Sero-prevalence and risk factors of *Toxoplasma gondii* infection among pregnant women attending antenatal care in Kigali, Rwanda

MUREBWAYIRE ESPERANCE

A DISSERTATION SUBMITTED FOR A STUDY IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY FROM UNIVERSITY OF NAIROBI, SCHOOL OF MEDICINE

2014

DECLARATION

I hereby declare that this is my original work and has not been presented in any other university for study leading to the award of a degree of Master of Science in Medical Microbiology

Esperance MUREBWAYIRE

H56/82379/2012

Sign…….. Date…….

SUPERVISORS

Prof. Walter Jaoko

Chairman,

Department of Medical Microbiology

University of Nairobi

Sign……..…….. Date…….

Dr H. Kariuki Njaanake

Lecturer,

Department of Medical Microbiology

University of Nairobi

Sign…….. Date……..

Prof. Kato Njunwa

Director of Postgraduate Studies

University of Rwanda

College of Medicine and Health Sciences/ Kigali Campus

Sign…….. Date……..

ACKNOWLEDGEMENT

First and foremost, I thank God for enabling me to undertake this study.

I am also very grateful to the Government of Rwanda through Ministry of Health for awarding me a scholarship for my postgraduate studies.

I am most appreciative to my supervisors Prof. Walter Jaoko, Dr H. Kariuki Njaanake, Prof Kato Njunwa for the guidance they offered throughout the process of writing this dissertation.

I also highly acknowledge the support from the entire academic staff in the department of Medical Microbiology, University of Nairobi for their dedication and helpful wise counsel.

I wish to register my heartfelt compliment to the administration of Rwanda Biomedical Center/National Reference Laboratory division for giving me the opportunity to carry out laboratory work at their facility, and I also recognize the staff in different areas such as in the Serology section for their assistance during laboratory analysis.

I also appreciate the administration of Muhima, Biryogo, Cor-Unum and Nyarugunga Health center for the support I got from staff especially those in ANC, during participants' recruitment.

The pregnant women who accepted to participate to the study, without whom it would not have been possible to conduct the study, I thank you so much.

To the Biokit company and the Africhem team for providing the kits and making sure they were delivered on time thank you.

To Festus Muriuki, the biostatistician who dedicated his time to transforming the data into valuable information, thank you.

Thank you so much Lucy Macharia for the advice and the endless encouragement and to my fellow post graduate students, I say you are wonderful.

Last but not least I thank my mother Josee, my sisters and brother for being a source of encouragement and inspiration.

DEDICATION

To my mother Nzamugurinka Josee, for her prayers and the challenges she overcame to secure me a place in school.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AIDS	Acquired Immunodeficiency Syndrome
ANC	Antenatal Care
CD4+	Cluster of Differentiation 4 positive
CFT	Complement fixation test
CSF	Cerebrospinal fluid
DNA	Deoxynucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
HIV	Human Immunodeficiency Virus
HRP	Horseradish Peroxidase
IFA	Indirect fluorescent antibody assay
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ml	Milliliter
NRL	National Reference Laboratory
OMS	Organisation Mondiale de la Santé
PMTCT	prevention of mother to child transmission
PV	Parasitophorous vacuole

rpm	rotation per minute
RT	PCR
SPSS	Statistical Package for Social Scientists
TE	Toxoplasmic encephalitis
USA	United States of America
µL	Microliter

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Background: Toxoplasmosis in pregnancy is associated with spontaneous abortions, low birth weight babies, congenital deformities and intrauterine deaths. In many developed countries, pregnant mothers are screened for infection with *Toxoplasma gondii*, the causative parasite, and treatment is offered early in order to prevent these complications. The disease is also associated with immunosuppressive disorders such as acquired immunodeficiency syndrome (AIDS). In most developing countries, including Rwanda, the burden and risk factors of *T. gondii* infection among pregnant women and among HIV infected persons is largely unknown thus making it difficult to plan and implement control measures.

Study Objective: This study aimed at determining the prevalence of *T. gondii* infections and their risk factors among pregnant women attending antenatal care clinics in Kigali, Rwanda.

Methodology: This was a cross-sectional descriptive study involving 384 pregnant women aged 18 years and above who were attending antenatal care clinics in Biryogo, Cor-Unum, Muhima and Nyarugunga health centres in Kigali city, Rwanda, between April and August 2014.

Venous blood samples were collected from study participants and screened for IgG and IgM antibodies against *T. gondii* using the ELISA technique whereas information on their HIV status and CD4+ cell count were obtained from their medical records. The participants were also interviewed about selected behaviors that predispose individuals to infections with *T. gondii*.

Results: The overall seroprevalence of antibodies against *T. gondii* among the women was 12.2%. Thirty seven (9.6%) of them were IgG seropositive and 15 (3.9%) were IgM seropositive. Ten of the 15 women who were positive for IgM were also positive for IgG, making the overall prevalence of pregnant women positive for both IgG and IgM 2.9%. The sero-positivity rate of *T. gondii*-specific antibodies was significantly higher among pregnant women who reported drinking untreated water than those who reported using treated water (22.4% versus 6.8%; OR=4.01, 95% CI: 2.08 - 7.69, p=0.001). Similarly, patients who reported eating undercooked meat had a significantly higher prevalence of anti-*T.gondii* seropositivity than those who reported ate well cooked meat (22.3% versus 8.1%; OR=3.32, 95% CI: 1.74-6.32, p<0.001).

Conclusion: The seroprevalence of *T. gondii* antibodies is relatively low among the pregnant women. Undercooked meat consumption and drinking untreated water are significantly associated with sero-prevalence of anti-*T. gondii* IgG and IgM among the pregnant women.

CHAPTER ONE: INTRODUCTION

T. gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis (J. L. Jones et al., 2001). It is estimated that about one third of the world's population is infected with *T. gondii* (Pappas, Roussos, & Falagas, 2009). In Africa, overall seroprevalence rate as high as 92.5% has been reported (Ayi et al., 2009). However, the prevalence of infection varies widely between countries (ranging between10 and 80%) and often within a given country or between different communities in the same region (Pappas et al., 2009). However, in most developing countries including Rwanda, the prevalence of toxoplasmosis is largely unknown.

In the immunocompetent *Toxoplasma* infection is often asymptomatic (Studenicova, Bencaiova, & Holkova, 2006), and frequently results in the chronic persistence of cysts within host tissues, that probably lie dormant for life (Malla, Sengupta, Dubey, Sud, & Dutta, 2005). In contrast, *Toxoplasma* infection in immunocompromised subjects is always life threatening. Various factors responsible for profoundly impaired cellular immunity can lead to severe toxoplasmosis, among which are HIV infection and immunosuppressive therapies (Botterel et al., 2002). Toxoplasmic encephalitis (TE) is the most predominant manifestation of the disease in these patients and can lead to various symptoms, ranging from headache, lethargy, incoordination, or ataxia to hemiparesis, loss of memory, dementia, or focal to major motor seizures, usually associated with fever (Luft & Remington, 1992). Furthermore, in these immunocompromised states such as in HIV infections, subjects are at risk of developing acute toxoplasmosis due to reactivation of the organism if their $CD4^+$ T-cell count decreases below 200 cells/µl (Sucilathangam G, Anna T, & Velvizhi G, 2013).

Majority of horizontal transmissions to humans is due to either the ingestion of tissue cysts in infected meat or by the ingestion of soil, water, or food contaminated with sporulated oocysts from the environment or, less frequently, directly from feline feces (Foulon et al., 1999). High prevalence of the infection have been reported among pregnant women and women of childbearing age from different foci in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa (Pappas et al., 2009) . This can lead to congenital toxoplasmosis, which is a serious disease that is associated with severe congenital deformities and intrauterine deaths especially when the infection is acquired early in pregnancy (Chen et al., 2005; Dunn et al., 1999; Foulon et al., 1999). Control of congenital toxoplasmosis is therefore important and, in the developed countries, includes screening of pregnant mothers for infection (Dunn et al., 1999).

Rwanda has a high fertility rate and the prevalence of HIV infection in general population aged between 15 to 45 years of 3%, while the prevalence of HIV among pregnant women is 4.3% (OMS, 2009-2013). HIV prevalence levels in the general population show disparities between urban area (7.3%) and rural area (2.2%), and between women (3.6%) and men (2.3%) (OMS, 2009-2013). It is therefore apparent that women more than men, and especially in the urban areas bear the greatest burden of HIV infection. Since *T. gondii* disease is most severe if the infection is acquired congenitally and the risk of infection is known to be higher in immunosuppression, there is need to determine the presence of toxoplasmosis infection among pregnant women and to explore if infection rates vary with HIV infection in this population.

Most studies on prevalence of T. gondii infections have relied on antibody detection. Although, antibody detection is associated with false positives. A more reliable way of diagnosing active infection is the use of molecular techniques such as PCR for diagnosing active infection using

blood (Dupouy-Camet et al., 1993; Filice, Hitt, Mitchell, Blackstad, & Sorensen, 1993),brain biopsy (Holliman et al., 1991; Holliman, Johnson, & Savva, 1990), CSF (Cristina et al., 1993; Lebech et al., 1992), and liver biopsy (Cristina et al., 1993). However, this technique is very expensive and not easily available in most developing countries making its use in routine diagnosis not feasible. It is for this reason that diagnosis of toxoplasmosis in most health facilities in these countries still largely rely on serology, and this is the method of diagnosis that was used in this study for purposes of being realistic with what is done in the field.

In line with this, the present study sought to determine the seroprevalence of *T. gondii* infections among pregnant women attending selected antenatal clinics in Rwanda, using ELISA tests and risk factors associated with the acquisition of toxoplasmosis.

1.1. RESEARCH QUESTION:

What is the prevalence of and risk factors for *T. Gondii* infections among pregnant women attending antenatal care clinics in Kigali, Rwanda?

1.2. OBJECTIVES

1.2.1. Broad objective

To determine the seroprevalence of and risk factors for *T. gondii* infections among pregnant women attending antenatal clinics in Kigali, Rwanda.

1.2.2. Specific objectives

1. To determine the prevalence of *T. gondii* among pregnant women in Kigali, Rwanda.
2. To determine risk factors associated with *T. gondii* infection among pregnant women in Kigali, Rwanda

1.3. STUDY JUSTIFICATION

Infections with *T. gondii* during pregnancy are important due to resultant congenital toxoplasmosis. Serological screening of pregnant women for *Toxoplasma gondii*-specific antibodies is however not routinely carried out during antenatal care in Rwanda. As a result, little is known about the prevalence of the parasite among pregnant mothers in the country. In a study done in two rural communities namely Ngenda and Nyarutovu in 1989, Rwanda about 50% of the adults in both communities had antibodies to *T. gondii* (Gascon, Torres-Rodriguez, Soldevila, & Merlos, 1989). Since then, there has been no follow-up study carried out nor any intervention programme implemented, especially for pregnant women and immunocompromised patients as groups at high risk of infections.

Antenatal serological screening of *T. gondii* infection based on the detection of IgG which indicates past infections and IgM indicating the acute or current infections (A. M. Deji-Agboola, O. S. Busari, O. A. Osinupebi, & Amoo, 2011). It is the mainstay in monitoring the risk for congenital toxoplasmosis and this study was conducted in order to determine the sero-prevalence of *T.gondii* among pregnant women attending antenatal care clinics in selected Health Centres in Kigali, Rwanda. In addition, this study was aimed at determining *T. gondii* infection associated factors to provide basic information on the prevalence of infections with *T. gondii* among pregnant women visiting public health facilities in Rwanda. The information obtained from the study may be useful in giving an estimate of the prevalence among pregnant women thus informing policy on preventive measures and for designing further research studies on toxoplasmosis in the country.

CHAPTER TWO: LITERATURE REVIEW

T. gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis. If primary toxoplasmosis occurs during pregnancy about one third of the cases may lead to congenital toxoplasmosis, with subsequent pathological effects (Zemene et al., 2012). In most immunocompetent adults the parasite does not cause serious illness, but it can cause blindness and mental retardation in congenitally infected children and devastating disease in immunocompromised individuals (Hill & Dubey, 2002).

2.1. EPIDEMIOLOGY

T. gondii has a worldwide distribution, but its prevalence varies greatly (J. L. Jones et al., 2001) and Table 6.

Sero prevalences of 43% and 28.3% have been reported in European countries such as France and Italy, respectively (Berger F, Desnclos JC, Le Strait Y, & Goulet V, 2008),(De Paschale, Agrappi, Manco, Cerulli, & Clerici, 2010). In Thailand, Nissapaton and others reported a 29.1% prevalence of toxoplasmosis in pregnant women (Nissapatorn, Suwanrath, Sawangjaroen, Ling, & Chandeying, 2011) whereas in Brazil Barbosa and others reported a prevalence rate of 66.3% among pregnant women (Barbosa, de Carvalho Xavier Holanda, & de Andrade-Neto, 2009). The marked variations in prevalence across regions and countries could be related to geographical factors - climate (rainfall, temperature). For example, it is thought that heat and humidity are important factors favoring the conservation of oocysts in the soil and thus participate maintaining a high prevalence. It is also known that this difference in prevalence may also be related to differences in dietary habits (Cenci-Goga, Rossitto, Sechi, McCrindle, & Cullor, 2011).

Data from several African countries suggest that the prevalence of toxoplasmosis during pregnancy varies from one country to another. For example, a seroprevalence rate of 60% and 43.7% were reported in Côte d'Ivoire and Nigeria respectively (Adou-Bryn, Ouhon, Nemer, Yapo, & Assoumou, 2004; Olusi, Gross, & Ajayi, 1996). In Senegal, Ndiaye and others reported 34.5% of *Toxoplasma* seroprevalence (Ndiaye et al., 2011). In Central African Republic, Morvan and others reported 60% seroprevalence rate,whereas in Gabon, Mpiga and others reported a seroprevalence rate of 56% . Morocco, El Mansouri and others reported a toxoplaasmosis seroprevalence rate of 50.6% (El Mansouri et al., 2007). Figure 1 illustrates that the geographical variation observed in other parts of the world is very similar in Africa. There is little known about *T. gondii* prevalence in Africa, table 7 shows the results of some studies carried out on this continent.

Besides varying between different continents and countries, *T. gondii* prevalence also can be heterogeneous within the same country. For example, a study by Jones and others showed that the seroprevalence in the USA varied from 29.2% in the northeast to 20.5% and 17.5% in the south-midwest and west respectively (J. L. Jones et al., 2001).

Likewise, the prevalence of toxoplasmosis seems to vary widely among HIV-positive and HIV negative adults. For example, a study conducted in 1991 in Zambia on HIV –positive and HIV – negative adults described a seroprevalence of 7%, while another study on a similar group in Ethiopia in 1991 showed a seroprevalence of 80% (Woldemichael et al., 1998; Zumla et al., 1991). The reason for this may not be clear but could include several factors such as socio-economic status, education, sanitary conditions and differences in dietary habits.

There is limited historical information about the disease in Rwanda, in a study done in two rural communities Ngenda and Nyarutovu now in Bugesera district eastern province (1989), 50% of the adults in both communities had antibodies to *T. gondii* (Gascon et al., 1989).

However, no more studies have been conducted on the prevalence of *Toxoplasma* infections in the general population or among the groups at high risk such as pregnant women and the immunocompromised patients.

2.2. BIOLOGY OF *TOXOPLASMA GONDII*

T. gondii is a non flagellated apicoplexan parasite. It belongs to the family of the Sarcocysdae in the class of the coccidian, and is the only species in the *Toxoplasma* genus.

T.gondii is classified as (from: http://www.ncbi.nlm.nih.gov/Toxonomy/):

Domain: Eukaryota

Kingdom: Alveolata

Phylum: Apicomplexa

Class: Coccidia

Subclass: Eucoccidiorida

Order: Eimeriorina

Family: Sarcocystidae

Genus: *Toxoplasma*

Species: *Toxoplasma gondii*

There are three infective stages of *T. gondii*: a rapidly dividing invasive tachyzoites, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected inside an oocyst. These infective stages are crescent-shaped cells, approximately 5µm long and 2µm wide, with a pointed apical end and a rounded posterior end. They are limited by a complex membrane, named the pellicle, which is closely associated with a cytoskeleton involved in the structural integrity and motility of the cell (Roos et al., 1999). They possess a nucleus, a mitochondrion, a Golgi complex, ribosomes, an endoplasmic reticulum, and a multiple-membrane-bound plastid-like organelle called the apicoplast (Roos et al., 1999). As for other members of the phylum *Apicomplexa*, they have an apical complex comprising of specialized cytoskeletal structures such as the conoid, which is involved in cell invasion, and numerous secretory organelles including rhoptries, dense granules, and micronemes (Robert-Gangneux & Darde, 2012).

2.2.1 LIFE CYCLE

T. gondii is a tissue-cyst-forming coccidium that alternates between definitive (sexual reproduction) and intermediate (asexual replication) hosts. The only known definitive hosts for *T. gondii* are members of family Felidae (domestic cats and their relatives). The parts of the sexual and asexual cycles and transmission dynamics in a given environment vary according to physical characteristics and according to the structures of both intermediate and definitive host populations. It is unique among this group because it can be transmitted not only between intermediate and definitive hosts (sexual cycle) but also between intermediate hosts via carnivorism (asexual cycle) or even between definitive hosts (Afonso, Thulliez, & Gilot-Fromont, 2006). This first step is followed by sexual development, with the formation of male and female gametes (gametogony)(Ferguson, 2002).

After the ingestion of cysts present in tissues of an intermediate host, the cyst wall is destroyed by gastric enzymes. Bradyzoites are the slow-growing, transmissible, and encysted form that are dormant, settle within enterocytes, where they undergo a self-limiting number of asexual multiplications, characterized by the development of merozoites within schizonts (Dubey, 1988),(Tenter, Heckeroth, & Weiss, 2000). After fertilization, oocysts formed within enterocytes are liberated by the disruption of the cell and excreted as unsporulated forms in cat feces. The process of sporogony occurs after a few days in the external environment. It implies a meiotic reduction and morphological changes leading to the formation of a sporulated oocyst with two sporocysts, each containing four haploid sporozoites. The shedding of oocysts begins 3 to 7 days after the ingestion of tissue cysts and may continue for up to 20 days. Infected cat can shed more than 100 million oocysts in its feces (J. L. Jones & Dubey, 2010). Oocysts can infect a wide range of intermediate hosts, virtually all warm-blooded animals, from mammals to birds, when ingested with food or water. Within intermediate hosts, the parasite undergoes only asexual development. After oocyst ingestion, sporozoites are liberated. They penetrate the intestinal epithelium, where they differentiate into tachyzoites, which are the rapidly growing, disease-causing forms. Tachyzoites quickly replicate inside a parasitophorous vacuole (PV) by endodyogeny inside any kind of cell and disseminate throughout the organism. As a result of the conversion from tachyzoite to bradyzoite, tissue cysts arise as early as 7 to 10 days postinfection and may remain throughout life in most hosts, predominantly in the brain or musculature. In the absence of an adequate immune response, tachyzoites will grow unabated and cause tissue destruction, which can be severe and even fatal (Blader & Saeij, 2009; Duncanson, Terry, Smith, & Hide, 2001). In addition, if the acute phase occurs during pregnancy, the parasite can cross the placenta and infect the fetus (congenital transmission) (Duncanson et al., 2001). (Figure 2)

2.3. TRANSMISSION AND RISK FACTORS

Transmission of the parasite can occur in several ways. Definitive hosts can be infected by ingestion of oocysts from the environment or cysts in prey. Transmission to humans can occur via accidental ingestion of infectious stages through faecal-oral route, drinking of contaminated water, consumption of contaminated food, infected undercooked meat, vertically from infected pregnant mothers, and rarely via organ transplantation or blood transfusion (Montoya & Liesenfeld, 2004).

In three European case-control studies, undercooked meat was shown to be the most important risk factor for *T. gondii* infection accounting for 30-60% of infections in pregnant women (Kijlstra & Jongert, 2008). After infection, most animals harbor parasites in their tissues. Serological studies on cattle show prevalences that can be as high as 92%, but tissue cysts are rarely found in beef as cattle are known to show a high resistance to *T. gondii* (Tenter et al., 2000). Pork was generally considered a major source of infection in Europe and the USA, but recent studies in the Netherlands, Austria and Germany have shown that *T. gondii* infections in pigs have dropped to less than 1% over the last ten years (Tenter et al., 2000). This is largely due to modern and more hygienic farming systems, as well as the increasing use of frozen meat (Kijlstra & Jongert, 2008). Free-ranging chickens are increasingly being identified as an important source of *T. gondii* infection, especially in developing countries, Kijlstra et el (2008) reported the sero-prevalences of up to 65%, and parasite presence was shown in 81% of these seropositive chickens (Kijlstra & Jongert, 2008).

The transmission of the parasite through contaminated water has generally been considered uncommon; however, the widespread infection of marine mammals indicates that contaminated

water may be a potential source of infection. Recent outbreaks of toxoplasmosis linked to contaminated water supplies provide further evidence for this (Dubey, 2004).

When a pregnant woman acquires a primary infection, tachyzoites can colonize placental tissues during the dissemination process and from there they gain access to the fetal compartment in about 30% of cases (Pfaff et al., 2007). The frequency of vertical transmission increases with the gestational age at maternal infection. At the beginning of pregnancy, the transplacental passage of tachyzoites is a rare event, but the consequences for the offspring are heavy (Pfaff et al., 2007).

As *T. gondii* tachyzoites can invade all nucleated cells, cysts can be found in virtually any organ. Therefore, in solid-organ transplantation, *Toxoplasma* infection can be transmitted through a cyst-containing organ from a donor with infection acquired in the distant past to a nonimmunized recipient. However, certain organs are more likely to harbor persistent cysts than others. Muscles commonly sustain parasite encystment; thus, heart transplant patients are at a higher risk for organ-related toxoplasmosis than are liver, lung, or kidney transplant patients (Robert-Gangneux & Darde, 2012).

The risk of transmitting infection through a blood transfusion is theoretically possible if the donor has recently acquired a *Toxoplasma* infection and is parasitemic at the time of blood sampling. Similarly, a risk associated with bone marrow is possible if the donor is parasitemic at the time of collection .

2.4. TOXOPLASMOSIS

European Union definition of toxoplasmosis: a zoonotic protozoan disease which presents with an acute illness with or more of the following: lymphadenopathy, encephalitis, chorioretinitis and dysfunction of the central nervous system. Congenital infections may also occur with

hydrocephalus, microcephalus intracerebral calcification, convulsions, cerebral retardation (EUROTOXO)(Estee T, Cooke F, & E., 2009). Over the last twenty years, it has also emerged as one of the most common opportunistic infections associated with HIV/AIDS, and is a major cause of mortality in AIDS patients in developing countries (Carruthers, 2002).

2.4.1. Clinical manifestations

The clinical manifestations of toxoplasmosis vary, depending on parasite characteristics such as virulence of the strain and number of parasite, as well as host factors such as genetic background and immune status (Montoya & Liesenfeld, 2004).

Toxoplasmosis in immunocompetent individuals is typically mild or asymptomatic and usually results in life-long immunity (Tenter et al., 2000). The most commonly symptoms include lymphadenopathy, which may be accompanied by headache, fever, fatigue, muscular or abdominal pain, with myocarditis, hepatitis and pulmonary necrosis as severe symptoms (Bhopale, 2003; Tenter et al., 2000). However, there are cases of the infection where overtly serious clinical symptoms may occur and include ocular toxoplasmosis, congenital toxoplasmosis, cerebral toxoplasmosis, and the reactivation of a latent infection in immunocompromised individuals.

2.4.2. Congenital toxoplasmosis

Transplacental transmission occurs when an immunocompetent woman acquires a primary infection during pregnancy (Hegab & Al-Mutawa, 2003), or may also be due to a reactivated infection in immunocompromised women (Dubey & Jones, 2008). Congenital infection can lead to a wide variety of manifestations in the fetus and infant including spontaneous abortion, still-birth, hydrocephalus or microcephalus, cerebral calcifications and retinochoroiditis (Gibbs, 2002; Goldenberg & Thompson, 2003). This occurs in between 1 and 10 per 10 000 live births in

Europe (Montoya & Liesenfeld, 2004; Tenter et al., 2000). Primary infection during pregnancy may result in severe damage or death of the foetus and long-term sequelae in the child. The risk of congenital infection increases from the first trimester (10-25%) to the third trimester (60-90%) with the development of a good blood flow (Dubey & Jones, 2008; Hegab & Al-Mutawa, 2003; J. L. Jones, Lopez, Wilson, Schulkin, & Gibbs, 2001b). Infection within the first two trimesters may result in death of the foetus in utero or spontaneous abortion. Infection in the last trimester usually results in newborns that are asymptomatic at birth, but may develop symptoms later in life (Montoya & Liesenfeld, 2004).

Most children born with congenital toxoplasmosis are asymptomatic at birth although 10–23% of infected newborns show clinical signs of toxoplasmosis at birth whereas approximately 80% of them develop neurological or ocular sequellae later in life and approximately 10% of prenatal infections result in abortion or neonatal death (Dubey & Jones, 2008; J. Jones, Lopez, & Wilson, 2003; Luft & Remington, 1992).

The classic triad of signs is hydrocephalus, retinochoroiditis and intracranial calcifications, and occurs in approximately 10% of all infected newborns ,however, there may be mild disease such as reduced vision, or it may cause severe abnormalities such as blindness, mental retardation and epilepsy (Tenter et al., 2000). Fabiana Maria Ruiz Lopes et al (2007)evaluated 2,126 pregnant women attended by the public health system of Rio Grande do Sul; they reported that 74.5% (1,583) were IgG positive, and among those, 3.6% (77) were also IgM positive. Among the IgG and IgM-positive pregnant women, 51 children were followed for at least one year of life; 28 were born from IgA positive mothers, most likely in the acute phase of infection and with risk of congenital transmission. Among these (IgG, IgM and IgA positive), three children (10.7%) had congenital infection confirmed, and one (3.6%) presented characteristic symptomatology.

Extrapolating these data to the population in their study, the authors found a transmission rate of 2.2 of every 1,000 births and of 0.7 among every 1,000 live births presenting symptomatology (Lopes, Goncalves, Mitsuka-Bregano, Freire, & Navarro, 2007).

2.4.3. Toxoplasmosis in immunocompromised host

Toxoplasmosis can be life-threatening and even fatal in immunocompromised patients such as those with HIV/AIDS, Hodgkin's disease or those undergoing immunosuppressive therapy, as a result of reactivation of chronic infection. High seroprevalence of latent *T. gondii* infection has been found among immunocompromised patients in China (Tenter et al., 2000; Zhou et al., 2011). Toxoplasmosis ranks high on the list of diseases which lead to death in patients with acquired immunodeficiency syndrome (AIDS). Approximately 10% of AIDS patients in the USA and up to 30% in Europe are estimated to die from toxoplasmosis (Luft & Remington, 1992). Although in AIDS patients any organ may be involved, toxoplasmic encephalitis (TE) occurs in approximately 40% of AIDS patients worldwide including the testis, dermis and the spinal cord, infection of the brain is most frequently reported (Tenter et al., 2000). Most AIDS patients suffering from toxoplasmosis have bilateral, severe and persistent headache which responds poorly to analgesics. As the disease progresses, the headache may give way to a condition characterized by confusion, lethargy, ataxia and coma. The predominant lesion in the brain is necrosis, especially of the thalamus (Renold et al., 1992). In immune compromised states such as in HIV infections, subjects are at risk of developing acute toxoplasmosis due to reactivation of the organism if their CD4+ T-cell count decreases below 200 cells/μL (Falusi et al., 2002; Jayawardena S, Singh S, Burzyantseva O, & H, 2008; Martinez E, Mago H, Rocha R, & M:, 2002).

2.4.4. Ocular toxoplasmosis

T. gondii is the most common cause of retinochoroiditis in humans worldwide, accounting for 28% to 55% of all cases of posterior uveitis (Bonfioli & Orefice, 2005; Lappalainen & Hedman, 2004). Retinochoroiditis commonly occurs as a result of congenital infection but can also be due to an acquired or reactivated infection (Bonfioli & Orefice, 2005; Dubey & Jones, 2008).

Active lesions usually present with a white focus of necrotizing retinochoroiditis close to old pigmented scars. These lesions are usually circular or oval in shape and they vary in size. In the congenital forms, the lesions are usually bilateral and central and in the acquired forms, the lesions are usually unilateral and solitary (Pavesio & Lightman, 1996). Parasites reach the eye as free tachyzoites or as cysts which rupture, releasing tachyzoites. Once infected cells lyse, tachyzoites invade the retina and multiply in the surrounding cells causing an inflammatory response (Rothova, 1993). Clinical manifestations of acute retinochoroiditis include tearing, pain, photophobia -and progressive loss of vision over time, especially when there is macular or optic nerve involvement (Dubey & Jones, 2008; Pavesio & Lightman, 1996).

2.5. DIAGNOSIS

Toxoplasmosis is frequently asymptomatic and clinical manifestations, when present, are usually non-specific and mimic other infections, making definitive clinical diagnosis very difficult (Hill & Dubey, 2002). Diagnosis is usually made by immunological testing, histological identification, isolation in tissue culture, molecular techniques or by a combination of these techniques. Cerebral toxoplasmosis can also be diagnosed using computerized tomography and magnetic resonance imaging (Sukthana, 2006). However, this technique is very expensive and not easily available in most developing countries making its use in routine diagnosis not feasible.

Serological- tests such as Sabin-Feldman dye test, which is the traditional gold standard, indirect fluorescent antibody assay (IFA), complement fixation test (CFT) and the enzyme-linked immunosorbent assay (ELISA) are widely used (Hill & Dubey, 2002).

These tests are used to detect anti-parasite antibody levels such as IgG, IgM, IgA and IgE (J. Jones et al., 2003). In a primary *T. gondii* infection, IgM appears a few weeks after infection, followed by IgA and IgE. These acute phase immunoglobulins peak after about two months and are usually undetectable by serological tests by six to nine months but can persist for longer periods (Montoya & Rosso, 2005; Sukthana, 2006). IgG, which appears after IgM, peaks after four months and persists at low levels throughout the duration of the host's life (Sukthana, 2006). In pregnant women, positive IgM results indicate the likely acquisition of infection during gestation and a positive IgG and negative IgM result indicates a previous infection (Montoya & Rosso, 2005).

Other diagnostic tests for toxoplasmosis are avidity tests which are based on the fact that during acute infections, IgG antibodies bind antigen relatively weakly and therefore have a low avidity. Chronic infections, however, have more strongly-binding antibodies and therefore have a high avidity (Lappalainen & Hedman, 2004; Montoya & Rosso, 2005).

Nucleic acid detection by PCR has both advantages and disadvantages. Advantages are that the detection of nucleic acid is not affected by the condition of the immune system, it is generally more sensitive and rapid than serological tests and diagnosis can be made from biopsies, blood, cerebrospinal fluid (CSF) and amniotic fluid. These advances in PCR techniques make it an invaluable diagnostic tool. Disadvantages are that false positive results due to contamination may occur, it may be too sensitive in detecting nonviable *T. gondii* remainders that do not cause

disease, and may yield false negative results due to inhibition (Johnson, Butcher, Savva, & Holliman, 1993). These problems with PCR can, however, be overcome and more rapid and sensitive methods are regularly being developed.

2.6. TREATMENT

Treatment for immunocompetent individuals is usually not necessary. Sulfadiazine plus pyrimethamine is the most commonly recommended therapy for congenital and ocular toxoplasmosis, as well as infection in immunocompromised individuals (Hill & Dubey, 2002; Montoya & Liesenfeld, 2004). Alternative treatments for patients intolerant to sulphonamides are also used. These include clindamycin plus pyrimethamine, clarithromycin plus pyrimethamine, and atovaquone (Arens, Barnes, Crowley, & Maartens, 2007). Maintenance therapy of half the dose of the therapeutic drugs is usually administered as the drugs are not effective against tissue cysts, which may be reactivated (Sukthana, 2006). Pregnant mothers suspected with acute toxopasmosis are treated with spiramycin, which reduces the risk of transmission to the fetus. However, as spiramycin does not cross the placenta, if fetal infection occurs treatment should be changed to pyrimethamine and sulfadiazine (Estee T et al., 2009).

2.7. PREVENTION AND CONTROL

Toxoplasmosis is a curable but potentially fatal disease. To prevent *T. gondii* infections in humans, a number of measures can be taken. Individuals should practice good hygiene. Washing is effective because the stages of *T. gondii* are killed by contact with soap and water. Hands should be washed thoroughly with soap and water after handling meat or soil. All fruits and vegetables must also be washed before they are to be consumed (Lopez, Dietz, Wilson, Navin, & Jones, 2000). All cutting boards, sink tops, knives and other materials coming in contact with uncooked meat should also be washed with soap and water. *T. gondii* organisms in meat can be

killed by exposure to extreme heat or cold. Tissue cysts in meat are killed by heating the meat throughout to 67 ^{0}C or by freezing to -13 ºC. Pregnant women should be especially careful, and should limit contact with cats, cat litter, soil and raw meat (Hill & Dubey, 2002; Lopez et al., 2000; Tenter et al., 2000).

In addition, the agent has been reported to be transmitted through blood transfusion and organ transplantation. Nevertheless, the risk of *T. gondii* transmission through blood transfusion is extremely low, and serologic testing of antibodies to *T. gondii* in blood donors appears to be unnecessary. It has been suggested that people who are at increased risk of toxoplasmosis, such as immunosuppressed individuals and pregnant women, receive *T. gondii* antibody-negative blood components for transfusion. Potential organ donors should be tested to prevent the spread of the parasite through transplanted organs. People with AIDS may be given highly active antiretroviral drugs to reduce the risk of toxoplasmosis (Gagandeep & Sehgal, 2010)

CHAPTER THREE: METHODOLOGY

3.1Study area

The study was carried out between April and August 2014 in four selected health centres in Kigali, Rwanda namely Biryogo, Cor-Unum, Muhima and Nyarugunga Health Centres. All these facilities are public health centres. Biryogo, Muhima and Cor-Unum are located in Nyarugenge while Nyarugunga is located in Kicukiro Districts, within Kigali city covering an area of 730 km^2 and with a population of approximately 1.1 million people. These health centres were selected using simple random sampling method. Each health centre has skilled nurses and socio-workers, laboratory technologists, doctors come on weekly bases. On average, each of Cor-Unum and Biryogo health centres caters for over 76 antenatal mothers per month, while Nyarugunga and Muhima health centres each caters for about 68 antenatal mothers per month. Each health center hosts voluntary counseling and testing (VCT) for HIV testing and antiretroviral (ARV) treatment for HIV patients. Each health center also has laboratory departments which are well equipped to carry out blood specimen collection and microscopic examination.

3.2 Study design

This was a cross-sectional descriptive study involving pregnant women attending antenatal care at selected health centres. Each participant was interviewed in private to promote confidentiality. Furthermore, no names were used on any data collection forms. Instead, each study participant was given a unique identifying number. Venous blood samples were drawn from each study participant, all specimens were processed in the NRL.

3.3 Study population

All pregnant women aged 18 years and above who were attending antenatal care at four selected health centres during study period were requested to enroll into the study.

3.4 Inclusion criteria

- Pregnant women 18 years and above, attending antenatal care and accepted to give their informed consent to participate in the study.
- Pregnant women who were willing to undergo HIV voluntary counseling and testing

3.5 Exclusion criteria

- Women below 18 years
- Pregnant women who declined to give a written informed consent to participate in the study.

3.6 Sample size and sampling method

All the pregnant women attending the selected health facilities that met the inclusion criteria were identified. After identification, the potential study participants were taken through the informed consent process during which the study objectives, risks, benefits and study procedures were explained in Kinyarwanda, English or a language of the participant's preference. A total of 384 pregnant women were recruited into the study.

Sample size calculation according to Fischer's formula

The minimum sample size for the study was calculated using the following formula given by Fischer

$$n = \frac{Z^2 pq}{l^2}$$ (Nigel, Pope, & Stanistreet, 2008)

n = required sample size for this study.

Z = Critical value from standard normal table. For a 95% CI, z=1.96

p = expected prevalence of infection with *T.gondii* in this case is 50% or 0.5

q = 1-p (probability of no event)

l = margin of error at 5% (standard value of 0.05)

$$n = \frac{1.96^2 \times 0.5\,(1 - 0.5)}{0.05^2} = \mathbf{384}$$

Sampling procedure for exit interview

A convenience sampling procedure was used. All the pregnant women who met the inclusion criteria were identified from those attending the antenatal care clinic. This was done after completing provision of antenatal care services. After identification, the potential study participants were taken through the informed consent process whereby the study objectives, risks, benefits and study procedures were explained in Kinyarwanda or English depending on the participant's preference. Only those who agreed to participate by signing the consent form were included in the study.

3.7 Data collection methods

After obtaining informed consent, the study participants were interviewed by administration of a standard questionnaire to obtain the socio-demographic and economic status information as well as epidemiological risk factors (Appendix 1).

The influential risk factors considered in the study included maternal age, gestation period, educational level (none, primary school ,secondary school and above), owning cats, sources of drinking water, and cooking and eating habits such as: eating raw or undercooked meat, tasting raw food while cooking, dining in restaurants. The level of knowledge regarding toxoplasmosis and sources of *T. gondii* infection were also evaluated. Laboratory results from the tests were entered using the same number as the one on the questionnaires. All the data were then entered into Excel spreadsheet and later exported into SPSS for statistical analysis.

3.8 Sample collection and sample processing

About 5mL of venous blood were collected aseptically from each of the 384 pregnant women. All specimens were transported to the NRL where plasma was separated from the whole blood by centrifugation at 3,000 rpm for 5 minutes. Plasma samples were numbered and tested for anti-*T. gondii* antibodies (IgM and IgG) using ELISA assays. Information on HIV status and CD4+ cell count of the participants was obtained from their medical records.

3.9 Laboratory assays

Bioelisa TOXO IgG (Biokit, Spain)

Ten microliters of plasma sample was diluted using 1000 µl of sample diluent. The diluted specimen was then incubated in *T. gondii* antigen-coated wells at 37^0 C for 1 hour. The wells were then washed with washing solution to remove residual test specimen. Enzyme-labeled

antibodies to human IgG (conjugate) were then added. After another washing using washing solution to eliminate unbound material an enzyme substrate solution containing a chromogen was added. This solution developed a blue colour if the sample contained anti-*T. gondii* IgG. The blue colour then changed to yellow after blocking the reaction with sulphuric acid. The intensity of the colour which is proportional to the amount of anti-*T. gondii* IgG in the test specimen was read using an ELISA reader at 450 nm wavelength. The concentration of antibodies in the sample was then determined by mean of a calibration curve.

Quality control

The following criteria were used to consider the results as valid:

1. Substrate blank: absorbance value was taken to be less than or equal to 0.100.

2. Negative control: absorbance value was taken to be less than 0.100 after subtracting the blank.

3. High positive calibrator: absorbance value was taken to be less than 0.600 after subtracting the blank.

4. Low positive calibrator: absorbance value was taken to be equal to or higher than 0.150 after subtracting the blank

5. Ratio high positive calibrator/low positive calibrator: equal to or greater than 2.5.

6. Ratio negative control/low positive calibrator: equal to or less than 0.5.

Qualitative results

1. The mean absorbance value of the low positive control (cut-off value) was calculated as:

Cut-off = LPCx

2. The sample absorbance value was divided by the cut-off value.

Positive: ratio absorbance/cut-off $\geq$ 1.0

Negative: ratio absorbance/cut-off < 0.9

Equivocal: ratio absorbance/cut-off $\geq$ 0.9 < 1.0

Bioelisa TOXO IgM (Immunocapture)

10µl of test serum was diluted using 1 mls of sample diluent and the diluted specimen incubated in microplate wells coated with rabbit antibodies anti-human IgM. The wells are then washed using washing solution to remove residual test sample, and 100 µl of Toxoplasma antigen labelled with peroxidase is added. The plates were then washed again using washing solution to eliminate unbound material. 100 ul of solution of enzyme substrate and chromogen were then added. This solution developed a blue colour if the sample contained anti-*T. gondii* IgM. The blue colour changed to yellow after blocking the reaction with sulphuric acid. The plates were then read using an ELISA reader at 450 nm wavelength.

Quality control

The following criteria were used to consider the results as valid:

1. Substrate blank: absorbance value was taken to be less than or equal to 0.100.

2. Negative control: absorbance value was taken to be less than 0.120 after subtracting the blank.

3. Low positive control: each of the individual absorbance values was not to differ more than 30% of the mean of the three values. The mean absorbance was taken to be equal to or higher than 0.200 after subtracting the blank.

4. High positive control: absorbance greater than or equal to 0.600 after subtracting the blank.

5. Ratio high positive control/low positive control greater than or equal to 1.5.

6. Ratio negative control/low positive control less than or equal to 0.5.

Qualitative results

1. The mean absorbance value of the low positive control (cut-off value) was calculated as:

Cut-off = LPCx

2. The sample absorbance value was divided by the cut-off value.

Positive: ratio absorbance/cut-off ≥ 1.0

Negative: ratio absorbance/cut-off < 0.9

Equivocal: ratio absorbance/cut-off ≥ 0.9 < 1.0

Quality Assurance Plan

Specimen collection, labeling, storage and transportation to the laboratory were in accordance to the standard operating procedures. Appendix 6.

Standard operating procedures were followed to carry out all laboratory procedures. All reagent preparation and testing procedure were done following the kits and equipments manufacturer instructions. Data quality was ensured at all stages of data collection, entry and analysis.

3.10 Data management and analysis

a. Data Cleaning and Preparation for Analysis:

Data collected were entered into an Excel spreadsheet in a password- protected computer. The filled questionnaires were in the safe custody of the principal investigator who had filled and stored them in a locked cabinet for verification during analysis. Further cleaning was carried out after entry using frequency distributions and cross-tabulations until no more errors were detected. There were no missing data.

The final step in the preparation for analysis was the creation of any composite variables from the cleaned data set.

b. Data Analysis Plan

In order to achieve the objectives of the study, data analysis which was done using Statistical Package for Social Sciences Programme (SPSS) version 17.0, was carried out using the following 3 steps:

i) Univariate analysis

The univariate analysis involved frequency distributions for categorical variables and descriptive statistics (means, medians, standard deviations) for continuous and discrete variables. Categorical variables were presented using frequency distribution tables. Univariate analysis was used to give an understanding of the characteristics of the sample that have been observed, as well as description of the response variables (*T. gondii* infection).

ii) Bivariate analysis

Bivariate analysis was used to investigate any association between the response variable (*T. gondii* infection) with socio demographic and other variables of interest (risk factors). The χ^2 test was used to test association between 2 variables if they were categorical and satisfy all the conditions. No variables were subjected to multivariable analysis.

3.11 Ethical considerations

Ethical approval for the study was sought from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (Reference number PG625/12/2013) appendix 7, Rwanda National Ethics Committee (Reference number 085/RNEC/2014) appendix 8 and the

Ministry of Education Rwanda (Reference number 985/12.00/2014) appendix 9. In addition, permission was obtained from all respective study sites before beginning the study. Informed written consent was sought from each pregnant woman prior to involvement in the study. Informed consent was obtained based on non-coercive approach from the study participants and confidentiality was always upheld. The study participants were taken through a process of obtaining informed consent whereby the study objectives, process, risks and benefits were explained to them before being enrolled into the study. Dignity of the study participants was upheld throughout the study. The study questionnaires were stripped off all identifying data to maintain participant's confidentiality. A number was assigned to each study participant. Potential risks for the study were minimal, commensurate with routine interrogation and relevant tests. These included time considerations and mild pain at the site of puncture for specimen collection. In case a participant was found to be infected with *T.gondii*, she was contacted confidentially and told to report to the antenatal care clinic for a medical attention.

3.12 Expected outcomes/Recommendations

It is expected that this study will lead to a better understanding of the presence and impact of *T. gondii* infections in pregnant women. This will also help to identify risk factors associated with *T. gondii* in pregnant women. The data obtained from the study will advise the implementation of systematic screening of *T. gondii* infections during antenatal care in Rwanda as a strategy to minimize congenital toxoplasmosis and will lead to better diagnosis and disease management.

This study will help to provide health education/ information about the risks of exposure to *T. gondii* to pregnant women in order to prevent primary infection during pregnancy.

CHAPTER FOUR: RESULTS

Study population

Samples were distributed according to the number of pregnant women seeking the antenatal care to the specific site. Biryogo, Cor-Unum, Muhima and Nyarugunga are public health centres, so it was expected that more pregnant women would come from this facility. Originally, the intention was to include private clinics so that mothers from high income areas could participate, but, one clinic (la clinic belle vie) moved from Kigali city whereas Hopital la Croix du Sud declined to give permission for participation by mothers from the facility. The number of participants from these facilities was therefore compensated for by taking more participants from the rest of the public initially targeted health facilities. The distribution of participants according to health facilities is shown in Table 1.

Table 1: Samples distribution according to the study sites

Health center	Average number per month	%	Expected samples
Biryogo HC	76	26	101
Cor-Unum HC	76	26	101
Muhima HC	68	24	91
Nyarugunga HC	68	24	91
Total	288	100	384

A total of 384 pregnant women were enrolled during the study period. The median age of the study population was 27 years with range from 18 to 45 years. The majority of women (51.0%) were aged between 26 and 35 years.

Most of the women were not married (57.8%), (63.5%) were unemployed, and (64.1%) were multigravid with 54% on second trimester of their pregnancy. Majority of women (71.1%) had primary education, and only (25.0%) had attained a higher level of education Table 2.

Table 2: Demographic characteristics of the study population

Demographic characteristics		n	%
Age Group	18-25	157	40.9
	26-35	196	51.0
	36-45	31	8.1
Marital status	Married	162	42.2
	Unmarried	222	57.8
Occupation	Employed	140	36.5
	Not Employed	244	63.5
Level of Education	None	15	3.9
	Primary	273	71.1
	Secondary and Above	96	25.0
Gravidity	Primigravid	138	35.9
	Multigravid	246	64.1
Trimester of pregnancy	First	80	20.8
	Second	207	54
	Third	97	25.2

T. gondii among pregnant women

The overall seroprevalence of *T. gondii* was 12.2%. Participants from Muhima Health Centre where 16 of the 91 examined had the highest sero-prevalence (17.6%). The lowest prevalence of infection was found among study participants attending Nyarugunga Health Centre where only 5 of the 91 women (5.5%) were positive for infection (Table 3).

Table 3: Facility based *T. gondii* sero-prevalence

Health Facility	Total	n (%)	p-value
Biryogo HC	101	16(15.8)	0.046
Cor-Unum HC	101	10(9.9)	
Muhima HC	91	16(17.6)	
Nyarugunga HC	91	5(5.5)	
Overall	**384**	47(12.2)	

Of the 384 pregnant women studied, 37 (9.6%) were positive for anti-*T. gondii*-specific IgG antibodies, indicating past infection and 15(3.9%) had positive IgM results indicating recent infection.

***T. gondii* infection among pregnant women in relation to their demographic status**

Of the total of 384 study participants, about 8.1% were within age range of 36-45 years. Amongst these age groups, 17.1% were positive for anti-*T. gondii* antibodies.

With regard to the association of seroprevalence with occupation of participants, 244/384 (63.5%) were unemployed of which 12.3% were positive. Regarding educational background of the participants 273/384(71.1%) of them attended primary school. Of these, about 21.1% were seropositive for *T.gondii*. About 222/384 (57.8%) of the pregnant women were married, with a seroprevalence of 12.6%. A total of 246/384 (64.1%) were multigravid, of these 12.2% were seropositive for *T.gondii*. About 54% of the pregnant women were within their second gestational period. The study showed that an increase in trimester had a corresponding increase in distribution of the infection. Table 4.

Table 4: Distribution of *T. gondii* sero-prevalence in relation to demographic characteristics among pregnant women

Characteristics	*T. gondii* sero-prevalence		Univariate	
	Positive n (%)	Negative n(%)	OR[95% CI]	p-value
Age (years)				
18-25	18(11.5)	138(88.5)		
26-35	23(11.9)	170(88.1)	1.586(0.58-4.342)	0.369
36-45	6(17.1)	29(82.9)	1.529(0.57-4.08)	0.396
Occupation				
Unemployed	30(12.3)	214(87.7)	0.986(0.52-1.86)	0.965
Employed	17(12.1)	123(87.9)		
Level of Education				
None	4(26.7)	11(73.3)		
Primary	33(21.1)	240(87.9)	0.320(0.09-1.19)	0.09
Secondary and above	10(10.4)	86(89.6)	0.846(0.40-1.79)	0.661
Marital Status				
Married	19(11.7)	143(88.3)	0.921(0.49-1.71)	0.794
Not Married	28(12.6)	194(87.8)		
Gravidity				
Primigravid	17(12.3)	121(87.7)	1.01(0.54-1.91)	0.972
Multigravid	30(12.2)	216(87.8)		
Trimester				
First	8(10.0)	72(90.0)		
Second	26(12.6)	181(87.4)	1.39(0.55-3.55)	0.487
Third	13(13.4)	84(86.6)	1.01(0.527-2.20)	0.838

Risk factors associated with *T. gondii* infection among pregnant women

In univariate analysis there was a significant difference in *Toxoplasma* sero-positivity rate among individuals in relation to the type of drinking water used. Pregnant women who used untreated water had a higher seropositivity rate to *T.gondii* specific antibodies than those who used treated water 22.4% versus 6.8% (OR: 3.95, 95% CI: 2.09 - 7.49, p<0.001). About 39/384 (10.2%) of participants reported to own a cat and their sero-prevalence for anti-*T. gondii* antibodies was7.7% compared to 12.8% with those who did not own a cat. This difference was however not statistically significant in univariate analysis (p=0.361).

About 30% of the study participants reported eating undercooked meat with 22.3% of them being positive for anti- *T.gondii* antibodies. The odds of anti-*T.gondii* seropositivity were significantly higher in those who ate undercooked meat compared to those did not (OR=3.27, 95% CI:1.75-6.09,p<0.001).

The data showed that 375(97.6%) of participants had never heard or seen information about toxoplasmosis prior to the interview. Although, data on CD4+ T cell count were obtained from medical records of pregnant mothers who were HIV positive, they were not subjected to further statistical analysis, because of the small number of HIV positive participants with *T. gondii* infection.

There were no significant associations between seroprevalence of *T. gondii* and other risk factors considered in the study. These influential factors included dining out doors in restaurants, tasting food while cooking, history of previous abortion, HIV status and being exposed to any kind of knowledge about toxoplasmosis (Table 5).

Table 5: Factors associated with *T. gondii* infections among pregnant women

Characteristics	T. gondii sero-prevalence		Univariate		Bivariate	
	Positive n(%)	Negative n(%)	OR[95% CI]	p-value	OR[95% CI]	p-value
Water Type						
Untreated	30(22.4)	104(77.6)	3.95(2.09-7.49)	**<0.001**	4.01(2.08 - 7.69)	**<0.001**
Treated	17(6.8)	233(93.2)				
Own a Cat						
Yes	3(7.7)	36(92.3)	0.57(0.17-1.93)	0.361		
No	44(12.8)	301(87.2)				
Under cooked meat						
Yes	25(22.3)	87(77.7)	3.27(1.75-6.09)	**<0.001**	3.32(1.74-6.32)	**<0.001**
No	22(8.1)	250(91.9)				
Eat in Restaurant						
Yes	26(15.9)	138(84.1)	1.79(0.97-3.30)	0.062		
No	21(9.5)	199(90.5)				
Taste food						
Yes	31(10.8)	256(89.2)	0.61(0.31-1.18)	0.139		
No	16(16.5)	81(83.5)				
Abortion						
Yes	12(16.4)	61(83.6)	1.55(0.76-3.16)	0.224		
No	35(11.3)	276(88.7)				
HIV status						
Positive	1(5.3)	18(94.7)	0.39(0.05-2.96)	0.341		
Negative	46(12.6)	319(87.4)				
T. *gondii* knowledge						
Yes	0	9(100)	1.14(1.10-1.19)	0.608		
No	47(12.5)	328(87.5)				

Seropositivity of Anti-*Toxoplasma* IgG in relation to type of drinking water and habit of eating of undercooked meat.

Pregnant women who used untreated water had a higher seroprevalence anti-*T. gondii*-specific IgG antibodies, than those who used treated water 17.9% versus 5.2% (OR: 3.99, 95% CI: 1.93 – 8.22, p<0.001). This difference was statistically significant in both univariate and bivariate analysis with a p-value< 0.001. About 29.2% of participants reported having a habit of eating undercooked meat and their sero-prevalence for anti-*T. gondii* specific antibodies was 17.9% compared to 6.3% with those who did not eat undercooked meat. This difference was statistically

significant in both univariate and Bivariate analysis (p=<0.001). There was no significant difference for other risk factors studied.

Table 6. Seropositivity of Anti-Toxoplasma IgG in relation to type of drinking water and habit of eating of undercooked meat.

Characteristics	IgG sero-prevalence		Univariate		Bivariate	
	Positive	Negative	OR[95% CI]	p-value	OR[95% CI]	p-value
	n(%)	n(%)				
Water Type						
Untreated	24(17.9)	110(82.1)	3.97(1.95, 8.11)	**<0.001**	3.99(1.93, 8.22)	**<0.001**
Treated	13(5.2)	237(94.8)				
Under cooked meat						
Yes	20(17.9)	92(82.1)	0.31(0.15, 0.61)	**<0.001**	0.306(0.15, 0.62)	**0.001**
No	17(6.3)	255(93.8)				

The sero-prevalence of *T. gondii*-specific IgM antibodies was higher in pregnant women who reported drinking untreated water (22.4%) than those who did not (6.8%) (OR=4.01, 95%CI: 2.09-7.69, p<0.001). Participants who reported had a habit of eating undercooked meat showed a higher sero-prevalence of T. gondii-specific antibodies (22.3%) than those who did not (8.1%) (OR=0.30, 95%CI: 0.16,-0.57, p=0.001).There was no significant differences for other risk factors studied.

Table 7. Seropositivity of Anti-Toxoplasma IgM in relation to type of drinking water and habit of eating of undercooked meat.

Characteristics	IgM sero-prevalence		Univariate		Bivariate	
	Positive	Negative	OR[95% CI]	p-value	OR[95% CI]	p-value
	n(%)	n(%)				
Water Type						
Untreated	30(22.4)	104(77.6)	3.96(2.09, 7.49)	**<0.001**	4.01(2.09, 7.69)	**<0.001**
Treated	17(6.8)	233(93.2)				
Under cooked meat						
Yes	25(22.3)	87(77.7)	0.31(0.16, 0.57)	**<0.001**	0.30(0.16, 0.57)	**0.001**
No	22(8.1)	250(91.9)				

CHAPTER FIVE: DISCUSSION

Few studies have looked at sero-prevalence of anti-T. *gondii* in Rwanda. The present study showed an overall seroprevalence of anti-*T. gondii* antibody among pregnant women in Kigali, to be 12.2%. This finding is lower than that previously reported in 1989 by Gascon *et al* in Nyarutovu and Ngenda (Gascon et al., 1989). In that sero-prevalence study, 50% of adults had evidence of *T. gondii* infections. However, the study by Gascon *et al* (1989) looked at the total population whereas in the present study, sero-prevalence of infection was confined to the pregnant mothers.

In addition, the observed differences in seroprevalence of anti-*T. gondii* infection may be due to differences in the two study populations. The present study was confined to an urban population in Kigali city, as opposed to the rural population that was studied by Gascon et al (1989).

In the present study, seropositivity of anti- *T. gondii* antibody increased with age of study participants. Although this observation was not statistically significant, it is in agreement with other previous similar studies (Al-Mohammad, Amin, Balaha, & Al-Moghannum, 2010; Markovich et al., 2014; Rosso et al., 2008). This could be explained by the fact that older women are more likely to have been exposed to any one of the risk factors than younger women as a result of longer exposure time.

The reported differences in seroprevalence between the current study and that by Gascon *et al* may also be due to gender differences in the two study populations. Gascon et al examined a population consisting of both males and females compared to the exclusively female population in the current study. Gender differences in sero-prevalence of *T.gondii* have previously been described. A study by Jones *et al* (J. L. Jones et al., 2001*)* showed a high seroprevalence of

T.gondii in men than in women. A similar finding was observed in a study by Lindova et al (Lindova et al., 2006). It should be noted however that gender differences in prevalence of *T. gondii* infection have not been observed in other studies. For example, Markovich *et al* (2014) in a study of the general population in Israeli found no significant gender differences in infection (Markovich et al., 2014).

Differences in seroprevalence of anti-T.*gondii* antibodies have also previously been attributed to differences in socioeconomic status of the populations. A study by Mwambe *et al* in Tanzania (Mwambe et al., 2013) showed that pregnant business women and employed women had higher infection rates of *T. gondii* than the unemployed. Although the reason for this difference is not clear, it can be speculated that these business women and employed women are of a higher socio-economic status which makes them to easily afford to eat poultry and pork which have been found to be a major source for *T. gondii* transmission (Yang et al., 2013). However, in the current study, seroprevalence in pregnant women who were employed or had their own business was quite similar with that of the unemployed. A similar observation has previously been made in Ethiopia (Gebremedhin et al., 2013).

Although the sero-prevalence of *T. gondii* in the total population was 12.2%, there was a significant variation from one health centre to another with the lowest being 5.5% and the highest being 17.9% with a p-value of 0.046. Regional variation of sero-prevalence of *T. gondii* infection has previously been described (J. L. Jones et al., 2001; Mwambe et al., 2013). The reason for this may not be clear but could include several factors such as socio-economic status, education, sanitary conditions and differences in dietary habits.

The fact that the study population had some individuals with IgM (3.9%) antibodies implies that transmission of *T. gondii* infection had either taken place during the pregnancy or the women had become pregnant shortly after acquiring *T. gondii* infection. Although the prevalence of acute infection was low at 3.9%, this is still an important finding since there is a risk of these women transmitting the infection to their unborn child thereby resulting in congenital toxoplasmosis, which is a more severe disease that may not only lead to abortion but also to congenital malformations in the new born. In contrast to developing countries, the prevalence of congenital toxoplasmosis in Africa is largely unknown. Whereas in developed countries congenital toxoplasmosis is preventable by screening of pregnant mothers for infection, this may not be feasible in resource constrained countries in Africa. In the absence of screening of pregnant women, hygienic measures and health education remain the cornerstone of toxoplasmosis prevention strategies for pregnant women.

The present study also showed a statistically significant association between the sero-prevalence of *T. gondii* infection and drinking of untreated water. This is not surprising as *T. gondii* infection can occur by ingestion of oocysts in contaminated water. Ishaku *et al* found a higher prevalence of *T. gondii* infection among pregnant women who drank water from the well compared to the women who drank piped water (Ishaku B, Ajogi I, Umoh J, & A, 2009). A similar finding showing an association between prevalence of *T. gondii* infection and source of drinking water was made by Montoya *et al* (Montoya & Liesenfeld, 2004).

Contact with cat litter may pose another risk for *T. gondii* infection. Although in the present study, there was no significant statistical difference in the seroprevalence of *T. gondii* infections between women who kept cats and those who did not, the number of women who kept cats was small thereby not allowing for an accurate assessment of the association between *T. gondii*

infection and keeping of cats. Other researchers have also not demonstrated the association between *T. gondii* infection and contact with cats (Nijem & Al-Amleh, 2009) (Ghoneim et al., 2010). In contrast to these studies, however, some other studies have shown a close association between the keeping of cats and the prevalence of *T. gondii* infection (Ghoneim et al., 2010; Zemene et al., 2012). The reason for conflicting results on this matter is not clear. Nevertheless, it can be asserted, that the risk of contracting *T. gondii* infection is not just the mere contact with cats but the way the cats' litter is handled by those who keep the cats which can make a great difference in infection rates.

In the present study, eating undercooked meat was a major risk factor in the transmission of *T.gondii* infection which is in agreement with studies conducted elsewhere (Alvarado-Esquivel, Estrada-Martinez, & Liesenfeld, 2011; Koskiniemi, Lappalainen, & Hedman, 1989; Lopez-Castillo, Diaz-Ramirez, & Gomez-Marin, 2005; Sroka et al., 2010). It is possible that one of the sources of *T. gondii* infection in this study population may be from eating meat with tachyzoites or bradyzoites hence leading to the infection transmission.

Although in the present study, it was observed that those who gave a history of eating in restaurants had a trend towards a higher seroprevalence of anti-*T. gondii* antibodies (OR= 1.79, 95% CI: 0.97-3.30, p=0.062) than those who did not, this difference was not statistically significant. However, some studies have demonstrated significant association between anti-*T.gondii* antibody seropositivity and behavior of eating in restaurants (Montoya & Liesenfeld, 2004).

The limited knowledge of the women on *T. gondii* is of great concern. The present study revealed that approximately 98% of participants were not aware of toxoplasmosis and its

association with congenital disease. About 12.5% of these were positive to anti-*T.gondii* antibodies, whereas among study participants who had some information on *T.gondii* none was found to be positive for anti-*T.gondii* antibodies. Although, it was not possible to compare the two groups because of the small number of participants who knew about *T.gondii,* this suggests that there is a need to launch an awareness program for the pregnant women about the risks of exposure to *T.gondii* in Kigali and possibly in the whole country.

There were no significant differences in seroprevalence of anti-*T.gondii* antibodies in relation to: tasting food while cooking, history of previous abortion, HIV status and being exposed to any kind of knowledge about toxoplasmosis. The absence of a statistically significant relationship between the prevalence of *Toxoplasma* infection among pregnant women in Kigali and many of the factors explored in this study, does not rule out the possibility of these factors having some influence on the transmission of toxoplasmosis. Probably a larger study might need being undertaken to explore further the role of the various risk factors for *T. gondii.*

Conclusion

The absence of a statistically significant relationship between the prevalence of *Toxoplasma* infection among pregnant women in Kigali and many of the factors explored in this study, does not necessarily mean that these factors have no influence on the transmission of toxoplasmosis in the country. Probably a lager sample size would have revealed significant relationships.

Undercooked meat consumption and drinking untreated water are significantly associated with the acquisition of *T. gondii* infection. The seroprevalence of *T. gondii* antibodies is relatively low among the pregnant women in this study.

Almost 98% of pregnant women who participated in the study had never heard or seen information about toxoplasmosis.

Recommendation

According to the findings of the present study, the prevalence of T. gondii infections in the pregnant mothers was 12.2%. However, almost 98% of pregnant women who participated in the study had never heard or seen information about toxoplasmosis. There is therefore the need to conduct public health education in order to create awareness on the disease and its transmission especially to pregnant women.

From the findings, it is also imperative that further studies on the burden of maternal and congenital toxoplasmosis be carried out in the country in order to advise policy on possible needs of instituting control programmes.

Screening of *T. gondii* infections during antenatal care should be considered in Rwanda as the main strategy to minimize congenital toxoplasmosis.

REFERENCES

A. M. Deji-Agboola, O. S. Busari, O. A. Osinupebi, & Amoo, A. O. J. (2011). Seroprevalence of Toxoplasma gondii Antibodies among Pregnant Women

Attending Antenatal Clinic of Federal Medical Center, Lagos, Nigeria. *International Journal of Biological & Medical Research, 2(4)*, 1135-1139.

Adou-Bryn, K. D., Ouhon, J., Nemer, J., Yapo, C. G., & Assoumou, A. (2004). [Serological survey of acquired toxoplasmosis in women of child-bearing age in Yopougon (Abidjan, Cote d'Ivoire)]. *Bull Soc Pathol Exot, 97*(5), 345-348.

Afonso, E., Thulliez, P., & Gilot-Fromont, E. (2006). Transmission of Toxoplasma gondii in an urban population of domestic cats (Felis catus). *Int J Parasitol, 36*(13), 1373-1382. doi: 10.1016/j.ijpara.2006.07.010

Al-Mohammad, H. I., Amin, T. T., Balaha, M. H., & Al-Moghannum, M. S. (2010). Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: seroprevalence and possible risk factors. *Ann Trop Med Parasitol, 104*(6), 493-504. doi: 10.1179/136485910x12786389891443

Alvarado-Esquivel, C., Estrada-Martinez, S., & Liesenfeld, O. (2011). Toxoplasma gondii infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study. *Parasit Vectors, 4*, 235. doi: 10.1186/1756-3305-4-235

Arens, J., Barnes, K., Crowley, N., & Maartens, G. (2007). Treating AIDS-associated cerebral toxoplasmosis - pyrimethamine plus sulfadiazine compared with cotrimoxazole, and outcome with adjunctive glucocorticoids. *S Afr Med J, 97*(10), 956-958.

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

Barbosa, I. R., de Carvalho Xavier Holanda, C. M., & de Andrade-Neto, V. F. (2009). Toxoplasmosis screening and risk factors amongst pregnant females in Natal, northeastern Brazil. *Trans R Soc Trop Med Hyg, 103*(4), 377-382. doi: 10.1016/j.trstmh.2008.11.025

Berger F, Desndos JC, Le Strait Y, & Goulet V. (2008). toxoplasmose chez les femmes enceintes en France : évolution de la séroprévalence et de l'incidence et facteurs associés, 1995-2003., 117-121, .

Bhopale, G. M. (2003). Pathogenesis of toxoplasmosis. *Comp Immunol Microbiol Infect Dis, 26*(4), 213-222. doi: 10.1016/s0147-9571(02)00058-9

Blader, I. J., & Saeij, J. P. (2009). Communication between Toxoplasma gondii and its host: impact on parasite growth, development, immune evasion, and virulence. *APMIS, 117*(5-6), 458-476. doi: 10.1111/j.1600-0463.2009.02453.x

Bonfioli, A. A., & Orefice, F. (2005). Toxoplasmosis. *Semin Ophthalmol, 20*(3), 129-141. doi: 10.1080/08820530500231961

Botterel, F., Ichai, P., Feray, C., Bouree, P., Saliba, F., Tur Raspa, R., . . . Romand, S. (2002). Disseminated toxoplasmosis, resulting from infection of allograft, after orthotopic liver transplantation: usefulness of quantitative PCR. *J Clin Microbiol, 40*(5), 1648-1650.

Carruthers, V. B. (2002). Host cell invasion by the opportunistic pathogen Toxoplasma gondii. *Acta Trop, 81*(2), 111-122.

Cenci-Goga, B. T., Rossitto, P. V., Sechi, P., McCrindle, C. M., & Cullor, J. S. (2011). Toxoplasma in animals, food, and humans: an old parasite of new concern. *Foodborne Pathog Dis, 8*(7), 751-762. doi: 10.1089/fpd.2010.0795

Chen, K. T., Eskild, A., Bresnahan, M., Stray-Pedersen, B., Sher, A., & Jenum, P. A. (2005). Previous maternal infection with Toxoplasma gondii and the risk of fetal death. *Am J Obstet Gynecol, 193*(2), 443-449. doi: 10.1016/j.ajog.2004.12.016

Cristina, N., Pelloux, H., Goulhot, C., Brion, J. P., Leclercq, P., & Ambroise-Thomas, P. (1993). Detection of Toxoplasma gondii in AIDS patients by the polymerase chain reaction. *Infection, 21*(3), 150-153.

De Paschale, M., Agrappi, C., Manco, M. T., Cerulli, T., & Clerici, P. (2010). Implementation of Screening for Toxoplasma gondii Infection in Pregnancy. *J Clin Med Res, 2*(3), 112-116. doi: 10.4021/jocmr2010.05.321w

Dubey, J. P. (1988). Long-term persistence of Toxoplasma gondii in tissues of pigs inoculated with T gondii oocysts and effect of freezing on viability of tissue cysts in pork. *Am J Vet Res, 49*(6), 910-913.

Dubey, J. P. (2004). Toxoplasmosis - a waterborne zoonosis. *Vet Parasitol, 126*(1-2), 57-72. doi: 10.1016/j.vetpar.2004.09.005

Dubey, J. P., & Jones, J. L. (2008). Toxoplasma gondii infection in humans and animals in the United States. *Int J Parasitol, 38*(11), 1257-1278. doi: 10.1016/j.ijpara.2008.03.007

Duncanson, P., Terry, R. S., Smith, J. E., & Hide, G. (2001). High levels of congenital transmission of Toxoplasma gondii in a commercial sheep flock. *Int J Parasitol, 31*(14), 1699-1703.

Dunn, D., Wallon, M., Peyron, F., Petersen, E., Peckham, C., & Gilbert, R. (1999). Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counselling. *Lancet, 353*(9167), 1829-1833. doi: 10.1016/s0140-6736(98)08220-8

Dupouy-Camet, J., de Souza, S. L., Maslo, C., Paugam, A., Saimot, A. G., Benarous, R., . . . Derouin, F. (1993). Detection of Toxoplasma gondii in venous blood from AIDS patients by polymerase chain reaction. *J Clin Microbiol, 31*(7), 1866-1869.

El Mansouri, B., Rhajaoui, M., Sebti, F., Amarir, F., Laboudi, M., Bchitou, R., . . . Lyagoubi, M. (2007). [Seroprevalence of toxoplasmosis in pregnant women in Rabat, Morocco]. *Bull Soc Pathol Exot, 100*(4), 289-290.

Estee T, Cooke F, & E., M. (2009). *Oxford handbook of infectious deseases and microbiology*.

Falusi, O., French, A. L., Seaberg, E. C., Tien, P. C., Watts, D. H., Minkoff, H., . . . Cohen, M. H. (2002). Prevalence and predictors of Toxoplasma seropositivity in women with and at risk for human immunodeficiency virus infection. *Clin Infect Dis, 35*(11), 1414-1417. doi: 10.1086/344462

Ferguson, D. J. (2002). Toxoplasma gondii and sex: essential or optional extra? *Trends Parasitol, 18*(8), 355-359.

Filice, G. A., Hitt, J. A., Mitchell, C. D., Blackstad, M., & Sorensen, S. W. (1993). Diagnosis of Toxoplasma parasitemia in patients with AIDS by gene detection after amplification with polymerase chain reaction. *J Clin Microbiol, 31*(9), 2327-2331.

Foulon, W., Pinon, J. M., Stray-Pedersen, B., Pollak, A., Lappalainen, M., Decoster, A., . . . Naessens, A. (1999). Prenatal diagnosis of congenital toxoplasmosis: a multicenter evaluation of different diagnostic parameters. *Am J Obstet Gynecol, 181*(4), 843-847.

Gagandeep, S., & Sehgal, R. (2010). Transfusion-transmitted parasitic infections. *Asian Journal of Transfusion Science, 4*(2), 73-77.

Gascon, J., Torres-Rodriguez, J. M., Soldevila, M., & Merlos, A. M. (1989). [Seroepidemiology of toxoplasmosis in 2 communities of Rwanda (Central Africa)]. *Rev Inst Med Trop Sao Paulo, 31*(6), 399-402.

Gebremedhin, E. Z., Abebe, A. H., Tessema, T. S., Tullu, K. D., Medhin, G., Vitale, M., . . . Dorny, P. (2013). Seroepidemiology of Toxoplasma gondii infection in women of child-bearing age in central Ethiopia. *BMC Infect Dis, 13*, 101. doi: 10.1186/1471-2334-13-101

Ghoneim, N. H., Shalaby, S. I., Hassanain, N. A., Zeedan, G. S., Soliman, Y. A., & Abdalhamed, A. M. (2010). Comparative study between serological and molecular methods for diagnosis of toxoplasmosis in women and small ruminants in Egypt. *Foodborne Pathog Dis, 7*(1), 17-22. doi: 10.1089/fpd.2008.0223

Gibbs, R. S. (2002). The origins of stillbirth: infectious diseases. *Semin Perinatol, 26*(1), 75-78.

Goldenberg, R. L., & Thompson, C. (2003). The infectious origins of stillbirth. *Am J Obstet Gynecol, 189*(3), 861-873.

Hegab, S. M., & Al-Mutawa, S. A. (2003). Immunopathogenesis of toxoplasmosis. *Clin Exp Med, 3*(2), 84-105. doi: 10.1007/s10238-003-0011-2

Hill, D., & Dubey, J. P. (2002). Toxoplasma gondii: transmission, diagnosis and prevention. *Clin Microbiol Infect, 8*(10), 634-640.

Holliman, R. E., Johnson, J. D., Gillespie, S. H., Johnson, M. A., Squire, S. B., & Savva, D. (1991). New methods in the diagnosis and management of cerebral toxoplasmosis associated with the acquired immune deficiency syndrome. *J Infect, 22*(3), 281-285.

Holliman, R. E., Johnson, J. D., & Savva, D. (1990). Diagnosis of cerebral toxoplasmosis in association with AIDS using the polymerase chain reaction. *Scand J Infect Dis, 22*(2), 243-244.

Ishaku B, Ajogi I, Umoh J, L. I., & A, R. (2009). Seroprevalence and Risk Factors for Toxoplasma Gondii Infection among Antenatal

Women in Zaria, Nigeria. *Research Journal of Medicine and Medical Sciences, 4(2)*, 483-488.

Jayawardena S, Singh S, Burzyantseva O, & H, C. (2008). Cerebral toxoplasmosis Cerebral toxoplasmosis in adult patients with HIV infection. . *Clinical medical journal Hospital Physician*, 17-24.

Johnson, J. D., Butcher, P. D., Savva, D., & Holliman, R. E. (1993). Application of the polymerase chain reaction to the diagnosis of human toxoplasmosis. *J Infect, 26*(2), 147-158.

Jones, J., Lopez, A., & Wilson, M. (2003). Congenital toxoplasmosis. *Am Fam Physician, 67*(10), 2131-2138.

Jones, J. L., & Dubey, J. P. (2010). Waterborne toxoplasmosis--recent developments. *Exp Parasitol, 124*(1), 10-25. doi: 10.1016/j.exppara.2009.03.013

Jones, J. L., Kruszon-Moran, D., Wilson, M., McQuillan, G., Navin, T., & McAuley, J. B. (2001). Toxoplasma gondii infection in the United States: seroprevalence and risk factors. *Am J Epidemiol, 154*(4), 357-365.

Jones, J. L., Lopez, A., Wilson, M., Schulkin, J., & Gibbs, R. (2001b). Congenital toxoplasmosis: a review. *Obstet Gynecol Surv, 56*(5), 296-305.

Kijlstra, A., & Jongert, E. (2008). Control of the risk of human toxoplasmosis transmitted by meat. *Int J Parasitol, 38*(12), 1359-1370. doi: 10.1016/j.ijpara.2008.06.002

Koskiniemi, M., Lappalainen, M., & Hedman, K. (1989). Toxoplasmosis needs evaluation. An overview and proposals. *Am J Dis Child, 143*(6), 724-728.

Lappalainen, M., & Hedman, K. (2004). Serodiagnosis of toxoplasmosis. The impact of measurement of IgG avidity. *Ann Ist Super Sanita, 40*(1), 81-88.

Lebech, M., Lebech, A. M., Nelsing, S., Vuust, J., Mathiesen, L., & Petersen, E. (1992). Detection of Toxoplasma gondii DNA by polymerase chain reaction in cerebrospinal fluid from AIDS patients with cerebral toxoplasmosis. *J Infect Dis, 165*(5), 982-983.

Lindova, J., Novotna, M., Havlicek, J., Jozifkova, E., Skallova, A., Kolbekova, P., . . . Flegr, J. (2006). Gender differences in behavioural changes induced by latent toxoplasmosis. *Int J Parasitol, 36*(14), 1485-1492. doi: 10.1016/j.ijpara.2006.07.008

Lopes, F. M. R., Goncalves, D. D., Mitsuka-Bregano, R., Freire, R. L., & Navarro, I. T. (2007). Toxoplasma gondii Infection in Pregnancy. *The Brazilian Journal of Infectious Diseases, 11*(5), 496-506.

Lopez-Castillo, C. A., Diaz-Ramirez, J., & Gomez-Marin, J. E. (2005). [Risk factors for Toxoplasma gondii infection in pregnant women in Armenia, Colombia]. *Rev Salud Publica (Bogota), 7*(2), 180-190.

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

Luft, B. J., & Remington, J. S. (1992). Toxoplasmic encephalitis in AIDS. *Clin Infect Dis, 15*(2), 211-222.

Malla, N., Sengupta, C., Dubey, M. L., Sud, A., & Dutta, U. (2005). Antigenaemia and antibody response to Toxoplasma gondii in human immunodeficiency virus-infected patients. *Br J Biomed Sci, 62*(1), 19-23.

Markovich, M. P., Shohat, T., Riklis, I., Avni, R., Yujelevski-Rozenblit, D., Bassal, R., . . . Rorman, E. (2014). Seroepidemiology of Toxoplasma gondii infection in the Israeli population. *Epidemiol Infect, 142*(1), 149-155. doi: 10.1017/s0950268813000903

Martinez E, Mago H, Rocha R, & M:, P. (2002). Epidemiological findings and prevalence of Toxoplasma gondii antibodies in HIV-positive patients in a Venezuelan hospital. *Valencia International Conference AIDS*, 7-14.

Montoya, J. G., & Liesenfeld, O. (2004). Toxoplasmosis. *Lancet, 363*(9425), 1965-1976. doi: 10.1016/s0140-6736(04)16412-x

Montoya, J. G., & Rosso, F. (2005). Diagnosis and management of toxoplasmosis. *Clin Perinatol, 32*(3), 705-726. doi: 10.1016/j.clp.2005.04.011

Mwambe, B., Mshana, S. E., Kidenya, B. R., Massinde, A. N., Mazigo, H. D., Michael, D., . . . Gross, U. (2013). Sero-prevalence and factors associated with Toxoplasma gondii infection among pregnant women attending antenatal care in Mwanza, Tanzania. *Parasit Vectors, 6*, 222. doi: 10.1186/1756-3305-6-222

Ndiaye, D., Sene, P. D., Ndiaye, M., Faye, B., Ndiaye, J. L., & Ndir, O. (2011). [Update on toxoplasmosis prevalence based on serological tests in pregnant women in Dakar, Senegal from 2002 to 2006]. *Med Trop (Mars), 71*(1), 101-102.

Nigel, B., Pope, D., & Stanistreet, D. (2008). *Quantitative Methods for Health Research*. England: John Wiley & Sons Ltd.

Nijem, K. I., & Al-Amleh, S. (2009). Seroprevalence and associated risk factors of toxoplasmosis in pregnant women in Hebron district, Palestine. *East Mediterr Health J, 15*(5), 1278-1284.

Nissapatorn, V., Suwanrath, C., Sawangjaroen, N., Ling, L. Y., & Chandeying, V. (2011). Toxoplasmosis-serological evidence and associated risk factors among pregnant women in southern Thailand. *Am J Trop Med Hyg, 85*(2), 243-247. doi: 10.4269/ajtmh.2011.10-0633

Olusi, T., Gross, U., & Ajayi, J. (1996). High incidence of toxoplasmosis during pregnancy in Nigeria. *Scand J Infect Dis, 28*(6), 645-646.

OMS. (2009-2013). STRATÉGIE DE COOPÉRATION DE L'OMS AVEC LES PAYS RWANDA (pp. 17).

Pappas, G., Roussos, N., & Falagas, M. E. (2009). Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. *Int J Parasitol, 39*(12), 1385-1394. doi: 10.1016/j.ijpara.2009.04.003

Pavesio, C. E., & Lightman, S. (1996). Toxoplasma gondii and ocular toxoplasmosis: pathogenesis. *Br J Ophthalmol, 80*(12), 1099-1107.

Pfaff, A. W., Abou-Bacar, A., Letscher-Bru, V., Villard, O., Senegas, A., Mousli, M., & Candolfi, E. (2007). Cellular and molecular physiopathology of congenital toxoplasmosis: the dual role of IFN-gamma. *Parasitology, 134*(Pt 13), 1895-1902. doi: 10.1017/s0031182007000200

Renold, C., Sugar, A., Chave, J. P., Perrin, L., Delavelle, J., Pizzolato, G., . . . Hirschel, B. (1992). Toxoplasma encephalitis in patients with the acquired immunodeficiency syndrome. *Medicine (Baltimore), 71*(4), 224-239.

Robert-Gangneux, F., & Darde, M. L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev, 25*(2), 264-296. doi: 10.1128/cmr.05013-11

Roos, D. S., Crawford, M. J., Donald, R. G., Kissinger, J. C., Klimczak, L. J., & Striepen, B. (1999). Origin, targeting, and function of the apicomplexan plastid. *Curr Opin Microbiol, 2*(4), 426-432. doi: 10.1016/s1369-5274(99)80075-7

Rosso, F., Les, J. T., Agudelo, A., Villalobos, C., Chaves, J. A., Tunubala, G. A., . . . Montoya, J. G. (2008). Prevalence of infection with Toxoplasma gondii among pregnant women in Cali, Colombia, South America. *Am J Trop Med Hyg, 78*(3), 504-508.

Rothova, A. (1993). Ocular involvement in toxoplasmosis *Br J Ophthalmol* (1993/06/01 ed., Vol. 77, pp. 371-377).

Sroka, J., Wojcik-Fatla, A., Szymanska, J., Dutkiewicz, J., Zajac, V., & Zwolinski, J. (2010). The occurrence of Toxoplasma gondii infection in people and animals from rural environment of Lublin region - estimate of potential role of water as a source of infection. *Ann Agric Environ Med, 17*(1), 125-132.

Studenicova, C., Bencaiova, G., & Holkova, R. (2006). Seroprevalence of Toxoplasma gondii antibodies in a healthy population from Slovakia. *Eur J Intern Med, 17*(7), 470-473. doi: 10.1016/j.ejim.2006.07.007

Sucilathangam G, Anna T, & Velvizhi G. (2013). Comparative Study of Elisa and Lat Techniques For the Serological Prevalence of Anti-Toxoplasma Antibodies Among Hiv Infected Patients. *International Journal of Scientific Research, 2*(10 October 2013).

Sukthana, Y. (2006). Toxoplasmosis: beyond animals to humans. *Trends Parasitol, 22*(3), 137-142. doi: 10.1016/j.pt.2006.01.007

Tenter, A. M., Heckeroth, A. R., & Weiss, L. M. (2000). Toxoplasma gondii: from animals to humans. *Int J Parasitol, 30*(12-13), 1217-1258.

Woldemichael, T., Fontanet, A. L., Sahlu, T., Gilis, H., Messele, T., Rinke de Wit, T. F., . . . Van Gool, T. (1998). Evaluation of the Eiken latex agglutination test for anti-Toxoplasma antibodies and seroprevalence of Toxoplasma infection among factory workers in Addis Ababa, Ethiopia. *Trans R Soc Trop Med Hyg, 92*(4), 401-403.

Yang, N., Mu, M. Y., Yuan, G. M., Zhang, G. X., Li, H. K., & He, J. B. (2013). Seroprevalence of Toxoplasma gondii in slaughtered horses and donkeys in Liaoning province, northeastern China. *Parasit Vectors, 6*, 140. doi: 10.1186/1756-3305-6-140

Zemene, E., Yewhalaw, D., Abera, S., Belay, T., Samuel, A., & Zeynudin, A. (2012). Seroprevalence of Toxoplasma gondii and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. *BMC Infect Dis, 12*, 337. doi: 10.1186/1471-2334-12-337

Zhou, P., Chen, Z., Li, H. L., Zheng, H., He, S., Lin, R. Q., & Zhu, X. Q. (2011). Toxoplasma gondii infection in humans in China. *Parasit Vectors, 4*, 165. doi: 10.1186/1756-3305-4-165

Zumla, A., Savva, D., Wheeler, R., Hira, S. L., Np; , Kaleebu, P., Sempala, S., . . . Holliman, R. (1991). Toxoplasma Serology In Zambian And Ugandan Patients Infected With The Human-Immunodeficiency-Virus. *T Royal Social Tropical Medicine H, 85 (2)85 (2) 227 - 22*, 227 - 229.

APPENDICES

APPENDIX 1. STUDY QUESTIONNAIRE

STUDY TITLE: Sero-prevalence and risk factors of *Toxoplasma gondii* infection among pregnant women attending antenatal care in Kigali, Rwanda.

NB. Put a tick (√) or a cross (×) in the appropriate box (mushyire ikimenyetso√ cg × ahabugenewe)

DATE OF INTERVIEW (Itariki) _____/____/2014

CODE OF THE PARTICIPANT (Umubare w'ibanga): _____

VISIT TYPE (Kwipimisha):FIRST VISIT(bwa mbere) [1] **REVISIT(Bwa kenshi)** [2]

SECTION A: SOCIODEMOGRAPHIC CHARACTERISTICS

1. Date of birth ____/____/_____(**igihe mwavukiye**)
2. Age of patient [ ][ ] (**imyaka**)
3. Where do you live? (**mutuye hehe**?)

District (**akarere**)_____Sector(**umurenge**) _____

4. Marital Status: (**irangamimerere**)

   [1] Married [1] **Ndubatse**

   [2]Unmarried [2] **Sinubatse.**

5. Occupation **(icyo mukora)**

   Student [1] **Umunyeshuri**

   Housewife [2] **Umugore wo mu rugo**

   Unemployed [3] **Ntakazi mfite**

Self employed	4	**Ndikorera**
Employed	5	**Umukoreshwa**

6. Level of education **(ikiciro cy'amashuri.)**

None	1	**Nta mashuri mfite**
Primary school	2	**Amashuri abanza**
Secondary school and above	3	**Amashuri yisumbuye no hejuru**

7. Gravidity **(imbyaro)**

Primigravid	1	**Inda ya mbere**
Multigravid	2	**Inda ya kabiri gusubiza hejuru**

8. Trimester of pregnancy **(igihembwe cy'umubyeyi)**

1st trimester	1	**Igihembwe cya 1**
2nd trimester	2	**Igihembwe cya 2**
3rd trimester	3	**Igihembwe cya 3**

Section B: Risk factors (Ibishobora gutera ingorane zo kwandura)

9. Which kind of water do you use? **Ni ayahe mazi mukoresha?**

(1)Untreated	1	**Adasukuye**
(2) Treated	2	**Asukuye**

10. Do you have cats at home? **Mufite injangwe mu rugo?**

Yes/**Yego** 1 No/**Oya** 2

11. do you eat raw or undercooked meat? **Mujya murya inyama mbisi cg zidatetse neza?**

Yes/**Yego** 1 No/**Oya** 2

12. Do you eat in restaurants? **Mujya murira muri restora?**

Yes/**Yego** 1 No/**Oya** 2

13. Do you test food during cooking? **Mujya mwumva ibiryo ko bihiye mu gihe mutetse?**

Yes/**Yego** 1 No/**Oya** 2

14. have you ever had abortion? **Mwigeze mukuramo inda?**

Yes/**Yego** 1 No/**Oya** 2

15. Do you know something about *Toxoplasma gondii*? **Hari icyo mwaba muzi kuri Toxoplasma gondii?**

Yes/**Yego** 1 No/**Oya** 2

16. **Have you been tested for HIV before? Mwigeze mwipimisha agakoko gatera SIDA?**

Yes/Yego 1 **No/Oya** 2

Positive 1 **Negative** 2

THANK YOU

MURAKOZE

APPENDIX 2. LABORATORY REPORT FORM

Code of participant:……..

Date of sample collection __dd___mm____yy

Date and time of arrival to the lab __dd___mm_____yy; Time_____

1. Toxo IgM ELISA	Positive	☐	Negative	☐
2. Toxo IgG ELISA	Positive	☐	Negative	☐
3. HIV test	Positive	☐	Negative	☐
4. $CD4^+$ count		.....		

APPENDIX 3. INFORMATION TO PARTICIPANTS AND CONSENT FORM

CODE OF THE PARTICIPANT: _____

TITLE OF STUDY: Sero-prevalence and risk factors of *Toxoplasma gondii* infection among pregnant women attending antenatal care in Kigali, Rwanda.

Investigator: Esperance MUREBWAYIRE

Supervisors: Prof. Jaoko Walter

Dr H. Kariuki Njaanake

Prof. Kato Njunwa

My name is Esperance Murebwayire and I am a post-graduate student from the University of Nairobi, Kenya. I wish to invite you to take part in this research. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

Purpose of the study

In this study we are interested in knowing how many women attending the antenatal care clinics in the selected health centres/hospitals in Kigali may be having toxoplasmosis infections and if there are some things that put one at risk. These infections are spread through ingestion of raw or undercooked infected meat or water contaminated with feline feces, and can also be transmitted from a mother to her baby. This can occur during pregnancy, and it can lead to blindness, mental retardation, congenital deformities and intrauterine deaths.

You are therefore being requested to take part in this study because you are attending the antenatal care clinic. We have obtained written permission from Rwanda National Ethical

Committee and the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee to conduct this study.

Study procedures

If you agree to take part in this research study, the interviewer will fill a form that will capture your personal details like your age, marital status, and education level. You will be asked further questions to assess if you have had any risk-related events in the past. 5 ml of blood will be collected from you. This procedure may cause slight discomfort. The samples will be taken to the National Reference Laboratory for analysis. In case of a positive result, you will be contacted and will be advised on how to get treated for the infection.

Confidentiality

We will not record your name or any identification anywhere in the questionnaire or laboratory form so no one will be able to tell who you are. It will be very confidential. Your form will only show a code that is assigned each participant.

Benefits and Risks

By choosing to participate in this study, you will not have any direct benefits from it other than that of a free test to know your health status. However the information obtained from the study will be useful to the country in general by giving information on the disease status of the people and can be used in planning for screening and management of toxoplasmosis infection in pregnant women. Other than the discomfort and pain in obtaining the blood samples, there are no other foreseeable risks that will arise from participating in the study.

In the case that you are found infected by *T. gondii*, you will be contacted confidentially and told to report to the antenatal care clinic for a medical prescription of the appropriate medication

Costs and Compensation

By choosing to participate in the study, you will not incur any extra monetary cost. You will however take about thirty minutes longer than your usual clinic visits to go through the study procedures. You will not be paid for taking part in the study.

Voluntary participation

Taking part in this study is voluntary. You have the right to choose not to take part in this study. If you decline to participate you will not be denied any services from this Health centre/hospital.

Questions

If you have any questions about this study now or later, you may contact the Principal Investigator, Esperance Murebwayire the following phone number +250783195125/+254705653119**.** If you have any questions about your rights as a study participant, you can contact the Chairperson of Rwanda National Ethics Committee Dr J.Baptiste Mazarati on these contacts +250788309807 or The secretary of RNEC Dr. Laetitia Nyirazinyoye on +250738683209 rnec@moh.gov.rw or my supervisor Prof. Kato Njunwa Email: njunwa@khi.ac.rw, knjunwa@yahoo.co.uk Phone:+250788490522 Kenyatta National Hospital/University of Nairobi Ethics and Research Committee on telephone number (+254) 020 2726300 or uonknh_erc@uonbi.ac.ke .

APPENDIX 4. CERTIFICATE OF CONSENT

I…… (Participant's name) have had the information on this form read and explained to me. I was free to ask any questions and they have been answered. I am exercising my free power of choice, and hereby give my consent to be included as a participant in this study of "**Sero-prevalence and risk factors of *Toxoplasma gondii* infection among pregnant women attending antenatal care in Kigali, Rwanda".**

Participant (Name)…… (Signature) ……. Date: … /...../2014

Investigator (Name)……. (Signature) …….. Date: …/…/2014

APPENDIX 4: Amakuru Agenewe uwemeye kugira uruhare mu bushakashatsi n'inyandiko ibyemeza

Umubare w'ibanga: _____

Umutwe w'ubushakashatsi: "Uburwayi bwa *TOXOPLASMA GONDII* n'ibifasha mu kubwandura mu bagore batwite I Kigali, Rwanda"

Umushakashatsi: Esperance MUREBWAYIRE

Abayobozi: Prof. Jaoko Walter

Dr H. Kariuki Njaanake

Prof. Kato Njunwa

Nitwa Esperance Murebwayire ndi umunyeshuri muri Kaminuza ya Nairobi, Kenya, mu kiciro cya gatatu cya Kaminuza. Nifuza kubatumira kugira uruhare muri ubu bushakashatsi. Amakuru akubiye muri iyi nyandiko arabafasha kwemera cyangwa kureka kugira uruhare muri ubu bushakashatsi. Ntimugire impungenge mukubaza icyo ari cyo cyose kirebana n'ubu bushakashatsi.

Intego y'ubushakashatsi

Muri ubu bushakashatsi turashaka kumenya abagore batwite bajya kubigo nderabuzima ndetse n'ibitaro byatoranijwe mu mugi wa Kigali, gukurikirana ubuzima bw'umubyeyi, baba bafite uburwayi bwa toxoplasmosis ndetse n'uburyo baba bayandura. Ubu burwayi bwanduzwa no kurya inyama mbisi cyangwa zidatetse neza zifite agakoko gatera **toxoplasmosis,** ubu burwayi kandi umubyeyi ashobora kubwanduza umwana igihe amutwite. Ku mwana wanduye akiri mu

nda bishobora kumuviramo ubuhumyi, gukererwa mu mikurire mu bwenge, kuvukana ubumuga, gupfira mu nda.

Rero mwasabwaga kugira uruhare muri ubu bushakashatsi kuberako muza gukurikirana ubuzima bw'umubyeyi utwite mu bigo nderabuzima n'ibitaro byatoranijwe. Dufite urwandiko rwanditse rutwemerera gukora ubu bushakashatsi twahawe n'ikigo cy'igihugu gishizwe ubushakashatsi mu Rwanda" Rwanda National Ethical and Research Committee".

Uburyo ubushakashatsi buzakorwa

Niba mwemeye kugira uruhare muri ubu bushakashatsi, umushakashatsi azuzuza urupapuro ku makuru aberekeye ho nk'imyaka, irangamimirere n' amashuri mwize. Muraza kubazwa ibindi bibazo kugirango tumenye niba mwarigeze muhura n'ibintu byabaviramo kwandura mu bihe byashize.Turaza kubasaba gutanga mililitiro eshanu (5) z'amaraso. Mushobora kugira ububabare budakabije mugihe cyo gufata amaraso.Ibizamini bizoherezwa muri Laboratwari nkuru y'igihugu kugirango bisuzumwe. Mu gihe bizagaragara ko mufite uburwayi, muzabimenyeshwa munagirwe inama y'uburyo mwavurwa.

Ibanga

Ntabwo tuzakoresha amazina cyangwa undi mwirondoro wanyu haba ku rupapuro rw'ibibazo cyangwa urupapuro rwa laboratwari, nta muntu n'umwe uzameya uwo uri we. Bizakorwa mu ibanga rikomeye.Urupapuro rwanyu ruzagaragaza umubare wihariye uzahabwa buri muntu uzitabira ubu bushakashatsi.

Inyungu ndetse n'ingaruka

Mu gihe muhisemo kugira uruhare muri ubu bushakashatsi, ntayindi nyungu muri bubone uretse gukorerwa ibizamini k'ubuntu no kumenya uko muhagaze kuri ubu burwayi. Icyakora, amakuru

azava muri ubu bushakashatsi azakoreshwa n'igihugu muri rusange, hatangwa amakuru agaragaza uko ubu burwayi buhagaze mu baturage, aya makuru azafasha mu guteganya uburyo abagore batwite bazajya basuzumwa bakanakurikiranwa kubyerekeye ubu burwayi. Uretse ububabare budakabije muri bugire mugihe hafatwa ikizamini cy'amaraso ntazindi ngaruka cyangwa impungenge zihari nyuma yo kugira uruhare muri ubu bushakashatsi.

Ikiguzi n'igihembo

Mugihe muhisemo kugira uruhare muri ubu bushakashatsi, nta mafaranga muzahabwa. Icyakora mushobora kumara iminota mirongo itatu (30) irenga ku gihe mwari musanzwe mumara kukigo nderabuzima cgagwa ibitaro. Nta gihembo giteganijwe ku umuntu uzitabira ubu bushakashatsi. Biramutse bigaragaye ko mufite uburwayi bwa Toxoplasmosis/HIV muzahamagarwa ku bitaro cg ikigonderabuzima mugirwe inama y'uburyo mwavurwa.

Uburenganzira bwo kwitabira ubushakashatsi

Ni k'ubushake bw'umuntu guhitamo kugira urahare muri ubu bushakashatsi. Mufite uburenganzira bwo kwanga kwitabira ubu bushashatsi.Muzahabwa serivisi zose mwahabwaga ku kigo nderabuzima/ibitaro, no mu gihe mwaba mutitabiriye ubu bushakashatsi.

Ibibazo

Mu gihe mwaba mufite ibibazo kuri ubu bushakashasti nonaha cyangwa nyuma , mwabaza umushakashatsi Esperance Murebwayire kuri telephone igendanwa +250783195125/ +25470563119. Niba mufite ibibazo k'uburenganzira bwanyu mwabaza umuyobozi uhagarariye ubushakashatsi mu Rwanda" Rwanda National Ethics Committee"Dr Mazarati J.Baptiste kuri telephone (+250788309807) cyangwa umunyamabanga wa RNEC Dr.Leatitia NYIRAZINYOYE +250738683209 P.O.Box 84 Kigali, Email: rnec@moh.gov.rw. Prof Kato

Njunwa Email: njunwa@khi.ac.rw, knjunwa@yahoo.co.uk Phone:+250788490522 .Kenyatta National Hospital/University of Nairobi -Ethics and Research Committee kuri telephone (+254)0202726300 cyangwa mukabandikira kuri uonknh_erc@uonbi.ac.ke.

MURAKOZE

APPENDIX 5. ICYEMEZO CY'UWEMEYE KWITABIRA UBUSHAKASHATSI

Jyewe…… (amazina y'uwitabiriye ubushakashatsi) numvise amakuru akubiye muri iyi nyandiko nyasomewe kandi nyasobanuriwe. Nari mfite umudendezo wo kubaza ibibazo kandi byashubijwe. Mfite uburenganzira bwose bwo guhitamo, nkaba nemeye kwitabira ubu bushakashatsi "**Uburwayi bwa *TOXOPLASMA GONDII* n'ibifasha mu kubwandura mu bagore batwite I Kigali, Rwanda"**

Amazinay'uwitabiriye ubushakashatsi……..…….

Umukono……Itariki:……./……./2014

Umushakashatsi (Amazina)…… (Umukono)…….Italiki……./……../2014

APPENDIX 6. STANDARD OPERATING PROCEDURE FOR SAMPLE LABELING, COLLECTION, TRANSPORT AND STORAGE

Labeling the sample

- Participants' identification number (personal code).
- The above MUST match the same on the informed consent form.
- Date, time and initials of the phlebotomist must be on the label of EACH tube.

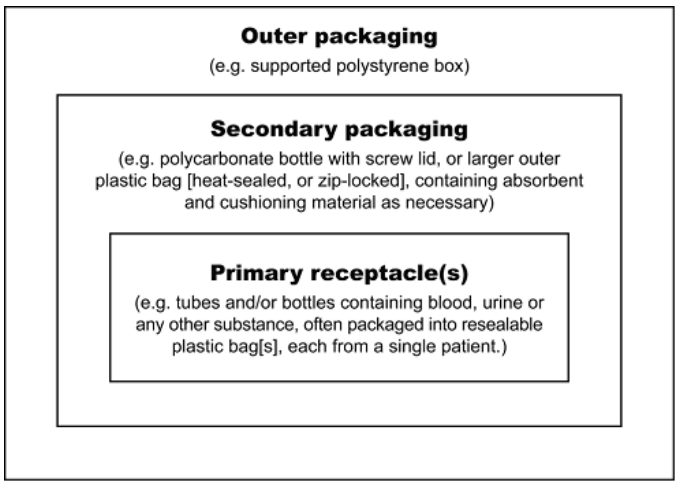
Performance of a venipuncture:

- Position the patient. The patient should sit in a chair. Hyperextend the patient's arm.
- Apply the tourniquet 7-10 centimeters above the selected puncture site. Do not place too tightly or leave on more than 2 minutes (and no more than a minute to avoid increasing risk for hemoconcentration). Wait 2 minutes before reapplying the tourniquet.
- The patient should make a fist without pumping the hand.
- Select the venipuncture site.
- Prepare the patient's arm using alcohol prep. Cleanse in a circular fashion, beginning at the site and working outward. Allow to air dry.
- Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. The needle should form a 15 to 30 degree angle with the surface of the arm. Swiftly insert the needle through the skin and into the lumen of the vein. Avoid trauma and excessive probing.
- Remove the tourniquet.
- Remove the needle from the patient's arm using a swift backward motion.

- Press down on the gauze once the needle is out of the arm, applying adequate pressure to avoid formation of a hematoma.
- Dispose of contaminated materials/supplies in designated containers.
- Mix and label all appropriate tubes.
- Deliver specimens promptly to the laboratory.

Packaging and transport

1. Triple Packaging



To fulfill the requirements of triple packaging, note that the common packaging arrangement shown in the above figure. The primary receptacles are placed inside an additional container (which could be as simple as a larger plastic bag with a zip-lock, or a heat sealed plastic bag) containing absorbent material sufficient to absorb any likely spill, before being placed in the outer packaging container.

- **Primary packaging**
  - Whole blood collected in EDTA tubes must be put in the first box

- Tubes should be maintained in the upright position.

- **Secondary packaging**

- The specimens collected in the EDTA tube should be put in a second box
- The tube containers are to be tightly capped and placed in a rack to maintain it in an upright position.
- The second box is to be put in a third box to fulfil triple packaging.
- Specimen containers and racks are placed in robust, leak-proof plastic or metal transport boxes with secure, tight fitting covers.

- **Thirdly packaging**

- The second box is packaged in the third box at study site and ready for transport to NRL.
- The transport box must be secured in the transport vehicle.

Specimen Storage

- Separate the plasma from the whole blood by centrifugation at 3,000 rpm for 5 minutes.
- Label separated plasma and keep it at -20°C until tested for (anti-*T.gondii* antibodies).
- Keep the plasma after being tested at -80^{o} C.

Table 8 : Seropositivity rates for toxoplasmosis in Europe, the Americas and Southeast Asia

Continents and countries	Year	Seropositivity (%)
Western Europe		
Austria	1998	43
Belgium	1997	50
France	2001	Up to 75
Germany	2004	26–54
Italy	2001	18–60
The Netherlands	2004	40.5
Spain	2004	28.6
Switzerland	1995	46
UK	1992	23–33
Scandinavia		
Denmark	1999	27.8
Finland	1995	20.3
Norway	1998	10.9
Sweden	2001	14.0–29.4
Central and Eastern Europe		
Croatia	2000	38.1
Poland	2001	46.4–58.5

Continents and countries	Year	Seropositivity (%)
Slovenia	2002	34
Yugoslavia	1998	57–93
North America		
USA	2004	16–40
Central America		
Costa Rica	1996	76
Cuba	1993	60
Mexico	2001	35
Panama	1988	90 (at 60 years of age)
South America		
Argentina	2001	72
Brazil	2001	59
West Indies	1991	29.7
Southeast Asia		
Indonesia	2000	58
Malaysia	2004	44.8
Thailand	1992, 1997, 2000, 2001	2.3–21.9

Table 9: Sero-prevalence rates for toxoplasmosis in Africa

Country	Year	Study population	Seroprevalence (%)	Reference
South Africa and Botswana	1978	See details in text (below)	20 11	Jacobs and Mason, 1978
Kenya	1991	HIV-positive and -negative adults	54	Brindle *et al.*, 1991
Sudan	1991	Apparently healthy individuals	41.7	Abdel-Hameed, 1991
Zambia	1991	HIV-positive and -negative adults	7	Zumla *et al.*, 1991
Cameroon	1992	Pregnant women	77.1	Ndumbe *et al.*, 1992
Senegal	1993	Women	40.3	Diallo *et al.*, 1996
Republic of Benin	1995	Pregnant HIV-negative women	53.6	Rodier *et al.*, 1995
Tanzania	1995	Pregnant women	35	Doehring *et al.*, 1995
Ethiopia	1998	HIV-positive and -negative adults	80	Woldemichael *et al.*, 1998
Burkina Faso	2004-2005	Pregnant women	25.3	Simpore *et al.*, 2006
Uganda	2006	HIV-positive adults	54	Lindström *et al.*, 2006

Figure 1: Toxoplasmosis in some African countries

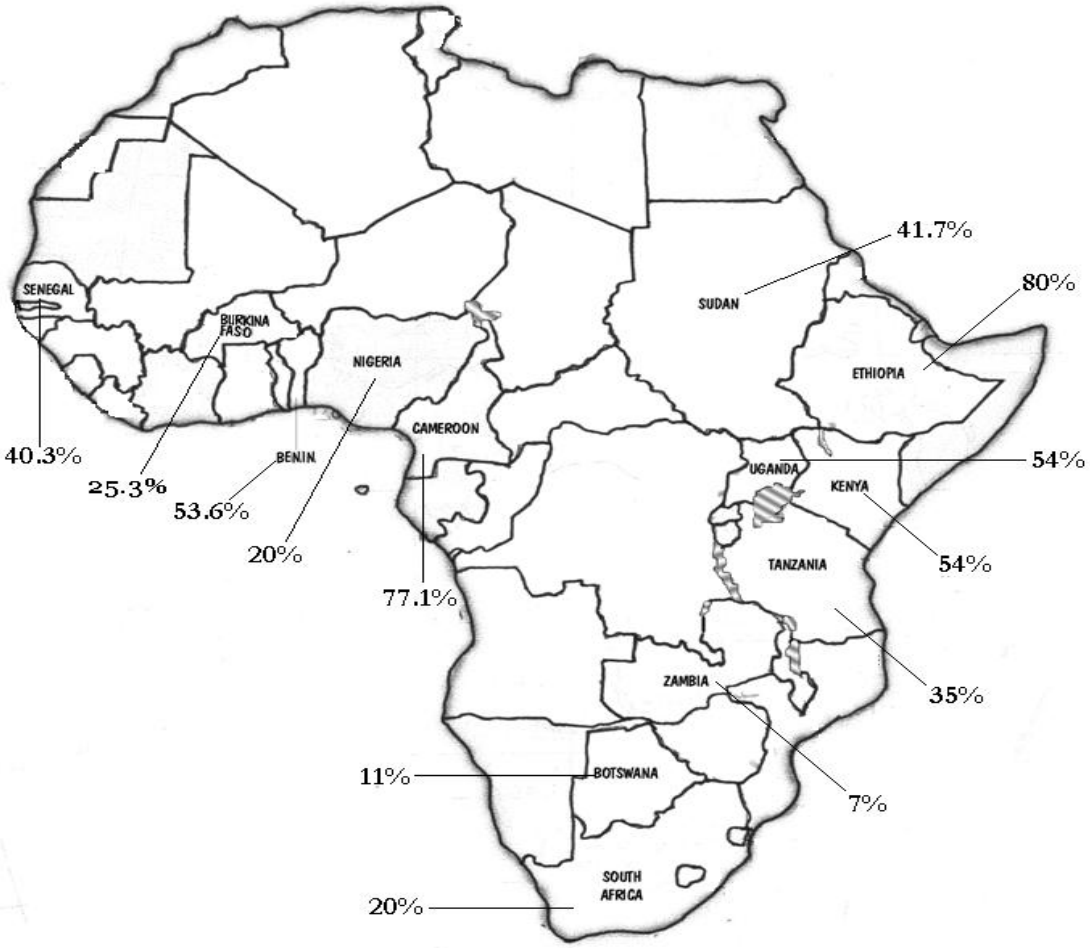
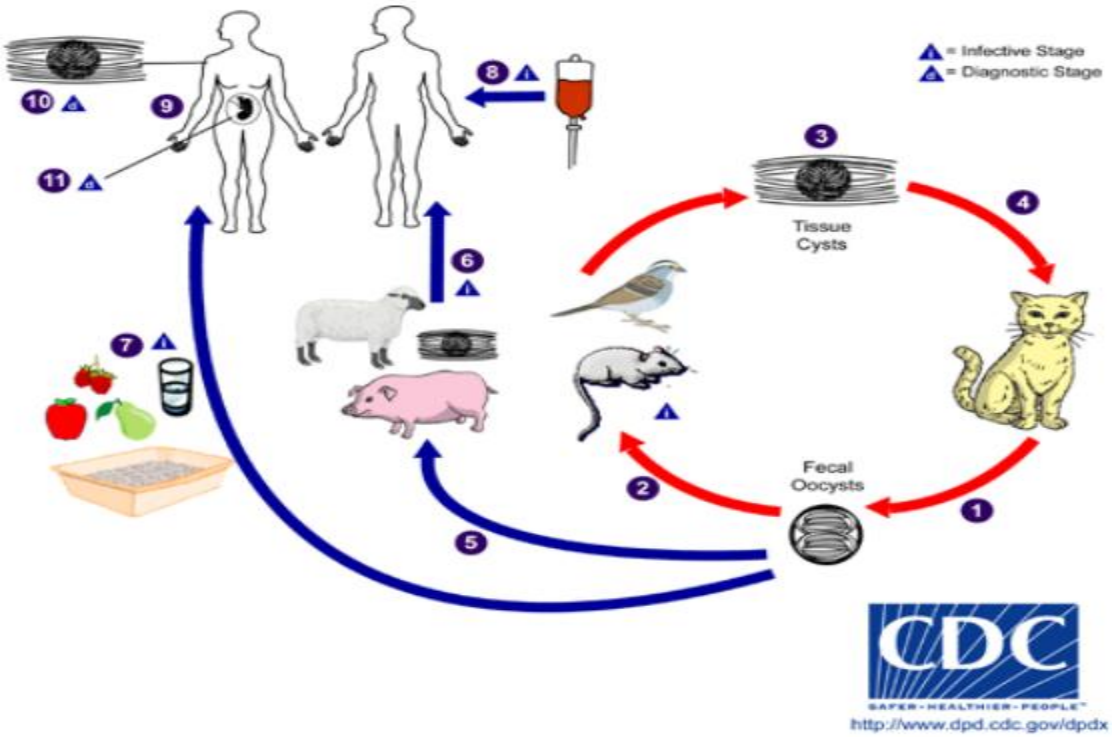


Figure 2 : Life cycle of *T. gondii*



APPENDIX 7: KNH-UoN-ERC approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 12 MAR 2014 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

Ref: KNH-ERC/A/53 Link:www.uonbi.ac.ke/activities/KNHUoN

12th March 2014

Murebwayire Esperance
Dept.of Medical Microbiology
School of Medicine
University of Nairobi

Dear Esperance

RESEARCH PROPOSAL: SERO-PREVALANCE AND RISK FACTORS OF *TOXOPLASMA GONDII* INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATALCARE IN KIGALI, RWANDA (P625/12/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 12th March 2014 to 11th March 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to Discover

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Chair, KNH/UoN-ERC
The Deputy Director CS, KNH
The Principal, College of Health Sciences, UoN
The Dean, School of Medicine, UoN
The Chairman, Dept. of Medical Microbiology, UoN
The Assistant Director, Health Information, KNH
Supervisors: Prof. Walter Jaoko, Dr. H. Kariuki Njaanake, Prof. Kato Njunwa

APPENDIX 8: RWANDA ERC APPROVAL

REPUBLIC OF RWANDA/REPUBLIQUE DU RWANDA

NATIONAL ETHICS COMMITTEE / COMITE NATIONAL D'ETHIQUE

Telephone: (250) 2 55 10 78 84
E-mail: info@rnecrwanda.org

Web site: www.rnecrwanda.org

Ministry of Health

P.O. Box. 84

Kigali, Rwanda.

FWA Assurance No. 00001973
IRB 00001497 of IORG0001100

April 04, 2014
No. 085/RNEC/2014

MUREBWAYIRE Esperance
Principal Investigator
(Student)

Your Project title: **"SERO-PREVALENCE AND RISK FACTORS OF TOXOPLASMA GONDII INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN KIGALI, RWANDA"** has been evaluated by the Rwanda National Ethics committee.

Name	Institute	Involved in the decision		
		Yes	No (Reason)	
			Absent	Withdrawn from the proceeding
Dr.Jean-Baptiste MAZARATI	Biomedical Services (BIOS)	X		
Prof. Eugène RUTEMBESA	National University of Rwanda	X		
Dr.Laetitia NYIRAZINYOYE	National University of Rwanda(school of public Health)	X		
Prof.Alexandre LYAMBABAJE	National University of Rwanda	X		
Ms.Françoise UWINGABIYE	Lawyer at Musanze	X		
Dr. Egide KAYITARE	National University of Rwanda	X		
Sr.Domitilla MUKANTABANA	Kabgayi Nursing and Midwife school	X		

Mr. David K. TUMUSIIME	Kigali Health institute		X	
Dr. Lisine TUYISENGE	Kigali Teaching Hospital	X		
Dr. Claude MUVUNYI	Biomedical Services (BIOS)	X		

After reviewing your protocol by expedited review procedures during the RNEC meeting of 22 March 2014 where quorum was met, and revisions made on the advice of the RNEC submitted on 01 April 2014, **Approval has been granted to your study.**

The committee also approved the requested waiver of consent from for participants under 21 year to participate in the study.

Please note that approval of the protocol and consent form is valid for **12 months**.
You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrollment of participants
3. All consent forms signed by subjects should be retained on file. The RNEC may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the RNEC in a timely fashion and before expiry of this approval.
5. Failure to submit a continuing review application will result in termination of the study.
6. Notify the Rwanda National Ethics committee once the study is finished.

Sincerely,

RWANDA NATIONAL ETHICS COMMITTEE
DATE 04/04/2014
COMITE NATIONAL D'ETHIQUE

Dr. Jean- Baptiste MAZARATI
Chairperson, Rwanda National Ethics Committee.

Date of Approval: April 04, 2014
Expiration date: April 03, 2015

C.C.
- Hon. Minister of Health.
- The Permanent Secretary, Ministry of Health.

APPENDIX 9: PERMISSION TO CARRY OUT RESEARCH IN RWANDA

REPUBLIC OF RWANDA

Kigali, 28/04/2014
Ref: 985/12.00/2014

MINISTRY OF EDUCATION
P.O BOX 622 KIGALI

Re: Permission to carry out research in Rwanda - No: MINEDUC/S&T/229/2014

Permission is hereby granted to **Ms. Esperance MUREBWAYIRE**, MSc student in Medical Microbiology at University of Nairobi, Kenya, to carry out research on: **"Sero-Prevalence and risk factors of Toxoplasma gondii infection among pregnant women attending antenatal care in Kigali, Rwanda".**

The research will be conducted in Muhima, Cor-num, Biryogo and Nyarugunga health centres; Clinic Belle-Vie and la Coix du sud Hospital, all located in Kigali City. She will need access to pregnant women's file (antenatal records). She will interview the Minister of Health, Head of RBC/IHDPC/Malaria and Neglected diseases Division and she will also need to interview the pregnant women on antenatal care.

The period of research is **from 15th April, 2014 to 15th April, 2015**. This period may be renewed if necessary, in which case a new permission will be sought by the researcher.

Please provide **Ms. Esperance MUREBWAYIRE** any support he may require in the course of conducting this research.

Yours sincerely,

MINISTRY OF EDUCATION
GASINGIRWA Marie Christine PhD
Director General Science
Technology & Research

Dr.Marie Christine GASINGIRWA
Director General,
Science, Technology and Research
Ministry of Education