

**Seroprevalence and Determinants of Toxoplasmosis in
Pregnant Women attending Antenatal Clinic at the
University Teaching Hospital, Lusaka, Zambia**

By

Christiana Frimpong

*A dissertation submitted in partial fulfillment of the requirements for the degree of Master of
Public Health (Epidemiology and Biostatistics)*

The University of Zambia

School of Public Health

2016

All rights reserved.
No part of this dissertation may be reproduced or transmitted in any manner without
permission in writing from the researcher or the University of Zambia

© 2016

DECLARATION

I, Christiana Frimpong, do hereby declare that this dissertation is a representation of my own work and that it has never been submitted for another degree in this or any other university or institution of higher learning.

Signed: _____ **Date:** _____

Candidate

Signed: _____ **Date:** _____

Supervisor

CERTIFICATE OF COMPLETION

I, **Christiana Frimpong**, hereby certify that this dissertation is the product of my own work. In submitting this dissertation in partial fulfillment of the requirement of the Master of Public Health degree programme, I further attest that it has not been submitted to another University in part or whole for the award of any programme.

Signature: _____

Date: _____

Having supervised and read this dissertation, I am satisfied that this is the original work of the author under whose name it is being presented. I confirm that the work has been completed satisfactorily and has been presented to the Board of Examiners.

Name of supervisor: Professor Charles Michelo

Signature: _____

Date: _____

Name of supervisor: Dr. Mpundu Makasa

Signature: _____

Date: _____

Name of supervisor: Ms. Lungowe Sitale

Signature: _____

Date: _____

CERTIFICATE OF APPROVAL

This dissertation by Christiana Frimpong has been approved as a partial fulfillment of the requirements for the award of the Master of Public Health (MPH) degree by the University of Zambia.

Examiner I

Signature: _____

Date: _____

Examiner II

Signature: _____

Date: _____

Examiner III

Signature: _____

Date: _____

Head, School of Public Health

Signature _____ Date: _____

Signed: _____ Date: _____

CHAPTER ONE: SUMMARY

Background: Toxoplasmosis is a neglected disease and in Africa its seroprevalence ranges from 18.7% - 92.5%. About 3% -10% HIV/AIDS - Toxoplasmosis co-infected patients die from the later. This study aimed to determine the seroprevalence and determinants of the disease among pregnant women attending antenatal clinic at the University Teaching Hospital (UTH).

Method: A cross-sectional study was employed where 411 pregnant women attending antenatal clinic at UTH were interviewed using closed ended questionnaires and their blood tested for *Toxoplasma gondii* IgG and IgM antibodies using the OnSite Toxo IgG/IgM Combo Rapid test cassettes by CTK Biotech, Inc, USA. Chi-square and Fisher's exact tests were used to test for associations whereas GLM for binary outcome, reporting odds ratios was used to determine significance.

Result: The overall seroprevalence of the infection (IgG) was 5.87%. There was no seropositive IgM result. Contact with cats showed 7.81 times the likelihood of contracting the infection in the pregnant women and being a farmer/being involved in construction work showed 15.5 times likelihood of contracting the infection. A graph of *Toxoplasma* infection plotted against socioeconomic status showed an inverse relationship, signifying association. However, though there were indications of association between contact with cats, employment type as well as socioeconomic status, there was not enough evidence to suggest these factors as significant determining factors of *Toxoplasma gondii* infection in our study population.

Conclusion: There is a low prevalence of *Toxoplasma gondii* infection among pregnant women in Lusaka, Zambia. Screening for the infection among pregnant women can be done once or twice during pregnancy to help protect both mother and child from the disease. Health promotion among women of child bearing age on the subject is of immense importance in order to help curb the situation. Further studies especially that of case-control and cohort studies should be carried out in the country in order to better ascertain the extent of the condition nationwide

ACKNOWLEDGEMENTS

My gratitude goes to the Education, Audio-visual and Culture Executive Agency Project of the European Commission that made it possible to conduct this study through the Intra-ACP (Africa, Caribbean & Pacific) Scholarship scheme. I am also grateful to my supervisors, Prof. Charles Michelo, Dr. Mpundu Makasa and Ms Lungowe Sitale, for their immense contributions and coaching throughout this study.

Also to Mr. John Chisoso, for helping in the conception of the study and supervising the laboratory work.

To the entire faculty members of the School of Public Health, to all friends and course mates who contributed to the success of this work, I say thank you!

To God be the glory!

TABLE OF CONTENTS

CHAPTER ONE: SUMMARY	6
CHAPTER TWO: BACKGROUND	10
2.1 Introduction.....	10
2.2 Brief History	10
2.3 Morphology, Life Cycle and Transmission	10
2.4 Epidemiology and Distribution of the Infection in Animals and Humans.....	12
2.5 Transmission from Mother to Child.....	13
2.6 Clinical Manifestations of <i>Toxoplasma gondii</i> Infection.....	13
2.7 Toxoplasmosis in HIV/AIDS patients	13
2.8 Determining factors associated with Toxoplasmosis in pregnant women	14
2.9 Screening, Diagnosis and Management of the disease	15
CHAPTER THREE: STUDY FOCUS	16
3.1 Rationale	16
3.2 Research Question	16
3.3 Main Objective.....	16
3.3.1 Specific Objectives	17
CHAPTER FOUR: METHODS	18
4.1 Study Design.....	18
4.2 Study Setting.....	18
4.3 Study Population.....	18
4.4 Sampling and Sample Size.....	19
4.5 Data collection	19
4.6 Data Analysis.....	20
4.7 Ethical Consideration.....	21
CHAPTER FIVE: RESULTS.....	22

CHAPTER SIX: DISCUSSION OF STUDY FINDINGS	29
6.1 Discussion	29
6.2 Conclusion	34
6.3 Recommendations	34
REFERENCES	35
Appendix 1: Budget	38
Appendix 2: Timeline	39
Appendix 3: Questionnaire	40
Appendix 4: Information Sheet and Consent form	45
Appendix 5: Consent form.....	48
Figure 1: Life Cycle of <i>Toxoplasma gondii</i>	11
Figure 2: Conceptual Framework (Self-Developed with Information Discovered from Literature)	14
Figure 3: Graph Showing an Inverse Relationship (Indicating Association) between Socio-Economic Status (SES) of Pregnant Women Attending Antenatal Clinic at UTH and their <i>Toxoplasma Gondii</i> Infection Status (toxoooutcome).....	28
Table 1: Characteristics of Pregnant Women who participated in the <i>Toxoplasma gondii</i> study at UTH	Error! Bookmark not defined.
Table 2: Prevalence and Factors Associated with <i>Toxoplasma gondii</i> Infection	25
Table 3: Predictors of <i>Toxoplasma gondii</i> Infection	27

CHAPTER TWO: BACKGROUND

2.1 Introduction

Toxoplasmosis is a neglected zoonotic disease caused by a blood protozoan parasite called *Toxoplasma gondii* (Tenter et al., 2000, Hotez and Kamath, 2009, Kijlstra and Jongert, 2009, Mazigo et al., 2013). The organism is found worldwide and it infects virtually all warm-blooded animals including human beings (Dubey, 2008b, Elmore et al., 2010). It is counted as the second most important pathogen after non-typhoid Salmonella, causing 28% and 24% food related deaths respectively (Scallan et al., 2011).

Once human beings contract the infection, they remain asymptotically infected for life (Dubey, 2008a) hence, the fierce cycle of transmitting the disease transplacentally to their unborn babies (Lucas and Gilles, 2005).

A steady IgG titre of *Toxoplasma gondii* antibodies indicates chronic or past infection whereas increased levels of IgM antibodies is said to indicate acute infections. Nonetheless, IgM titres can stay high for more than a year and four times rise in the titres of IgG antibodies in two different blood samples tested on the same day in the same laboratory also indicates acute infection (Van kessell and Eschenbach, 2014).

2.2 Brief History

Two scientists; Nicolle and Manceaux, first discovered the organism in 1908 in a hamster-like rodent (Dubey, 2008b). The medical importance of this organism however, remained unknown until it was isolated from the tissue of a one month old dead baby in 1939 (Dubey, 2008b). In 1948, *T. gondii* specific antibody test called the Sabin–Feldman dye test was discovered, and it gave much insight on the organism. The veterinary importance of the organism also came into light in 1957, when the organism caused abortion storms in sheep and was isolated in the placentas and fetuses in numerous mysterious abortions in ewes (Dubey, 2008b).

2.3 Morphology, Life Cycle and Transmission

The organism belongs to the genus *Toxoplasma* and the species *gondii*, thus the binomial name *Toxoplasma gondii* (Dubey, 2008b). It has three clonal lineages; type I, II and III which have

different virulence and all three types have been identified in chickens in Africa (Lindström et al., 2006).

The life cycle of *T. gondii* constitutes the sexual reproduction component which occurs in both wild and domestic cats (definitive hosts) and the asexual component which occurs basically in all warm-blooded animals including human beings (intermediate hosts) (Weiss and Kim, 2011, Nissapatorn, 2009). The organism has four parasitic stages namely the tachyzoite stage which is a multiplicative stage in its intermediate host (Weiss and Kim, 2011, Hogan et al., 1960); the merozoite stage, which is a multiplicative stage in the cat prior to sexual reproduction, the bradyzoite stage; a gradually dividing stage to form tissue cysts (Weiss and Kim, 2011) and then the oocyst protected sporozoite stage which is found in the environment (Hogan et al., 1960).

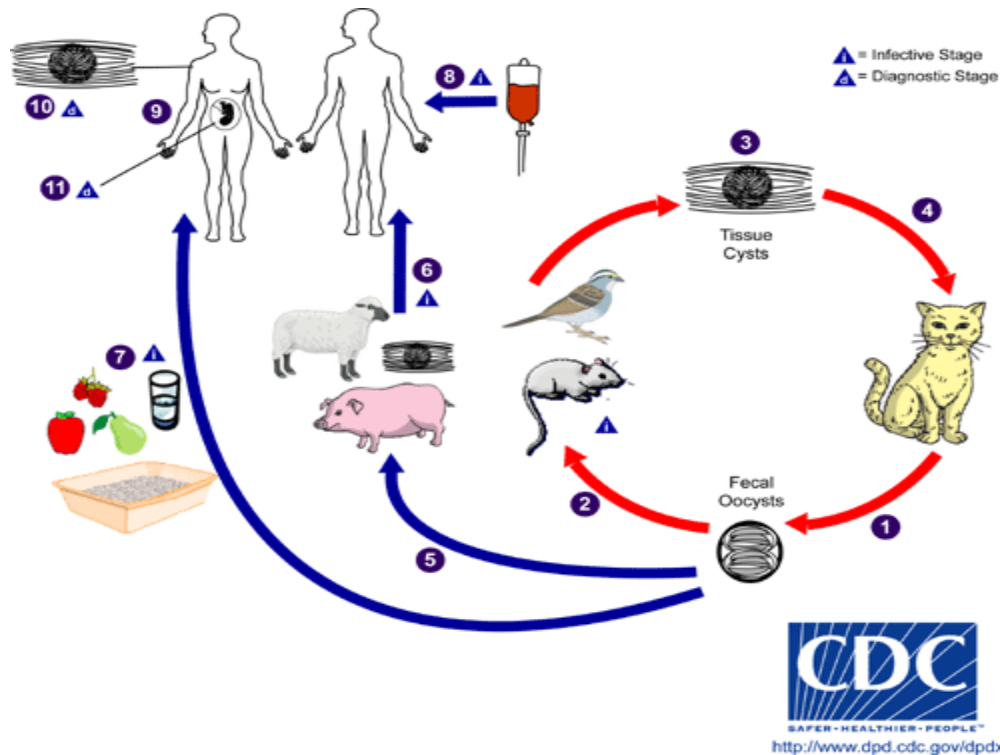


Figure 1: Life Cycle of *Toxoplasma gondii*

From the figure above, fecal oocysts from an already infected cat contaminate the environment, then through animals like rats and birds the cat gets re-infected when it preys on them. Humans get infected directly through handling contaminated cat litter boxes, meat and vegetables (Lucas and Gilles, 2005), blood transfusion (Singh, 2003) and organ transplants (Nissapatorn, 2009,

Dubey, 2008a, Singh, 2003) or indirectly through eating improperly cooked contaminated meat or vegetables (Nissapatorn, 2009, Lucas and Gilles, 2005). Once in the human being, the organism lodges in the muscle tissues and then into the womb to infect the fetus; in the case of a pregnant woman (Nissapatorn, 2009, Singh, 2003).

2.4 Epidemiology and Distribution of the Infection in Animals and Humans

Toxoplasma gondii is found more commonly in tissues of food animals (Nissapatorn, 2009, Lucas and Gilles, 2005, Dubey, 1996) such as pigs, sheep and goats but also from pig feet and soils in hog cages with prevalence range of 2.1%-68% (Dubey, 1996). According to Ayi et al. (2009), Toxoplasmosis seroprevalence is 39% in pigs, 26.8% in goats and 33.2% in sheep. A current study in Brazil to genotype Toxoplasmosis also uncovered a new genotype (ToxoDB#65) which had been previously identified in farm / pet animals in the country and this elaborates further, the significance of animal involvement in human infections (Vijaykumar et al., 2016).

A third of the global human population is believed to have had exposure with the organism and may be chronically infected with it (Pappas et al., 2009). Most people who get infected remain asymptomatic until the immune system is weakened which paves way for clinical conditions to set in (Weiss and Dubey, 2009). This is why Toxoplasmosis is said to range from being asymptomatic to overt disease and can actually cause outbreaks (Dubey, 2008a, Ayi et al., 2009, Van kessell and Eschenbach, 2014).

Seroprevalence of Toxoplasmosis in pregnant women varies geographically. Aqeely et al. (2014) reports 3.7% in Korea, Flatt and Shetty (2013) reports 17.3% in London, 68.6% is reported in Brazil (Sroka et al., 2010), 24.1% in Saudi Arabia (Aqeely et al., 2014), 92.5% in Ghana (Ayi et al., 2009), 30.9% in Tanzania (Mwambe et al., 2013), 6.4% in South Africa (Kistiah et al., 2011), 28.3% in Thailand (Nissapatorn et al., 2011), 53.7% in HIV positives and 5.3% in HIV negatives in Thailand (Wanachiwanawin et al., 2001), 38% in HIV positives and 32% in HIV negatives in Nigeria and 31.3% in HIV positives and 10.9% in HIV negatives in Mozambique (Sitoe et al., 2010).

2.5 Transmission from Mother to Child

Vertical transmission of the infection from mother to child is dependent on gestational age, immunologic development of the fetus and the virulence of the parasite (Aqeely et al., 2014, Van kessell and Eschenbach, 2014, Singh, 2003). Late maternal infections cause rapid transmission of parasites to fetuses, hence higher incidence (Van kessell and Eschenbach, 2014, Singh, 2003) but less severe clinical conditions (Van kessell and Eschenbach, 2014, Weiss and Dubey, 2009, Singh, 2003) and vice versa. Women who seroconvert at 24 to 30 weeks gestational period are at the highest risk of having severely congenitally infected babies (Van kessell and Eschenbach, 2014).

2.6 Clinical Manifestations of *Toxoplasma gondii* Infection

Some general clinical manifestations of the infection are ocular disease, lymphadenopathy (most common), encephalitis and generalized infection in immunocompromised people (Weiss and Dubey, 2009, Wanachiwanawin et al., 2001). There is also spontaneous abortion of fetuses as well as stillbirths (Van kessell and Eschenbach, 2014). Surviving babies on the other hand develop neurological diseases such as epileptic seizures, choroidoretinitis, hydrocephalus, intracerebral calcification, mental retardation and deafness at a stage in their lifetime (Wanachiwanawin et al., 2001, Singh, 2003, Van kessell and Eschenbach, 2014).

2.7 Toxoplasmosis in HIV/AIDS patients

Toxoplasmosis is an opportunistic infection which causes further severe health conditions in HIV/AIDS patients (Nissapatorn, 2009, Singh, 2003, Wanachiwanawin et al., 2001). It is the most prevalent disorder that affects the central nervous system of HIV patients (Vijaykumar et al., 2016, Lindström et al., 2006) and it causes cerebral toxoplasmosis which is fatal to this group of people (Vijaykumar et al., 2016, Nissapatorn, 2009, Navia et al., 1986). According to Weiss and Dubey (2009) and Nissapatorn (2009), the occurrence of the disease in HIV/AIDS patients is as a result of reactivation of latent infection and not necessarily new infections, hence, seroprevalence of *T. gondii* infection is high in HIV endemic countries, (Lindström et al., 2006). This disease is the cause of death in 3%-10% AIDS patients (Singh, 2003) and about 2.5 million people are co-infected with HIV/AIDS and Toxoplasmosis in Africa and are therefore at the risk

of dying from the later (Lindström et al., 2006). Compared to HIV negative mothers with Toxoplasmosis, infants born to HIV - Toxoplasmosis co-infected mothers are more susceptible to congenital toxoplasmosis, hence, a higher risk of disease and death among these children (Wanachiwanawin et al., 2001).

2.8 Determining factors associated with Toxoplasmosis in pregnant women

There are a number of factors associated with toxoplasmosis in pregnancy. According to Baril et al. (1999), Cook et al. (2000) and Boyer et al. (2005), eating undercooked or cured meat is a determining factor for the disease. Having a pet cat is also significant according to studies by Baril et al. (1999) and Boyer et al. (2005). Contact with soil (Cook et al., 2000); educational level and occupation (Jones et al., 2001, Berger et al., 2009); age and crowded conditions (Jones et al., 2001); being foreign born / race (Jones et al., 2001, Nissapatorn et al., 2003); Parity (Nissapatorn et al., 2003) and eating raw vegetables (Baril et al., 1999) are all determining factors of the disease.

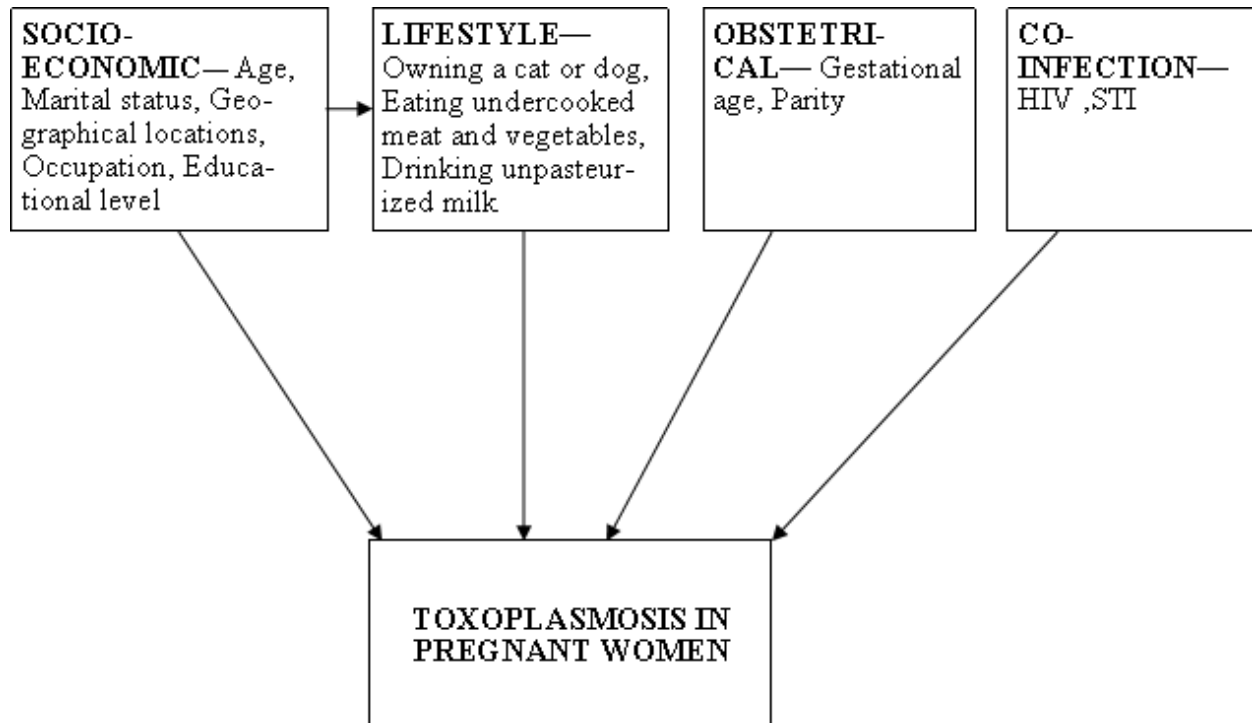


Figure 2: Conceptual Framework (Self-Developed with Information Discovered from Literature)

According to the diagram above, conditions such as age, geographic location (Mwambe et al., 2013), marital status, occupation and educational level of an individual (Berger et al., 2009, Jones et al., 2007) influence the individual's susceptibility to contract Toxoplasmosis. At the same time, these socio-economic conditions can lead to lifestyles such as owning a cat (Boyer et al., 2005, Baril et al., 1999) or dog, drinking unpasteurized milk, eating undercooked meat and vegetables which in turn also increase the likelihood of an individual to contract this disease. Very important also in the transmission of Toxoplasmosis is obstetrical conditions such as parity (Nissapatorn et al., 2003) and gestational age (Van kessell and Eschenbach, 2014). Co-infection such as HIV/AIDS also increases the risk of Toxoplasmosis infection in an individual (Wanachiwanawin et al., 2001, Singh, 2003).

2.9 Screening, Diagnosis and Management of the disease

Most serological data on acute toxoplasmosis in pregnancy is from France and Austria due to the implementation of routine screening of pregnant women for the disease (Wong and Remington, 1994, Singh, 2003). Screening helps to detect the infection before it manifests (Remington et al., 2004, Breugelmans et al., 2004) and diagnosis depends largely on identifying specific antibodies in the blood of an infected individual as well as by isolating the DNA of the parasite from the amniotic fluid after birth (Sukthana, 2006, Singh, 2003).

Management of congenital toxoplasmosis is by termination of the pregnancy at the legal gestational age or by administering spiramycin, or pyrimethamine and sulfadiazine not to cure but to reduce the risk of transmission of the disease to the fetus (Van kessell and Eschenbach, 2014, Kijlstra and Jongert, 2009, Breugelmans et al., 2004).

CHAPTER THREE: STUDY FOCUS

3.1 Rationale

The exact burden of Toxoplasmosis in pregnancy in Zambia is unknown and this is so because screening for *Toxoplasma gondii* in pregnant women is not routinely done in the country. Meanwhile, the Burden of the disease is severe in Immune compromised populations such as pregnant women and HIV patients (Remington et al., 2004). This not only keeps the country in the dark on the subject but also makes it difficult to detect and protect unborn babies from transplacental infections which bring about congenital Toxoplasmosis. As a result of this, babies, infants and children may suffer from disease conditions and complications and even death, which on the other hand could have been prevented.

Compounding the situation further has been the lack of information on the determinants of the disease here in the country.

This study sought to and has provided the prevalence of *Toxoplasma gondii* infection in UTH, Lusaka, Zambia. It has shed light on possible determining factors of the disease. It has set the foundation for further studies to be carried out in this area and it has brought a degree of awareness on the disease. These will further create avenue for management and treatment of this disease.

Preventive measures such as screening of pregnant women and neonates could be put in place to protect both mother and child from the infection as is done routinely in pregnant women in France and Austria (Van kessell and Eschenbach, 2014, Aspöck and Pollak, 1991) and in neonates in Denmark and USA (Antsaklis et al., 2002).

3.2 Research Question

What is the prevalence of and factors associated with Toxoplasmosis among pregnant women attending antenatal clinic at the University Teaching Hospital in Lusaka, Zambia (UTH)?

3.3 Main Objective

To determine the prevalence of and explore factors associated with Toxoplasma infection among pregnant women who attend antenatal clinic at UTH

3.3.1 Specific Objectives

1. To determine the proportion of women attending antenatal clinic at UTH who have Toxoplasmosis.
2. To compare the prevalence of *T. gondii* infection in HIV positive and HIV negative pregnant women.
3. To explore the socio-demographic characteristics of the pregnant women with Toxoplasmosis at UTH

CHAPTER FOUR: METHODS

4.1 Study Design

A Cross-sectional study was employed for this research.

4.2 Study Setting

The study was carried out at the maternity wing (B02) of the University Teaching Hospital (UTH), Lusaka, Zambia. The hospital is a referral hospital and the biggest public hospital in the country. It has bed space of 1800 and four clinical departments namely; Internal Medicine, Obstetrics and Gynaecology, Neo neonatal and Paediatrics and Department of Surgery. It also has patient attendance of 310,527 per year with the greater number of the admissions being in the Department of Maternity and Gyneacology (31,811) (UTH, 2015).

This setting was purposively chosen because it is the country's biggest referral hospital and it has a lot of patients in attendance at any given time.

4.3 Study Population

The study population was all pregnant women attending antenatal clinic at UTH and the period for data collection was from August to October 2015. The average number of antenatal (ANC) attendance per month at the hospital was 1,200.

4.3.1 Inclusion Criteria

The study included consenting pregnant women of all stages of pregnancy.

Both HIV positive and HIV negative pregnant women were allowed to participate in the study.

4.3.2 Exclusion Criteria

Women who did not consent to participate were not included in the study.

4.4 Sampling and Sample Size

The study employed estimation for simple proportion to calculate the sample size for the study. In sampling, the first participant was randomly picked, after which every third consenting pregnant woman was interviewed and tested for the infection.

Sample size estimation

$$n = (z/\Delta)^2 p (1-p) \text{ (Wayne D, 2010)}$$

Where;

n = Sample size

Z = 1.96 (95% confident interval)

Δ = precision

P = proportion

Using p=31% = 0.31(Mwambe, 2013) and a change of 0.05 (Wayne D, 2010);

$$n = (1.96/ 0.05)^2 0.31(1-0.31)$$

$$n = 329$$

$$n = 411 \text{ (with response rate of 80\%)}$$

4.5 Data collection

4.5.1 Tools

Data was collected between August and October 2015. The primary method applied in this study was the use of structured interview with structured closed-ended questionnaires. The questionnaire was developed from information reviewed in literature (from some studies such as Nissapatorn et al. (2003), Boyer et al. (2005) and Berger et al. (2009). It gathered information on socio-demographic factors, behavioral, obstetrical and health status of the pregnant women. It also gathered information on awareness of Toxoplasmosis among pregnant women.

4.5.2 Specimen Collection

In addition to the questionnaire, blood samples were taken for blood analysis for *Toxoplasma gondii* infection. Blood was taken in two different ways. About 2mls of blood from the blood

drawn from the women by the hospital was taken and tested. For pregnant women who were not undergoing routine blood testing by the hospital, about 2mls of venous blood was drawn from them and tested for the infection.

The OnSite Toxo IgG/IgM Combo Rapid test cassettes, (a lateral flow chromatographic immunoassay) manufactured by the CTK Biotech, Inc. USA were used in the laboratory to detect the presence of Toxoplasma antibodies in the blood samples. The cassettes were removed from the pouches, placed on a clean, flat surface and labeled with the participants' identity numbers. Then with the help of plastic droppers, blood drops from each participant were dispensed (dropper held in vertical position and avoiding air bubbles) in the sample wells of the rapid test cassettes (one cassette for each blood sample). The blood migrated by capillary action across the cassettes and results indicated in the results window of these cassettes were recorded within fifteen (15) minutes (following manufacturer's instruction), with help from a set timer.

The devices and left over specimens were discarded after use in a bio-safety plastic bag for laboratory wastes.

4.6 Data Analysis

Data was analysed using STATA version 12 (StataCorp, Texas, USA). The quantile-quantile Q-Q plot was used to check the normality of the continuous variable 'age' before it was categorised.

The data was normally distributed, hence the mean age and standard deviation was recorded. Chi-square test was used to determine associations between the infection and the characteristics of the pregnant women through cross-tabulations but where appropriate, Fishers exact test was used instead of Chi-square test.

Generalized linear model for binary outcome, in this case, Toxoplasma infection; present or absent, and reporting odds ratios was used in univariate analysis. This was to determine significance of individual characteristics/variables to the infection. After univariate analysis, all variables which came out significant, together with priori of the study such as 'contact with cats', 'HIV status', 'eating meat' etc were put into the final model for multivariable analysis as was done by Flatt and Shetty (2013). The model was run and corresponding odds ratios, confidence

intervals and p-values ($p < 0.05$ was considered statistically significant) were recorded at 95% confidence interval.

4.7 Ethical Consideration

Ethical issues with this study were that of confidentiality with participants' information, concerns with drawing blood, disclosure of participants' HIV status; which is an invasion of privacy and anxiety about knowing one's test results.

These concerns were addressed by explaining into details the essence and importance of the study to participants and letting them understand how the study will contribute knowledge to the entire population. Procedures and risk were explained thoroughly to them after which consent was obtained from them for participation in the study. The pregnant women were assured of anonymity, for those whose left-over blood was going to be obtained and tested for the infection and both anonymity and safety of method of drawing blood for those who were not due for blood test by the hospital and their blood was going to be drawn for the study. Participants were assured of minimal risk in the procedure and that was with the prick involved in the drawing of blood. They were given their privacy during both the interview period and the time for drawing of blood and their answered questionnaires and blood test results were kept confidential. They were allowed to quit the study at any time and there was no consequence for quitting the study. The benefit of the study to the participants was that they got to know if they were reactive to the test or not and in the occasion where they were reactive to the test, they were referred to their doctors in the hospital to advice. It was further explained to them that others in the community will also benefit in future should there be measures put in place to curb the infection levels depending on the results that would come out of this study. These were all communicated to participants before obtaining their consent.

All the information gathered on the pregnant women were kept confidential on a secured laptop and filing cabinet and they were assured of this.

Ethical clearance was sought from the Excellence in Research Ethics and Science (ERES Converge IRB) and permission was sought from the Hospital Management to allow the study to take place at the maternity wing (BO2) of the hospital.

CHAPTER FIVE: RESULTS

Description of study population

The results section comprises of the prevalence of Toxoplasmosis and various explored determinants, as well as the extent of association of the explored determinants with the infection. The sample size for this study was four-hundred and eleven and this number was attained during data collection. Table 1 highlights the population characteristics: Most of the pregnant women (59.6%) were in the age group of 25-34years. A greater number (51.7%) had up to secondary education and were employed (51.7 and 62.7%) and out of which 63.6% worked as professionals or in administrative positions. Most of the pregnant women (42.3%) lived in low cost residential areas which imply highly dense settlements. These pregnant women were classified under an income categorization of 'Below K3000', 'Between K3000 and K5000' and 'Above K5000', based on classification adopted from a recent research done by the National AIDS Council of Zambia: Most of them (43.6%) were in the category 'Between K3000 and K5000. About 65% of them had no children and 16.8% were HIV positive.

Twenty-four of the pregnant women out of the 411 who participated in the study were reactive to *Toxoplasma gondii* IgG antibodies. This represents an overall prevalence of 5.87% of the total number of pregnant women who attend antenatal clinic at the university teaching hospital. None of these pregnant women were reactive to *Toxoplasma gondii* IgM antibodies.

Table 1: Characteristics of Pregnant Women who participated in the *Toxoplasma gondii* study at UTH

CHARACTERISTICS	RESPONSE (%) n
1. SOCIO-DEMOGRAPHIC	
AGE IN YEARS	
15-24	(18.7) 77
25-34	(59.6) 245
35-49	(21.7) 89
LEVEL OF EDUCATION	
Up to secondary*	(51.7) 209
Tertiary	(48.3) 195
MARITAL STATUS	
Married	(88.3) 361
Never been married	(5.38) 22
Others	(6.36) 26
EMPLOYED	
In Employment	(62.7) 257
Unemployed	(37.3) 153
EMPLOYMENT TYPE	
Farming and construction	(2.7) 7
Professional/Administrative	(63.6) 164
Trading/ other businesses	(33.7) 87
INCOME	
Below K3000	(37.6) 94
Between K3000 and K5000	(43.6) 109
Above K5000	(18.8) 47
RESIDENCE	
High cost residential area	(18.5) 76
Medium cost residential area	(39.2) 161
Low cost residential area	(42.3) 174
2.OBSTETRICAL FACTORS	
GESTATIONAL AGE	
First Trimester	(20.5) 84
Second Trimester	(32.6) 134
Third Trimester	(47.0) 192
PARITY	
No child (first pregnancy)	(64.5) 262
One child	(29.3) 119
Two and above	(6.16) 25
3. BEHAVIORIAL/ LIFESTYLE FACTORS	
CONTACT WITH CATS	
Yes	(10.0) 41
No	(90.0) 369
CONTACT WITH DOGS	
Yes	(22.2) 91
No	(77.8) 319

EATING MEAT	
Yes	(97.6) 401
No	(2.43) 10
LENGTH OF COOKING MEET	
Under 30 minutes	(12.6) 49
Within 30 to 60 minutes	(55.9) 218
Within 60 to 120 minutes	(18.0) 70
Over 120 minutes	(13.6) 53
EATING CURED MEAT	
Yes	(87.0) 347
No	(13.0) 52
EATING RAW VEGETABLES	
Yes	(81.3) 334
No	(18.7) 77
DRINKING UNPASTEURIZED MILK	
Yes	(8.76) 36
No	(91.2) 375
4. CO-INFECTION	
HIV STATUS	
Positive	(16.8) 69
Negative	(82.2) 338
5. CLINICAL FACTORS	
ORGAN TRANSPLANT	
Yes	(0.49) 2
No	(99.5) 409
BLOOD TRANSFUSION	
Yes	(4.14) 17
No	(95.9) 394

Note: 'n' is the number of participants per each category; n total =411

* = the response for 'never attended school' and 'attended primary school' was together less than 10, so we combined these categories up to secondary level in order to have comparative numbers.

Age as a continuous variable was normally distributed with the mean age of the pregnant women being 29.87 and a standard deviation of 5.77.

Table 2: Prevalence and Factors Associated with *Toxoplasma gondii* Infection

UNIVARITE ANALYSIS				
CHARACTERISTICS	PREVALENCE (%)and CATEGORY TOTAL (n)	OR	95% CI	P-VALUE
AGE IN YEARS				
15-24	11.7 (77)	3.11	1.21-7.96	0.02
25-34	4.08 (245)	1.00	1.00	1.00
35-49	5.62 (89)	1.40	0.46-4.21	0.55
LEVEL OF EDUCATION				
Up to secondary	6.22 (209)	1.00	1.00	1.00
Tertiary	4.62 (195)	0.73	0.30-1.75	0.48
MARITAL STATUS				
Married	4.99 (361)	1.00	1.00	1.00
Never been married	18.2 (22)	3.46	1.08-11.1	0.04
Others	7.69 (26)	1.91	0.41-8.79	0.41
EMPLOYMENT TYPE				
Farming and construction	28.6 (7)	8.97	1.47-54.6	0.02
Professional/Administrative	6.90 (87)	1.66	0.54-5.11	0.38
Trading/other businesses	4.27 (164)	1.00	1.00	1.00
INCOME				
Below K3000	10.64 (94)	3.13	0.95-10.3	0.06
Between K3000 and K5000	3.67 (109)	1.0	1.00	1.00
Above K5000	2.13 (47)	0.57	0.06-5.25	0.62
RESIDENCE				
Low cost residential area	8.62 (174)	1.0	1.00	1.00
Medium cost residential area	3.73 (161)	2.30	0.64-8.18	0.20
High cost residential area	3.95 (76)	0.94	0.23-0.13	0.93
GESTATIONAL AGE				
First Trimester	3.57 (84)	0.68	0.18-2.53	0.56
Second Trimester	8.21 (134)	1.64	0.68-4.00	0.28
Third Trimester	5.18 (193)	1.0	1.00	1.00
PARITY*				
No child (first pregnancy)	4.62 (262)	1.77	0.23-13.9	0.59
One child	9.09 (119)	0.83	0.09-7.80	0.87
Two and above	3.47 (25)	1.0	1.00	1.00
CONTACT WITH CATS				
Yes	7.32 (41)	1.31	0.37-4.59	0.68
No	5.69 (369)	1.0	1.00	1.00
CONTACT WITH DOGS				
Yes	6.59 (91)	1.18	0.45-3.07	0.73
No	5.64 (319)	1.0	1.00	1.00
EATING MEAT				
Yes	5.99 (401)	1.0	1.00	1.00
No	0 (10)	2.65*10 ⁻⁰⁶	0	0.99

LENGTH OF COOKING MEAT					
	Under 30 minutes	4.08 (49)	0.62	0.14-2.82	0.54
	Within 30 to 60 minutes	6.42 (218)	1.00	1.00	1.00
	Within 60 to 120 minutes	4.29 (70)	0.65	0.18-2.34	0.51
	Over 120 minutes	3.77 (53)	0.57	0.13-2.60	0.47
EATING CURED MEAT					
	Yes	5.19 (347)	1.00	1.00	1.00
	No	11.5 (52)	2.38	0.90-6.32	0.08
DRINKING UNPASTEURIZED MILK					
	Yes	8.33 (36)	1.53	0.43-5.41	0.51
	No	5.60 (375)	1.00	1.00	1.00
BOILING RAW MILK BEFORE DRINKING					
	Yes	5.77 (104)	0.72	0.26-2.01	0.53
	No	7.86 (140)	1.00	1.00	1.00
HIV STATUS					
	Positive	8.70 (69)	1.69	0.65-4.43	0.28
	Negative	5.33 (338)	1.0	1.00	1.00
TAKEN SULPHADOXINE+PYRIMETHYMNENE (FANCIDA)					
	Yes	4.92 (244)	1.0	1.00	1.00
	No	7.19 (167)	1.50	0.66-3.42	0.34

Note: Associations were calculated with Chi-square, and Fisher's exact test where appropriate, where as GLM for binary outcome reporting odds ratios was used for the univariate analysis

* =the category 'two and above' was created because the participants who had three to eight (maximum) children were few hence the groups were not very comparative

The generalized linear model for binary outcome, reporting odds, was employed in univariate analysis (table 2). This was to determine the outcome of infections (present or absent), the significance of the variables in determining the risk of the infection as well as the extent of the risk (odds) they pose.

Results of the model provided in table 2 shows three variables were significant, namely; age, marital status and employment type and one, income, showing a border line significance of p-value 0.06. The younger age category, 15-24 years, showed significance of p-value 0.02 and 3.11 times the likelihood of contracting the infection among this age group as compared to the older groups which showed no significance with the infection. Those who had never been married, under marital status, also showed significance of p-value 0.04, with 3.5 times risk of contracting the disease as compared to the married and others. Economic indicators, farming/ construction, (under employment type) and income level of less than K3000 (under income) exhibited significance of p-value 0.02 and 0.06 (border line significance) respectively. These illustrated relatively high risks of 8.97 and 3.13 times the risk of contracting the infection respectively.

The rest of the variables did not show significance at this level though expected.

Table 3: Predictors of *Toxoplasma gondii* Infection

MULTIVARIABLE ANALYSIS				
CHARACTERISTICS	PREVALENCE (%)and CATEGORY TOTAL (n)	OR	95% CI	P-VALUE
AGE IN YEARS				
15-24	11.7 (77)	0.11	0.00-7.41	0.30
25-34	4.08 (245)	1.00	1.00	1.00
35-49	5.62 (89)	1.91	0.25-14.7	0.53
LEVEL OF EDUCATION				
Up to secondary*	6.22 (209)	0.60	0.10-3.57	0.57
Tertiary	4.62 (195)	1.00	1.00	1.00
MARITAL STATUS				
Married	4.99 (361)	1.46	0.01-271	0.89
Never been married	18.2 (22)	10.9	0.06-1955	0.37
Others	7.69 (26)	1.00	1.00	1.00
EMPLOYMENT TYPE				
Farming and construction	28.6 (7)	15.5	0.23-1019	0.20
Professional/Administrative	6.90 (87)	0.71	0.08-6.02	0.75
Trading/other businesses	4.27 (164)	1.00	1.00	1.00
RESIDENCE				
Low cost residential area	8.62 (174)	2.84	0.20-39.3	0.44
Medium cost residential area	3.73 (161)	0.40	0.03-5.77	0.50
High cost residential area	3.95 (76)	1.00	1.00	1.00
CONTACT WITH CATS				
Yes	7.32 (41)	7.81	0.99-61.8	0.05
No	5.69 (369)	1.00	1.00	1.00
EATING CURED MEAT				
Yes	5.19 (347)	1.00	0.02-43.6	1.00
No	11.5 (52)	1.00	1.00	1.00
HIV STATUS				
Positive	8.70 (69)	0.39	0.04-3.47	0.40
Negative	5.33 (338)	1.00	1.00	1.00

Note: Variables, ‘eating meat’, parity, ‘length of cooking meat’, ‘drinking unpasteurised milk’ and income were omitted in the final model due to their small numbers

The generalized linear model for binary outcome, reporting odds, was again employed in the multivariable analysis. All significant variables at univariate analysis level, together with the priori of the study such as ‘contact with cats, ‘eating meat’, ‘eating cured meat’, ‘level of education’, ‘HIV status, employment type as well as ‘residence’ were put in the final model in order to come up with the determinants of toxoplasmosis. At multivariable analysis (Table 3), there was no significant outcome but for ‘contact with cats’ which demonstrated a borderline significance of p-value 0.05.

Employment type and contact with cats though not showing significant p-values, illustrated

associations with the infection showing high risks of 15.5 times and 7.81 times the risk of contracting the infection in the pregnant women respectively. These two variables gave the highest odds ratios (risk of the infection) in this study. At the same time, variables such as ‘eating meat’, ‘length of cooking meat’ and income were omitted in the final output by stata because of their small numbers, hence they are not shown in the table.

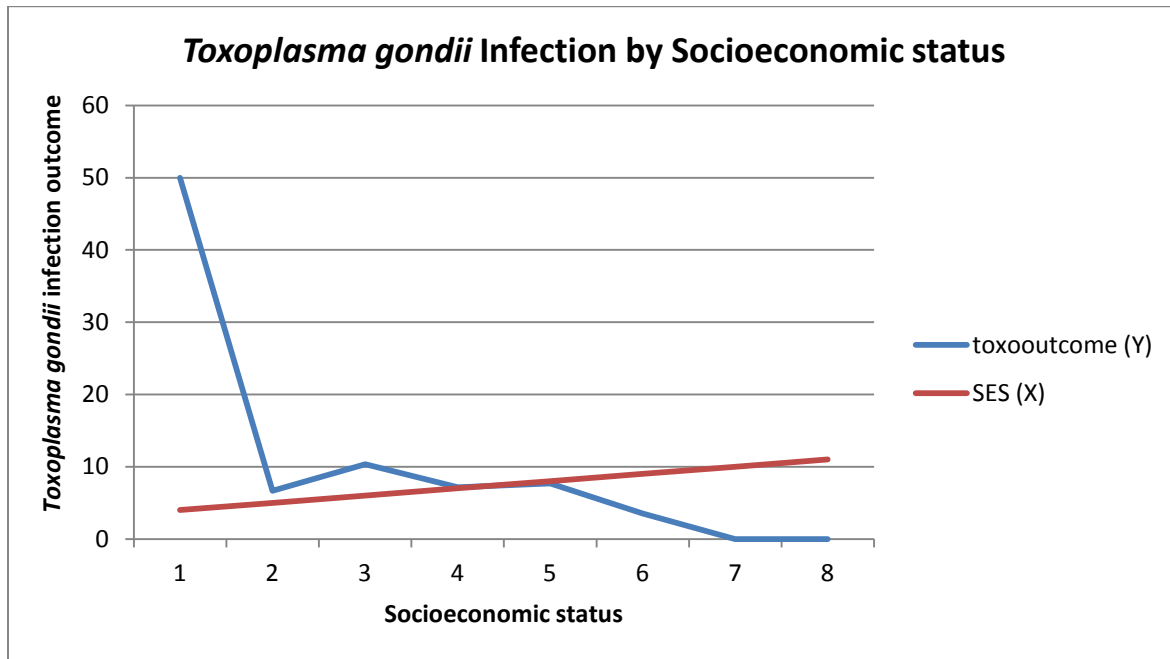


Figure 3: Graph Showing an Inverse Relationship (Indicating Association) between Socio-Economic Status (SES) of Pregnant Women Attending Antenatal Clinic at UTH and their Toxoplasma Gondii Infection Status (toxoutcome)

This graph (fig.3) gives a picture of the interaction between Toxoplasmosis and socio-economic status of the pregnant women in this study. Individual factors indicative of socio-economic status of the pregnant women such as their residence, income level, educational level and employment type were put together to create the variable ‘socio-economic status’. From the graph, it is realised that infections are highest where the socio-economic status of the pregnant women is lowest and the infection reduces with increase in socio-economic status generally.

CHAPTER SIX: DISCUSSION OF STUDY FINDINGS

6.1 Discussion

This study has shown that the overall seroprevalence of *Toxoplasma gondii* infection among pregnant women attending antenatal clinic in UTH, Lusaka, Zambia is 5.87% and that ‘contact with cats’, ‘employment type’ and socio-economic status are associated with the infection.

There are studies with comparatively low seroprevalence of the infection in pregnant women such as in the United Kingdom, 9%, (Aqeely et al., 2014), South Africa, 6.4%, (Kistiah et al., 2011) and Korea, 3.7%, (Aqeely et al., 2014). However, the seroprevalence in this current study is also low. This is in comparison with results from other studies done in the region such as 18.7% in Mozambique (Siteo et al., 2010), 30.9% in Tanzania (Mwambe et al., 2013), 92.5% in Ghana (Ayi et al., 2009), and globally, 24.1% in Saudi Arabia, (Aqeely et al., 2014), 28.3% in Southern Thailand (Nissapatorn et al., 2011) and 17.3% in London (Flatt and Shetty, 2013). Nonetheless, we find of epidemiological importance the low prevalence and agree with statements by Kistiah that “A low prevalence means that more previously unexposed people are at risk of acquiring an acute infection, which may cause congenital disease in pregnant women, or which, in reactivation form may ultimately be life-threatening in HIV/AIDS patients” (Kistiah et al., 2011) and by Andiappan that, “The seroprevalence may vary in a global view, but the risk of this parasitic infection in human populations, especially in pregnant women, still holds a great interest” (Andiappan et al., 2014). So though the prevalence turned out to be low, it is still of medical importance.

The low prevalence in this study could have been due to the urban setting under which the study was carried out. Studies in rural settings may yield a higher prevalence. Nonetheless, a study such as Ayi et al. (2009) was carried out in an urban setting, but still reported very high prevalence of the infection.

There was no IgM result found in this study. Studies such as Flatt and Shetty (2013), Kistiah et al. (2011) and Ayi et al. (2009) found IgM antibodies but consistent with this study are studies such as Mwambe et al. (2013), Siteo et al. (2010) and Ertug et al. (2005) which found no IgM antibodies. Most studies report mainly no or few IgM antibodies as compared to IgG. Probably

more IgM antibodies can be discovered using methods such as the ELISA which detects early IgM infections even within the first two weeks of infection (Van kessell and Eschenbach, 2014).

As mentioned in the first paragraph of this chapter, this study set out to establish in addition to the prevalence, the determinants of Toxoplasmosis in pregnant women and the results though indicative of association between *Toxoplasma gondii* infection and the explored determinants mentioned above, were without enough evidence to suggest these factors as important determining factors of the disease in pregnant women.

Cats are believed to be the main carriers and transmitters of *Toxoplasma gondii* infection to man, due to the fact that they are the definitive hosts of the organism (Weiss and Kim, 2011). Studies such as Ayi et al. (2009) and Baril et al. (1999) have also established that they are significantly associated with Toxoplasmosis. This current study however, did not establish significance between the infection and ‘contact with cats’. Nonetheless, the risk of contracting the infection where there was contact with cats was found to be 7.81 times more than where there was no contact with cats in this current study. Studies such as Cook et al. (2000), Nissapatorn et al. (2003), Ertug et al. (2005), Flatt and Shetty (2013) and Mwambe et al. (2013) were consistent with our study finding in terms of not establishing significant association between *Toxoplasma gondii* infection and cats. With this result withstanding, suffice it to say, that the risk of contracting Toxoplasmosis is as a result of the way the cat litter is handled and not necessarily simple contact with cats (Mwambe et al., 2013, Shao et al., 2015). More so, excretion of oocysts (which is infective) by infected cats lasts only few weeks (Cook et al., 2000). These we believe are the reasons for the non-significance which was found in this current study as well as other studies which share same results.

Eating meat and eating undercooked meat were not significantly associated with Toxoplasmosis in this study. Interestingly however, all pregnant women who were reactive to the *Toxoplasma gondii* test in this study happen to eat meat and none of those who do not eat meat have the infection. Studies such as Baril et al. (1999), Cook et al. (2000), Boyer et al. (2005) and Flatt and Shetty (2013) report that eating undercooked meat is a determining factor of Toxoplasmosis, but the divergent result of this current study turns to concur with studies such as Nissapatorn et al. (2003) and Ertug et al. (2005), which did not find ‘eating undercooked meat’ to be significantly associated with Toxoplasmosis. One reason for the disparity arises from the different types of

meat that were investigated into in the various studies (Avelino et al., 2004); this current study investigated into beef, mutton, chevon, chicken, pork and 'others' which comprised bush meat or game meat. In addition to the aforementioned, the different prevalence of the disease in food producing animals in the various affected regions plays a significant role in the infection in man (Pappas et al., 2009), as well as the different hygienic conditions under which the meat are kept and handled before consumption (Jones et al., 2007). What's more, the definition of undercooked meat may differ among these studies due to the different settings (culture and preference) under which these studies were carried out. This study considered undercooked meat to be meat cooked in less than thirty minutes: This is not standardized. Undercooked meat in some European countries refers to rare meat which still has blood in it and this is uncommon in our setting. It is uncertain the criteria the other studies used. We believe this could be the reason why this variable did not come out as an important determining factor of the infection in this study.

This study has also found that prevalence of *Toxoplasma gondii* infection among HIV positive pregnant women is higher than that in HIV negative ones. However, the difference was not statistically significant. The difference in the prevalence between these two groups is consistent with studies such as Ogoina et al. (2013), Siteo et al. (2010) and Wanachiwanawin et al. (2001), but, Siteo et al. (2010) and Wanachiwanawin et al. (2001) reported significant association between HIV positive status and Toxoplasmosis which this current study, Shao et al. (2015) and Ogoina et al. (2013) could not establish. One of the reasons for the non-significance could be as a result of the Anti-Retroviral Treatment(ART) which some of the HIV positive pregnant women were on as argued by Shao et al. (2015). Nonetheless, Ogoina et al. (2013) went further in their study to compare the prevalence of the infection in HIV- Toxoplasmosis co-infected individuals on ART and HIV- Toxoplasmosis co-infected individuals who were not on treatment and found no significant association. One way that both studies by Siteo et al. (2010) and Wanachiwanawin et al. (2001) differ from this study is in terms of the choice of test kits used. Both studies used ELISA tests, which are known to be comparatively the most sensitive and specific tests on the market for such tests, although the Onsite Toxo Rapid test kit that we used in our study is also accurate and certified. We are of the view that this could be a reason for the different results.

'Employment type' was also found to have association with the infection but was not established to be of significant association in this study. Contrary to this study's finding, Jones et al. (2001)

found soil related occupation to be significant to acquiring the infection. Andiappan et al. (2014) also established that 'being a labourer' in his study was significantly associated with Toxoplasmosis whereas Mwambe et al. (2013) discovered in his study that employed/business women were more likely to be infected than peasants and this was significant. In this current study however, being a farmer/being involved in construction work showed 15.5 times likelihood of contracting the infection as compared to trading/other businesses and professional/administration which had a reduced risk of the infection, but this was without enough evidence to suggest significance. Cook et al. (2000), also reported weak association with 'working with animals' but studies such as Shao et al. (2015) and Aqeely et al. (2014) found occupation to have no association with the infection at all. There is an extent of diversity when it comes to 'occupation', as different studies investigate different economic activities under occupation and others do not specify exactly which activities investigated, but from results in this study and some of the aforementioned studies, the infection seems higher with environment and animal related activities.

Again in this study, there was increased risk of the infection with increase in age as was reported in studies such as Jones et al. (2001), Ertug et al. (2005) and Mwambe et al. (2013), however, these studies found this association to be significant contrary to our current study's finding. Studies such as Ayi et al. (2009) and Ogoina et al. (2013) also did not establish a significant association between the two. We believe that results in this study is accurate, in that age appears to be associated to the infection because time of exposure increases with age (Ertug et al., 2005) and not that age has direct association with the infection. We believe that age alone is neither sufficient no necessary for the infection to occur.

This study, Ayi et al. (2009) and Shao et al. (2015) could not establish significant association between pregnancy related risk factor such as gestational age and *Toxoplasma gondii* infection. One possible explanation to this is that the exact time of infection was not defined in these studies, including ours (Shao et al., 2015). However, the highest infections discovered generally among the second trimester pregnancies, and in the third and second trimesters respectively in the HIV positive pregnant women in this study imply higher transmission of the infection to the unborn babies. Hence, higher risk of congenital Toxoplasmosis should there be seroconversion

as is common in HIV-Toxoplasmosis co-infection cases (Van kessell and Eschenbach, 2014, Ayi et al., 2009, Singh, 2003).

Another variable, 'socio-economic status', created out of all variables indicative of socio-economic status in this study, (income, residence, employment type and education) has also shown that pregnant women of low socio-economic status have higher risk of the infection. Other studies consistent with this finding are Andiappan et al. (2014), Sroka et al. (2010) and Rosso et al. (2008). We believe that pregnant women of low socio-economic status are more prone to live in highly dense areas with bad sanitary conditions as well as work under these conditions, lack good education and may lack knowledge on good hygienic practices in general, and these conditions brood and pose them to such infections.

These factors discussed above appear to be sufficient but not necessary to produce the disease.

Strengths and Limitations of the study

There are a few limitations to this study. To begin with, these results cannot be generalised to the entire population in Zambia due to the fact that it was carried out in only pregnant women and again it was carried out in just the referral hospital in Lusaka. Secondly, the quantity of anti *T. Gondii* antibodies in the blood specimen which are normally below detection limits of the assay or detectable antibodies that are not present during the stage of the disease at the time of blood collection shows negative results. Furthermore, though a negative test result indicates absence of the infection, it does not rule out possibility of exposure to or with the infection. Again, some specimen containing unusually high titer of heterophile antibodies or rheumatoid factor may affect the expected results.

Though these limitations exist, this study is the first of its kind in UTH and it was carefully and accurately done following all necessary precautions. The test kits were bought and shipped straight from the manufacture and the guidelines for use for both the kits and the laboratory where we worked were keenly adhered to under careful supervision. The manufacturing dates as well as expiring dates of the test kits were checked to ensure that they were not expired. The study population was also a true susceptible population where Toxoplasmosis is concerned hence the need for the study.

6.2 Conclusion

There is low prevalence of *T. gondii* infection among pregnant women attending antenatal clinic in UTH, Lusaka, Zambia, with a prevalence of 5.87%. Contact with cats, employment type and socio-economic status have association with *Toxoplasma* infection.

The low prevalence in this investigated area could be due to the fact that fewer women own or have contact with cats and also that farming / construction is not an activity greatly engaged in by most women in the urban city, Lusaka.

Recommendations

Toxoplasmosis screening can be carried out twice (during first and third trimesters) or at least once (during first trimesters) for every pregnant woman in hospitals. We recommend this because as highlighted early in this study, vertical transmission of the infection during the first trimester is critical and causes severe clinical conditions in the fetus, and third trimester infections have rapid transmission rate of parasites to fetuses, hence causing higher incidence of disease. This when practised, will help save the lives of both mother and child. Health promotion among women of childbearing age is also of immense importance in order to create awareness of the disease in this group and help curb it. In as much as we have given a good picture of the infection among pregnant women in Lusaka, we recommend that more robust studies such as cohort and case-control studies be carried out in the country in order to better ascertain the extent of the condition nationwide.

REFERENCES

- ANDIAPPAN, H., NISSAPATORN, V., SAWANGJAROEN, N., CHEMOH, W., LAU, Y. L., KUMAR, T., ONICHANDRAN, S., SUWANRATH, C. & CHANDEYING, V. 2014. Toxoplasma infection in pregnant women: a current status in Songklanagarind hospital, southern Thailand. *Parasit. Vectors*, 7, 239.
- ANTSAKLIS, A., DASKALAKIS, G., PAPANTONIOU, N., MENTIS, A. & MICHALAS, S. 2002. Prenatal diagnosis of congenital toxoplasmosis. *Prenatal diagnosis*, 22, 1107-1111.
- AQEELY, H., EL-GAYAR, E. K., PERVEEN KHAN, D., NAJMI, A., ALVI, A., BANI, I., MAHFOUZ, M. S., ABDALLA, S. E. & ELHASSAN, I. M. 2014. Seroepidemiology of Toxoplasma gondii amongst Pregnant Women in Jazan Province, Saudi Arabia. *Journal of tropical medicine*, 2014.
- ASPÖCK, H. & POLLAK, A. 1991. Prevention of prenatal toxoplasmosis by serological screening of pregnant women in Austria. *Scandinavian journal of infectious diseases. Supplementum*, 84, 32-37.
- AVELINO, M. M., CAMPOS JÚNIOR, D., PARADA, J. B. D. & CASTRO, A. M. D. 2004. Risk factors for Toxoplasma gondii infection in women of childbearing age. *Brazilian Journal of Infectious Diseases*, 8, 164-174.
- AYI, I., EDU, S., APEA-KUBI, K., BOAMAH, D., BOSOMPEN, K. & EDOH, D. 2009. Sero-epidemiology of toxoplasmosis amongst pregnant women in the greater Accra region of Ghana. *Ghana medical journal*, 43.
- BARIL, L., ANCELLE, T., GOULET, V., THULLIEZ, P., TIRARD-FLEURY, V. & CARME, B. 1999. Risk factors for Toxoplasma infection in pregnancy: a case-control study in France. *Scandinavian journal of infectious diseases*, 31, 305-309.
- BERGER, F., GOULET, V., LE STRAT, Y. & DESENCLOS, J.-C. 2009. Toxoplasmosis among pregnant women in France: risk factors and change of prevalence between 1995 and 2003. *Revue d'épidémiologie et de santé publique*, 57, 241-248.
- BOYER, K. M., HOLFELS, E., ROIZEN, N., SWISHER, C., MACK, D., REMINGTON, J., WITHERS, S., MEIER, P., MCLEOD, R. & GROUP, T. S. 2005. Risk factors for Toxoplasma gondii infection in mothers of infants with congenital toxoplasmosis: implications for prenatal management and screening. *American journal of obstetrics and gynecology*, 192, 564-571.
- BREUGELMANS, M., NAESSENS, A. & FOULON, W. 2004. Prevention of toxoplasmosis during pregnancy—an epidemiologic survey over 22 consecutive years. *Journal of perinatal medicine*, 32, 211-214.
- COOK, A., HOLLIMAN, R., GILBERT, R., BUFFOLANO, W., ZUFFEREY, J., PETERSEN, E., JENUM, P., FOULON, W., SEMPRINI, A. & DUNN, D. 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. *Commentary: Congenital toxoplasmosis—further thought for food. Bmj*, 321, 142-147.
- DUBEY, J. 1996. Strategies to reduce transmission of Toxoplasma gondii to animals and humans. *Veterinary parasitology*, 64, 65-70.
- DUBEY, J. A. J., JL 2008a. Toxoplasma gondii infection in humans and animals in the United States. *International journal for parasitology*, 38, 1257-1278.
- DUBEY, J. P. 2008b. The history of Toxoplasma gondii—the first 100 years. *Journal of Eukaryotic Microbiology*, 55, 467-475.
- ELMORE, S. A., JONES, J. L., CONRAD, P. A., PATTON, S., LINDSAY, D. S. & DUBEY, J. 2010. Toxoplasma gondii: epidemiology, feline clinical aspects, and prevention. *Trends in parasitology*, 26, 190-196.
- ERTUG, S., OKYAY, P., TURKMEN, M. & YUKSEL, H. 2005. Seroprevalence and risk factors for toxoplasma infection among pregnant women in Aydin province, Turkey. *BMC public health*, 5, 66.
- FLATT, A. & SHETTY, N. 2013. Seroprevalence and risk factors for toxoplasmosis among antenatal women in London: a re-examination of risk in an ethnically diverse population. *The European Journal of Public Health*, 23, 648-652.

- HOGAN, M. J., YONEDA, C., FEENEY, L., ZWEIGART, P. & LEWIS, A. 1960. Morphology and culture of Toxoplasma. *Archives of ophthalmology*, 64, 655-667.
- HOTEZ, P. J. & KAMATH, A. 2009. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Negl Trop Dis*, 3, e412.
- JONES, J. L., KRUSZON-MORAN, D., SANDERS-LEWIS, K. & WILSON, M. 2007. Toxoplasma gondii infection in the United States, 1999–2004, decline from the prior decade. *The American Journal of Tropical Medicine and Hygiene*, 77, 405-410.
- JONES, J. L., KRUSZON-MORAN, D., WILSON, M., MCQUILLAN, G., NAVIN, T. & MCAULEY, J. B. 2001. Toxoplasma gondii infection in the United States: seroprevalence and risk factors. *American journal of epidemiology*, 154, 357-365.
- KIJLSTRA, A. & JONGERT, E. 2009. Toxoplasma-safe meat: close to reality? *Trends in parasitology*, 25, 18-22.
- KISTIAH, K., WINIECKA-KRUSNELL, J., BARRAGAN, A., KARSTAEDT, A. & FREAN, J. 2011. Seroprevalence of Toxoplasma gondii infection in HIV-positive and HIV-negative subjects in Gauteng, South Africa. *Southern African Journal of Epidemiology and Infection*, 26, 225-228.
- LINDSTRÖM, I., KADDU-MULINDWA, D. H., KIRONDE, F. & LINDH, J. 2006. Prevalence of latent and reactivated Toxoplasma gondii parasites in HIV-patients from Uganda. *Acta tropica*, 100, 218-222.
- LUCAS, A. O. A. & GILLES, H. M. 2005. *Lucas and Gilles Short Textbook of Public Health Medicine for the Tropics*, New York, Oxford University Press, New York.
- MAZIGO, H. D., MORONA, D., KWEKA, E. J., WAIHENYA, R., MNYONE, L. & HEUKELBACH, J. 2013. Epilepsy and tropical parasitic infections in Sub-Saharan Africa: a review. *Tanzania Journal of Health Research*, 15.
- MWAMBE, B., MSHANA, S. E., KIDENYA, B. R., MASSINDE, A. N., MAZIGO, H. D., MICHAEL, D., MAJINGE, C. & GROß, U. 2013. Sero-prevalence and factors associated with Toxoplasma gondii infection among pregnant women attending antenatal care in Mwanza, Tanzania. *Parasit Vectors*, 6, 222.
- NAVIA, B. A., PETITO, C. K., GOLD, J. W., CHO, E. S., JORDAN, B. D. & PRICE, R. W. 1986. Cerebral toxoplasmosis complicating the acquired immune deficiency syndrome: clinical and neuropathological findings in 27 patients. *Annals of neurology*, 19, 224-238.
- NISSAPATORN, V. 2009. Toxoplasmosis in HIV/AIDS patients: a living legacy. *Microbes, Viruses and Parasites in AIDS Process*, ed. by V. Zajac (InTech, Croatia, 2011), 307-352.
- NISSAPATORN, V., NOOR AZMI, M., CHO, S., FONG, M., INIT, I., ROHELA, M., KHAIRUL ANUAR, A., QUEK, K. & LATT, H. 2003. Toxoplasmosis: prevalence and risk factors. *Journal of Obstetrics & Gynecology*, 23, 618-624.
- NISSAPATORN, V., SUWANRATH, C., SAWANGJAROEN, N., LING, L. Y. & CHANDEYING, V. 2011. Toxoplasmosis-serological evidence and associated risk factors among pregnant women in southern Thailand. *The American journal of tropical medicine and hygiene*, 85, 243-247.
- OGOINA, D., ONYEMELUKWE, G. C., MUSA, B. O. & OBIAKO, R. O. 2013. Seroprevalence of IgM and IgG Antibodies to Toxoplasma infection in healthy and HIV-positive adults from Northern Nigeria. *The Journal of Infection in Developing Countries*, 7, 398-403.
- PAPPAS, G., ROUSSOS, N. & FALAGAS, M. E. 2009. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. *International journal for parasitology*, 39, 1385-1394.
- REMINGTON, J. S., THULLIEZ, P. & MONTOYA, J. G. 2004. Recent developments for diagnosis of toxoplasmosis. *Journal of clinical microbiology*, 42, 941-945.
- ROSSO, F., LES, J. T., AGUDELO, A., VILLALOBOS, C., CHAVES, J. A., TUNUBALA, G. A., MESSA, A., REMINGTON, J. S. & MONTOYA, J. G. 2008. Prevalence of infection with Toxoplasma gondii

- among pregnant women in Cali, Colombia, South America. *The American journal of tropical medicine and hygiene*, 78, 504-508.
- SCALLAN, E., HOEKSTRA, R. M., ANGULO, F. J., TAUXE, R. V., WIDDOWSON, M.-A., ROY, S. L., JONES, J. L. & GRIFFIN, P. M. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*, 17.
- SHAO, E. R., NDAZANA, S. G., CHACHA, W., MASENGA, G., TOLBERT, S., MOSHA, D., KIFARO, E. G. & NYOMBI, B. M. 2015. Sero-Prevalence and Factors Associated with Toxoplasma Gondii Infection among Pregnant Women Attending Antenatal Care in the Referral Hospital in Tanzania: Cross Sectional Study. *Annals of Clinical and Laboratory Research*.
- SINGH, S. 2003. Mother-to-child transmission and diagnosis of Toxoplasma gondii infection during pregnancy. *Indian journal of medical microbiology*, 21, 69.
- SITOE, S. P. B. L., RAFAEL, B., MEIRELES, L. R., ANDRADE JR, H. F. D. & THOMPSON, R. 2010. Preliminary report of HIV and Toxoplasma gondii occurrence in pregnant women from Mozambique. *Revista do Instituto de Medicina Tropical de São Paulo*, 52, 291-295.
- SROKA, S., BARTELHEIMER, N., WINTER, A., HEUKELBACH, J., ARIZA, L., RIBEIRO, H., OLIVEIRA, F. A., QUEIROZ, A. J. N., ALENCAR, C. & LIESENFELD, O. 2010. Prevalence and risk factors of toxoplasmosis among pregnant women in Fortaleza, Northeastern Brazil. *The American journal of tropical medicine and hygiene*, 83, 528-533.
- SUKTHANA, Y. 2006. Toxoplasmosis: beyond animals to humans. *Trends in parasitology*, 22, 137-142.
- TENTER, A. M., HECKEROTH, A. R. & WEISS, L. M. 2000. Toxoplasma gondii: from animals to humans. *International journal for parasitology*, 30, 1217-1258.
- UTH. 2015. *University Teaching Hospital, Ministry of Health, Government of the Republic of Zambia* [Online]. UTH. Available: <http://www.uth.gov.zm/> [Accessed 07/06/2015 2015].
- VAN KESSELL, K. A. & ESCHENBACH, D. A. 2014. *Toxoplasmosis In Pregnancy* [Online]. Available: www.glowm.com/section_view/heading/Toxoplasmosis%20in%20Pregnancy/item/187 [Accessed 22nd April 2015].
- VIJAYKUMAR, B., LEKSHMI, S. U., KANT, R. S., VAIGUNDAN, D., MAHADEVAN, A., RAJENDRAN, C., SHANKAR, S. & JAYSHREE, R. 2016. Genetic characterization of Toxoplasma gondii from autopsy proven cases of AIDS associated cerebral toxoplasmosis in South India. *Infection, Genetics and Evolution*.
- WANACHIWANAWIN, D., SUTTHENT, R., CHOKEPHAIBULKIT, K., MAHAKITTIKUN, V., ONGROTCHANAKUN, J. & MONKONG, N. 2001. Toxoplasma gondii antibodies in HIV and non-HIV infected Thai pregnant women. *Asian Pacific Journal of Allergy and Immunology*, 19, 291-294.
- WEISS, L. M. & DUBEY, J. P. 2009. Toxoplasmosis: A history of clinical observations. *International journal for parasitology*, 39, 895-901.
- WEISS, L. M. & KIM, K. 2011. *Toxoplasma gondii: the model apicomplexan. Perspectives and methods*, Academic Press.
- WONG, S.-Y. & REMINGTON, J. S. 1994. Toxoplasmosis in pregnancy. *Clinical Infectious Diseases*, 853-861.

Appendix 1: Budget

ITEMS	UNIT	QUANTITY	UNIT PRICE (KWACHA)	TOTAL (KWACHA)
1.0 STATIONERY				
1.2 Printing, photocopying, binding				3000
1.3 Poster				1000
1.4 Test kits		420		7500
2.0 Graduate Forum				
2.1 Graduate forum at Livingstone (1 st year)				1000
2.2 Graduate forum at Livingstone (2 nd year)				1000
3.0 Publication				
Publishing fee				7000
4.0 Graduation				
4.1 Graduation gown				1500
4.2 Graduation fees/expenses				1000
5.0 Compensation for Nurses During data collection				2500
SUBTOTAL				22,500
6.0 Miscellaneous				2000
GRAND TOTAL				24,550

Appendix 2: Timeline

ACTIVITY	YEARS IN QUARTERS (Q)					
	YEAR 1				YEAR 2	
	2014	2015			2015	2016
Registration	3 RD QUARTER	1 ST QUARTER	2 ND QUARTER	3 RD QUARTER	4 TH QUARTER	1 ST QUARTER
Proposal writing						
Proposal presentation						
Ethical approval						
Data collection						
Data management and Analysis						
Thesis defense and submission						

Appendix 3: Questionnaire

SECTION 1: BACKGROUND INFORMATION

I am going to begin by asking you some basic questions about yourself.

No.	Questions	Coding Categories		SKIP
Q101	In what month and year were you born?	Month [][]	Year [][][][]	
Q102	How old were you at your last birthday?	[][]		
Q103	Have you ever attended school?	Yes No	1 2	If No: Q201
Q104	What is the highest level of education you have attained?	None Primary Secondary Tertiary	00 1 2 3	
Q105	Which religion do you belong to?	Christianity Muslim Hindu Other Specify _____	1 2 3 4	
Q106	What is your current marital status?	Married Cohabiting (staying together but not married) Never been married Divorced or separated Widowed Other: _____	1 2 3 4 5 66	
Q107	As you know, some people take up jobs for which they are paid in cash or kind. Others sell things, have a small business or work on the family farm or in the family business. In the last 3 months, have you done any of these things or any other work?	Yes No	1 2	If no: Skip to Q108

Q108	What is your occupation, that is, what kind of work do you mainly do? (Probe: Anything else?) Multiple responses ok. Circle all mentioned.	Farming / Agriculture Other labor, e.g., construction Sell things at market Professional/Administrative Other: _____	1 2 3 4 66	
Q109	How much do you earn per month?	Below K3000 Between K3000 and K5000 Above K5000	1 2 3	
Q110	What tribe are you?	Tonga Bemba Nyanja Lozi Kaonde Luvale Other Specify _____	1 2 3 4 5 6	
Q111	Where do you live?	_____		
Q112	How long have you lived in your current residence?	_____		
Q113	How old is your pregnancy?	Gestation in Weeks _____ First trimester Second trimester Third trimester	1 2 3	
Q114	How many children have you given birth to			
Q115	Have you received organ transplant or blood transfusion before? (Multiple response possible)	Organ transplant Blood transfusion	Yes No [1] [2] [1] [2]	
Q116	When did you receive it/them?			

--END OF SECTION 1--

SECTION 2: HIV STATUS (HIV STATUS IS NECESSARY FOR THE STUDY SO INFORMATION WILL BE GATHERED WITH PERMISSION FROM PARTICIPANTS)

	Questions	Coding Categories		SKIP
Q201	What is the HIV status of the respondent? (check from patient files since it is indicated)	Positive	1 2 3	
		Negative		
		Results not indicated		

--END OF SECTION 2--

SECTION 3: ASSESSMENT OF EXPOSURE TO TOXOPLASMOSIS

	Questions	Coding Categories		SKIP
Q301	Do you have a cat or dog at home? (Multiple response possible)	Cat Dog	Yes No [1] [2] [1] [2]	
Q302	Do you eat meat?	Yes No	1 2	If No: Skip to Q307
Q303	How often do you eat meat in a week?			
Q303	What types of meat do you like eating? (Circle all mentioned)	Beef Mutton Chevon (goat meat) Chicken meat Pork Other Specify _____	Yes No [1] [2] [1] [2] [1] [2] [1] [2]	

			[1] [2]	
Q304	How long do you cook your meat before eating? (Ask for time in minutes)	_____		
Q305	Do you eat cured meat?	Yes No	1 2	If No: Skip to Q307
Q306	How often do you eat cured meat in a week?			
Q307	Do you eat raw vegetables?	Yes No	1 2	If No: Skip to Q309
Q308	How often do you eat raw vegetables?	_____		
Q309	Do you drink raw fresh milk?	Yes No	1 2	If No: Skip to Q312
Q310	How often do you drink raw fresh milk?	_____		
Q311	Do you boil your fresh milk before drinking?	Yes No	1 2	
Q312	What is your main source of drinking water?	_____		
Q313	Do you treat or boil your water before drinking?	Yes, always Yes, sometimes Not at all	1 2 3	

--END OF SECTION 3--

SECTION 4: KNOWLEDGE ON TOXOPLASMOSIS

No.	Questions	Coding Categories		SKIP
Q401	Have you heard about the disease called Toxoplasmosis?	Yes No	1 2	If No: Skip to Q408
Q402	If yes, What is it?	_____		
Q403	Do you know how Toxoplasmosis is transmitted?	Yes No	1 2	If No: Skip to Q405
Q404	If yes, how is Toxoplasmosis transmitted?	_____		
Q405	Have you been tested for Toxoplasmosis before?	Yes No	1 2	if No: Skip to Q408
Q406	If yes, how long ago were you tested for toxoplasmosis?	_____		
Q407	What was the result of the test?	Positive Negative	1 2	
Q408	Have you been taking your Sulphadoxine+ Pyrimethime (Fancida)?	YES NO Specify.....1 st , 2 nd , 3 rd	1 2 3	
Q409	Has the respondent been tested for Toxoplasmosis?	YES NO	1 2	
Q410	What are the Toxoplasmosis test results?	Positive Negative	1 2	
	Thank you for making time to take part in this study...			

--END --

Appendix 4: Information Sheet and Consent form

UNIVERSITY OF ZAMBIA SCHOOL OF MEDICINE;

DEPARTMENT OF PUBLIC HEALTH

INFORMATION SHEET

Study Title: Seroprevalence and determinants of Toxoplasmosis in pregnant women attending Antenatal Clinic at the University Teaching Hospital, Lusaka, Zambia

Principal Investigator: Christiana Frimpong

IRB No.:

Purpose of research project

This study is part of my programme of training in Public health with the University of Zambia medical school. The study is to find out the number of pregnant women attending antenatal clinic who have Toxoplasmosis. I also want to find out what causes it.

I want to find out if the disease is high in HIV positive pregnant women more than HIV negative pregnant women or not.

Why you are being asked to participate?

People needed for this study are pregnant women coming for antenatal clinic. You are asked to take part in this study because you are a pregnant woman.

A total of 411 pregnant women are needed for this study.

Procedures

If you agree to take part in this study:

- You will be interviewed. This interview will take about 15 minutes. The questions are to find out what you know about the disease. Again, to know how you got it if you do have it. It will be done in a private place. With your permission, 2mls of venous blood will be drawn from you for testing in the lab. With a Toxoplasma serological test kit, the blood will be tested for the disease Toxoplasmosis. If the hospital is going to draw blood from you for routine tests, then with your permission, 2mls of blood will be taken from what they draw from you for the testing. Again, with your permission, your HIV status will be checked and recorded from your folder. Your results will be confidential. You will get to know your results through your doctor later.

Risks/discomforts

This exercise will involve just a little pain due to the needle prick in the drawing of blood. It will be the only discomfort in this study.

Benefits

Participants will get to know their test results through their doctors and also those who will test positive will be referred to the doctors for treatment.

This study is of benefit to all pregnant women and their babies because it can help put in place structures to protect them from the disease in future as well.

Payment

There is no payment for participating in this study

Protecting data confidentiality

Steps will be put in place to protect the information you give us. Nobody outside the study team will get to know your information or results. The collected data will be locked in a secured place and they will be destroyed within 3 years.

What happens if you do not want to participate in the study?

You are free to decide whether you want to take part in this study or not. This will not affect the care you receive.

Who do I call if I have questions or problems?

- In case of any problem contact me, Christiana Frimpong (Investigator)

Address: University of Zambia School of Medicine

Department of Public Health

P.O Box 50110

Lusaka

Telephone: +260-968-131927

- ERES Ethics Committee office for any ethical queries. The Ethics Committee contact information is:

Address: 33 Joseph Mwilwa, P/B, 125, Lusaka

Telephone: +26095515563

E-mail: eresconverge@yahoo.co.uk

Appendix 5: Consent form

CONSENT TO PARTICIPATE IN RESEARCH

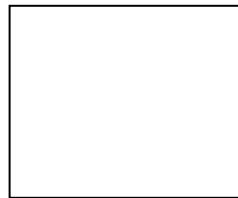
SEROPREVALENCE AND DETERMINANTS OF TOXOPLASMOSIS IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT THE UNIVERSITY TEACHING HOSPITAL (UTH), LUSAKA, ZAMBIA

I have read the information concerning the study on Seroprevalence and Determinants of Toxoplasmosis in Pregnant Women Attending Antenatal Clinic at the University Teaching Hospital (UTH), Lusaka, Zambia. I have been taken through the details of the study; the purpose, procedures, benefits and risks, and I have been given the chance to ask questions and my questions have been answered to my satisfaction.

I am also aware that I am free to withdraw from this study at any time.

By signing this document, I am agreeing to participate in this study. I have been given a copy of this form.

Signature: _____ or



Date: _____

Signature of Witness: _____

Date: _____

The Chairperson

Eres Converge IRB. Plot No: 33 Joseph Mwila Rd. Roadspark. Lusaka

+260977796839

eresconverge@yahoo.co.uk

The principal Investigator (PI) Christiana Frimpong

096-8131927

Christy.frimpong@gmail.com

University of Zambia, Lusaka Zambia