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……………………………………..
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DEDICATION

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

DECLARATION

ACKNOWLEDGEMENT

DEDICATION

LIST OF ABBREVIATIONS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

CHAPTER ONE: INTRODUCTION

  1.1. RESEARCH QUESTION:

  1.2. OBJECTIVES

     1.2.1. Broad objective

     1.2.2. Specific objectives

  1.3. STUDY JUSTIFICATION

CHAPTER TWO: LITERATURE REVIEW

  2.1. EPIDEMIOLOGY

  2.2. BIOLOGY OF *TOXOPLASMA GONDII*

     2.2.1. LIFE CYCLE

  2.3. TRANSMISSION AND RISK FACTORS

  2.4. TOXOPLASMOSES

     2.4.1. Clinical manifestations

     2.4.2. Congenital toxoplasmosis

     2.4.3. Toxoplasmosis in immunocompromised host

     2.4.4. Ocular toxoplasmosis

  2.5. DIAGNOSIS

  2.6. TREATMENT
CHAPTER THREE: METHODOLOGY .................................................................21

3.1 Study area .........................................................................................21

3.2 Study design .....................................................................................21

3.3 Study population ..............................................................................22

3.4 Inclusion criteria ................................................................................22

3.5 Exclusion criteria ...............................................................................22

3.6 Sample size and sampling method .....................................................22

3.7 Data collection methods ....................................................................24

3.8 Sample collection and sample processing ..........................................24

3.10 Data management and analysis .........................................................28

3.11 Ethical considerations .......................................................................29

3.12 Expected outcomes/Recommendations ............................................30

CHAPTER FOUR: RESULTS .........................................................................31

CHAPTER FIVE: DISCUSSION ......................................................................38

Conclusion .............................................................................................42

Recommendation ....................................................................................43

REFERENCES ............................................................................................44

APPENDICES ..............................................................................................49

APPENDIX 1. STUDY QUESTIONNAIRE ..................................................49

APPENDIX 2. LABORATORY REPORT FORM .............................................52

APPENDIX 3. INFORMATION TO PARTICIPANTS AND CONSENT FORM ............53

APPENDIX 4. CERTIFICATE OF CONSENT ................................................56

APPENDIX 5. ICYEMEZO CY’UWEMEYE KWITABIRA UBUSHAKASHATSI ..........60

APPENDIX 6. STANDARD OPERATING PROCEDURE FOR SAMPLE LABELING,
COLLECTION, TRANSPORT AND STORAGE ..................................................61
APPENDIX 7: KNH-UoN-ERC approval..........................................................................................69

APPENDIX 8: RWANDA ERC APPROVAL..................................................................................70

APPENDIX 9: PERMISSION TO CARRY OUT RESEARCH IN RWANDA.................................72
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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</thead>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
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<tr>
<td>ANC</td>
<td>Antenatal Care</td>
</tr>
<tr>
<td>CD4+</td>
<td>Cluster of Differentiation 4 positive</td>
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<td>CFT</td>
<td>Complement fixation test</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DNA</td>
<td>Deoxynucleic Acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HRP</td>
<td>Horseradish Peroxidase</td>
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<td>IFA</td>
<td>Indirect fluorescent antibody assay</td>
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<td>IgA</td>
<td>Immunoglobulin A</td>
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<td>ml</td>
<td>Milliliter</td>
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<tr>
<td>NRL</td>
<td>National Reference Laboratory</td>
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<tr>
<td>OMS</td>
<td>Organisation Mondiale de la Santé</td>
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<tr>
<td>PMTCT</td>
<td>prevention of mother to child transmission</td>
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<tr>
<td>PV</td>
<td>Parasitophorous vacuole</td>
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<td>--------------</td>
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<td>rpm</td>
<td>rotation per minute</td>
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<tr>
<td>RT</td>
<td>PCR</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for Social Scientists</td>
</tr>
<tr>
<td>TE</td>
<td>Toxoplastic encephalitis</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
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<tr>
<td>μL</td>
<td>Microliter</td>
</tr>
</tbody>
</table>


LIST OF TABLES

Table 1: Samples distribution according to the study sites .......................................................... 31
Table 2: Demographic characteristics of the study population ...................................................... 32
Table 3: Facility based *T. gondii* sero-prevalence ...................................................................... 33
Table 4: Distribution of *T. gondii* sero-prevalence in relation to demographic characteristics among pregnant women .................................................................................................................. 34
Table 5: Factors associated with *T. gondii* infections among pregnant women ......................... 36
Table 6. Seropositivity of Anti-Toxoplasma IgG in relation to type of drinking water and habit of eating of undercooked meat .................................................................................................................. 37
Table 7. Seropositivity of Anti-Toxoplasma IgM in relation to type of drinking water and habit of eating of undercooked meat .................................................................................................................. 37
Table 8: Seropositivity rates for toxoplasmosis in Europe, the Americas and Southeast Asia .......... 64
Table 9: Sero-prevalence rates for toxoplasmosis in Africa ............................................................ 66
LIST OF FIGURES

Figure 1: Toxoplasmosis in some African countries ..............................................................67
Figure 2: Life cycle of T. gondii ..................................................................................................68
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 between 10 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, McRindle, & 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, Ouohon, 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 toxoplasmosis 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 \textit{T. gondii} (Gascon et al., 1989).

However, no more studies have been conducted on the prevalence of \textit{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 \textbf{TOXOPLASMA GONDII}

\textit{T. gondii} is a non flagellated apicomplexan parasite. It belongs to the family of the Sarcocysdae in the class of the coccidian, and is the only species in the \textit{Toxoplasma} genus.

\textit{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:** \textit{Toxoplasma}
- **Species:** \textit{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 al (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, stillbirth, 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 sequelae 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 toxoplasmosis 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 °C or by freezing to -13 °C. 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

3.1 Study 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 km² 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)

\[ n = \frac{1.96^2 \times 0.5 \times (1 - 0.5)}{0.05^2} = 384 \]

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°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:

   \[ \text{Cut-off} = \text{LPCx} \]
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
**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:

\[ \text{Cut-off} = \text{LPCx} \]

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 \( \geq 0.9 < 1.0 \)

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 $\chi^2$ 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

<table>
<thead>
<tr>
<th>Health center</th>
<th>Average number per month</th>
<th>%</th>
<th>Expected samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biryogo HC</td>
<td>76</td>
<td>26</td>
<td>101</td>
</tr>
<tr>
<td>Cor-Unum HC</td>
<td>76</td>
<td>26</td>
<td>101</td>
</tr>
<tr>
<td>Muhima HC</td>
<td>68</td>
<td>24</td>
<td>91</td>
</tr>
<tr>
<td>Nyarugunga HC</td>
<td>68</td>
<td>24</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>288</td>
<td>100</td>
<td>384</td>
</tr>
</tbody>
</table>

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

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

*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

<table>
<thead>
<tr>
<th>Health Facility</th>
<th>Total</th>
<th>n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biryogo HC</td>
<td>101</td>
<td>16(15.8)</td>
<td>0.046</td>
</tr>
<tr>
<td>Cor-Unum HC</td>
<td>101</td>
<td>10(9.9)</td>
<td></td>
</tr>
<tr>
<td>Muhima HC</td>
<td>91</td>
<td>16(17.6)</td>
<td></td>
</tr>
<tr>
<td>Nyarugunga HC</td>
<td>91</td>
<td>5(5.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>384</strong></td>
<td><strong>47(12.2)</strong></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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 was 7.7% 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

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

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.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IgG sero-prevalence</th>
<th>Univariate</th>
<th>Bivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive n(%)</td>
<td>Negative n(%)</td>
<td>OR[95% CI]</td>
</tr>
<tr>
<td>Water Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>24(17.9)</td>
<td>110(82.1)</td>
<td>3.97(1.95, 8.11)</td>
</tr>
<tr>
<td>Treated</td>
<td>13(5.2)</td>
<td>237(94.8)</td>
<td></td>
</tr>
<tr>
<td>Under cooked meat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20(17.9)</td>
<td>92(82.1)</td>
<td>0.31(0.15, 0.61)</td>
</tr>
<tr>
<td>No</td>
<td>17(6.3)</td>
<td>255(93.8)</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IgM sero-prevalence</th>
<th>Univariate</th>
<th>Bivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive n(%)</td>
<td>Negative n(%)</td>
<td>OR[95% CI]</td>
</tr>
<tr>
<td>Water Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>30(22.4)</td>
<td>104(77.6)</td>
<td>3.96(2.09, &lt;0.001)</td>
</tr>
<tr>
<td>Treated</td>
<td>17(6.8)</td>
<td>233(93.2)</td>
<td>7.49</td>
</tr>
<tr>
<td>Under cooked meat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>25(22.3)</td>
<td>87(77.7)</td>
<td>0.31(0.16, &lt;0.001)</td>
</tr>
<tr>
<td>No</td>
<td>22(8.1)</td>
<td>250(91.9)</td>
<td>0.57</td>
</tr>
</tbody>
</table>
CHAPTER FIVE: DISCUSSION

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

In the present study, seropositivity of anti- \textit{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 \textit{et al.}, 2014; Rosso \textit{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 \textit{et al} may also be due to gender differences in the two study populations. Gascon \textit{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 \textit{T. gondii} have previously been described. A study by Jones \textit{et al} (J. L. Jones \textit{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 cat ownership.
infection and keeping of cats. Other researchers have also not demonstrated the association between \textit{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 \textit{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 \textit{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 \textit{T. gondii} infection which is in agreement with studies conducted elsewhere (Alvarado-Esquivel, Estrada-Martinez, \& Liesenfeld, 2011; Koskineni, L rappalainen, \& Hedman, 1989; Lopez-Castillo, Diaz-Ramirez, \& Gomez-Marin, 2005; Sroka et al., 2010). It is possible that one of the sources of \textit{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-\textit{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-\textit{T. gondii} antibody seropositivity and behavior of eating in restaurants (Montoya \& Liesenfeld, 2004).

The limited knowledge of the women on \textit{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 larger 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


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


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 × ahabugenewo)

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 (akare re)______________Sector (umuren ge) ______________

4. Marital Status: (irang am iner re)

   [1] Married 1 Ndubatse

5. Occupation (icyo muko ra)

   Student 1 Umunyeshuri
   Housewife 2 Umugore wo mu rugo
   Unemployed 3 Ntakazi mfite

49
<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Self employed</strong></td>
<td>4</td>
</tr>
<tr>
<td><strong>Employed</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Level of education</strong></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
</tr>
<tr>
<td>Primary school</td>
<td>2</td>
</tr>
<tr>
<td>Secondary school and above</td>
<td>3</td>
</tr>
<tr>
<td><strong>Gravidity</strong></td>
<td></td>
</tr>
<tr>
<td>Primigravid</td>
<td>1</td>
</tr>
<tr>
<td>Multigravid</td>
<td>2</td>
</tr>
<tr>
<td><strong>Trimester of pregnancy</strong></td>
<td></td>
</tr>
<tr>
<td>1st trimester</td>
<td>1</td>
</tr>
<tr>
<td>2nd trimester</td>
<td>2</td>
</tr>
<tr>
<td>3rd trimester</td>
<td>3</td>
</tr>
<tr>
<td><strong>Section B: Risk factors</strong></td>
<td></td>
</tr>
<tr>
<td>Which kind of water do you use?</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>1</td>
</tr>
<tr>
<td>Treated</td>
<td>2</td>
</tr>
<tr>
<td><strong>Do you have cats at home?</strong></td>
<td></td>
</tr>
</tbody>
</table>
11. Do you eat raw or undercooked meat? *Mujya murya inyama mbisi cg zidatate 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? *Mwigze 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? *Mwigze 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 rnce@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 Agene we uwemeye kugira uruhare mu bushakashatsi n’inyandiko ibye meza

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


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 nbisi 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’IBUTIYO 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


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 urete 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 bakanakurikirana kubyrekeye 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’igihe mbo


Uburenganzira bwo kwitabira ubushakashatsi

Ni k’ubushake bw’umuntu guhitamo kugira urahare muri ubu bushakashatsi. Mufite uburenganzira bwo kwanga kwitabira ubu bushashantsi. 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 igandanwa +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: rneg@moh.gov.rw. Prof Kato
MURAKOZE

APPENDIX 5. ICYEMEZO CY’UWEMEYE KWITABIRA UBUSHAKASHATS

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)……………….Itariki……/……/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°C.
Table 8: Seropositivity rates for toxoplasmosis in Europe, the Americas and Southeast Asia

<table>
<thead>
<tr>
<th>Continents and countries</th>
<th>Year</th>
<th>Seropositivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Western Europe</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>1998</td>
<td>43</td>
</tr>
<tr>
<td>Belgium</td>
<td>1997</td>
<td>50</td>
</tr>
<tr>
<td>France</td>
<td>2001</td>
<td>Up to 75</td>
</tr>
<tr>
<td>Germany</td>
<td>2004</td>
<td>26–54</td>
</tr>
<tr>
<td>Italy</td>
<td>2001</td>
<td>18–60</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>2004</td>
<td>40.5</td>
</tr>
<tr>
<td>Spain</td>
<td>2004</td>
<td>28.6</td>
</tr>
<tr>
<td>Switzerland</td>
<td>1995</td>
<td>46</td>
</tr>
<tr>
<td>UK</td>
<td>1992</td>
<td>23–33</td>
</tr>
<tr>
<td><strong>Scandinavia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denmark</td>
<td>1999</td>
<td>27.8</td>
</tr>
<tr>
<td>Finland</td>
<td>1995</td>
<td>20.3</td>
</tr>
<tr>
<td>Norway</td>
<td>1998</td>
<td>10.9</td>
</tr>
<tr>
<td>Sweden</td>
<td>2001</td>
<td>14.0–29.4</td>
</tr>
<tr>
<td><strong>Central and Eastern Europe</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Croatia</td>
<td>2000</td>
<td>38.1</td>
</tr>
<tr>
<td>Poland</td>
<td>2001</td>
<td>46.4–58.5</td>
</tr>
<tr>
<td>Continents and countries</td>
<td>Year</td>
<td>Seropositivity (%)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Slovenia</td>
<td>2002</td>
<td>34</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>1998</td>
<td>57–93</td>
</tr>
<tr>
<td><strong>North America</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>2004</td>
<td>16–40</td>
</tr>
<tr>
<td><strong>Central America</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costa Rica</td>
<td>1996</td>
<td>76</td>
</tr>
<tr>
<td>Cuba</td>
<td>1993</td>
<td>60</td>
</tr>
<tr>
<td>Mexico</td>
<td>2001</td>
<td>35</td>
</tr>
<tr>
<td>Panama</td>
<td>1988</td>
<td>90 (at 60 years of age)</td>
</tr>
<tr>
<td><strong>South America</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>2001</td>
<td>72</td>
</tr>
<tr>
<td>Brazil</td>
<td>2001</td>
<td>59</td>
</tr>
<tr>
<td>West Indies</td>
<td>1991</td>
<td>29.7</td>
</tr>
<tr>
<td><strong>Southeast Asia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indonesia</td>
<td>2000</td>
<td>58</td>
</tr>
<tr>
<td>Malaysia</td>
<td>2004</td>
<td>44.8</td>
</tr>
<tr>
<td>Country</td>
<td>Year</td>
<td>Study population</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>South Africa and Botswana</td>
<td>1978</td>
<td>See details in text (below)</td>
</tr>
<tr>
<td>Kenya</td>
<td>1991</td>
<td>HIV-positive and -negative adults</td>
</tr>
<tr>
<td>Sudan</td>
<td>1991</td>
<td>Apparently healthy individuals</td>
</tr>
<tr>
<td>Zambia</td>
<td>1991</td>
<td>HIV-positive and -negative adults</td>
</tr>
<tr>
<td>Cameroon</td>
<td>1992</td>
<td>Pregnant women</td>
</tr>
<tr>
<td>Senegal</td>
<td>1993</td>
<td>Women</td>
</tr>
<tr>
<td>Republic of Benin</td>
<td>1995</td>
<td>Pregnant HIV-negative women</td>
</tr>
<tr>
<td>Tanzania</td>
<td>1995</td>
<td>Pregnant women</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>1998</td>
<td>HIV-positive and -negative adults</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>2004-2005</td>
<td>Pregnant women</td>
</tr>
<tr>
<td>Uganda</td>
<td>2006</td>
<td>HIV-positive adults</td>
</tr>
</tbody>
</table>
Figure 1: Toxoplasmosis in some African countries
Figure 2: Life cycle of *T. gondii*
APPENDIX 7: KNH-UoN-ERC approval

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

Dear Esperance

RESEARCH PROPOSAL: SERO-PREVALENCE AND RISK FACTORS OF TOXOPLASMA GONDII INFECTION AMONG PREGNANT WOMEN ATTENDING ANTENATAL CARE IN KIGALI, RWANDA (P629/12/2013)

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.
D.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 Njumwa
# 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: “SEERO-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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Institute</th>
<th>Involved in the decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Jean-Baptiste MAZARATI</td>
<td>Biomedical Services (BIOS)</td>
<td>X</td>
</tr>
<tr>
<td>Prof. Eugène RUTEMBESA</td>
<td>National University of Rwanda</td>
<td>X</td>
</tr>
<tr>
<td>Dr. Laetitia NYIRAZINYOYE</td>
<td>National University of Rwanda (school of public Health)</td>
<td>X</td>
</tr>
<tr>
<td>Prof. Alexandre LYAMBABAJE</td>
<td>National University of Rwanda</td>
<td>X</td>
</tr>
<tr>
<td>Ms. Françoise UWINGABIYE</td>
<td>Lawyer at Musanze</td>
<td>X</td>
</tr>
<tr>
<td>Dr. Egide KAYITARE</td>
<td>National University of Rwanda</td>
<td>X</td>
</tr>
<tr>
<td>Sr. Domitilla MUKANTABANA</td>
<td>Kabgayi Nursing and Midwife school</td>
<td>X</td>
</tr>
</tbody>
</table>
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,

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

MINISTRY OF EDUCATION
P.O BOX 622 KIGALI

Ref: G. T. 270/12/2014/2014

Re: Permission to carry out research in Rwanda - No: MINEUC/SX/1/229/2014

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 she may require in the course of conducting this research.

Yours sincerely,

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