Again, Ayeh-Kumi *et al*. (2010) and Kwofie (2012) reported lower seroprevalence of anti-*T. gondii* IgG of 32.7% and 37.6% respectively compared to the current study.

Similarly, 39.1% (43/110) was observed for *T. gondii* IgM antibodies. The significance of the seropositive *T. gondii* IgM antibodies indicates a recent exposure to *T. gondii.* The seroprevalence of *T. gondii* IgM antibodies of the current study is higher compared to 29.7% and 0.0% reported by Ayeh-Kumi *et al.* (2010) and Kwofie (2012) in the Greater Accra region (Accra) respectively. However, Ayi *et al.* (2009) in Accra reported the seroprevalence of anti-*T. gondii* IgM of 76.1% which is higher.

IgG immunoglobulins is a marker of chronic (old) infection and shows that an individual has been previously infected with infection (toxoplasmosis) whereas IgM

immunoglobulin, a marker of acute (recent) infection, is used in determining the time of infection. The higher seroprevalence of *T. gondii* IgG antibodies as obtained from the study is comparable to a study by Selamawit (2004) which sought to show that, IgG are the most abundant immunoglobulin that are secreted in the body. The presence of high levels of anti-*T. gondii* IgG in the body merely indicated past exposure to *T. gondii*. However, that does not distinguish recent infection from past infection of toxoplasmosis. The more seronegative anti-*T. gondii* IgM with a corresponding high seropositive anti-*T. gondii* IgG shows *T. gondii* infection of at least six months earlier. This is because IgM immunoglobulins appear much earlier following an infection than IgG immunoglobulins and disappear faster relative to IgG antibodies following recovery. This means IgM immunoglobulins are detected earlier during acquired primary infection. However, the titers of IgM immunoglobulins decrease and become negative within some few months. These assertions are consistent with a report by Hill and Dubey (2002) and Montoya (2002).

From the study it was also observed that, 27.3% (30/110) tested seropositive to both *T. gondii* IgG and IgM antibodies. This is because some seropositive anti-*T. gondii* IgM titers, although some disappear, may reactivate during the chronic stage of the infection and hence test seropositive for anti-*T. gondii* IgG. This is comparable to a study by Montoya (2002).

The high seropositive of both anti-*T. gondii* IgG and anti-*T. gondii* IgM of 87.3% (69/79) and 79.1% (34/43) respectively for pregnant women in their third trimester of gestation poses health risk to the pregnancies and newborns. This is because contraction of congenital toxoplasmosis prior to pregnancy is in most cases less fatal. This is attributed to the inability of the *T. gondii* to pass from the mother to the foetus *in utero* following

establishment of immunity to the *T. gondii* infection. The condition may be fatal for pregnant women infected by *T. gondii* for the first time. This is because, as a result of lack of immunity, *T. gondii* may cross the placenta where they can infect the foetus. This may pose health risk to the foetus. It is for this reason that early diagnosis during pregnancy is most desirable so as to offer prompt management, including treatment. This may reduce the likelihood of placental transmission of *T. gondii* and eventually avoid risk to the foetus. Furthermore, 44.5% (49/110) and 11.8% (13/110) were reported for pregnant women with only *T. gondii* IgG and IgM antibodies respectively. The overall seroprevalence for the study was 83.6% (92/110). The overall seroprevalence of *T. gondii* IgG and IgM antibodies of 83.6% is higher than the global seroprevalence of 46.1% as reported by Jacquire (1995) and 49.7% in the Greater Accra region (Accra) as reported by AyehKumi *et al*. (2010). However, the overall seroprevalence of the current study is similar to studies reported by Partisani (1991), which estimated the seroprevalence of latent *T. gondii* infection in Europe, Africa and Latin America as 75-90% and 92.5% in the Greater Accra region (Accra) as reported by Ayi *et al.* (2009).

The seroprevalence among the seropositives was observed to be 96.2% (76/79) and 95.3% (41/43) for pregnant women aged between 21 and 40 years for *T. gondii* IgG and IgM antibodies respectively. The results of the study suggest a general increase in seropositivity with age from between 15 and 20 years to 21 and 30 years for both *T. gondii* IgG and IgM antibodies. This result is consistent to that published by Nester *et al.* (2004) and Garcia *et al.* (1999) which reported that the distribution of toxoplasmosis increases with age in women of child-bearing age. There was however a decrease in seropositivity with age from between 21 and 30 years and between 31 and 40 years for both *T. gondii* IgG and IgM antibodies.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study was to determine the seroprevalence of *T. gondii* infection among pregnant women ANC clinic at the Manhyia District hospital. The study showed an overall

seroprevalence of 83.6% among pregnant women. It was also established that 71.8% and 39.1% of the pregnant women had *T. gondii* IgG and IgM antibodies respectively present in their blood. Moreover, 27.3% of the pregnant women were seropositive for both *T. gondii* IgG and IgM antibodies. Again, 44.5% of the pregnant women were seropositive for only anti-*T. gondii* IgG against 11.8% for only anti-*T. gondii* IgM.

6.2 Recommendations

Since risk factors for toxoplasmosis was not assessed and discussed for the study, it is recommended a further study to determine the risk factors for toxoplasmosis infection, especially among pregnant women in the Ashanti region be conducted. It is again recommended to policy stakeholders in healthcare delivery that, screening of pregnant women for toxoplasmosis be included in the diagnostic laboratory tests routinely done during antenatal care.

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APPENDICES

Appendix I a: Raw Data for ELISA *Toxoplasma gondii* IgG

Sample ID	Optical Density (OD) values	Interpretation
001	2.416	POS

002	2.384	POS
003	0.499	NEG
004	0.447	NEG
005	2.438	POS
006	0.465	NEG
007	0.898	NEG
008	1.103	POS
009	0.835	NEG
010	2.120	POS
011	2.339	POS
012	0.428	NEG
013	2.107	POS
014	2.268	POS
015	2.671	POS
016	1.352	POS
017	2.462	POS
018	2.082	POS
019	2.293	POS
020	1.461	POS
021	1.727	POS
022	0.815	NEG
023	2.256	POS
024	0.462	NEG
025	0.446	NEG
026	2.317	POS
027	2.179	POS
028	2.051	POS
029	0.802	NEG
030	2.365	POS
031	0.784	NEG
032	2.189	POS
033	2.462	POS
034	2.259	POS
035	2.214	POS
036	1.285	POS
037	2.204	POS
038	1.906	POS
039	1.180	POS

040	2.163	POS
041	2.416	POS
042	0.360	NEG
043	2.252	POS
044	2.102	POS
045	1.602	POS
046	2.227	POS
047	1.451	POS
048	0.892	NEG
049	2.638	POS
050	2.082	POS
051	2.117	POS
052	1.403	POS
053	0.659	NEG
054	2.122	POS
055	0.425	NEG
056	2.548	POS
057	0.899	NEG
058	0.479	NEG
059	0.666	NEG
060	2.403	POS
061	1.329	POS
062	1.344	POS
063	2.671	POS
064	2.490	POS
065	2.638	POS
066	0.881	NEG
067	0.196	NEG
068	2.607	POS
069	2.380	POS
070	2.666	POS
071	3.035	POS
072	2.314	POS
073	2.638	POS
074	0.398	NEG
075	2.515	POS
076	0.056	NEG
077	0.367	NEG
078	2.490	POS
079	2.370	POS
080	0.448	NEG
081	2.416	POS
082	2.384	POS

083	0.726	NEG
084	2.403	POS
085	0.375	NEG

086	0.060	NEG
087	0.511	NEG
088	0.701	NEG
089	2.115	POS
090	2.458	POS
091	2.170	POS
092	2.522	POS
093	0.399	NEG
094	1.984	POS
095	2.061	POS
096	2.433	POS
097	2.157	POS
098	1.666	POS
099	2.242	POS
100	2.220	POS
101	0.423	NEG
102	2.222	POS
103	2.584	POS
104	2.433	POS
105	2.333	POS
106	2.115	POS
107	2.138	POS
108	2.300	POS
109	2.197	POS
110	2.360	POS

Positive (79) Negatives (31)

Appendix I b: Raw Data for ELISA *Toxoplasma gondii* IgM

Sample ID	**Optical Density (OD) values (Results)**	**Interpretation**
001	0.644	NEG

002	0.792	NEG
003	1.058	POS
004	1.228	POS
005	0.990	NEG
006	0.138	NEG
007	0.122	NEG
008	1.252	POS
009	0.130	NEG
010	0.237	NEG
011	0.309	NEG
012	0.230	NEG
013	1.151	POS
014	1.155	POS
015	0.119	NEG
016	2.666	POS
017	0.766	NEG
018	1.246	POS
019	0.399	NEG
020	1.243	POS
021	1.002	POS
022	1.010	POS
023	0.465	NEG
024	1.034	POS
025	0.152	NEG
026	0.883	NEG
027	0.100	NEG
028	0.137	NEG
029	1.314	POS
030	1.278	POS
031	0.860	NEG
032	0.226	NEG
033	1.103	POS
034	0.177	NEG
035	1.001	POS
036	1.201	POS
037	1.197	POS
038	1.143	POS
039	0.398	NEG
040	1.085	POS
041	0.854	NEG

042	0.323	NEG
043	0.717	NEG
044	1.325	POS
045	0.100	NEG
046	1.210	POS
047	0.876	NEG
048	0.559	NEG
049	1.336	POS
050	0.435	NEG
051	0.259	NEG
052	1.293	POS
053	1.225	POS
054	1.304	POS
055	0.453	NEG
056	0.170	NEG
057	1.168	POS
058	0.117	NEG
059	1.337	POS
060	0.237	NEG
061	0.136	NEG
062	0.193	NEG
063	1.327	POS
064	0.431	NEG
065	0.398	NEG
066	0.864	NEG
067	1.220	POS
068	1.252	POS
069	0.262	NEG
070	0.091	NEG
071	1.245	POS
072	1.201	POS
073	1.300	POS
074	1.299	POS
075	1.152	POS
076	1.060	POS
077	0.116	NEG
078	1.137	POS
079	0.065	NEG
080	0.123	NEG
081	1.093	POS
082	1.113	POS
083	0.120	NEG
084	1.191	POS

085	1.215	POS
086	0.232	NEG
087	1.127	POS

088	0.128	NEG
089	0.073	NEG
090	0.134	NEG
091	0.173	NEG
092	1.100	POS
093	0.475	NEG
094	0.367	NEG
095	0.421	NEG
096	0.483	NEG
097	0.216	NEG
098	0.263	NEG
099	0.737	NEG
100	0.303	NEG
101	0.762	NEG
102	0.336	NEG
103	0.324	NEG
104	0.292	NEG
105	0.619	NEG
106	0.331	NEG
107	0.279	NEG
108	0.365	NEG
109	0.243	NEG
110	0.228	NEG

Positive(43) Negative(67)

Appendix II: Complete Components of the ELISA Kit

The following reagents/materials were provided with the commercial ELISA kits (Teco Diagnostics, 1268 N, Lakeview Ave., Anaheim, CA 92807, USA).

a. Anti-Toxoplasma gondii IgG ELISA kit (Catalog Number : IGGT-96)

i. Microtiter wells: Toxoplasma antigen-coated wells (12X8 wells)

ii. Enzyme conjugate Reagent (Red colour): Red cap. 1 vial (12ml)

iii. Sample diluent (Green colour): 1 bottle (22ml) iv. Negative calibrator: 0.IU/ml. Natural cap. (100µL/vial)

v. Cut-off calibrator: 32IU/ml. Yellow cap. (100 µL/vial)
vi. Positive calibrator: 100IU/ml. Red cap. (100 µL/vial) vii. Positive calibrator: 300IU/ml. Green cap. (100 µL/vial) viii. Negative control: Range stated on label. Blue cap. (100 µL/vial) ix. Positive control: Range stated on label. Purple cap. (100 µL/vial)

x. Wash Buffer concentrate (20X): 1 bottle (50ml)

xi. TMB Reagent (One-step): 1 vial (11ml) xii. Stop solution: 1N HCl. Natural cap. 1 vial (11ml).

xiii. 1 set of operators manual/leaflet (Manufacturer's Instruction)

b. Anti-*Toxoplasma gondii* IgM ELISA kit (Catalog Number : IGMT-96)

i. Microtiter wells: Toxoplasma antigen-coated wells (12X8 wells) ii. Enzyme conjugate Reagent (Red colour): Red cap. 1 vial (12ml) iii. Sample diluent (Blue colour): 1 bottle (22ml) iv. Negative calibrator: 0.IU/ml. Natural cap. (100µL/vial)

v. Cut-off calibrator: 32IU/ml. Yellow cap. (100 µL/vial) vi. Positive calibrator: 100IU/ml. Red cap. (100 µL/vial) vii. Positive calibrator: 300IU/ml. Green cap. (100 µL/vial) viii. Negative control: Range stated on label. Blue cap. (100 µL/vial) ix. Positive control: Range stated on label. Purple cap. (100 µL/vial)

x. Wash Buffer concentrate (20X): 1 bottle (50ml) xi.

TMB Reagent (One-step): 1 vial (11ml) xii. Stop

solution: 1N HCl. Natural cap. 1 vial (11ml).

xiii. 1 set of operators manual/leaflet (Manufacturer's Instruction)

Appendix III: Structured Questionnaire for pregnant women

STRUCTURED QUESTIONNAIRE FOR PREGNANT WOMEN

General characteristics of pregnant women. Please tick (√) the appropriate box

Questionnaire Code …… Date of visit (dd/mm/yr) …….

Name of participant:……..……. Place

of residence: …… Contact

address: ……...

Telephone Number: …….....

1. Age of participant	Tick
a. 15- 20 years	
b. 21-30 years	
c. 31-40 years	

2. Marital Status	Tick
a. Married	
b. Single	
c. Divorced	
d. Widowed	

3. Educational Status	Tick
a. Basic education	
b. Secondary/Middle school	
c. Tertiary education	
d. No formal education	

4. Occupation of Respondent	Tick
a. Casual worker	
b. Civil servant	
c. Farming	
d. Trading	
e. Self employed	
f. Unemployed	
g. Others (Specify in space provided below)	

If others, please specify

_____

_____

_____

5. What is the duration of your pregnancy?

a. First trimester ☐ b. Second trimester ☐ c. Third trimester ☐

6. Have you been screened of Toxoplasmosis before?

a. Yes ☐ b. No c. No idea ☐ ☐

(If Yes continue from 7, otherwise Thanks for your time)

7. What was the result of the screening?

b. Positive ☐ b. Negative ☐ c. No idea ☐

8. When were you infected with toxoplasmosis?

a. Less than a year ago ☐ b. A year ago ☐

c. More than a year ☐ d. No idea ☐

9. How was the diagnosis done?

a. Laboratory diagnosis ☐ b. Medical Officer's/Clinician's discretion ☐

c. Others (For ☐ others, Please specify in space below)

……

……

10. Were you put on treatment/medication?

a. Yes ☐ b. No ☐ c. No idea ☐

11. What was the treatment regimen/medication used for you?

a. Administration of Pyrimethamine and Sulphonamide e.g. Sulphodiazine ☐

b. Spiramycin ☐ c. Others (For ☐ others please specify below) **MANY THANKS FOR YOUR TIME.**

Appendix IV: Consent form for pregnant women

CONSENT FORM FOR PREGNANT WOMEN

Title: Seroprevalence of *Toxoplasma gondii* infection among pregnant women in the

Ashanti Region of Ghana: Evidence from the Manhyia District Hospital, Kumasi.

Principal Researcher: Sefah-Boakye, Justine, B. Sc (Hons), M.Sc Clinical Microbiology candidate

Address: 1. Department of Clinical microbiology, School of Medical Sciences, KNUST, Kumasi

2. P.O. Box KS 10224, Prempeh II Street, Adum, Kumasi, Ghana.

Dear Madam,

I am a final year postgraduate student at the Kwame Nkrumah University of Science and Technology, Kumasi pursuing an M.Sc Clinical Microbiology at the Department of Clinical Microbiology of the School of Medical Sciences. In partial fulfilment for an award of the masters' degree, I am conducting a research on the topic "**Seroprevalence of *Toxoplasma gondii* infection among pregnant women in the Ashanti Region of Ghana: Evidence from the Manhyia District Hospital, Kumasi**. I humbly request your full participation voluntarily to assist me in undertaking the study.

Purpose of study: Determination of the seroprevalence of *Toxoplasma gondii* infection among pregnant women in the Ashanti Region of Ghana is the principal reason that necessitated the study. Evidence for the study shall be obtained from the Manhyia District Hospital, a hospital with high antenatal care attendance in Kumasi.

Study Background: Toxoplasmosis is a protozoan infection that affects warm-blooded animals, including humans. Human infection of toxoplasmosis occurs through eating of

tissue cysts in raw or poorly cooked meat, ingestion of food or water contaminated with mature oocysts faeco-orally, transplacental (vertical) transmission from mother to foetus *in utero* and in rare cases, needlestick injury, blood transfusion and organ transplantation from seropositive donors.

Toxoplasmosis is an infection that raises public health and global concerns in most countries, especially developing countries. Toxoplasmosis is estimated to be carried by nearly a third of the global population.

Congenital toxoplasmosis, a deadly form of toxoplasmosis, occurs when unborn and developing foetus becomes infected with *T. gondii* through the placenta *in utero*. Maternal-fetal transmission of *T. gondii* occurs between one and four months after placenta has been colonized by tachyzoites. This has dire health consequences on pregnancies and newborns. Moreover, the risk of congenital infection from acute *T. gondii* infection in pregnancies ranges between 20% and 50% when strict treatment regimens are not adhered.

Consequences such chorioretinitis, hydrocephalus, intracranial calcification, encephalitis and convulsions are commonly related to *T. gondii* infection in pregnancies. Intrauterine growth restrictions and intrauterine foetal death are common signs of *in utero* infection in severe cases.

How it will be done: You will be required to complete structured questions from an easy-to-complete questionnaire and also about 3 milliliters (mls) of venous blood drawn aseptically by an expert phlebotomist. Samples taken will be tested for *T. gondii* antibodies. Results for the test shall confidentially be communicated to only you if you wish. Be assured of your liberty to discontinue participating in the study if you so wish at any point in time.

Potential Benefit

By participating in the study, your health status on toxoplasmosis could be known and when needed appropriate medication given at no cost to you as a participant of the study.

Possible Risks and Discomforts: There is no known risk/discomfort associated except the temporal pain you might feel while drawing the blood.

Confidentiality: Information obtained shall be treated with strict confidentiality and shall be used for research purposes only. However, participants who test positive shall be attended to by a qualified physician if you so wish.

Contacts for Additional Information

If you have any questions about the study, you may contact any of the following persons/group:

Mr. Sefah-Boakye, Justine (P.O. Box KS 10224, Sir Prempeh II street, Adum, Kumasi; Tel 0243419217/0501346499/0261852667)

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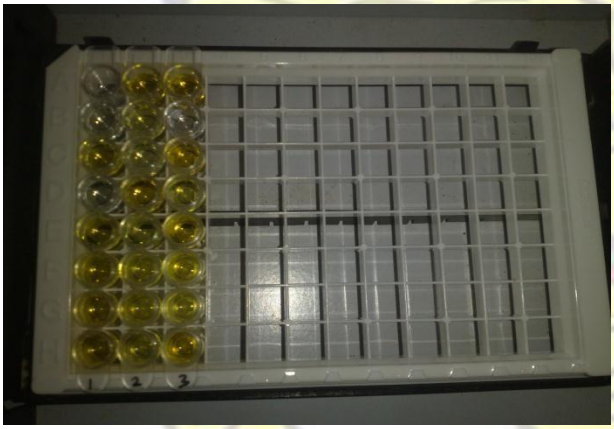
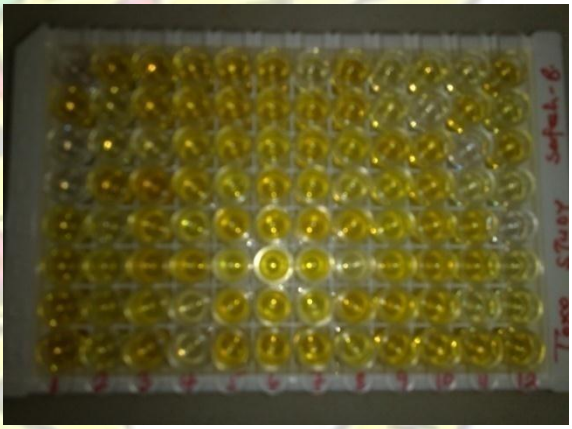
Name of Study participant Signature/Thumb print Date

**NB:** Do you wish to know the results for your toxoplasmosis test?

YES ☐ NO ☐

**Appendix V: ELISA Plates from the showing results of Anti-*Toxoplasma gondii* IgG and IgM.**

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