SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN IN THE ASUNAFO NORTH DISTRICT, BRONG-AHAFO REGION, GHANA

By

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DECLARATION

I hereby Declare that this submission is my own work towards the MSc 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 University, except where due acknowledgement has been made in the text.

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(Head of Department) Signature Date

DEDICATION

I dedicate this thesis to my dear wife Nana Adjoa Achiamaah Aryee and my lovely daughters Charlene Ampofo-Adu Gyamaah Aryee and Elizabeth Yeboah Aryee.

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I first and foremost express my sincere gratitude to God Almighty. I could not have come this far without His grace and guidance. I glorify His name.

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ABSTRACT

Toxoplasma gondii is the causative organism of toxoplasmosis and it can be acquired orally or congenitally. Congenital infection of neonates is found to be the cause of several neurological, brain and ophthalmic disorders later in life. The objectives of this study were to determine the seroprevalence of *T. gondii* among pregnant women attending antenatal clinic at Asunafo North district of the Brong-Ahafo region, Ghana.

One hundred and forty-six (146) pregnant women aged 16 to 40 years voluntarily took part in this study. Blood samples were collected and questionnaires were administered to them. ELISA test was done to detect anti-*T. gondii*IgG and IgM antibodies in their blood samples. The data collected was analysed using SPSS (Version 16). Analysis of the data collected showed 23.3% (34/146) and 67.8% (99/146) were positive for anti-*T. gondii*IgM and IgG respectively. Furthermore, a breakdown of the analysed data is as follows: 10.3% (15/146) tested positive for only anti-*T. gondii*IgM, 54.8% (80/146) also tested positive for only anti-*T. gondii*IgG and 13% (19/146) tested positive for both anti-*T. gondii*IgM and IgG.This gave a total prevalence of *anti-T gondii* antibodies tested to be 78.1% (114/146).This implies that 23.3% of the respondents were recently infected and 67.8% of the respondents were old infection.

Further studies need to be done to determine the rate of mother-to-child transmission of *T. gondii* infection in Ghana.

CHAPTER ONE

INTRODUCTION

1.1 Background

Toxoplasmosis is a zoonotic parasitic disease caused by the protozoan parasite *Toxoplasma gondii* (Dubey, 1998). The primary host of the parasite is the felid (cat) family but the parasite also infects most genera of warm-blooded animals, including humans (Dubey and Beattie, 1988). About 20% to 90% of the world's adult population in different regions are reported to have had contact with the parasite (Galván-Ramirez *et al.,* 1998). Though *Toxoplasma gondii* has been known as a potential human parasite for many years, it was only around 1969-70 that it was discovered as a coccidian parasite (Smyth, 1996). It is an obligate intracellular parasite and was first discovered in 1908 in a desert rodent, the Gondi rat (Dubey J.P, 2008).

Toxoplasma infection may be acquired orally or congenitaliy (Thulliez *et al.,*1992). The disease is important for its serious implications in immuno-suppressed individuals including pregnant women (Halomen and Weiss, 2013). It also has severe consequences on foetuses in congenital transmission (Cenci-Goga *et al.*, 2011). Toxoplasmosis is asymptomatic and is often associated with short self-limiting illnesses in immunocompetent individuals (Halomen and Weiss, 2013). This is typical of both the acute and latent forms of the disease. The situation is however different in immunocompromised persons such as HIV/AIDS patients (Agyei and Lartey, 2004; Meisheri*et al*., 1997), persons undergoing chemotherapy, organ transplant recipients, and sometimes in pregnant women in which the disease is usually deadly to their foetuses (Stepick-Biek *et al.,* 2002).

Transmission of the infection can be acquired (horizontal) or congenital (vertical) (Thulliez *et al.,* 1992). Toxoplasmosis can be acquired through the ingestion of oocysts shed in infected cats' faeces (Dubey, 2008). Other means of acquiring toxoplasmosis is ingesting soil, vegetables, and fruits contaminated with *T. gondii* oocysts, tachyzoites in unpasteurised milk, cysts in raw or undercooked infected meat (Dubey, 2008). Although beef and vegetables are potential sources of *T. gondii* infection, pork and lamb are known to be the most common sources of contamination (Boothroyd, 2009). Insects such as flies and cockroaches can also carry oocysts from cat faeces to food (Wallace, 1971). Needle stick injuries, cuts, blood transfusion and organ transplantation have also been mentioned as possible infection risk factors (Motoya *et al.,* 2004).

Congenital toxoplasmosis is a clinical state of the disease in the foetus that results from an acute primary infection acquired by the mother during pregnancy (Kaye, 2011). The mother-to-foetus transmission rates vary according to gestational age at the time of maternal infection (Stepick-Biek *et al.,* 2002) and the severity of congenital toxoplasmosis vary with the trimester during which infection is acquired (CDC, 2002). Maternal infection in the first trimester of gestation results in a transmission rate of 10-15%, which rises up to 68% in the third trimester (Thulliez *et al.,* 1992). This implies that maternal infections occurring early in pregnancy are less likely to be transmitted to the foetus than infections acquired later in pregnancy. This can result in severe consequences such as spontaneous abortion, still-birth, or the child may be born with some degree of abnormalities in the central nervous system including hydrocephalus, mental retardation and chorioretinitis(McAuley *et al.,* 1994; Guerina *et al.,* 1994). It is however dependent on the age of the foetus when it is infected and the virulence of the *Toxoplasma* species(McAuley *et al*., 1994; Guerina *et al.,* 1994).Toxoplasmosis can be prevented by washing vegetables and fruits before eating, and avoiding uncooked or improperly cooked

meat of infected animals (Dunn *et al.,* 1999).The practice of good personal hygiene should greatly be encouraged (Dunn *et al.,* 1999). The prevalence of *Toxoplasma gondii* infection in man varies depending on the alimentary habits, hygienic conditions, the presence of the definitive host (cat) and the climate (Walle *et al.*, 2013; Caballero-Ortega *et al.*, 2012; Dabritz and Conrad, 2010).

It is estimated that between 30% and 65% of all people worldwide are infected with toxoplasmosis (Tenter *et al.,* 2000). However, there is a large variation between countries; for example in France, the prevalence rate of total *T. gondii* antibodies lies at 88%, and in Germany, the Netherlands and Brazil, prevalence rates are around 80%, over 80% and 67% respectively. In Britain about 22% are carriers, and South Korea's rate is 4.3% (Zimmer, 2006).

Despite the above mentioned facts, early diagnosis of toxoplasmosis can help treat and manage the disease effectively (Garin and Eyles, 1958). Diagnoses in health centres usually involve anti-*Toxoplasma* antibody detection and parasite DNA detection (Araujo and Remington, 1990; Decoster *et al.,* 1988;Switaj *et al.,* 2005). Treatment regimen following diagnosis involves the use of anti-parasitic agents. Pyrimethamine and sulfadiazine have been known to be the best over the years (Remington *et al.,* 2006; Mui *et al.,* 2008).

Studies in Ghana however, show varying but high sero-prevalence values (51.2% to 92.5%) in humans, especially in pregnant women (Anteson *et al.,* 1978a; Anteson *et al.,* 1978b; Anteson *et al.,* 1980; Ayi *et al.,* 2005; Ayi *et al.,* 2009) and in animals (Arko-Mensah *et al.,* 2000; Van der Puije *et al.,* 2000). Ayi *et al.,*(2005) reported a high sero-prevalence of 89% among eye patients with eye lesions. Maternal toxoplasmosis as a risk factor for spontaneous abortion was investigated by Al-Hamdani and Mahdi(1997) and it was found to be more frequent in women with habitual abortion (18.5%) than in the

normal pregnancy group (5.9%). Furthermore, the study reported *Toxoplasma*antibodies were more prevalent in women having cats at home than in women who do not possess cats (Al-Hamdani and Mahdi, 1997). Toxoplasmic eye lesions in adults have been known to be a consequence of congenital infection at birth (McAuley *et al.*, 1994; Guerina *et al.*, 1994). A mother-to-child transmission risk of 38.4% was reported in 2012 in the Greater Accra region of Ghana (Kwofie *et al*., 2012).

1.2 Problem Statement

Limited research has been conducted on toxoplasmosis in Ghana and the few conducted are in urban settings and southern parts of the country (Ayi *et al*., 2005; Ayi *et al*., 2009, Ayeh-Kumi *et al*., 2010, Kwofi *et al*., 2012). The seroprevalence of toxoplasmosis among pregnant women in the Asunafo North district has not been established. This study is the first ever conducted in the Brong-Ahafo Region which lies in the northern sector of the country. It will serve as reference information for the northern sector of the country and also for rural settings in future studies. Despite the fact that congenital *T. gondii* infections have potential significant health risks on the foetuses of women who acquire infection during pregnancy there is significantly high sero-prevalence among pregnant women in Ghana (Ayi *et al*., 2009;Kwofi *et al*., 2012), awareness of the disease among Ghanaians is very low; even among the educated group.

Generally, toxoplasmosis is mild for the mother as compared to the infected foetus (Kwofie *et al*., 2012). Congenital toxoplasmosis could have serious socioeconomic and psychological effects on affected families (Kwofie *et al*., 2012). The pain caused by the disease and the cost of care, especially with the case of hydrocephalus, mental retardation and even blindness could be very high (Kwofie *et al*., 2012). Apart from the above mentioned risks, the psychological trauma of having miscarriage(s) or stillbirth(s) cannot

be overemphasized. This study and similar ones in future may inform policies makers to make it a policy to routinely screen pregnant women as part of their antenatal care and this will greatly reduce the rate of the congenital form of the disease.

In Ghana, despite the risk of toxoplasmosis to both mother and child, screening of pregnant women for *Toxoplasma* infection is not included in the free-of-charge antenatal care policy, due mainly to ignorance and lack of information. Such a lack of screening for risk of congenital toxoplasmosis predisposes countless newborns throughout the country to intra-uterine infection and development of mild to severe consequences, which could have been minimized by an early post-natal intervention. The neglect of toxoplasmosis as a disease of minimum or no public health importance has resulted in the ignorance of the public about the disease and its potential threats on the society.

1.3 Justification

Though some research has been conducted on *T. gondii* infection in Ghana, they were only sited in the coastal or southern parts of the country and the results have been generalised for the whole country despite differences in environmental conditions and population behaviour. Not much has been done to determine the real situation of the disease in the remaining two ecological zones of the country. This study will provide data on the prevalence of risk of *Toxoplasma* infection in pregnant women in the Asunafo North district of the Brong-Ahafo region of Ghana and its association with exposure to possible infection risk factors. Also the knowledge derived from this study will add up to existing information and enhance the prospects of policy formulation to routinely screen pregnant women and women of child bearing age for *Toxoplasma* infection to reduce the effects of the disease which could be pronounced in the later years of the child.

1.4 Objectives

1.4.1 Main objective

- To determine the prevalence of *Toxoplasma gondii* infection among pregnant women in the Asunafo North district.

1.4.2 Specific objectives

- To estimate the prevalence *T. gondii* infection among pregnant women in the Asunafo North district.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TOXOPLASMOSIS AND *TOXOPLASMA GONDII*

2.1.1 Epidemiology

Toxoplasmosis is a zoonotic parasitic disease caused by the protozoan parasite *Toxoplasma gondii* (Dubey, 1998). The parasite infects most genera of warm-blooded animals, including humans, but the primary host is the felid or cat family (Dubey and Beattie, 1988). About 20% to 90% of the world's adult population in different regions is reported to have had contact with the parasite (Galván-Ramirez *et al.,* 1998). However, there are large variations in prevalence between and within different countries in animals and humans. The prevalence is directly proportional with age (Nester *et al.*, 2004).

Local fauna, environmental conditions, cultural habits, and social and economic patterns may influence human prevalence: in France, for example, around 88% of the population is carrier, probably due to a high consumption of raw and lightly cooked meat (Berger *et al.*, 2009). Germany, the Netherlands and Brazil have also shown to have high human prevalence of around 80%, over 80% and 67% respectively (Kortbeek, 1999).In Britain, about 22% are carriers and that of South Korea's rate is only4.3% (Lafferty, 2006).The Centre for Disease Control and Prevention noted that the overall seroprevalence in the United States as determined with specimens collected by the third National Health and Nutritional Assessment Survey (NHANES III) between1988 and 1994 was found to be 22.5%, with seroprevalence among women of childbearing age (15 to 44 years) to be15% (McQuillan*et al*., 2004).West Africa might be widely endemic with human toxoplasmosis (Adou-Bryn et al., 2004) even though there has been no large scale survey to establish the prevalence. This accession is supported by case reports of probable toxoplasmosis infection (Watson and Zinsstag, 2000).Even though there has been no large scale survey to

establish the human prevalence of toxoplasmosis in Ghana, cross-sectional studies conducted in urban settings in the southern sector of the country suggest a higher prevalence (Ayi *et al*., 2005; Ayi *et al*., 2009;Ayeh-Kumi*et al*., 2010).

2.1.2 A Historical Perspective

Though *Toxoplasma gondii* had been known as a potential human parasite for many years, its true nature as a coccidian was discovered only around 1969-70(Smyth, 1996). *T. gondii* is an obligate intracellular parasite (Roberts and Janovy, 2000) and was first discovered in 1908 in a desert rodent *Ctenodactylus gundii* by Nicolle and Manceaux (Nicolle and Manceaux, 1909). The parasite was named based on its morphology (mod. L. Toxo: arc or bow, plasma: life) and host (Nicolle and Manceaux, 1909).

The first human *T. gondii* infection was identified by three pathologists in an infant girl in New York, USA, in 1938 (Wolf *et al*., 1939). The girl developed convulsive seizures when she was just three days old and lesions were noted in the maculae of both eyes through an ophthalmoscope. She died at one month old and an autopsy was performed. At post mortem, brain, spinal cord, and right eye were removed for examination. Free and intracellular *T. gondii* were found in lesions of encephalomyelitis and retinitis of the girl. In addition, viable *T. gondii* was isolated in animals inoculated with tissues from the girl. It was later found that the child became infected congenitally. Sabin (1942) made an extensive review of congenital toxoplasmosis and proposed that hydrocephalus or microcephalus, intracerebral calcification, and chorioretinitis are typical clinical signs of congenital toxoplasmosis. Prior to then, Sabin had reported toxoplasmosis in a six year old boy with the initials R. H. in Cincinnati, OH (Sabin, 1941). The strain isolated from the boy took its name after the initials (RH), hence becoming the famous RH strain. These events led to the discovery of other strains such as ocular toxoplasmosis (Sabin, 1941;

Silveira *et al.*, 1988) and toxoplasmosis in other animals (Hartley and Marshall, 1957; Dubey and Beattie, 1988; Dubey, 2001).

2.1.3 Classification of *T. gondii*

Toxoplasma gondii is a member of the kingdom Protoctista, phylum Apicomplexa which consists of intracellular parasites that are characterised by polarized cell structure and a complex cytoskeletal and organelle arrangement at their apical end (Dubey, 1998). Other members of this phylum include the human pathogens *Plasmodium* (the causative agent of malaria) and *Cryptosporidium* as well as the animal pathogens *Eimeria* (the causative agent of chicken coccidiosis) and *Sarcocystis*.

T. gondii belongs to the class Conoidasidaea of which members are obligate intracellular parasites (they generally complete the sexual stage of their life cycles within a host's intestinal tract) and subclass Coccidiasina(Dubey, 1998). *T. gondii* belongs to order Eucoccidiorida (consists of parasites of humans, domesticated animals, wild animals, and birds) and the suborder Eimeriorina (includes many species that predominantly parasitize domestic animals, with few human parasites). It also belongs to the family Sarcocystidae (carry out a life cycle that requires more than one obligate host) (Dubey, 1998). *Toxoplasma* is one of the three well known genera; this genus requires a member of the *felidae* (Frenkel, 1970) to complete its sexual life cycle. Finally, it belongs to the species *gondii*; the only species in the genus *Toxoplasma* (Dubey, 1998).

2.1.4 Genetic diversity of *T. gondii*

The structure of *T. gondii* is highly clonal, despite a sexual phase in its life cycle (Howe and Sibley 1995; Sibley and Boothroyd, 1992; Sibley, 2003). Three major clonal types identified are types I, II, and III (Sibley, 2003). These were derived from recombination

between two highly similar ancestral lineages (Grigg *et al*., 2001; Su *et al.*, 2003). Research shows that Type II strains are the causative agents of more than 70% of human cases of toxoplasmosis in the United States and France (Ajzenberg, 2002; Darde´, 1992; Howe and Sibley, 1995). Though Type II strains are relatively avirulent in mice they readily establish chronic infections which are characterized by tissue cysts that are highly infectious by the oral route in humans (Sibley, 2003; Su *et al*., 2003).

Type I strains on the other hand, are more virulent and have a greater capacity to cross tissue barriers in vitro and in vivo (Su *et al*., 2002, Sibley and Boothroyd, 1992; Barragan and Sibley, 2002).

2.1.5 Morphology of *T. gondii*

Toxoplasma gondii has three forms based on the developmental stages of its life cycle. These morphological forms are the tachyzoites (actively dividing Forms), bradyzoites (slowly dividing forms in tissue cysts) and oocysts (Dubey and Beattie, 1988). A detailed study of the various forms reveals the basic characteristic differences in their structures as shown in Figure 1.

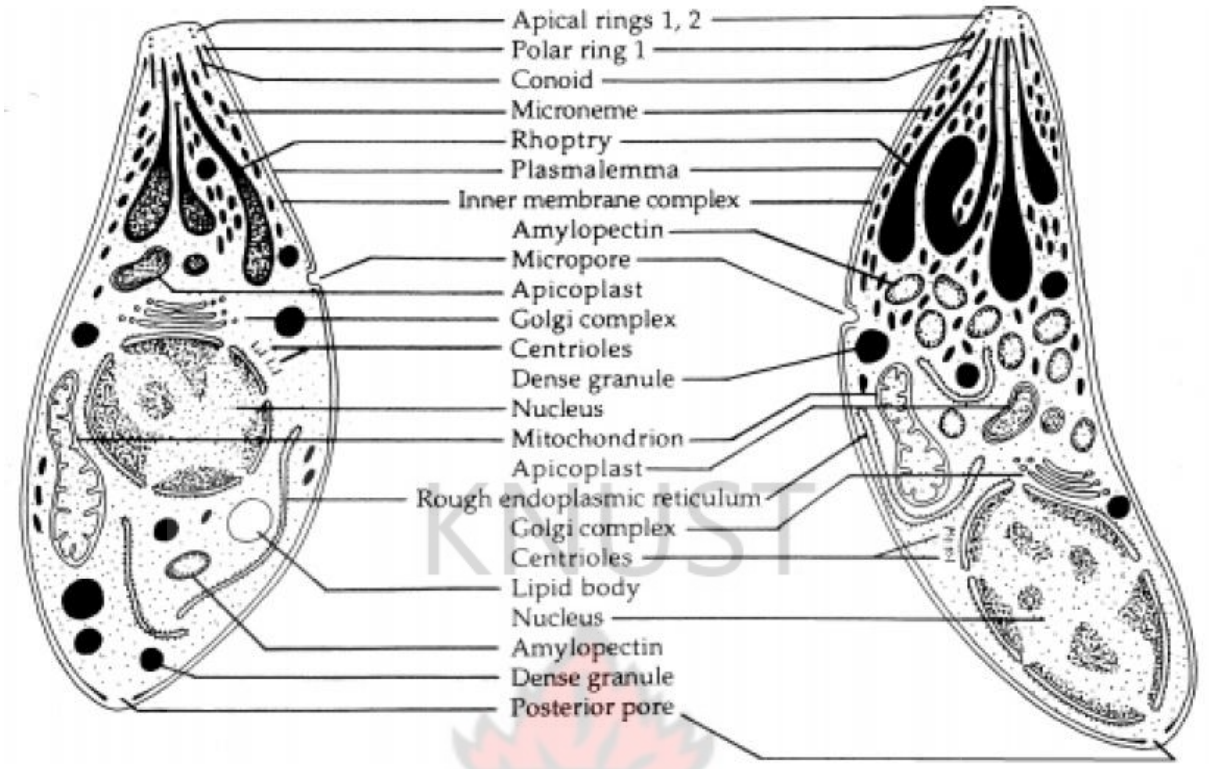

Figure 1: Schematic drawings of a tachyzoite (left) and a bradyzoite (right) of *T. gondii*. The drawings are composites of electron micrographs (Dubey *et al.*, 1998).

2.1.5.1 Tachyzoites

Frenkel(1973) described the stages of the parasite with observations he made on the behaviour of the parasite at each stage of development. The stage at which the parasite rapidly multiplied in any cell of the intermediate host and in the non-intestinal epithelial cells of the definitive host was described as the "tachyzoite" (tachos; speed in Greek). The tachyzoite is often crescent shaped, approximately 2mm by 6 mm (Fig. 1), with a pointed anterior (conoidal) end and a rounded posterior end. The internal structures of the tachyzoite consists of various organelles and inclusion bodies including a pellicle (outer covering), apical rings, polar rings, conoid, rhoptries, micronemes, micropore, mitochondrion, subpellicular microtubules, endoplasmic reticulum, golgi complex, ribosomes, rough and smooth endoplasmic reticula, micropore, nucleus, dense granules,

amylopectin granules (which may be absent), and a multiple-membrane-bound plastid-like organelle which has also been called a golgi adjunct or apicoplast (Dubey *et al.*, 1998).

2.1.5.2 Bradyzoites and tissue cysts

On the other hand, Frenkel used the term "bradyzoite" (brady; slow in Greek) to describe the stage of the parasite which multiplied slowly within a tissue cyst(Frenkel, 1973). Hundreds of crescent-shaped bradyzoites are enclosed by thin (<0.5 μm thick) and elastic tissue cyst walls (Plate 1), each approximately 7μm by 1.5μm in size (Melhorn and Frenkel, 1980). The tissue cysts develop within the host cell cytoplasm and remain intracellular as the bradyzoites divide by endodyogeny (Ferguson and Hutchison, 1987). Tissue cysts vary in size; young tissue cysts may be as small as 5μm in diameter and contain only two bradyzoites, while older ones may contain hundreds of bradyzoites (Dubey *et al*., 1998).

Bradyzoites are slightly structurally different from tachyzoites (Fig 1). They have a nucleus situated toward the posterior end, whereas the nucleus in tachyzoites is more centrally located. Bradyzoites are more slender than tachyzoites. Bradyzoites are less susceptible to destruction by proteolytic enzymes as compared to tachyzoites (Jacobs *et al*., 1960). The prepatent period in cats after consumption of bradyzoites is shorter than that after consumption of tachyzoites (Dubey and Frenkel, 1967). Cysts are usually formed in neural tissues such as the eye and the brain, and the muscular tissues (Dubey and Frenkel, 1967).However, visceral organs including the lungs, kidneys, and liver can also be infected (Dubey and Frenkel, 1967). Intact tissue cysts probably do not cause any harm and can persist for the life of the host without causing a host inflammatory response (Dubey and Frenkel, 1967).

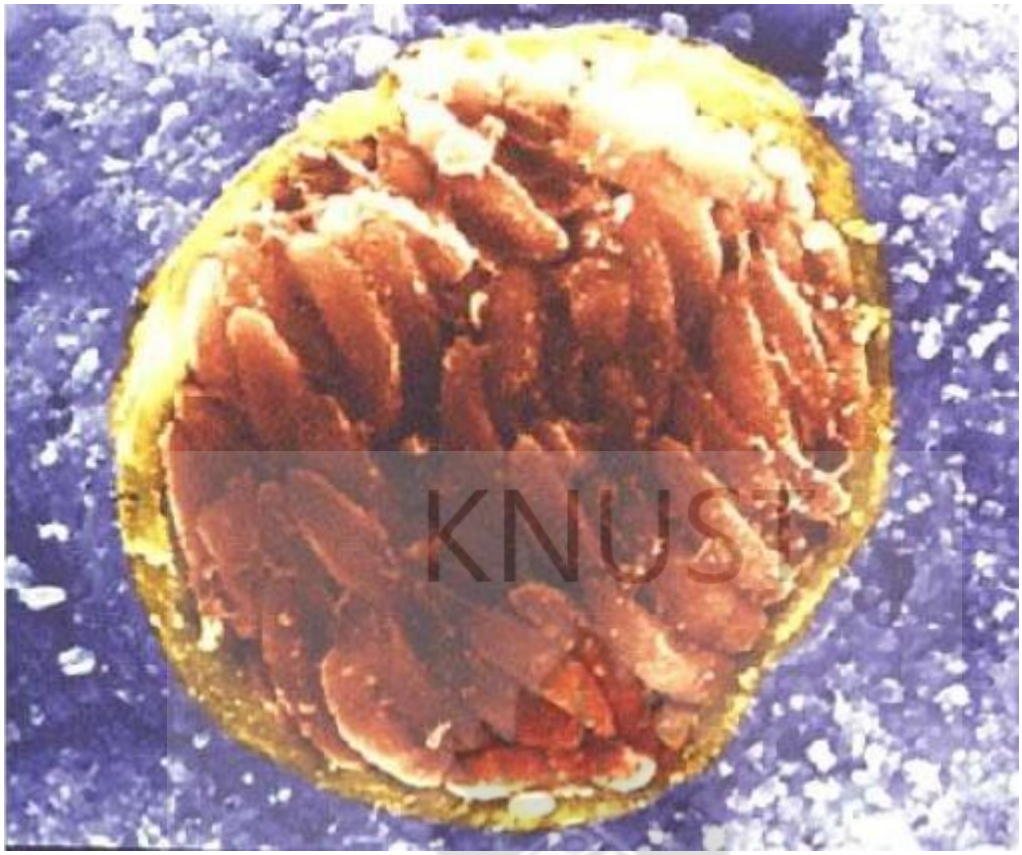

***Plate 1: Toxoplasma* bradyzoites in tissue cyst (Source: Ferguson, 1987)**

2.1.5.3 Oocysts

Oocysts of *Toxoplasma gondii* are shed only in the faeces of their definitive hosts (domestic and wild felids) (Dubey and Beattie, 1988). Unsporulated oocysts are almost spherical (10mm by 12 mm in diameter) (CDC, 2002). Under the light microscope, the oocyst wall consists of two colourless layers (Plate 2A). Polar granules are absent but there are numerous sporonts in the oocyst. The oocyst does not survive in arid cool climates and can be destroyed by heating (Wilson and McAuley, 1999).

Under favourable environmental conditions such as aeration and temperature (4^{o}C-37^{o}C) sporulation takes place outside the cat within 1 to 5 days after excretion (Dubey and Beattie, 1988). Sporulated oocysts range between subspherical and ellipsoidal in shape (11 by 13 µm in diameter) (CDC, 2002). Each oocyst contains two ellipsoidal sporocysts

which lack Stieda bodies (Plate 2A). Sporocysts measure 6mm by 8 mm (CDC, 2002). A sporocyst residuum is present but oocyst residuum is absent (CDC, 2002). Each sporocyst contains four sporozoites (CDC, 2002). After sporulation the oocysts become infectious and can retain its infectious state for approximately a year under favourable conditions i.e., in warm, moist soil (Dubey *et al*., 1998).

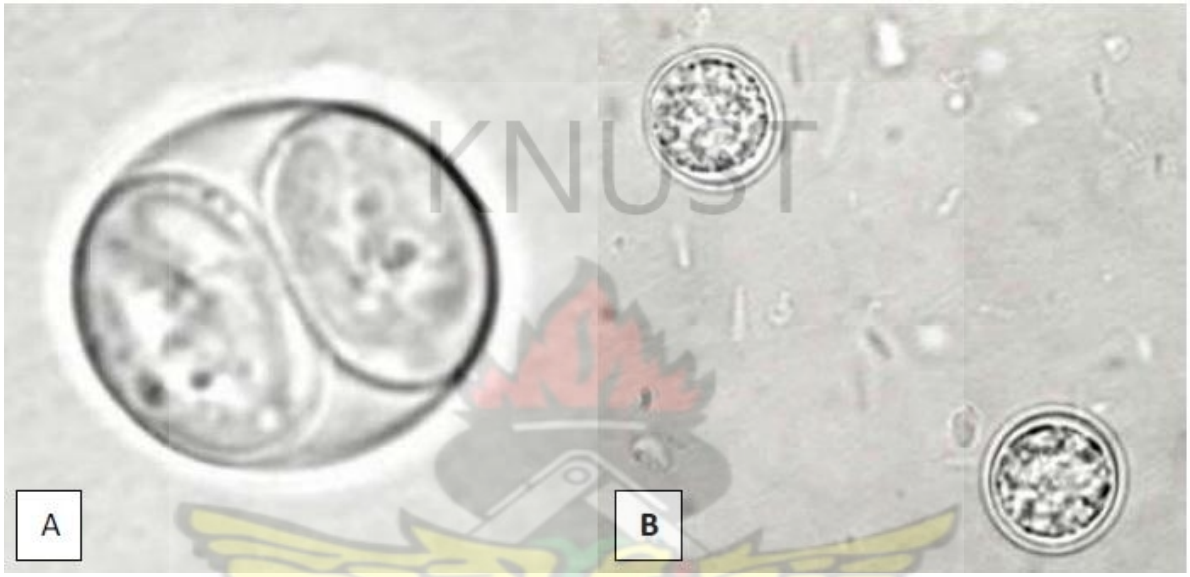

Plate 2A*: Toxoplasma gondii* unsporulated oocyst in an unstained wet mount.Plate2B:*Toxoplasma gondii* sporulated oocyst in an unstained wet mount(Source: CDC, 2002)

2.1.6 Life cycle of *T. gondii*

Toxoplasma gondii is capable of undergoing alternative generation (Dubey, 1998). Its life cycle consists of two phases, the sexual phase and the asexual phase (Dubey, 1998). The sexual phase takes place only in cats (both domestic and wild) and this makes cats the parasite's definitive host (Figure 2) (Dubey and Beatie, 1988). The asexual phase can take place in other warm-blooded animals including cats, mice, humans, and birds (Dubey and Beatie, 1988). These are called the intermediate host. Rodents are the typical intermediate hosts (Dubey and Beatie, 1988).

The definitive hosts of *T. gondii*(cats) become infected by ingesting sporulated oocysts or usually infected animals containing tachyzoites, bradyzoites or tissue cysts or by congenital infection (Dubey and Beatie, 1988).

Bradyzoites are released into the stomach and the small intestine by the action of proteolytic enzymes in the digestive tract (Jacobs *et al*., 1960). The bradyzoites are transformed into tachyzoites through a process unknown asendodyogeny (Dubey, 1998). Endodyogeny is essentially a budding process in which two daughter cells are formed inside the original or mother cell, which is then consumed by the developing daughter cells (Sheffield and Melton, 1968).

After about two to five asexual generations in the cat, a sexual generation of micro – (male) and macro – (female) gametes is also found throughout the small intestine mostly in the ileum 3 – 15 days of infection (Dubey *et al.,* 1998).Fertilization occurs and the zygote develops into a thick walled oocyst (Dubey *et al.,* 1998). Mature oocysts are discharged through the intestinal lumen in the cat's faeces by the rapture of intestinal epithelial cells (Dubey *et al.,* 1998). The oocysts become infectious after sporulation and can remain infectious for months even in cold and dry climates (Dubey, 1977).

The asexual life cycle occurs in all intermediate hosts; including humans and felines (Dubey *et al.,* 1998). Ingestion of tissue cysts or oocysts is followed by infection of the intestinal epithelial cell by bradyzoites or sporozoites respectively (Dubey *et al.,* 1998). Through the same process of endodyogeny, these become tachyzoites (Dubey, 1998). Tachyzoites enter host cells, mainly nucleated cells such as immature red blood cells, white blood cells, and macrophages by actively penetrating through the host cell plasmalemma or by phagocytosis (Bonhomme *et al*., 1992). Upon entry into 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 (Dubey, 1998). Inside the

vacuole, the tachyzoite multiplies itself by endodyogeny until the infected cell is filled with parasites and bursts, releasing other tachyzoites which infect other cells and tissues (Dubey and Beatie, 1988; Barragan *et al*., 2002). Infected cells distribute tachyzoites throughout the body via the blood or lymph first to the mysentric lymph nodes, followed by the liver and lungs (Figure 2) (Dubey and Beatie, 1988; Barragan *et al.,* 2002). As a response to host acquired immunity in immunocompetent persons, pseudocysts are formed enclosing tachyzoites that eventually differentiate into bradyzoites (Isrealski *et al.,* 1993). These cysts are found predominantly in tissues with low immuno-activity, such as the central nervous system and muscle tissues where they may reside for the life of the host, hence the name tissue cysts (Black *et al*., 2000, Dubey and Frenkel, 1972).

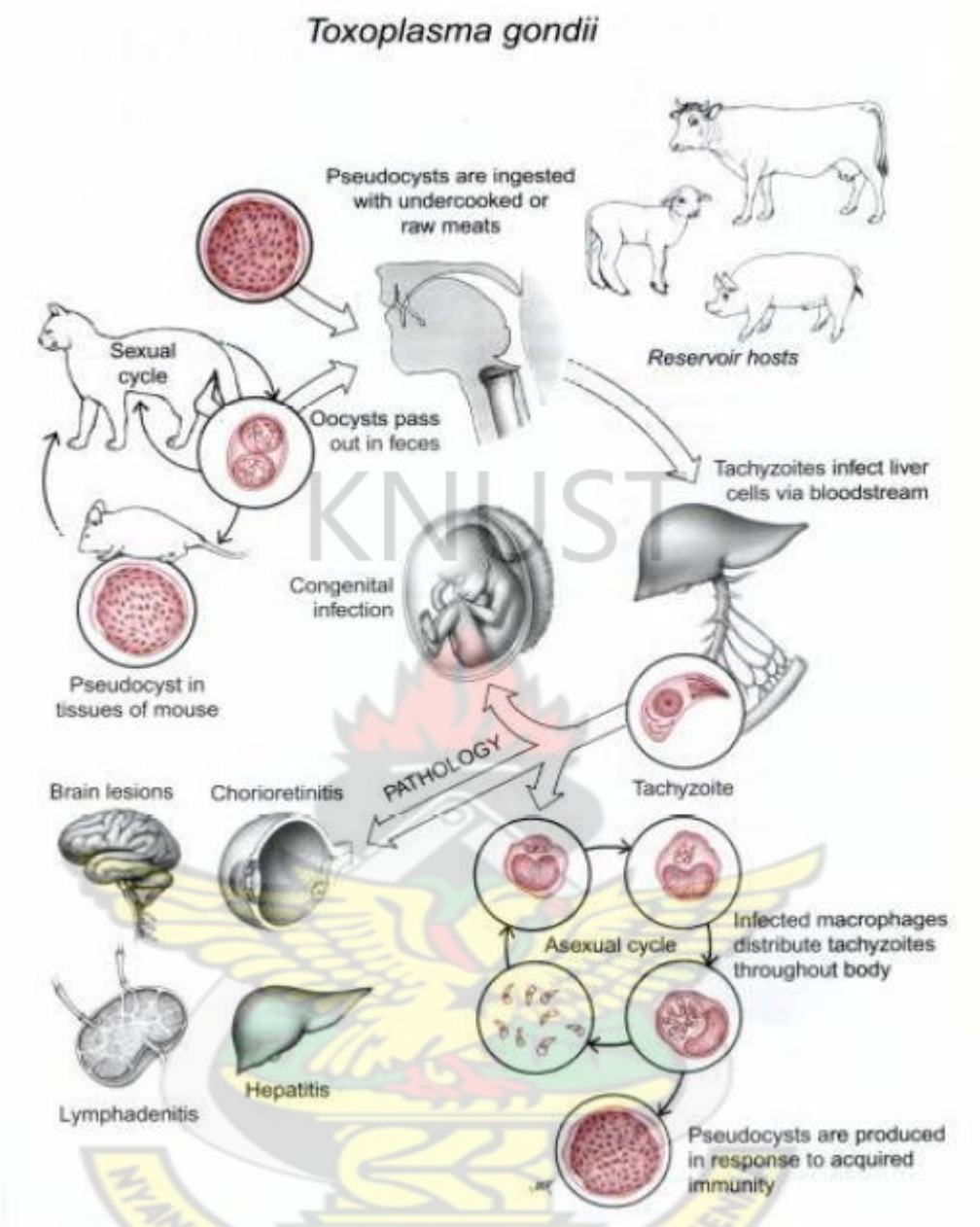

Figure 2: T. gondii life cycle and pathogenesis (Source: Racaniello, 2010)

2.1.7 Transmission of *T. gondii*

T. gondii is Transmitted by three principal routes: horizontally through tissue cysts and oocysts from cats and vertically (congenital). *Toxoplasma gondii* tissue cysts are transmitted horizontally to humans by the ingestion of infected raw or inadequately cooked meat such as pork, mutton, beef or that of any other warm-blooded animal (Dubey,

1994). One can also be infected through uncooked foods that have come in contact with infected meat (Dubey, 1994).

Apart from above route of infection, the parasite is also horizontally transmitted to humans when they inadvertently ingest oocysts that cats have passed in their faeces, either from a litter box or from soil (e.g., garden soil), on unwashed fruits or vegetables, or in unfiltered water (Remington *et al*., 2001). The third route of transmission occurs when acutely infected pregnant women pass the infection vertically to the foetus in their womb. This can cause serious disease in the foetus (Remington *et al*., 2001).

Transmission of the parasite is dependent on factors such as climate and rate of exposure to sources of *T. gondii* infection. *Toxoplasma* sero-positivity is reportedly higher in hot and humid climates (Feldman, 1974). Some parts of the world are therefore likely to have higher levels of toxoplasmosis prevalence than others. A high prevalence of 92.5% was reported for Ghana (Ayi *et al*., 2009),in France for example, around 88% of the population is carrier (Berger *et al*., 2009). Germany, the Netherlands and Brazil have also shown to have high human prevalence of around 80%, over 80% and 67% respectively (Kortbeek, 1999).In Britain, about 22% are carriers and that of South Korea's rate is only4.3% (Lafferty, 2006).The Centre for Disease Control and Prevention notedthat the overall seroprevalence in the United States as determined with specimens collected by the third National Health and Nutritional Assessment Survey (NHANES III) between1988 and 1994 was found to be 22.5%, with seroprevalence among women of childbearing age (15 to 44 years) to be15% (McQuillan*et al*., 2004). Some countries have reported high prevalence to *T. gondii* infection because of their level of exposure to the sources of infection. The very high seroprevalence of *Toxoplasma* in France is attributed to the eating of undercooked meat (Dubey, 1988). According to Krick and Remington, the frequency of *T. gondii* infection increases from 0.5% to 1.0% per year of age (Krick and Remington,

1973). Al-Hamdani *et al* reported in 1997 that the overall prevalence of antibodies gradually increases with age, reaching a peak value of 23.7% in the age group 35-45years (Al-Hamdani *et al*., 1997).There is significant association between seroprevalence of *Toxoplasma gondii* antibodies and gender, with the male sex being at increased risk of *Toxoplasma gondii* seropositivity (Ayeh-Kumi, 2010).

2.1.8 Pathogenesis and clinical manifestations

Toxoplasma gondii infection is usually asymptomatic in both the definitive and intermediate hosts. In immunocompetent persons a powerful cell-mediated immune response develops against the tachyzoites to bring the infection under control. As a result, the tachyzoites go into the tissue cyst or bradyzoite stage (Gazzinelli *et al*., 1993). Severe disease in humans is usually observed only in congenitally infected children and in immunosuppressed individuals, including patients with acquired immune deficiency syndrome (AIDS) (Dubey and Beatie, 1988).

The tachyzoite stage is responsible for tissue damage since there is often no toxin produced during *Toxoplasma gondii* infection (Remington *et al*., 1995). Necrosis is therefore caused by intracellular multiplication of tachyzoites. The severity of clinical signs depends on the number of tachyzoites released, the ability of the host immune system to limit tachyzoites spread, and the organs damaged by the tachyzoites. Due to the ability of adult immunocompetent animals to control tachyzoites spread efficiently, toxoplasmosis is usually asymptomatic. However, in immunocompromised persons, particularly foetuses and persons with AIDS, tachyzoites spread systemically and cause interstitial pneumonia, myocarditis, hepatic necrosis, meningoencephalomyelitis, encephalitis, chorioretinitis, lymphadenopathy, and myositis (Remington *et al*., 1995). Lymphadenitis is a common manifestation in humans. Infected nodes are tender and

discrete but not painful; the infection subsides in weeks or months. Other clinical signs include fever, malaise, fatigue, muscle pains, sore throat, headache, diarrhoea, cough, dyspnea, icterus, seizures, and death. *T. gondii* is also an important cause of abortions and stillbirths in humans and other intermediate hosts (Daffos *et al.*, 1988).

2.1.9 Pregnancy and congenital toxoplasmosis

The 2002 CDC report states that about 15% of women of childbearing age are immune to toxoplasmosis (CDC, 2002). Although pregnant women are not immunosuppressed in the classic sense, immunologic changes of pregnancy may induce a state of increased susceptibility to certain intracellular pathogens, including viruses, intracellular bacteria, and parasites (Daffos *et al.*, 1988).

Women infected with *T. gondii* infection before conception are with a rare exception of not transmitting the infection to their foetuses (Vogel *et al.,* 1996). Unfortunately, women infected with *T. gondii* during pregnancy can transmit the infection across the placenta to their foetuses. The undeveloped immune system of foetuses makes them highly venerable when their mothers become infected for the first time during pregnancy. The risk of congenital transmission is lowest (10–25%) when acute maternal infection occurs during the first trimester and highest (60–90%) when acute maternal infection occurs during the third trimester (Remington *et al.*, 2001; Foulon *et al.*, 1999; Dunn *et al.*, 1999).

However, the severity of disease is worse if infection is acquired in the first trimester (Remington *et al.,* 2001, Holliman, 1995). The overall risk of congenital infection from acute *T. gondii* infection during pregnancy is 20% to 50%. After infection of a pregnant woman, tachyzoites spread through the bloodstream to the placenta, causing cell destruction. Infection acquired during the first trimester may lead to spontaneous abortion, still birth, while infection acquired later during pregnancy is usually asymptomatic in the

neonate (Daffos *et al.*, 1988). Tachyzoites may also infect the foetus, causing damage in multiple organs (Guerina *et al.*, 1994). Congenitally acquired *T. gondii* often infects the brain and retina and can cause a wide spectrum of clinical disease. Mild disease may consist of slightly diminished vision, whereas severely diseased children may exhibit classical signs such as retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcifications (Remington *et al.*, 1995). Hydrocephalus is the least common but most dramatic lesion of congenital toxoplasmosis (Plate 3). Ocular disease is the most common sequel (Georgie, 1994).

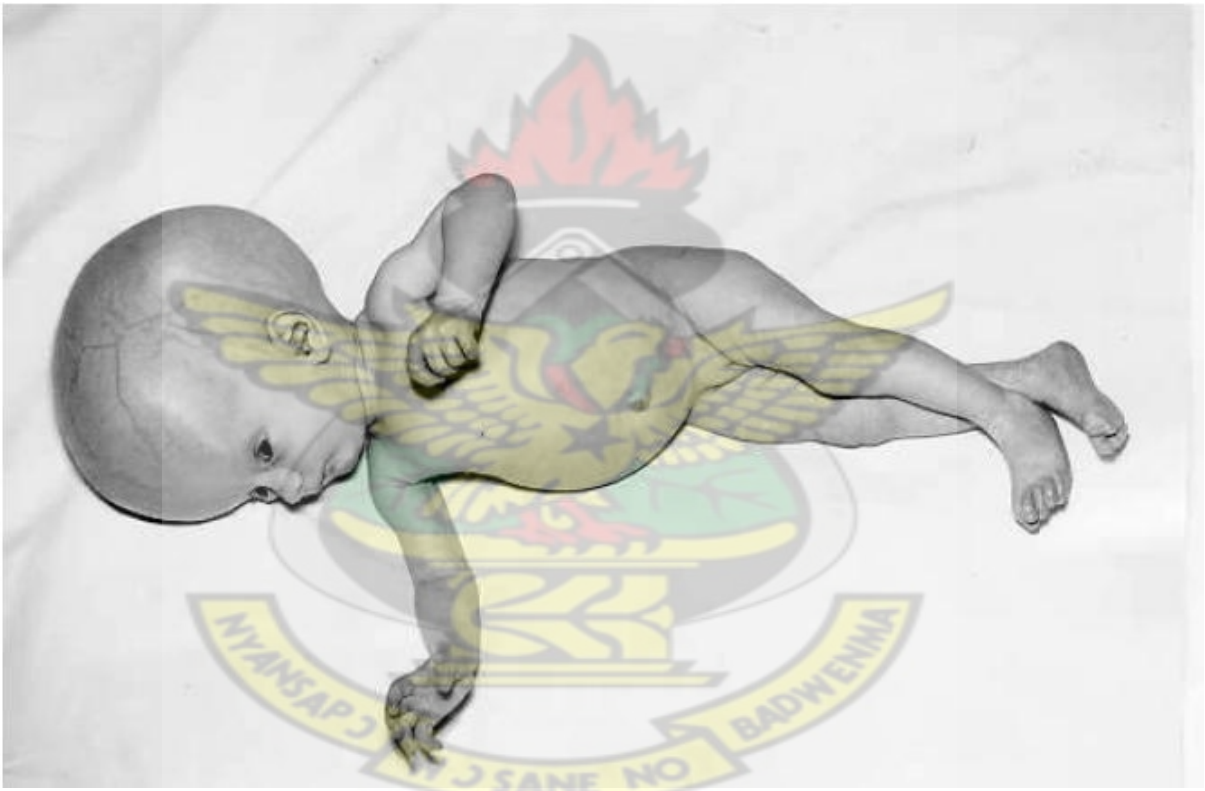

Plate 3: A girl with hydrocephalus due to congenital toxoplasmosis (Dubey and Beattie, 1988)

2.1.10 Diagnosis of *T. gondii* infection

It is very important to diagnosis *T. gondii* in pregnant women especially those who acquire their infection during gestation and in foetuses and new-borns who are congenitally infected (Thulliez *et al.*, 1992; Montoya, 2002; Remington *et al.*, 2001). Diagnosis can be done in the laboratory indirectly by serological methods and directly by polymerase chain reaction (PCR), hybridisation, and histological methods.

2.1.10.1 Serological diagnosis

There are diverse established serological tests for the diagnosis of *T. gondii* infection. Examples include Sabin-Feldman Dye Test, Indirect Flourenscent Antibody Test for IgG and/or IgM detection (IFA), Agglutination Test, Enzyme Immuno-assay(EIA), IgG-avidity for distinguishing between recent and acute infection and Enzyme Linked Immunosorbent Assay (ELISA). Immunoglobulin G (IgG) and ImmunoglobulinM (IgM), and in some cases, Immunoglobulin A (IgA) antibodies are measured for the detection of *T. gondii* (Aurojo and Remington, 1990; Decoster *et al*., 1988). IgM are the first set of antibodies to appear when an infection occurs for the first time. They persist for a limited time, maybe six months, indicating an acute infection (Del Bono *et al*., 1989). IgG titres rise shortly after IgM in a case of acute infection, but declines slowly afterwards and persists for a long time to build immunity against another future parasitemia (Figure3).

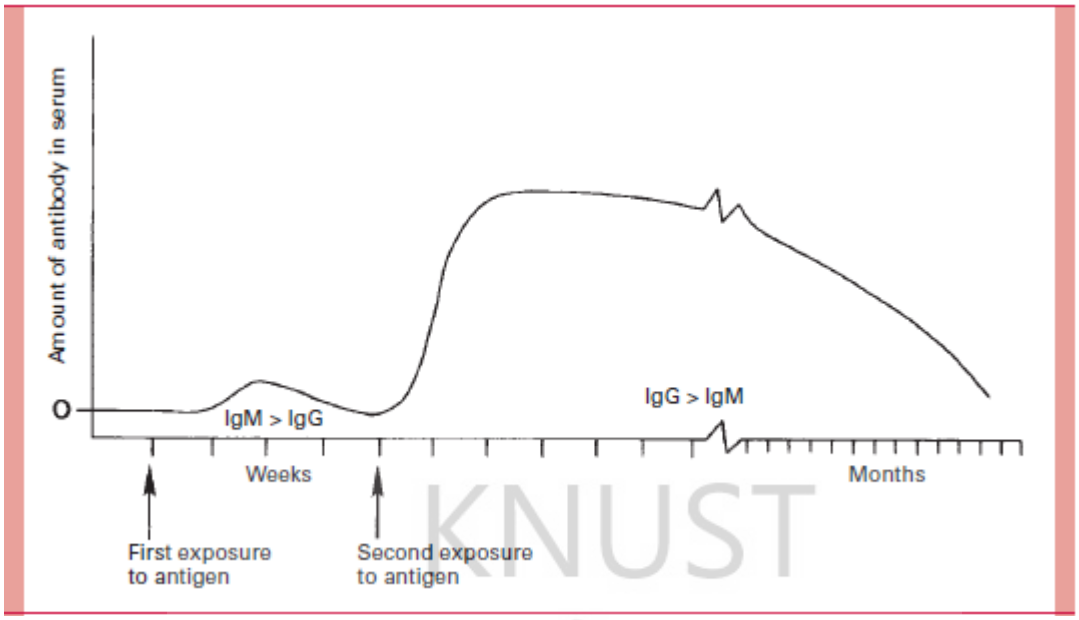

Figure 3: Primary and secondary immunologic responses (Source: Sherris *et al.*, 2004)

Diagnosis of acute toxoplasmosis is based on the demonstration of a significant increase in specific IgG antibody levels and/or the presence of specific IgMantibodies (Brooks *et al.*, 1987; Remington and Klein 1990). Serological tests on blood sample of a new born baby or of an umbilical cord can help detect a congenital infection or otherwise, although sometimes results can be ambiguous. IgG are passed from the mother to the baby through the placenta and could be of maternal origin. On the other hand, IgM cannot pass the placenta, so their presence in the baby is suggestive of an infection in the new-born. Nevertheless, not all infected babies produce IgM (Boyer, 1996). Thus the absence of IgM does not exclude congenital toxoplasmosis. In such a case, a follow-up is highly recommended. IgG-titres of maternal origin naturally decline in the first six months of a new-born baby. Persisting (or rising) IgG-titres prove congenital infection of the baby (Boyer, 1996). Therefore serological testing should be repeated until IgG turns negative,

exclusion of congenital toxoplasmosis cannot be confirmed until this is done (Trojovsky *et al*., 1998).

2.1.10.2 Molecular diagnosis

The weak or lack of immunity in newly born babies and patients with immunodeficiency makes serological diagnosis impossible in such cases. The use of molecular diagnostic techniques is appropriate for such patients, as these techniques do not depend on the immunological status of the host (Switaj *et al*., 2005). The presence of *T. gondii* in a biological sample can be diagnosed by molecular techniques aimed at detecting its genetic material. The sensitivity and specificity of PCR-based methods depend on an appropriate technique for isolation of genetic material (DNA) from samples, the characteristics of the DNA sequence chosen for amplification, and the parameters of the amplification reaction itself (Switaj *et al*., 2005).

The sequence mostly used for detection of *T. gondii*is the B1 gene which was first identified in 1989 by Burg *et al.*, (Burg *et al.*, 1989).The B1 gene has 35 copies in the genome. Although its function is not yet known, it is known to be very specific. Homan *et al* (2000) identified a 529-bp sequence, also specific for *T. gondii*, which has over 300 copies in the genome (85). A real time PCR amplification of the two sequences revealed a ten-fold specificity of the 529-bp sequence than the B1 sequence (Reischl *et al*., 2003). Several other single-copy sequences, including the SAG1 SAG2, SAG3, SAG4, and GRA4 genes have been used as PCR targets (Pelloux *et al*., 1998; Howe *et al*., 1997; Rinder *et al*., 1995). Su *et al*.,(2006)have also developed a set of markers which can provide a high resolution for the detection and genotyping of *T. gondii*. These markers are capable of distinguishing the three clonal lineages of *T. gondii* include SAG2, SAG3, BTUB and GRA6 among others. Dubey *et al.*,(2007)also developed a nested PCR-RFLP

protocol using these three way multilocus markers. The nested PCR gives higher resolution for the detection and genotyping of *T. gondii.* In their work with brain tissues of Arctic foxes in 2008 Prestrud *et al.,* (2008) confirmed the reproducibility of the protocol.

2.1.11 Treatment

Research on *T. gondii* has identified some chemotherapeutic agents that can restrict the growth of actively proliferating parasites which destroy cells and tissues and thereby, preventing damage to the brain and eye (Garin *et al.*, 1985, Araujo *et al.*, 1992, McLeod *et al.*, 1992; Derouin, 2001; Remington *et al.*, 2006; Meneceur *et al.*, 2008).

Pyrimethamine and sulfadiazine have been the main drugs administered for the treatment of toxoplasmosis (Garin *et al.*, 1985). Pyrimethamine interferes with the conversion of folic acid to folinic acid through dihydropteroate synthase (DHPS), whereas sulfadiazine interferes with the formation of folic acid from para-amino benzoic acid (Meneceur *et al.*, 2008). Human beings unlike *T.gondii* can utilize exogenous folinic acid for their cells (Meneceur*et al.,* 2008). The combined therapy of pyrimethamine and sulfadiazine has been seen to be much more active than either pyrimethamine or sulfadiazine alone and has been the "gold standard" to which other antimicrobial agents (single or combine) have been compared (Remington *et al*., 2006; Mui *et al*., 2008).

In 1958, Garin and Eyles discovered spiramycin as an antitoxoplasmic agent in mice (Garin and Eyles, 1998). Spiramycin has since been used as a prophylactic in women during pregnancy to reduce transmission of the parasite from mother to foetus. This is because it is non-toxic and does not cross the placenta (Garin and Eyles, 1998).

2.1.12 Prevention and control

Knowledge about the life cycle of *T. gondii* has aided in the prevention and control of *T. gondii* infection (Frenkel, 1973). Though *T. gondii* infection can be transmitted through various routes, the exposure risk of toxoplasmosis can be minimized by improved personal and environmental hygiene, meat processing standards and health education (Dunn *et al.*, 1999). Research reveals that *T. gondii* oocysts in infected meat can be killed when frozen to a temperature of about -20^{o}C, heated to about 70^{o}C or exposure to gamma radiation (Frenkel *et al*., 1970). The information is being used by regulatory bodies to control the spread of infection through public education.

Several general sanitation and food safety measures are considered in the control of *Toxoplasma* infection. Among these are, proper hand washing, especially, after handling or having contact with a cat and its litter box, raw meat, fresh unwashed fruits and vegetables and soil, and before eating. Proper treatment of fruits and vegetables before eating is highly encouraged. All fruits and vegetables eaten raw should be well washed and peeled off before eating them. Drinking of treated water should be ensured.

Routine diagnosis of pregnant women is paramount in managing and controlling congenital infection. In other parts of the world (USA, Germany and France) immunisation of babies against *T. gondii* infection is a norm (Araujo, 1994).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study Site

The antenatal clinic at the Municipal Hospital, Goaso in the Asunafo North district of Brong-Ahafo was the study site. The Municipal Hospital is the main referral hospital in the district; and also for the newly created Asunafo South district and part of the Western region. The site is in the northern sector of Ghana and is rural hence is appropriate for my problem statement.

3.2 Study Design

The study was a cross-sectional one involving screening of pregnant women during their antenatal care. Approval and Ethical clearance was obtained from the Committee on Human Research Publication and Ethics, SMS KNUST. Permission for the study was also obtained from the authorities at the Municipal Hospital, Goaso. Study participants were recruited based on informed consent. Questionnaires were administered to seek personal information, toxoplasmosis related knowledge, and exposure to *Toxoplasma* infection risk factors. Maternal venous blood was taken from volunteer pregnant women. The blood samples were processed appropriately and tested for *T. gondii* IgM and IgG using ELISA technique. The results were entered into computer data processing software and analysed appropriately according to the study objectives.

3.3 Study Population

The study population consisted of pregnant women attending antenatal clinic during the study period.

3.4 Study Participants

The study participants consisted of self-volunteered pregnant women within the study population. The participants were recruited prospectively and consecutively.

3.5 Sample Size

A hundred and seven pregnant women or more was required in this study for the cross-sectional screening. This number, based on the previous prevalence of 92.5% reported by Ayi *et al* in 2009, was generated using the formula:

$N= [Z^2 (P) (1-P)] / (Error)^2$

Where N= Sample size, Z= 1.96, Error= 5%, P= 92.5%

$N= [(1.96)^2(0.925)(1-0.925)]/(0.05)^2$

$N= [(3.8416)(0.925)(0.075)]/0.0025$

$N= 0.66511/0.0025$

$N= 106.6044$

$N\approx 107$

3.6 Informed consent and Questionnaire administration

Written consent was sought after the study had been explained to the participants and closed-ended questionnaires administered by the interviewer method following informed consent.

3.7 Sample Collection

3.7.1 Maternal Blood

About 3ml venous blood samples were drawn from participant's arm using sterile disposable hypodermic vacutainer needles and tubes. Serum was obtained by centrifugation of venous blood at 14000 rpm for 20 min and stored at -80^{o}C until further use.

3.8 Analyses of Samples

3.8.1 ELISA

Commercial anti-*Toxoplasma* antibodies detection Enzyme Linked Immunosorbent Assay (ELISA) kits (CTK Biotech, Inc, USA) was used to detect IgG and IgM antibodies qualitatively from the serum samples according to manufacturer's instructions.

3.8.1.1 Anti-Toxoplasma IgG Test

Ninety-six well antigen-coated Microtitre plates provided in the kits was removed and allowed to attain room temperature. ELISA plate maps were designed and samples were dispensed into the wells according to the plate maps. All sample-designated wells were filled with 100µl of sample diluent and 10µl of each sample respectively. Positive and negative controls designated wells were filled with 100µl of positive and negative controls respectively. Plates were rocked for about twenty seconds to mix the samples.

Covered plates were incubated at 37^{o}C for 30min. Plates were then washed five times with wash buffer (PBS with Tween 20) and excess liquid absorbed using an absorbent material. Wells were then filled with 100µl of Horse Radish Peroxidase (HRP)-anti human IgG conjugate. Covered plates were then incubated for another 30 min at 37^{o}C. 100µl of TMB substrate were added to each well. Covered plates were incubated at 37^{o}C for 30min in darkness. The reaction was stopped by adding 100µl of stop buffer to each well. The plates were then read for absorbance at a wavelength of 450nm against the blank well within 15 minutes after adding the stop solution using the Spectrophotometer, Multiskan assent (Thermo systems).

3.8.1.2 Anti-Toxoplasma IgM Test

The Procedure was the same for the anti- *Toxoplasma* IgG test, as per manufacturer's instructions.

3.8.1.3 Cut-off Value

The cut-off value was set using the formula: 0.15 + N. where N is the mean optical density (OD) of the negative controls, as instructed by the manufacturer.

3.8.1.4 Calculation of specimen OD ratio

The OD ratio of each specimen was calculated by dividing the OD value by the cut-off value as provided by the manufacturer and shown below:

Specimen OD ratio = Specimen OD

Cut-off Value

3.8.1.5 Assay Validation and Interpretation of results

The mean OD of the Toxo IgG and IgM positive controls must be ≥ 1.00/0.80 respectively (required by international standards). The mean OD of the Toxo IgG and IgM negative controls also must be ≤ 0.10 (required by international standards). Once these requirements are met the results were considered valid and interpreted as follows:

Positive samples were those whose specimen OD ratios were greater than or equal to 1.0 while samples with specimen OD ratio less than 1.0 were considered negative.

3.9 Data Analyses

A descriptive statistical analysis of the serological data in relation to the demographic data collected was done using SPSS (Statistical Package for Social Scientists) version 16 software.

CHAPTER FOUR

RESULTS

4.1 Seroprevalence of Anti – *T. gondii* IgG and IgM

ELISA test conducted on respondents' blood samples to detect anti-*T. gondii*IgM and IgG showed 23.3% (34/146) and 67.8% (99/146) were positive respectively (Figure 4). A breakdown of the analysed data was as follows: 10.3% (15/146) tested positive for only anti-*T. gondii*IgM, 54.8% (80/146) also tested positive for only anti-*T. gondii*IgG and 13% (19/146) tested positive for both anti-*T. gondii*IgM and IgG. This gave a total prevalence of anti-*T gondii* antibodies testing positive to be 78.1% (114/146) (Figure 4).

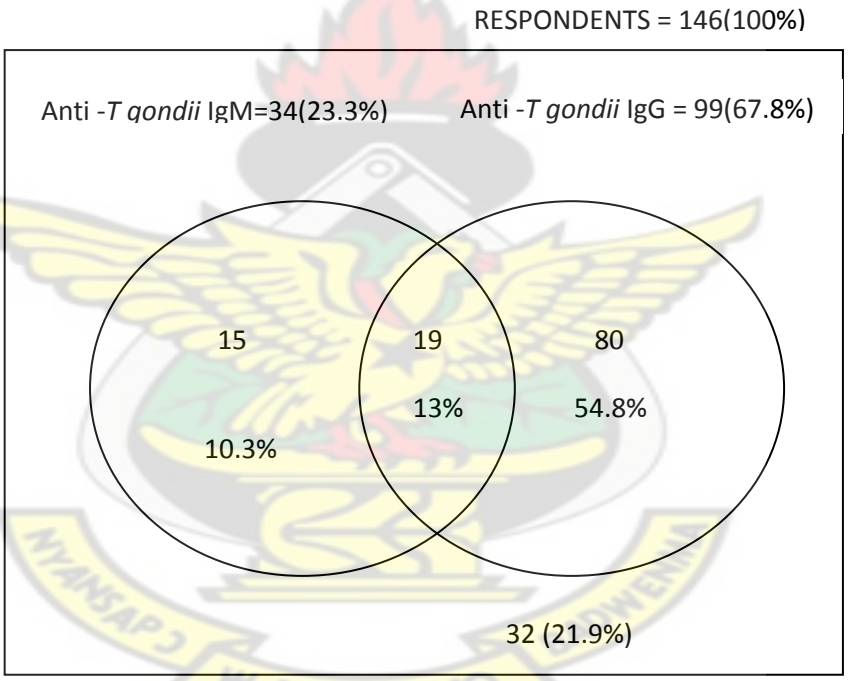

Figure 4: Venn diagram illustration of anti-T gondii anti-bodies prevalence

4.2 General Characteristics of Study Participants

A total of one hundred and forty six (146) women aged 16 to 40 years were involved in the study. The results showed 17.1%(25/146), 37.7%(55/146), 19.2%(28/146),

14.4%(21/146), and 11.6%(17/146) were in the age brackets 16-20, 21-25, 26-30, 31-35, and 36-40 respectively(Table 4.1).

Table 4.1: Age distribution of respondents

Age ranges	Frequency	Percent
16-20	25	17.1
21-25	55	37.7
26-30	28	19.2
31-35	21	14.4
36-40	17	11.6
Total	146	100.0

Analysis of the data collected showed 56.2% (82/146) had attained elementary school, 28.8% (42/146) had no formal education, 11% (16/146) had some second cycle education, and 4.1% (6/146) had tertiary education (Table 4.2).

Table 4.2: Distribution of the educational status of respondents

Educational status	Frequency	Percent
ELEMENTARY	82	56.2
NO FORMAL EDUC.	42	28.8
SECOND CYCLE EDUC.	16	11.0
TERTIARY EDUC.	6	4.1
Total	146	100.0

Regarding their employment, the results revealed that 44.5% (65/146) were farmers, 22.6% (33/146) were traders, 5.5% (8/146) do some kind of office work, 12.3% (18/146) also are vocationally employed and 15.1% (22/146) were unemployed (Table 4.3).

Table 4.3: Occupational distribution of respondents

Occupation	Frequency	Percent
FARMING	65	44.5
OFFICE WORK	8	5.5
TRADING	33	22.6
VOCATIONAL JOB	18	12.3
UNEMPLOYED	22	15.1
Total	146	100.0

Of the total population 88.4% (129/146) were multi-gravid (Table 4.4).

Table 4.4: Distribution previous number of pregnancies of respondents

Previous number of pregnancy	Frequency	Percent
0	17	11.6
1	40	27.4
2	23	15.8
3	27	18.5
Above 3	39	26.7
Total	146	100.0

The results also showed that 24.7% (36/146), 41.6% (61/146) and 33.6% (49/146) of the respondents were in their first, second and third trimester of pregnancy respectively (Table 4.5).

Table 4.5: Distribution of respondents' stage of pregnancy

Trimester of respondent's pregnancy	Frequency	Percent
First trimester	36	24.7
Second trimester	61	41.8
Third trimester	49	33.6
Total	146	100.0

The seroprevalence of anti-*T gondii* IgM and IgG among age groups of the participating pregnant women are shown in Table 4.6 and Table 4.7 respectively with the 21-25 age group range having the highest prevalence of *T. gondii* antibodies tested.

Table 4.6: Seroprevalence of anti-*T. gondii* IgM among age groups

AGE	anti-*T gondii* IgG		
	NEGATIVE	POSITIVE	Total
16-20	20	5	25
21-25	38	17	55
26-30	26	2	28
31-35	15	6	21
36-40	13	4	17
Total	112	34	146

Table 4.7: Seroprevalence of anti-*T. gondii* IgG among age groups

AGE	anti-*T gondii* IgG		
	NEGATIVE	POSITIVE	Total
16-20	12	13	25
21-25	20	35	55
26-30	4	24	28
31-35	5	16	21
36-40	6	11	17
Total	47	99	146

Of the actively infected respondents 41.2% (14/34), 8.8% (3/34), 17.6 (6/34), 20.6% (7/34), and 11.8% (4/34) were farmers, office workers, traders, unemployed and vocationally employed respectively (Table 4.8).

Table 4.8: Relationship between occupation and anti-*T gondii* IgM

OCCUPATION	anti-*T gondii* IgM		
	NEGATIVE	POSITIVE	Total
Farming	51	14(41.2)	65
Office work	5	3(8.8)	8
Trading	27	6(17.6)	33
Unemployed	15	7(20.6)	22
Vocational	14	4(11.8)	18
Total	112	34(100)	146

*percentage in parenthesis

Of the respondents who had past infection, 48.5% (48/99) were farmers, 6.1% (6/99) were office workers, 22.2% (22/99) were traders, 11.1% (11/99) were unemployed and 12.1% (12/99) do some kind of vocational job (Table 4.16).

Table 4.9: Relationship between occupation and anti *T gondii* IgG

Occupation	anti *T gondii* IgG		
	NEGATIVE	POSITIVE	Total
Farming	17	48(48.5)	65
Office work	2	6(6.1)	8
Trading	11	22(22.2)	33
Unemployed	11	11(11.1)	22
Vocational	6	12(12.1)	18
Total	47	99(100)	146

It was also observed that respondents in their second trimester of pregnancy recorded the highest percentage of *T. gondii* infection (Table 4.10 and Table 4.11).

Table 4.10: Relationship between stage of pregnancy and anti *T gondii* IgG

STAGE OF CURRENT PREGNANCY		anti *T gondii* IgG		
		NEGATIVE	POSITIVE	Total
	1st trimester	9	27	36
	2nd trimester	23	38	61
	3rd trimester	15	34	49
Total		47	99	146

Table 4.11: Relationship between stage of pregnancy and anti-*T gondii* IgM

STAGE OF CURRENT PREGNANCY		anti-*T gondii* IgM		
		NEGATIVE	POSITIVE	Total
	1st trimester	26	10	36
	2nd trimester	46	15	61
	3rd trimester	40	9	49
Total		112	34	146

CHAPTER FIVE

DISCUSSION

Though acquisition of *Toxoplasma gondii* infection by pregnant women during gestation and its effects on both the mother and the foetus is preventable, it still remains the cause of tragic disease in the newborn (Remington *et al*, 2006). The aim of this study was to determine the seroprevalence of anti-*T. gondii*IgM and IgG among pregnant women in the Asunafo North district of the Brong-Ahafo region, Ghana. An anti-*T gondii* IgG sero-prevalence value of 67.8% (99/146) was found among the pregnant women. This sero-prevalence value is lower compared to the 73.6% IgG prevalence reported by Ayi *et al*(2009) among pregnant women in the Greater Accra region but greater than the 37.6% value reported by Kwofie*et al*(2012)also among pregnant women in their third trimmest of pregnancy in the Greater Accra region of Ghana. The seroprevalence of 67.8% anti-*T gondii*IgG among pregnant implies that this high percentage have had *T. gondii* infection in the past. The presence of anti-*T gondii* IgG antibodies in the sera of the pregnant women may also indicate latent infections, and therefore signifying the potential reoccurrence of the disease condition among the pregnant women putting their foetuses at risk. The study also recorded 23.3% (34/146) anti-*T gondii* IgM seroprevalence among the pregnant women. This implies that 23.3% of the pregnant women have had a recent infection. The very high seroprevalence of active infection could be as a result of primary infection or a reactivated past infection (especially, in the 54.8% that tested positive for both anti-*T. gondii* IgG) which is reported to occur in immuno-compromised individuals. However, antibodies, particularly IgM may not rise during reactivation of the encysted form of the parasite (Holliman, 1995). The presence of anti-*T gondii* IgM strongly suggests the presence of parasite antigens in the circulatory system of the pregnant women, implying possible presence of parasites, specifically tachyzoites, also in their

circulatory system. Although prepregnancy *Toxoplasma* infection status of the pregnant women is not known, it could be deduced from the combination of antibodies detected and age of pregnancy that 6.9% (10/146) in their first trimester could have acquired the infection up to 3 months before conception or during pregnancy.

Montoya and Remington(2008) advised that if serological test results suggest a recently acquired infection, an effort should be made to determine whether the infection was likely acquired during gestation or shortly before conception in order to determine if the foetus is at risk. This implies that 6.9% (10/146) of the respondents who were in their first trimester of pregnancy stand 10% -15% risk of transmitting the infection congenitally with severe consequences to their foetus and this risk will rise up to 68% in the third trimester of pregnancy (Thulliez *et al.,* 1992)

Congenital infection of the feotus occurs if the mother acquires acute infection during pregnancy. An acute infection may result from a primary infection or re-activation of latent (chronic) infection in any case of immuno-depression (Luft and Remington, 1992; Isrealski and Remington, 1993). In a situation where the women got infected before the pregnancy, the presence of tissue cysts still poses a high risk of congenital transmission by increasing the chances of re-activation, i.e, the release of bradyzoites (which will later be transformed into tachyzoites) into the bloodstream, worsened by a suppression of one's immune system.

Follow-up on babies born to these mothers for about a year could help to really conclude on infectivity, as was done and published by Trojovsky *et al* (1998). They followed up on babies with anti-*T. gondii*IgG at birth for about a year. Babies that turned sero-negative eventually were declared negative while those that remained sero-positive were declared positive and treated. It is only through adequate follow-up screening that congenital transmission can be excluded (Trojovsky *et al.,* 1998).The prevalence of 78.1% observed

indicates that most cases of newborns with toxoplasmosis will be detected. The high prevalence of toxoplasmosis may be due to consumption of undercooked meat, poor hygienic practises associated with food handling (Jones J.L. *et al*., 2007) and soil contamination in Ghana (Afonso*et al*., 2008).The distribution of toxoplasmosis is known to increase according to age (Nester E.W. *et al*., 2004).However, in this study no significant association existed between the various age groups for IgG(P = 0.086) and IgM (P = 0.174).The p-value for the association between age and prevalence was greater than 0.05 (p>0.05) hence the conclusion that there is no significant association between age and prevalence of *Toxoplasma gondii* antibodies.

CHAPTER SIX

6.1 CONCLUSIONS AND RECOMMENDATIONS

This study has revealed that at least a third of the participating pregnant women have *T. gondii* infection. Most importantly, it has given a description of the *T. gondii* infection among pregnant women in the northern sector of Ghana and rural settings. These results indicate high risk of congenital transmission of *T. gondii* in Ghana and also provide baseline data for future work ascertain the rate of mother-to-child transmission in Ghana. The p-value for the association between age and prevalence was greater than 0.05 ($p>0.05$) hence the conclusion that there is no significant association between age and prevalence of *Toxoplasma gondii* antibodies. It would be appropriate to replicate this study in other districts of Ghana. Considering the outcome and health implications of this investigation, there is the need for further studies to determine the prevalence of toxoplasmosis among groups of people such as pregnant women and women of child bearing age, people living with HIV and AIDS, blood donors and the general population. It must be mentioned however that some of the cases negative for IgM antibodies may be early infections with very low levels of antibody response. It must also be noted that by being based on antibody detection, the assays performed in this study may have shown false negatives. Hence, seroprevalence analyses could be a low estimate of disease burden. Polymerase Chain Reaction (PCR) may be a more accurate method in delineating the infection rate.

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APPENDICES:APPENDIX Ia: Raw data of ELISA TEST RESULTS ELISA WORKSHEET 1 (*anti T gondii* IgG)

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK OD=0.062	002 OD=0.117 NEGATIVE	010 OD=2.365 POSITIVE	018 OD=2.241 POSITIVE	026 OD=2.189 POSITIVE	034 OD=1.729 POSITIVE	042 OD=0.892 NEGATIVE	050 OD=1.991 POSITIVE	058 OD=1.237 POSITIVE	066 OD=0.564 NEGATIVE	074 OD=2.066 POSITIVE	082 OD=1.158 POSITIVE
B	POSITIVE CONTROL OD=1.569	003 OD=1.826 POSITIVE	011 OD=2.935 POSITIVE	019 OD=1.431 POSITIVE	027 OD=2.425 POSIVE	035 OD=1.389 POSITIVE	043 OD=0.569 NEGATIVE	051 OD=1.409 POSITIVE	059 OD=2.060 POSITIVE	067 OD=2.591 POSITIVE	075 OD=2.688 POSITIVE	083 OD=0.163 NEGATIVE
C	NEGATIVE CONTROL OD=0.193	004 OD=0.635 NEGATIVE	012 OD=2.336 POSITIVE	020 OD=1.959 POSITIVE	028 OD=1.241 POSITIVE	036 OD=1.799 POSITIVE	044 OD=1.946 POSITIVE	052 OD=1.870 POSITIVE	060 OD=2.423 POSITIVE	068 OD=0.693 NEGATIVE	076 OD=2.66 POSITIVE	084 OD=1.160 POSITVE
D	CALIBRATOR1 (0.1IU/ml) OD=0.125	005 OD=1.311 POSITIVE	013 OD=2.423 POSITIVE	021 OD=1.929 POSITIVE	029 OD=2.154 POSITIVE	037 OD=2.365 POSITIVE	045 OD=0.483 NEGATIVE	053 OD=2.079 POSITIVE	061 2.048 POSITIVE	069 OD=1.930 POSITIVE	077 1.747 POSITIVE	085 OD=1.159 POSITIVE
E	CALIBRATOR2 (32IU/ml) OD=0.791	006 OD=0.362 NEGATIVE	014 OD=2.106 POSITIVE	022 OD=1.097 POSITIVE	030 OD=2.211 POSITIVE	038 OD=1.067 POSITIVE	046 OD=2.396 POITIVE	054 OD=2.309 POSITIVE	062 OD=2.396 POSITIVE	070 OD=0.184 NEGATIVE	078 OD=2.008 POSITIVE	086 OD=0.153 NEGATIVE
F	CALIBRATOR3 (100IU/ml) OD=0.814	007 OD=0.317 NEGATIVE	015 OD=2.309 POSITIVE	023 OD=1.034 POSITIVE	031 2.447 POSITIVE	039 2.049 POSITIVE	047 OD=0.491 NEGATIVE	055 OD=0.472 NEGATIVE	063 OD=0.695 NEGATIVE	071 OD=0.728 NEGATIVE	079 OD=0.425 NEGATIVE	087 OD=0.152 NEGATIVE
G	CALIBRATOR4 (300IU/ml) OD=1.146	008 OD=1.731 POSITIVE	016 OD=1.363 POSITIVE	024 OD=0.449 NEGATIVE	032 2.200 POSITIVE	040 OD=2.230 POSITIVE	048 OD=2.357 POSITIVE	056 OD=0.664 NEGATIVE	064 OD=0.590 NEGATIVE	072 OD=0.299 NEGATIVE	080 OD=2.922 POSITIVE	088 OD=0.163 NEGATIVE
H	001 1.579 POSITIVE	009 OD=2.621 POSITIVE	017 OD=1.338 POSITIVE	025 OD=2.797 POSITIVE	033 OD=1.200 POSITIVE	041 OD=2.357 POSITIVE	049 OD=1.865 POSITIVE	057 OD=1.428 POSITIVE	065 OD=0.695 NEGATIVE	073 OD=2.144 POSITIVE	081 OD=0.161 NEGATIVE	089 OD=0.166 NEGATIVE

ELISA WORKSHEET 2 (*anti T gondii*IgG)

	1	2	3	4	5	6	7	8	9	10	11
A	BLANK OD=0.035	091 OD=2.011 POSITIVE	099 OD=1.461 POSIVE	107 OD=1.397 POSITIVE	115 OD=0.157 NEGATIVE	123 OD=0.199 NEGATIVE	131 OD=1.078 POSITIVE	139 OD=0.701 NEGATIVE			
B	POSITIVE CONTROL OD=2.316	092 OD=2.268 POSITIVE	100 OD=0.274 NEGATIVE	108 OD=1.067 POSITIVE	116 OD=1.432 POSITIVE	124 OD=1.237 POSITIVE	132 OD=0.399 NEGATIVE	140 OD=1.313 POSITIVE			
C	NEGATIVE CONTROL 0.077	093 2.755 POSITIVE	101 OD=0.263 NEGATIVE	109 OD=0.213 NEGATIVE	117 OD=0.579 NEGATIVE	125 OD=0.694 NEGATIVE	133 OD=1.117 POSITIVE	141 OD=1.388 POSITIVE			
D	CALIBRATOR1 (0.1IU/ml) OD=0.075	094 OD=3.014 POSITIVE	102 OD=1.337 POSITIVE	110 OD=0.323 NEGATIVE	118 OD=1.095 POSITIVE	126 OD=1.138 POSITIVE	134 OD=0.389 NEGATIVE	142 OD=1.286 POSITIVE			
E	CALIBRATOR2 (32IU/ml) OD=1.366	095 OD=2.376 POSITIVE	103 OD=1.367 POSITIVE	111 OD=0814 NEGATIVE	119 OD=1.244 POSITIVE	127 OD=1.193 POSITIVE	135 OD=0.782 NEGATIVE	143 OD=1.306 POSITIVE			
F	CALIBRATOR3 (100IU/ml) OD=1.806	096 OD=2.621 POSITIVE	104 OD=0.519 NEGATIVE	112 OD=1.357 POSITIVE	120 OD=0.377 NEGATIVE	128 OD=0.216 NEGATIVE	136 OD=0.319 NEGATIVE	144 OD=1.192 POSITIVE			
G	CALIBRATOR4 (300IU/ml) OD=1.929	097 OD=1.106 POSITIVE	105 OD=0.309 NEGATIVE	113 OD=0.179 NEGATIVE	121 OD=1.018 POSITIVE	129 OD=1.245 POSITIVE	137 OD=1.204 POSITIVE	145 OD=1.162 POSITVE			
H	090 OD=0.166 NEGATIVE	098 OD=1.141 POSITIVE	106 OD=1.204 POSITIVE	114 OD=1.254 POSITIVE	122 OD=0.821 NEGATIVE	130 OD=1.305 POSITIVE	138 OD=1.286 POSITIVE	146 OD=1.181 POSITIVE			

ELISA WORKSHEET 3(anti *T gondii*IgM)

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK OD=0.031	005 OD=0.628 NEGATIVE	013 OD=0.286 NEGATIVE	021 OD=0.507 NEGATIVE	029 OD=0.312 NEGATIVE	037 OD=0.325 NEGATIVE	045 OD=0.285 NEGATIVE	053 OD=0.476 NEGATIVE	061 OD=0.481 NEGATIVE	069 OD=0.582 NEGATIVE	077 OD=0.503 NEGATIVE	085 OD=0.105 NEGATIVE
B	POSITIVE CONTROL OD=1.139	006 OD=0.578 NEGATIVE	014 OD=0.305 NEGATIVE	022 OD=0.306 NEGATIVE	030 OD=0.323 NEGATIVE	038 OD=0.610 NEGATIVE	046 OD=0.730 NEGATIVE	054 OD=0.590 NEGATIVE	062 OD=0.544 NEGATIVE	070 OD=0.186 NEGATIVE	078 OD=0.630 NEGATIVE	086 OD=0.603 NEGATIVE
C	NEGATIVE CONTROL OD=0.073	007 OD=0.578 NEGATIVE	015 OD=0.515 NEGATIVE	023 OD=0.721 NEGATIVE	031 OD=0.351 NEGATIVE	039 OD=0.408 NEGATIVE	047 OD=0.307 NEGATIVE	055 OD=0.436 NEGATIVE	063 OD=0.399 NEGATIVE	071 OD=0224 NEGATIVE	079 OD=0.455 NEGATIVE	087 OD=0.737 NEGATIVE
D	CALIBRATOR (0.1IU/ml) OD=0.558	008 OD=0.377 NEGATIVE	016 OD=0.041 NEGATIVE	024 OD=0.670 NEGATIVE	032 OD=0.764 NEGATIVE	040 OD=0.313 NEGATIVE	048 OD=0.433 NEGATIVE	056 OD=0.316 NEGATIVE	064 OD=0.390 NEGATIVE	072 OD=0352 NEGATIVE	080 OD=0.608 NEGATIVE	088 OD=0.575 NEGATIVE
E	001 OD=0.593 NEGATIVE	009 OD=.566 NEGATIVE	017 OD=0.628 NEGATIVE	025 OD=0.531 NEGATIVE	033 OD=0.384 NEGATIVE	041 OD=0.819 NEGATIVE	049 OD=0.602 NEGATIVE	057 OD=0.707 NEGATIVE	065 OD=0.349 NEGATIVE	073 OD=0451 NEGATIVE	081 OD=0.887 NEGATIVE	089 OD=0.883 NEGATIVE
F	002 OD=0.859 NEGATIVE	010 OD=0.694 NEGATIVE	018 OD=0.330 NEGATIVE	026 OD=0.478 NEGATIVE	034 OD=0.340 NEGATIVE	042 OD=0.731 NEGATIVE	050 OD=0.190 NEGATIVE	058 OD=0.109 NEGATIVE	066 OD=0.728 NEGATIVE	074 OD=1.091 POSITIVE	082 OD=0.785 NEGATIVE	090 OD=1.998 POSITIVE
G	003 OD=0.789 NEGATIVE	011 OD=0.531 NEGATIVE	019 OD=0.376 NEGATIVE	027 OD=0.638 NEGATIVE	035 OD=0.510 NEGATIVE	043 OD=1.993 POSITIVE	051 OD=0.533 NEGATIVE	059 OD=0.430 NEGATIVE	067 OD=0.720 NEGATIVE	075 OD=0.091 NEGATIVE	083 OD=1.098 POSITIVE	091 OD=0.553 NEGATIVE
H	004 OD=0.449 NEGATIVE	012 OD=0.486 NEGATIVE	020 OD=0.567 NEGATIVE	028 OD=0.496 NEGATIVE	036 OD=0.816 NEGATIVE	044 OD=1.801 POSITIVE	052 OD=0.448 NEGATIVE	060 OD=0.501 NEGATIVE	068 OD=0.454 NEGATIVE	076 OD=0.307 NEGATIVE	084 OD=0.105 NEGATIVE	092 OD=0.387 NEGATIVE

ELISA WORKSHEET 4 (anti *T gondii*IgM)

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK OD=0.004	096 OD=0.795 NEGATIVE	103 OD=1.100 POSITIVE	110 OD=1.018 POSITIVE	117 OD=1.042 POSITIVE	124 OD=1.210 POSITIVE	131 OD=1.004 POSITIVE	138 OD=1.065 POSITIVE	145 OD=1.383 POSITIVE			
B	POSITIVE CONTROL OD=1.139	097 OD=0.692 NEGATIVE	104 OD=1.628 POSITIVE	111 OD=0.866 NEGATIVE	118 OD=1.345 POSITIVE	125 OD1.020 POSITIVE	132 OD=0.664 NEGATIVE	139 OD=1.722 POSITIVE	146 OD=1.015 POSITIVE			
C	NEGATIVE CONTROL OD=0.073	098 OD=0.738 NEGATIVE	105 OD=0.841 NEGATIVE	112 OD=0.747 NEGATIVE	119 OD=1.071 POSITIVE	126 OD=0.636 NEGATIVE	133 OD=1.192 POSITIVE	140 OD=1.268 POSITIVE				
D	CALIBRATOR (0.1IU/ml) OD=0558	099 OD=0783 NEGATIVE	106 OD=0.791 NEGATIVE	113 OD=1.910 POSITIVE	120 OD=0.665 NEGATIVE	127 OD=0.755 NEGATIVE	134 OD=1.926 POSITIVE	141 OD=1.027 POSITIVE				
E	093 OD=0.638 NEGATIVE	100 OD=1.293 POSITIVE	107 OD=0.748 NEGATIVE	114 OD=0.645 NEGATIVE	121 OD=1.985 POSITIVE	128 OD=1.934 POSITIVE	135 OD=0.854 NEGATIVE	142 OD=1.375 POSITIVE				
F	094 OD=0.721 NEGATIVE	101 OD=0.800 NEGATIVE	108 OD=1.198 POSITIVE	115 OD=1.134 POSITIVE	122 OD=1.244 POSITIVE	129 OD=1.153 POSITIVE	136 OD=0.802 NEGATIVE	143 OD=0.633 NEGATIVE				
G	095 OD=0.750 NEGATIVE	102 OD=0.699 NEGATIVE	109 OD=1.490 POSITIVE	116 OD=1.650 POSITIVE	123 OD=0.517 NEGATIVE	130 OD=0.411 NEGATIVE	137 OD=1.364 POSITIVE	144 OD=0.605 NEGATIVE				

NUMBER POSITIVE=34

APPENDIX Ib: Raw data of questionnaire matched with ELISA test results

ID	AGE	EDUCATION	OCCUPATION	PNP	NS/A	SPP	PCH/N	HRM	MEATC	PREPM	TYPEM	KC	TK	VEGC	PREPV	IgM	IgG
1	40	O' LEVEL	FARMER	>3	0	7 to 9	yes	yes	yes	cooked till soft	pork, mutton,beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
2	16	ELEMENTARY	STUDENT	0	0	4 to 6	yes	yes	yes	cooked till soft	mutton,beef, chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
3	18	ELEMENTARY	vocational	1	1	1to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	yes	Mutton, beef	yes	cooked	NEGATIVE	POSITIVE
4	31	ELEMENTARY	MARKET WOMAN	>3	1	7 to 9	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
5	25	ELEMENTARY	UNEMPLOYED	3	0	7 to 9	No	yes	yes	cooked till soft	pork, mutton, beef, chicken	yes	Mutton, beef	yes	cooked	NEGATIVE	POSITIVE
6	16	ELEMENTARY	STUDENT	1	0	7 to 9	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
7	22	ELEMENTARY	TRADER	2	2	1 to 3	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
8	20	NO FORMAL EDU	FARMER	2	0	4 to 6	No	yes	yes	cooked till soft	pork, mutton,beef, chicken	yes	Mutton, beef	yes	cooked	NEGATIVE	POSITIVE
9	35	ELEMENTARY	FARMER	>3	0	1 to 3	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
10	33	NO FORMAL EDU	TRADER	>3	0	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	yes	Mutton, beef	yes	cooked	NEGATIVE	POSITIVE
11	18	ELEMENTARY	STUDENT	0	0	7 to 9	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
12	20	ELEMENTARY	FARMER	1	0	7 to 9	yes	yes	Yes	cooked till soft	mutton,beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
13	29	ELEMENTARY	AGRO INDUSTRY	3	0	1 to 3	No	No	Yes	cooked till soft	mutton, beef, chicken	yes	mutton	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
14	31	SECONDARY	MARKET WOMAN	1	0	1 to 3	No	yes	yes	cooked till soft	pork, mutton,beef, chicken	yes	Mutton	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE

15	23	NO FORMAL EDU	FARMER	>3	0	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
16	29	ELEMENTARY	FARMER	>3	0	7 to 9	No	No	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
17	20	NO FORMAL EDU	FARMER	3	0	4 to 6	No	yes	yes	cooked till soft	Beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
18	24	ELEMENTARY	FARMER	1	0	1 to 3	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	Mutton, beef, chicken	yes	cooked	NEGATIVE	POSITIVE
19	38	ELEMENTARY	FARMER	>3	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
20	23	ELEMENTARY	FARMER	2	0	7 to 9	yes	yes	yes	cooked till soft	pork, mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
21	40	ELEMENTARY	FARMER	>3	0	4 to 6	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
22	21	ELEMENTARY	STUDENT	1	1	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
23	18	ELEMENTARY	UNEMPLOYED	0	0	4 to 6	No	No	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
24	19	NO FORMAL EDU	FARMER	0	0	4 to 6	No	No	yes	cooked till soft	pork, mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
25	30	TERTIARY	TEACHER	3	3	1 to 3	NO	yes	yes	cooked till soft	pork, mutton, beef, chicken	yes	pork, mutton, beef, chicken	yes	steamed	NEGATIVE	POSITIVE
26	18	NO FORMAL EDU	vocational	2	2	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE

27	24	ELEMENTARY	UNEMPLOYED	1	0	7 to 9	No	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	Mutton	yes	cooked	NEGATIVE	POSITIVE
28	29	ELEMENTARY	FARMER	>3	0	4 to 6	yes	yes	yes	cooked till soft	pork, mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
29	25	ELEMENTARY	FARMER	2	0	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
30	29	ELEMENTARY	FARMER	>3	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
31	25	ELEMENTARY	FARMER	>3	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
32	25	NO FORMAL EDU	vocational	1	0	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
33	30	NO FORMAL EDU	FARMER	>3	2	7 to 9	No	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
34	28	NO FORMAL EDU	FARMER	>3	1	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
35	30	NO FORMAL EDU	FARMER	>3	0	7 to 9	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
36	26	NO FORMAL EDU	FARMER	>3	2	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
37	27	TERTIARY	TEACHER	3	2	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
38	29	ELEMENTARY	FARMER	3	1	7 to 9	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
39	35	ELEMENTARY	vocational	2	0	4 to 6	No	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE

40	37	ELEMENTARY	FARMER	>3	0	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
41	35	ELEMENTARY	TRADER	>3	0	7 to 9	yes	yes	yes	cooked tough/soft	Beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
42	18	ELEMENTARY	vocational	1	1	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
43	23	NO FORMAL EDU	FARMER	3	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
44	23	NO FORMAL EDU	FARMER	1	1	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
45	22	NO FORMAL EDU	FARMER	2	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
46	33	NO FORMAL EDU	FARMER	3	1	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
47	22	ELEMENTARY	FARMER	2	2	4 to 6	No	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
48	25	SECONDARY	FARMER	3	0	1 to 3	No	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
49	23	NO FORMAL EDU	FARMER	3	1	7 to 9	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
50	19	SECONDARY	TRADER	0	0	1 to 3	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
51	34	NO FORMAL EDU	FARMER	>3	1	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
52	28	ELEMENTARY	vocational	3	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE

53	20	ELEMENTARY	UNEMPLOYED	0	0	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
54	24	ELEMENTARY	TRADER	1	1	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
55	22	NO FORMAL EDU	FARMER	2	0	4 to 6	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
56	35	NO FORMAL EDU	vocational	>3	0	7 to 9	yes	yes	yes	cooked tough/soft	chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
57	28	NO FORMAL EDU	vocational	>3	0	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
58	18	NO FORMAL EDU	FARMER	1	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
59	22	ELEMENTARY	vocational	0	0	1 to 3	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
60	22	NO FORMAL EDU	MARKET WOMAN	1	0	4 to 6	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
61	21	NO FORMAL EDU	FARMER	3	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
62	40	NO FORMAL EDU	FARMER	>3	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
63	32	NO FORMAL EDU	FARMER	2	0	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
64	38	ELEMENTARY	TRADER	>3	1	1 to 3	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
65	25	NO FORMAL EDU	FARMER	2	0	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
66	28	NO FORMAL EDU	TRADER	2	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE

67	24	NO FORMAL EDU	FARMER	2	2	4 to 6	No	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
68	28	ELEMENTARY	TRADER	2	0	4 to 6	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
69	26	TERTIARY	OFFICE WORK	2	2	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
70	37	ELEMENTARY	FARMER	>3	0	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
71	23	ELEMENTARY	FARMER	2	0	7 to 9	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
72	18	SECONDARY	STUDENT	0	0	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
73	22	SECONDARY	STUDENT	0	0	1 to 3	No	yes	yes	cooked tough/soft	pork, mutton,beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
74	23	ELEMENTARY	TRADER	0	0	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
75	24	ELEMENTARY	TRADER	2	1	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
76	23	ELEMENTARY	vocational	1	1	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
77	24	ELEMENTARY	TRADER	3	0	1 to 3	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
78	25	TERTIARY	OFFICE WORK	1	0	7 to 9	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
79	24	ELEMENTARY	TRADER	1	1	7 to 9	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE

80	27	SECONDARY	TRADER	1	0	7 to 9	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
81	28	ELEMENTARY	FARMER	3	1	4 to 6	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
82	27	TERTIARY	OFFICE WORK	1	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
83	24	ELEMENTARY	UNEMPLOYED	1	0	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	steamed	POSITIVE	NEGATIVE
84	28	NO FORMAL EDU	FARMER	1	1	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
85	26	ELEMENTARY	FARMER	3	0	7 to 9	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
86	24	ELEMENTARY	vocational	1	0	4 to 6	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
87	23	NO FORMAL EDU	MARKET WOMAN	3	0	7 to 9	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
88	37	ELEMENTARY	FARMER	>3	0	7 to 9	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
89	36	NO FORMAL EDU	FARMER	>3	0	4 to 6	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
90	21	SECONDARY	UNEMPLOYED	0	0	7 to 9	yes	yes	yes	cooked till soft	Beef, chicken	yes	chicken	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
91	30	ELEMENTARY	FARMER	>3	0	7 to 9	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE

92	25	NO FORMAL EDU	FARMER	3	0	4 to 6	yes	yes	yes	cooked till soft	mutton, beef, chicken	yes	mutton	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
93	24	SECONDARY	UNEMPLOYED	1	1	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
94	31	NO FORMAL EDU	FARMER	3	0	7 to 9	yes	yes	yes	cooked till soft	chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
95	40	SECONDARY	FARMER	>3	1	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
96	21	ELEMENTARY	vocational	3	0	1 to 3	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
97	30	NO FORMAL EDU	vocational	3	0	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
98	37	ELEMENTARY	MARKET WOMAN	>3	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	chicken	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
99	24	ELEMENTARY	MARKET WOMAN	1	1	7 to 9	yes	No	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
100	20	ELEMENTARY	UNEMPLOYED	1	0	7 to 9	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
101	23	ELEMENTARY	MARKET WOMAN	1	0	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
102	32	ELEMENTARY	MARKET WOMAN	3	0	7 to 9	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
103	24	SECONDARY	UNEMPLOYED	1	1	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
104	40	ELEMENTARY	FARMER	>3	0	4 to 6	No	yes	yes	cooked till soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
105	24	ELEMENTARY	MARKET WOMAN	2	1	4 to 6	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE

106	26	NO FORMAL EDU	MARKET WOMAN	2	0	7 to 9	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
107	28	NO FORMAL EDU	MARKET WOMAN	2	0	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
108	24	ELEMENTARY	MARKET WOMAN	1	0	1 to 3	yes	yes	yes	cooked till soft	pork, mutton, beef, chicken	yes	mutton	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
109	24	SECONDARY	OFFICE WORK	0	0	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	steamed, cooked	POSITIVE	NEGATIVE
110	23	ELEMENTARY	vocational	0	0	4 to 6	yes	yes	yes	cooked till soft	pork, mutton, beef, chicken	yes	Mutton, beef, chicken	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
111	20	ELEMENTARY	UNEMPLOYED	1	0	4 to 6	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
112	19	ELEMENTARY	STUDENT	0	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
113	24	ELEMENTARY	TRADER	3	1	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
114	25	ELEMENTARY	FARMER	2	0	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
115	25	ELEMENTARY	FARMER	3	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
116	20	ELEMENTARY	STUDENT	1	1	7 to 9	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
117	18	ELEMENTARY	STUDENT	0	0	4 to 6	No	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE

118	27	TERTIARY	OFFICE WORK	0	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
119	37	NO FORMAL EDU	FARMER	>3	0	4 to 6	No	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	pork, mutton, beef, chicken	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
120	35	ELEMENTARY	TRADER	>3	1	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
121	31	ELEMENTARY	TRADER	1	0	4 to 6	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
122	31	ELEMENTARY	vocational	1	0	1 to 3	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	Mutton, beef, chicken	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
123	19	ELEMENTARY	STUDENT	1	0	7 to 9	No	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	NEGATIVE
124	32	NO FORMAL EDU	FARMER	2	0	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	yes	beef	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
125	20	ELEMENTARY	STUDENT	1	1	1 to 3	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
126	35	ELEMENTARY	TRADER	3	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	mutton, beef	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
127	39	ELEMENTARY	TRADER	3	3	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	yes	Mutton, beef, chicken	yes	Fresh, steamed, cooked	NEGATIVE	POSITIVE
128	39	ELEMENTARY	FARMER	>3	1	1 to 3	No	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
129	22	SECONDARY	FARMER	>3	2	4 to 6	yes	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
130	18	ELEMENTARY	MARKET WOMAN	1	1	7 to 9	yes	yes	yes	cooked till soft	chicken	No	NA	yes	cooked	NEGATIVE	POSITIVE
131	23	SECONDARY	FARMER	1	1	7 to 9	yes	yes	yes	cooked tough/soft	beef	yes	chicken	yes	fresh and raw	POSITIVE	POSITIVE
132	23	ELEMENTARY	vocational	2	2	7 to 9	yes	yes	yes	cooked tough/soft	chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
133	40	NO FORMAL EDU	FARMER	>3	1	7 to 9	yes	yes	yes	cooked tough/soft	chicken	No	NA	yes	cooked	POSITIVE	POSITIVE

134	22	ELEMENTARY	OFFICE WORK	1	0	7 to 9	No	yes	yes	cooked tough/soft	mutton	yes	mutton	yes	steamed	POSITIVE	NEGATIVE
135	30	ELEMENTARY	FARMER	3	0	7 to 9	yes	yes	yes	cooked tough/soft	chicken	No	NA	yes	cooked	NEGATIVE	NEGATIVE
136	18	SECONDARY	UNEMPLOYED	1	0	7 to 9	No	yes	yes	cooked till soft	chicken	No	NA	yes	steamed	NEGATIVE	NEGATIVE
137	27	ELEMENTARY	FARMER	>3	0	1 to 3	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
138	35	ELEMENTARY	MARKET WOMAN	>3	0	1 to 3	No	yes	yes	cooked tough/soft	mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	POSITIVE
139	17	ELEMENTARY	FARMER	0	0	4 to 6	yes	yes	yes	cooked tough/soft	pork, mutton, beef, chicken	No	NA	yes	Fresh, steamed, cooked	POSITIVE	NEGATIVE
140	25	NO FORMAL EDU	vocational	1	1	7 to 9	yes	yes	yes	cooked till soft	beef	No	NA	yes	cooked	POSITIVE	POSITIVE
141	31	ELEMENTARY	FARMER	3	1	7 to 9	yes	yes	yes	cooked till soft	mutton, beef, chicken	No	NA	yes	cooked	POSITIVE	POSITIVE
142	24	ELEMENTARY	FARMER	1	0	1 to 3	yes	yes	yes	cooked till soft	chicken	No	NA	yes	fresh. Steamed, cooked	POSITIVE	POSITIVE
143	31	NO FORMAL EDU	FARMER	>3	0	4 to 6	No	yes	yes	cooked till soft	mutton, chicken	No	NA	No	NA	NEGATIVE	POSITIVE
144	38	NO FORMAL EDU	FARMER	>3	0	4 to 6	No	No	yes	cooked till soft	mutton	No	NA	yes	cooked	NEGATIVE	POSITIVE
145	33	ELEMENTARY	vocational	>3	0	4 to 6	No	No	yes	cooked till soft	pork, mutton, beef, chicken	No	NA	yes	steamed	POSITIVE	POSITIVE
146	23	SECONDARY	MARKET WOMAN	1	0	1 to 3	No	No	yes	cooked till soft	mutton, chicken	yes	mutton	yes	cooked	POSITIVE	POSITIVE

APPENDIX II: Questionnaire for pregnant women

SEROPREVALENCE OF *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN IN THE ASUNAFO NORTH DISTRICT OF BRONG-AHAFOREGION

QUESTIONNAIRE FOR PREGNANT WOMEN

Kindly provide the needed information in the questionnaire below. You may tick the appropriate box or boxes (_) as indicated. Thank you.

PART A: PERSONAL INFORMATION

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

1. Name:…… Age (years):.....
2. Area of residence: …… Duration at residence:…….….
3. Contact address: …… Telephone No:……..
4. Educational background
   - ☐ No formal education
   - ☐ Elementary
   - ☐ Secondary/Vocational
   - ☐ Tertiary (Polytechnic, university, etc)
   - ☐ Other (specify): ……..
5. Where do you work?
   - ☐ Office (bank, school, etc)
   - ☐ Hospital (labour ward, theatre, accident/emergency centre, blood bank)
   - ☐ Hospital (records, OPD, nurse/mid-wife/theatre, wards)
   - ☐ Slaughter House

☐ Garden/farm

☐ Market (sell vegetables, raw meat)

☐ Vocational Centre (carpentry, fitting mechanic)

☐ Other (specify)……

PART B: TOXOPLASMOSIS-RELATED INFORMATION

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

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

If your answer to Question (Qu.) 7. is "No", please skip Qu. 8, 9 and 10. Go to Qu. 11
If "Yes", please answer Qu. 8, 9 and 10 before continuing with Qu. 11

8. When was the test conducted?

☐ 3 to 6 months ago ☐ Up to a year ago ☐ More than a year ago

☐ Other (specify)……..

9. What was the result? ☐ Positive ☐ Negative ☐ No idea

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

11. Would you like to be tested for *Toxoplasma* infection during this pregnancy?

☐ Yes ☐ No

12. How many pregnancies have you had before the current pregnancy?

None (this is my first)

☐ One ☐ Two ☐ Three ☐ More than three

13. Have you ever had a stillbirth, spontaneous abortion(s) [miscarriage(s)]?

☐ Once ☐ Twice ☐ Three times or more ☐ Never

14. How old is your current pregnancy?

☐ 1 to 3 months ☐ 4 to 6 months ☐ 7 to 9 months

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

If "Yes" to Qu. 15, please answer Qu. 16. If "No" skip Qu. 16

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

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

18. Do you eat meat? ☐ Yes ☐ No, I am a vegetarian

If your answer to Qu. 18 is "No" please skip and go to Qu. 21

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

☐ Cooked but tough ☐ Cooked till soft ☐ Cooked tough or soft

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

☐ Pork ☐ Goat meat/mutton ☐ Beef ☐ Chicken ☐ No idea

☐ Others (specify)……

21. Do you eat khebabs? ☐ Yes ☐ No

If yes, what type do you enjoy most?

☐ Pork ☐ Goat meat/mutton ☐ Beef ☐ Chicken ☐ No idea

☐ Others (specify)……

22. Are you a lover of vegetables? ☐ Yes ☐ No

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

☐ Fresh and raw ☐ Steamed ☐ Cooked

24. Will you allow a blood sample to be taken from you the purpose of this study?

☐ Yes ☐ No

Thank you very much

APPENDIX III: 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): _____