SEROPREVALENCE AND RISK FACTORS OF TOXOPLASMOSIS IN INDIVIDUALS ATTENDING CHIPOKOTAMAYAMBA CLINIC IN NDOLA, ZAMBIA.

BY

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DECLARATION

The contents of the dissertation are the author's own works. The dissertation has not previously been submitted for the award of a degree to any University

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CERTIFICATE OF APPROVAL

This dissertation submitted by **Victor M. Daka** has been approved as fulfillment for the award of the degree of MASTERS OF SCIENCE IN ONE HEALTH ANALYTICAL EPIDEMIOLOGY at the University of Zambia.

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ABSTRACT

Toxoplasmosis is a zoonotic parasitic infection caused by *Toxoplasma gondii*. It is estimated to infect a third of the world's population although the majority of the infections are largely asymptomatic. In HIV/ AIDS patients toxoplasmosis is capable of causing debilitating disease and is of significance when vertically transmitted to the unborn fetus causing serious disease sequale. There is limited data on the epidemiology of toxoplasmosis in Zambia which is important for shaping public health policy. In this study we determined seroprevalence of *Toxoplasma gondii* IgG among individuals attending Chipokotamayamba clinic located in Ndola, Zambia.

The study was cross-sectional; employed the Enzyme Linked Immunosorbent Assay (ELISA) method, to determine individual serostatus to *Toxoplasma gondii* IgG and a structured questionnaire to collect the data on potential risk factors. Seroprevalence was determined through frequency distribution of serostatus, and statistical significance of the potential risk factors was assessed using multivariate logistic regression.

Four hundred and eight (408) individuals participated in the study. Seroprevalence was 10.8%. The seroprevalence of toxoplasmosis by HIV status was 9.5% and 12.4% in HIV negative and HIV positive individuals respectively. Seroprevalence of toxoplasmosis was 9.2% among women of child bearing age (15-44 years). No statistically significant difference in *T. gondii* seroprevalence was demonstrated among sexes (p=0.44), occupation (p=0.58), HIV status (p=0.12) and residence (p=0.54).No significant association between *T. gondii* seroprevalence and history of contact with cats, cleaning the cat litter box, eating raw or undercooked vegetables, eating raw or undercooked meat and being in regular contact with soil or a soil related occupation was found.

The findings from this study indicate that toxoplasmosis is endemic in the population attending Chipokotamayamba clinic and could be of clinical significance in the management of individuals at risk of cerebral and congenital toxoplasmosis. Most women attending Chipokotamayamba clinic are susceptible to acute *Toxoplasma* infection and should be educated about ways to minimize exposure to *T. gondii.*

We recommend health education on ways to avoid *T. gondii* infection as well as regular screening for toxoplasmosis in HIV positive individuals and pregnant mothers attending Chipokotamayamba clinic. More research is needed in Zambia to clearly define the epidemiology of Toxoplasmosis.

DEDICATION

This dissertation is dedicated to my wonderful wife, my parents and all the other family members and friends for believing in me and for sacrificing their time and effort in supporting me in my education.

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LIST OF ABBREVIATIONS

%	Percent
AIDS	Acquired Immunodeficiency Virus
ART	Anti-Retroviral Therapy
CD4	Cluster Differentiation
CI	Confidence Interval
CNS	Central Nervous System
CSO	Central Statistical Office
CT	Cerebral toxoplasmosis
DAT	Direct Agglutination test
DNA	Deoxyribonucleic Acid
DRGS	Directorate of Research and Graduate Studies
DT	Dye Test
ELISA	Enzyme Linked Immunosorbent Assay
Fig	Figure
HIV	Human Immunodeficiency Virus
IFA	Indirect flurescent antibody
IgA	Immunoglobulin A

IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
MAT	Modified Agglutination test
MRI	Magnetic Resonance Imaging
NAC	National AIDS council
NDHMT	Ndola District Health Management Team
OIE	World organization for animal health
OPD	Out Patient's Department
OR	Odds Ratio
PCR	polymerase Chain Reaction
SACIDS	Southern African Centre of Infectious Disease Surveillance
SP	Sulfadoxine Pyrimethamine
TDRC	Tropical Diseases Research Centre
μm	Micrometer
UNZA	University of Zambia

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CHAPTER 1

1.1 INTRODUCTION

Toxoplasmosis is a zoonotic parasitic infection caused by an obligate intracellular coccidian protozoan parasite *Toxoplasma gondii* (*T. gondii*) belonging to the phylum Apicomplexa, class Sporozoasida, and order Eucoccidiida (Ogoina, *et al*., 2013). It is a facultative heteroxenous parasite whose definitive hosts are members of the *Felidae* species, but is capable of infecting mammals, birds and reptiles as intermediate hosts (Kristiah, 2009).

Toxoplasma gondii has a ubiquitous distribution being able to develop in a wide variety of hosts such as humans, dogs, pigs, rodents, cattle among others (Sedlak & Bartova, 2006). It is estimated that more than one billion people worldwide are infected by toxoplasmosis (Switaj, *et al*., 2005). It has been regarded as the one of the most successful parasites on Earth because of its broad host range, worldwide distribution, high infection rates and its ability to maintain a benign existence in its host (Kristiah, *et al*., 2011).

Transmission to humans is through direct contact with fecal material infected with *T. gondii* oocysts, consumption of undercooked meat or vegetables contaminated with *T. gondii* oocysts and congenital transmission from an infected mother to the unborn foetus (Muhie & Keskes, 2014). Infection in humans is usually as a consequence of consumption of contaminated undercooked meat and contact with soil infected with *T. gondii* oocysts in individuals involved in gardening (Stray-Pederson, 1992). Children that come into contact with contaminated soil and sandboxes may also get infected (Overton & Bennet, 2010). Infected pigs have been known the most likely meat source of *T. gondii* infection for people in many countries (da Silva, *et al*.,

2010). Infection is possible in as much as 25% of pork, 15% of lamb and 5% of beef (Stray-Pederson, 1992). Unwashed fruits and berries may have *T. gondii* oocyts on their surface and may be a source of infection to humans (Overton & Bennet, 2010). Consumption of unpasteurized milk from infected animals has been known to be a risk factor for *T. gondii* infection as well as ingestion of oocysts from contaminated hands that have come into contact with infected cat faeces (Boyer & Mcleod, 2004). Although very few cases have been reported, blood transfusion and organ transplants have been cited as potential sources of toxoplasmosis infection (Holliman, 2003). Stroking and playing with domestic cats is not infectious but contact with cat litter can be (Overton & Bennet, 2010). Trans-placental transmission to the unborn foetus may result in congenital disease leading to potentially serious sequela such as microcephaly, hydrocephalus, blindness and mental retardation (Boyer & Mcleod, 2004). The risk of transmission to the foetus depends on the gestation age of the mother when infection occurs, with risk highest in the last part of the third trimester when up to ninety percent (90%) of the fetuses can be infected compared to 10% in the first trimester (Remington & Desmonts, 1990). Very early infections (e.g. in the first trimester) may result in death *in-utero* or birth of a newborn with severe central nervous system (CNS) involvement such as cerebral calcifications and hydrocephalus (Ferguson, *et al*., 2013). However, the risk of sequela is less as the pregnancy advances with maternal infection in the latter stages of the pregnancy producing asymptomatic infants with latent infection (Overton & Bennet, 2010).

Co-infection with other pathogens in humans infected with the Human Immunodeficiency Virus-1 (HIV-1) may enhance progression of the disease to the Acquired Immunodeficiency Syndrome (AIDS) (Lin & Bowman, 1992). Toxoplasmosis, in individuals infected with HIV infection, is as a result of reactivation of latent infection and is one of the most frequent opportunistic infections

causing focal intercerebral lesions that complicate AIDS (Nissapatorn, *et al*., 2004). The clinical presentation of toxoplasmosis in individuals with HIV/AIDS is called cerebral toxoplasmosis (CT) and presents diagnostic and therapeutic challenges for health personnel attending to them especially in developing countries where infrastructure is limited but the number of HIV infected individuals continues to increase (Israelski & Remington, 1992). CT is undoubtedly a serious life threatening infection but can be treated provided there is prompt diagnosis and treatment (Nissapatorn, *et al*., 2004).

1.2 PROBLEM STATEMENT

Cerebral Toxoplasmosis is one of the most common CNS opportunistic infections in HIV infected individuals and also the most common cause of focal deficits in patients with AIDS (Porter & Sande, 1992). Human Immunodeficiency Virus prevalence in Ndola district is estimated to be 12.3% (CSO, 2007) providing an inherent risk of CT in individuals that may be co-infected with HIV and toxoplasmosis.

Detailed recent demographic and epidemiological data of groups at risk of toxoplasmosis or that of the general population in Zambia is missing and more knowledge, such as seroprevalence of infection, is needed at regional level to implement solutions aimed at reducing the risk for this disease in Zambia. Currently, differential diagnosis of toxoplasmosis is difficult and is merely based on clinical symptoms in the new-borns and in HIV/AIDS patients. The diagnosis of toxoplasmosis is complicated and may involve the use of multiple investigative techniques in combination with clinical signs which poses a serious diagnostic challenge. In addition to this, the clinical signs of toxoplasmosis are non-specific and may mimic several other infectious diseases (Hill & Dubey, 2002) making toxoplasmosis a difficult disease to manage.

This study will provide the much needed information on the epidemiology of toxoplasmosis. This information will enable clinicians and policy makers to be aware of the prevailing prevalence of toxoplasmosis in patients attending Chipokotamayamba clinic thereby helping in the initiating, planning and implementing interventions that will ultimately prevent and control toxoplasmosis among at-risk populations in Zambia. This study will also help policy makers plan adequately for the management of toxoplasmosis among patients attending Chipokotamayamba clinic.

1.3 OBJECTIVES

1.3.1 General Objective

To investigate the seroprevalence of toxoplasmosis and its risk factors among HIV positive and HIV negative individuals attending the outpatient's department at Chipokotamayamba clinic in Chifubu in Ndola district.

1.3.2 Specific Objectives

1. To determine the seroprevalence of toxoplasmosis among HIV positive and HIV negative patients attending Chipokotamayamba clinic in Ndola district
2. To determine risk factors associated with toxoplasmosis seropositivity among HIV positive and HIV negative patients attending Chipokotamayamba clinic

CHAPTER 2

2.1 LITERATURE REVIEW

2.1.1 Historical perspectives of toxoplasmosis

Toxoplasma gondii was discovered by scientists Nicole and Manceaux while conducting research in Leishmaniasis in Tunisia in 1908 (Dubey & Jones, 2008). At about the same time, a similar organism was found in Brazil by Splendor (Weiss & Dubey, 2009). The pathogenic potential of *T. gondii* was only recognized in the 1920s and 1930s in children presenting with symptoms such as intraocular lesions, encephalitis, retinochoroiditis and hydrocephalus (Innes, 2010). For the next thirty years, organisms similar to *T. gondii* were found in several other hosts (Dubey, 2008). In the 1950s, *T. gondii* was found to be a major cause of abortion in sheep leading to theories about its transmission and also about the possible impact on congenital transmission in humans (Innes, 2010). The discovery of the cat as the definitive host in the 1960s helped to elucidate the life cycle (Weiss & Kim, 2014). The shedding of oocysts and their subsequent sporulation with spores surviving in the environment for months helped to understand transmission and survival mechanisms of *T. gondii* (Dubey, 1996). In the 1980s, coinciding with the advent of HIV and AIDS, toxoplasmosis emerged as the major cause of death in patients with AIDS (Nissapatorn, *et al*., 2004).

2.1.2 Life Cycle and transmission of *Toxoplasma gondii*

Toxoplasma gondii exists in three morphological forms (Wertheim, *et al.*, 2004). These are: (i) the sporozoite stage found in oocysts in cat faeces, (ii) the rapidly dividing tachyzoite stage

found during an acute infection, and (iii) the slowly dividing bradyzoite stage which is found encysted in tissues such as muscle and brain during a chronic latent infection (Petersen & Dubey, 2001). The tachyzoite is crescent shaped, with a pointed anterior end and a rounded posterior end (Fig 2). Ultrastructurally, it contains organelles such as apical rings, polar rings, conoid, rhoptries, micronemes, micropore, rough endoplasmic reticulum, golgi complex, centrioles, nucleus, mitochondrion, lipid bodies, amylopectin and dense granules (Dubey, *et al.*, 1988). Multiplication is mainly by repeated endodyogeny in which two daughter cells are produced within the parent consuming it (Dubey, *et al*., 1998). Tachyzoites spread rapidly through the blood stream infecting tissues such as the brain, muscle and the placenta and are responsible for the acute phase of the disease as they illicit a strong inflammatory response in the host (Kristiah, 2009). Gastric digestion is destructive to tachyzoites as they are sensitive to proteolytic enzymes found in the stomach (Montoya & Leinsenfeld, 2004).

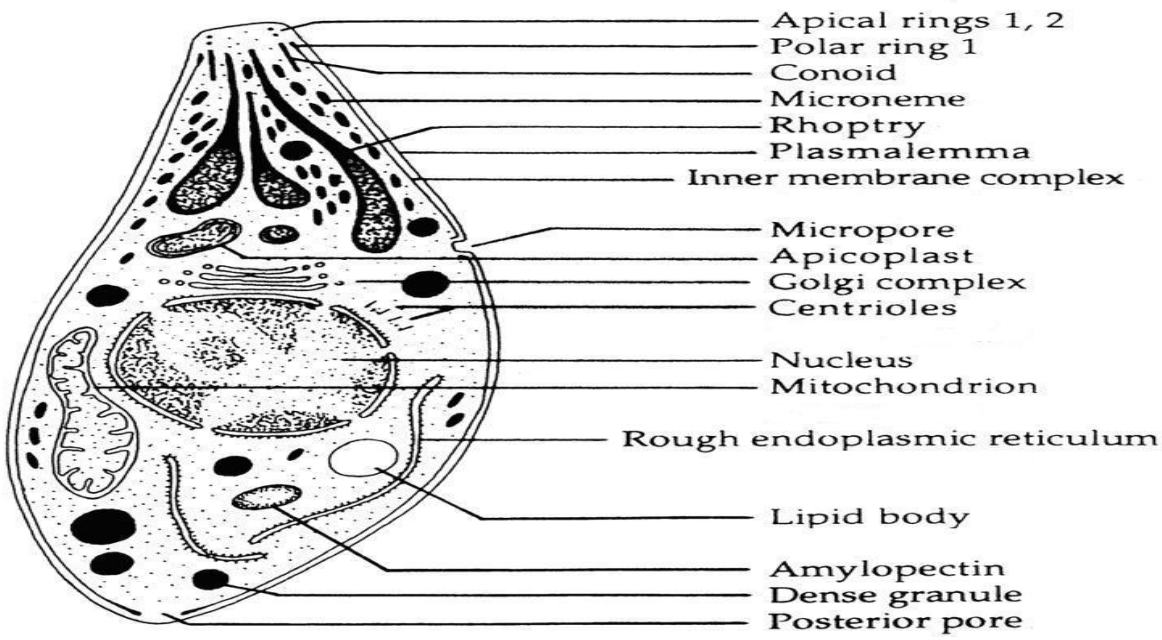

Figure 1: Diagram of *T. gondii* tachyzoite showing organelles (Adapted from Dubey, *et al*., 1998).

The development of humoral antibodies one to two weeks after infection by *T. gondii* is usually associated with destruction of tachyzoites or transformation of bradyzoites (Tenter, *et al.*, 2000). Bradyzoites, shown in figure 2, are usually found lodged in tissues and are thus called 'tissue cysts'. In contrast to tachyzoites, bradyzoites are resistant to digestion by gastric juices (pepsin and trypsin) and can persist in tissues for the lifetime of an organism allowing for transmission to another host if the organism or any of its infected parts are consumed (Dubey, 2008). Bradyzoites differ only slightly to tachyzoites, i.e. in the number of specific organelles such as rhoptries, but have essentially the same organelles (Bresciani, *et al*., 2013).

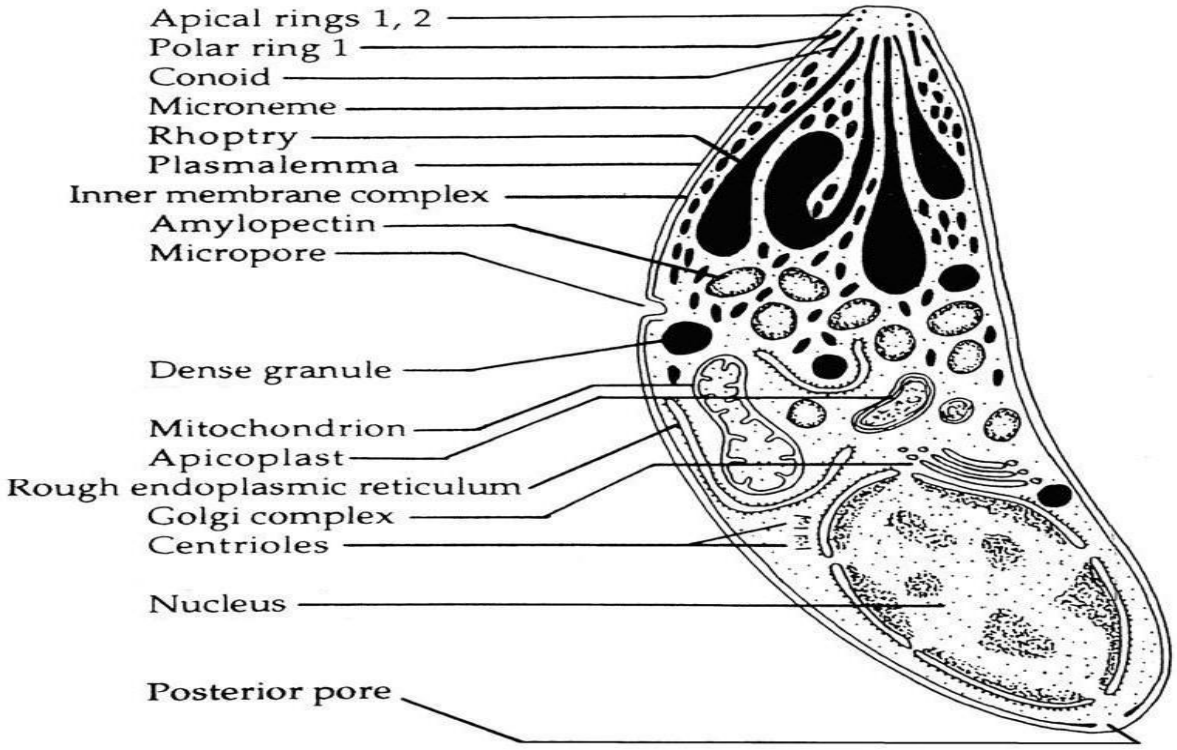

Figure 2: Diagram of *T. gondii* bradyzoites showing organelles (Adapted from Dubey, *et al*., 1998)

Oocysts develop in the intestines of the cat of the family *Felidae* (while other mammals including humans are accidental hosts) and are the form that maintains the life cycle of *T. gondii*

by sexual differentiation (Subauste, 2006). They are subsequently shed in the faeces of the cat. They are oval in shape and measure about 10-12 µm in diameter (Tenter, *et al*., 2000). The morphological features of oocysts consist of two sporocysts containing four sporozoites (figure 3).

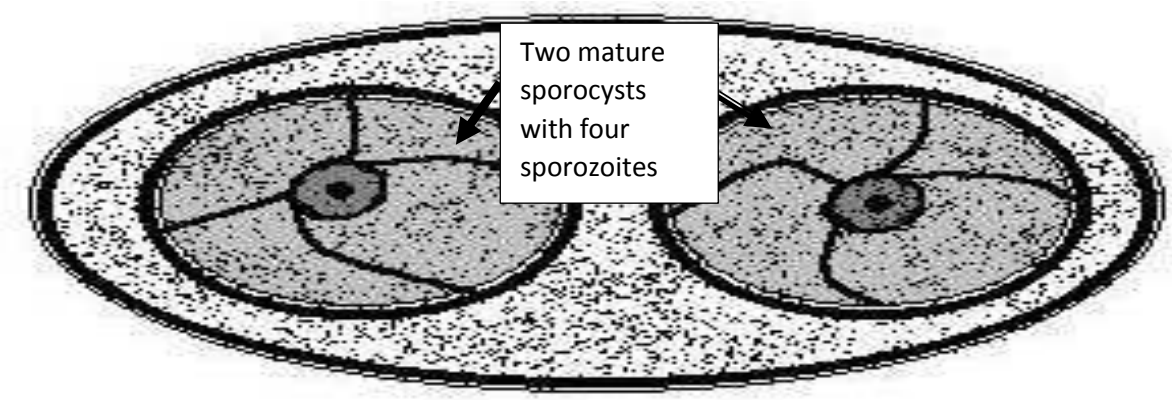

Figure 3: Diagram of a *T. gondii* oocyst (Adapted from Mohamed, 2013).

The life cycle of *T. gondii* was elucidated in 1970 (Dubey, 1996) and consists of two stages: the first being sexual reproduction in the cat which is the definitive host and the other being asexual multiplication in the intermediate host which can be any mammal such as humans or sheep or rodents (Muhie & Keskes, 2014). Figure 4 shows the life cycle of *T. gondii*

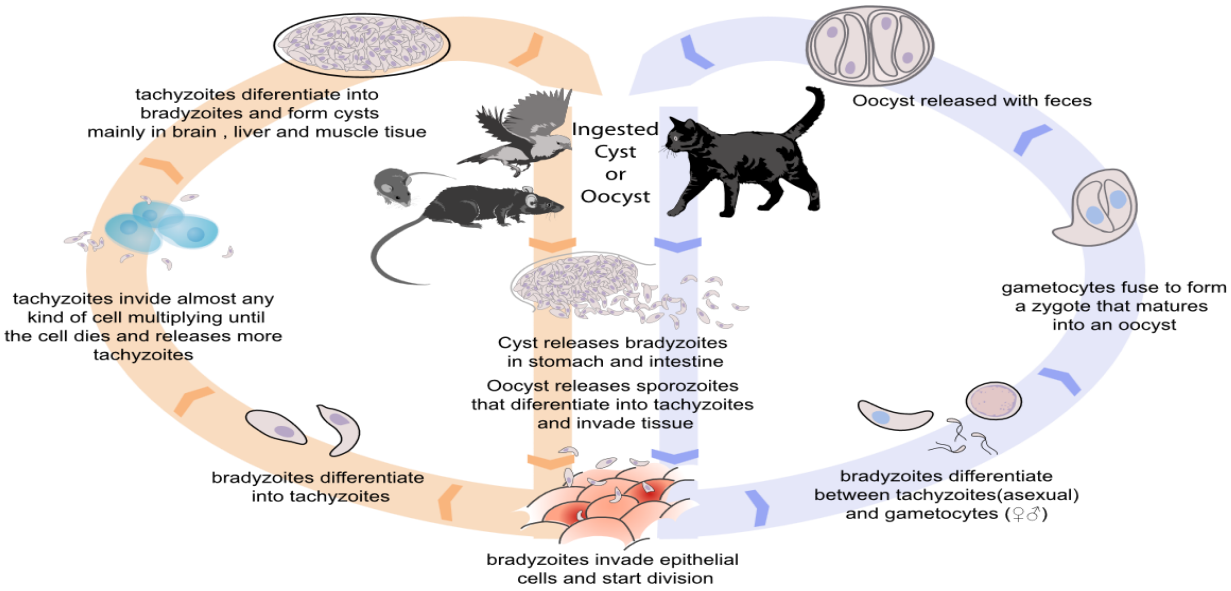

Figure 4: Life cycle of *T. gondii* (courtesy of LOH, 2014)

Cats may ingest sporulated oocysts through direct contact or may ingest tissue cysts through consumption of rodents, birds or meat contaminated with *T. gondii* (Evans, 1992). These will enter tissues and start dividing and differentiating forming zygotes and eventually oocysts which are eventually passed in cat faeces (OIE, 2008). Freshly shed oocysts are not infective but depending on the temperature and humidity of the environment may take 1-5 days to mature forming sporulated oocysts. These are the infective forms of *T. gondii* (Muhie & Keskes, 2014). The sporulated oocysts may be transmitted to humans directly through contamination by direct contact with fecal oocysts or indirectly as bradyzoites by consumption of contaminated water or food (Robert-Gangeux & Darde, 2012). Oocysts or bradyzoites transform to tachyzoites rapidly invading cells in neighboring tissues. Tachyzoites will rapidly multiply with the host cells leading to rupture of the cells, thereby releasing the parasite into the blood stream or lymphatics (Bhopale, 2003). As the host immunity gets established, the tachyzoites disappear from visceral tissues and form the latent stage of the parasite i.e. the tissue cysts. These can persist in the

intermediate host for years to life with no clinical symptom being observed (Dubey & Beattie, 1988).

The transmission of *T. gondii* takes place by one of two ways: horizontal and vertical transmission (Amuta, *et al.*, 2012). Horizontal transmission is through oral ingestion of infectious oocysts from the immediate environment or by ingestion of undercooked meat containing tissue cysts (Robert-Gangeux & Darde, 2012). Vertical transmission may occur by transplacental transfer of tachyzoites from an acutely infected mother to the unborn fetus (Dubey, 1991).

2.1.3 Pathogenesis and clinical signs of toxoplasmosis in animals

Feline toxoplasmosis is a multi-systemic disease which may cause clinical disease in domestic and wild animals (Dubey, 2010)**.** It is a major cause of abortion and mortality in animals leading to economic losses as well as lowering the food basket with effects on family health (Jadoon, *et al*., 2009). In cats, which are the definitive hosts, toxoplasmosis is usually asymptomatic (Darabus, *et al*., 2011). In a study done in Iran on wild and domestic dogs, it was found that dogs, unlike cats, are mechanical vectors and do not show specific clinical signs (Shadfar, *et al*., 2012). Toxoplasmosis has been known to be a major cause of abortion and stillbirth in sheep and goats (Jadoon, *et al*., 2009; EFSA, 2007). Prevalence in herbivores has been seen to be highest in spring while in autumn and winter the prevalence has been seen to be highest in pigs (Darabus, *et al*., 2011). In swine, toxoplasmosis causes reproductive disorders such as premature birth with pneumonia, myocarditis and encephalitis (EFSA, 2007). However, most cases of Toxoplasmosis may be asymptomatic with mild non-specific symptoms such as hyperthermia, anorexia and tachypnea (Dubey & Beattie, 1988). Infectivity rates of toxoplasmosis in cattle are low with

clinical signs not usually observed. In some rare instances, however, *T. gondii* has been isolated in aborted fetuses (Dubey, *et al.*, 2012).

2.1.4 Pathogenesis and clinical signs of toxoplasmosis in humans

Toxoplasmosis in humans is a debilitating disease whose clinical presentation may vary from being asymptomatic to serious disease sequealae such as central nervous system (CNS) signs to death in infected individuals (Bujor-Moraru & Ispas, 2011). The clinical manifestation of toxoplasmosis depends on several factors such as; innoculum dose, virulence of the toxoplasma strain, genetic background of the individual, and the immunocompetency of the individual infected (Montoya & Liensenfeld, 2004). In many hosts infected by toxoplasmosis and harboring latent tissue cysts, the parasite has an affinity for neural and muscular tissues though it may be found in visceral organs such as the liver, lung and kidneys (Dubey, *et al*., 1998). In humans whose immune system is not compromised, *toxoplasma* infection rarely causes infection eliciting mild symptoms such as headache, generalized lymphadenopathy, fatigue, myocarditis, muscular pain, hepatitis and pulmonary necrosis (EFSA, 2007). These symptoms are largely ignored in a clinical setting with alternative clinical diagnosis being employed (EFSA, 2007).

In patients with AIDS, *T. gondii* has been implicated as the most common opportunistic infection causing severe life threatening debilitating disease in individuals with a CD 4 count less than 200 cells/µl (Nissapatorn, *et al*., 2004). Cerebral toxoplasmosis (CT) is the most common cause of cerebral focal lesions in individuals with AIDS thereby complicating the course of the disease (Kristiah, 2009). Disease in individuals with AIDS is due to reactivation of latent infection. Tissue cysts rupture with released bradyzoites multiplying and spreading to other organs (Nissapatorn, *et al*., 2004). Clinical signs may manifest as severe disseminated disease with encephalitis, meningoencephalitis, focal signs and other CNS complications (EFSA, 2007).

Severe and persistent headache which does not respond to analgesics is usually seen in AIDS patients with toxoplasmosis (Muhie & Keskes, 2014). As the disease progresses, the headache gives way to a condition characterized by confusion, lethargy, ataxia, and coma (Muhie & Keskes, 2014). Failure of the disease to resolve results in death of individuals (Nissapatorn, *et al.*, 2004).

Vertical transmission occurs from primary infection of the mother during pregnancy (Jones, *et al*., 2001). The risk of transmission increases with gestational age while, in contrast, the risk of serious disease sequel in the unborn fetus reduces with gestational age (Costa, *et al*., 2012). Clinical symptoms vary with early infection causing pre-natal or post-natal death or severe damage to the fetus (Muhie & Keskes, 2014). Later infection in gestation can cause generalized disease in-utero, subsequent infection of the CNS, birth of children with disease and sequelae such as hydrocephaly, chorioretinitis or cerebral calcification (EFSA, 2007). Infection in the later stages of the third trimester or just before birth may result in the newly born being born with apparent infection, fever eruptions, hepatomegaly, splenomegaly or pneumonia (Montoya & Liensenfeld, 2004).

In 25% of patients infected with toxoplasmosis, patients may experience a permanent loss of vision in affected eyes clinically presents as ocular lesions and necrotizing retinitis (EFSA, 2007). *T gondii* is also the most common cause of retinochoroiditis in humans infected with toxoplasmosis accounting for 28% to 55% of posterior uveitis (Pavesion & Lightman, 1996).

2.1.5 Epidemiology and risk factors of toxoplasmosis

T. gondii has a worldwide distribution with varying prevalence in humans and animals among countries as well as variations between geographical locations within a country (Kristiah, *et al.*,

2011). Figure 6 shows variations in seroprevalence rates among countries in Africa.

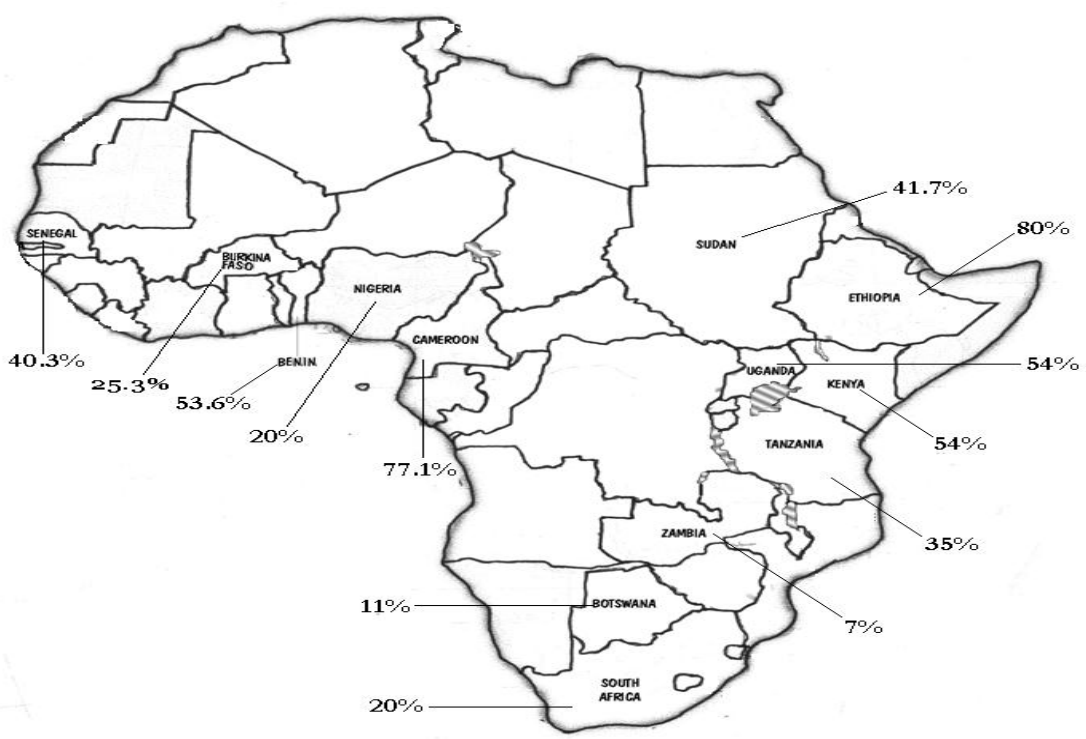

Figure 6: Variations in seroprevalence rates in humans among countries in Africa (Courtesy of Kristiah, 2009)

Epidemiological surveys in pre-teen children in lower socio-economical economies in Brazil showed that the environment was highly contaminated with toxoplasmosis with seven out of 31 soil samples contaminated with *T. gondii* oocysts (Dubey, *et al*., 2012). A similar study to investigate toxoplasmosis in free range chickens found a *T. gondii* seroprevalence of 38.4% which was a sign of environmental contamination on the farms they came from (Tilahun, *et al.*, 2013).

A study in western Romania showed that toxoplasmosis is a highly endemic disease in sheep and cats with a higher seroprevalence being found in rural regions compared to urban regions (Darabus, *et al*., 2011). A *T. gondii* seroprevalence of 33.75% was found in cats in a study in the Czech Republic (Sedlak & Bartova, 2006).

The mechanism of how dogs become infected by *T. gondii* is relatively unknown but their close association with humans serves as a good indicator of environmental contamination (Dubey, *et al*., 2012). A higher seroprevalence in stray and farm dogs compared to pet dogs possibly indicated that eating infected prey was an important source of infection (Shadfar, *et al*., 2012).

Among the food animals, infected pigs are the most likely meat source of *T. gondii* infection for people in many countries (da Silva, *et al*., 2010). Eating homemade sausages has long been considered a source of *T. gondii* infection (Glasner, *et al*., 1992b).

The epidemiology of toxoplasmosis in Zambia is relatively undefined. A study conducted to determine the seroprevalence in HIV positive and HIV negative individuals found a seroprevalence of 4% and 11% in the two groups respectively (Zumla, *et al*., 1991). Another study conducted in HIV positive individuals showed a seroprevalence of 17.9% (Sinyangwe, 2009).

Several risk factors have been identified in the transmission of *T. gondii*. These are: owning cats, eating raw or uncooked pork, lamb, mutton, beef, game or mincemeat products, eating raw or undercooked vegetables or fruits and frequent consumption of vegetables outside the home (Jones, *et al*., 2001). Other risk factors include; having poor hand hygiene, contact with soil, being in a soil related occupation, washing kitchen knives frequently and cleaning the cat litter box (Kapperud, *et al*., 1988).

2.1.6 Diagnosis of toxoplasmosis

The difficulty in diagnosing toxoplasmosis is complex with symptoms mimicking a host of other diseases (Kompalic-Cristo, *et al.*, 2004). Diagnosis is made by two broad methods i.e. identification of the agent and by serological testing (EFSA, 2007). Identification of *T. gondii*

can be made by isolation from tissues, oocyst detection in drinking water, brain imaging and the polymerase chain reaction (PCR). Histological testing is the best method for testing fetal membranes to determine if vertical transmission has occurred (OIE, 2008).

Brain imaging can be used to identify cerebral toxoplasmosis using computerized tomography (CT) and magnetic resonance imaging (MRI) in patients suspected to be infected by toxoplasmosis (Hill & Dubey, 2002).*Toxoplasma gondii* oocysts have been detected in drinking water using the method that relies on the collection of a large-volume sample of water and passing it through a cartridge filter. The filtrate is then inoculated into rodents for identification (Isaac-Renton, *et al*., 1998).

PCR has an advantage over other diagnostic methods as it is not affected by the condition of the immune system but may be prone to contamination and therefore may give false positive results (Johnson, *et al*., 1993). More recently, a real-time PCR has been developed to allow simultaneous quantification, amplification and visualization of DNA. It is very similar to existing PCR methods. After each round of amplification, fluorescent dyes intercalate with the double-stranded DNA and the results, shown on an amplification plot, allow quantification of the parasite DNA in the sample (EFSA, 2007). The real-time PCR is a highly sensitive and specific method, however it is expensive and requires specialized detection systems and therefore may only be cost-effective in laboratories where analysis of large numbers of samples is carried out in batches (OIE, 2008).

Serological tests are used to detect increased antibody levels such as IgG and IgM in the serum of patients with toxoplasmosis with elevated levels of IgG antibodies indicating infection occurred at some point (Tekkesin, 2012). Several serological tests are available for the detection of *T. gondii* antibodies namely; the Dye test (DT), Indirect Fluorescent antibody (IFA) test,

Direct agglutination test (DAT) and the Enzyme linked immunosorbent assay (ELISA) (OIE, 2008). The first to appear is IgM which peaks after two weeks followed by IgA and IgE (Montoya, 2002). IgG peaks after four months and persists at low levels throughout the entirety of one's life. Serological teats are not without limitations. Detection of antibodies in immunocompromised individuals may be difficult due to the deterioration of the immune system (Kristiah, 2009).

2.1.7 Treatment of toxoplasmosis

In healthy individuals toxoplasmosis resolves without the need for drug intervention (Muhie & Keskes, 2014). The need for possible treatment in immunocompetent individuals with mild symptoms has not been demonstrated (EFSA, 2007). The treatment of choice in immunocompromised individuals with toxoplasmosis is a combination of pyrimethamine and sulfadiazine (EFSA, 2007). This treatment should be given continually until there is improvement in their condition. For AIDS patients, the continuation of the medication for the rest of their life or while they are immunocompromised may be necessary (Nissapatorn, *et al*., 2004).

Pregnant women, newborns and infants can be treated with pyrmethamine and sulfadoxine although the infection is not eradicated completely (Muhie & Keskes, 2014). Spiramycine (a macrolide) is the antibiotic used for treatment of toxoplasmosis for most pregnant women to prevent infection to the unborn fetus (Overton & Bennet, 2010). In latent infection, treatment is not effective as antibiotics are not able to reach bradyzoites in sufficient concentrations (Muhie & Keskes, 2014; EFSA, 2007). Atovaquone has been used for treatment against *Toxoplasma* cysts in AIDS patients (Muhie & Keskes, 2014).

In Zambia, HIV positive patients with a CD 4 count less than 100 and presenting with focal neurologic symptoms are treated for cerebral toxoplasmosis and monitored before treatment for other possible conditions (Erbelding & Ghanem, 2009).

2.1.8 Prevention and control

The primary aim of any prevention and control method against toxoplasmosis is to avoid contact with potential infection sources such as cats, soil, and contact with raw meat. This is particularly important in pregnant women and individuals that are immunocompromised (MDH, 2012).. Control of rodents, flies and cockroaches in human habitats is important to prevent them from serving as mechanical vectors in the transmission of toxoplasmosis (Muhie & Keskes, 2014). Other preventive measures include; washing kitchen utensils regularly, washing hands after contact with cat litter, keeping cats away from areas where children play and treatment of mothers identified with acute *Toxoplasma* infection to prevent possible congenital transmission (OIE, 2008). Educating at-risk groups such as HIV positive individuals and pregnant women on the risk factors for toxoplasmosis has been known to be effective in prevention of toxoplasmosis (Kravetz & Federman, 2005).

CHAPTER 3

3.1 MATERIALS AND METHODS

3.1.1 Study Area

This study was conducted from January to April, 2014 at Chipokotamayamba clinic in Ndola. Chipokotamayamba clinic is situated in Chifubu in Ndola district in the Copperbelt province of Zambia (Figure 7). Ndola is the second largest city in Zambia lying 12°56'0"South, 28°37'0"East with an estimated population of 455,194 (CSO, 2010). It is a high density area with the population being generally middle class in socio-economic status.

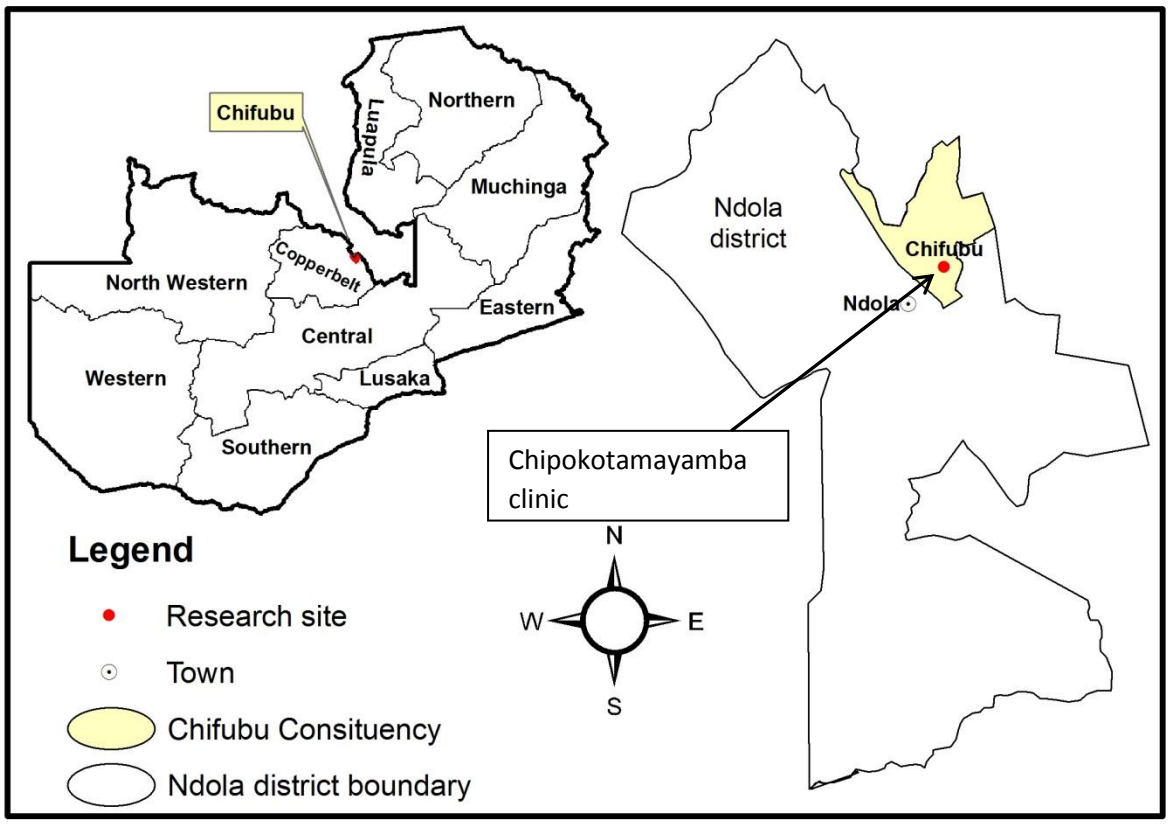

Figure 7: Map showing the location of study site

Chifubu is representative of urban situations throughout Zambia with its population living in small, closely spaced houses built on standard one or two-bedroomed floor plans. The houses have electricity, piped city water, and a flush toilet but the infrastructure is often in a poor state

of repair due to the stress placed on the ageing and unmaintained structures. Civic authorities identified crowding as their most important problem (Stuebling, 1997). The catchment area for the clinic is mainly the population residing in Chifubu but a good number also come from surrounding areas such as Kansenshi, Kansenshi Extension, Pamodzi, Kawama and Mitengo rendering the population fairly homogenous in distribution. The Bemba language is the most commonly used form of communication among the inhabitants of Chifubu followed by English.

Chipokotamayamba clinic provides general medical services to patients such as routine screening and testing for malaria, TB, HIV and other minor but significant cases. Specialist services such as the antenatal clinic and anti-retroviral services (ART) are also provided.

3.1.2 Study design and Sampling

The study was a facility-based cross-sectional study involving patients attending the Outpatient Department (OPD) at Chipokotamayamba clinic in Ndola. The clinic receives approximately 500 patients every week for various services. Systematic sampling was used to recruit participants into the study but only those individuals who consented or assented to participate in the study were included in the study. A semi-structured questionnaire was administered to consenting individuals followed by blood collection for HIV and *toxoplasma* testing.

3.1.3 Sample Size Calculation

The sample size required for this study was calculated using the sample size formula for comparing two proportions (Eng, 2003) represented by the formula below:

$$n = \frac{2(\bar{p})(1-\bar{p})(Z_{\beta} + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

Where: n is the sample size required in each group for the study

P_1 is referential estimate of the prevalence of population 1

P_2 is the referential estimate of the prevalence of population 2

ṕ is the average of the two estimates P_1 and P_2

Z_β represents the desired statistical power

$Z_{\alpha/2}$ represents the desired level of statistical significance

The referential proportions used to obtain the required sample size were based on the prevalence by Zumla *et al* (1991), where the prevalence for the HIV positive group taken as P_1 in this case was 4% while the prevalence for the HIV negative group taken as P_2 in this case was 11%. A statistical power of 80% and a 95% confidence level was used to come up with a required sample size of 219 for each group to be studied (Select, 2013). The required sample size was not met for the HIV positive group but this was mitigated by using the benchmark used of an attrition rate of 20% for missing data (Mason, 1999).

3.1.4 Ethical Considerations

Approval to conduct the study was obtained from the Directorate of Research and Graduate studies (DRGS) through the Assistant Dean, Postgraduate (Appendix 9). Ethical clearance to conduct the study was sought and granted from ERES converge IRB (IRB No 00005948) (Appendix 10). Permission to conduct the study at Chipokotamayamba clinic was granted by the Permanent Secretary from the ministry of Community Development Mother and Child Health (Appendix 11) and finally from the Ndola District Health Management Team (NDHMT) through the Provincial Health Office (Appendix 12).

Patient information and results were treated with the utmost confidentiality and all the information in the study was restricted to the researcher, supervisors and clinicians involved in

the study only. Study participant's serum was used for testing for HIV status and ELISA analysis only with prior full disclosure to the study participants. The study questionnaire, blood and serum samples were assigned and identified by means of a study number for confidentiality. The patient's file number was indicated on the questionnaire to allow for tracing back only when necessary and with consent by the principle investigator and clinic staff involved in the study. The study participants were provided with an information sheet by a community health worker who explained the details of the study in the language of their choosing before obtaining informed consent (Appendix 1-3 and 5-7).

3.1.5 Study Questionnaire Administration

A semi-structured questionnaire was first piloted at Lubuto clinic, which is of a similar setting as Chipokotamayamba clinic, for consistency, logic and clarity. Five individuals were chosen at random after which the questionnaire was administered. These did not form part of the final analysis of the main study. The questionnaire was then administered to study participants in the main study asking and probing questions on demographics, and potential risk factors for toxoplasmosis in the language of their choosing (Appendix 4 and 8).

3.1.6 Blood Collection and Aliquoting

Four millilitres of venous whole blood was collected from the antecubital vein into sterile plain vacutainer containers. Blood was allowed to clot and then centrifuged for 10 minutes at 1500 rpm. Serum was separated and transferred to cryovials. An aliquot was made of the serum and used for HIV testing. The study participants who were interested in knowing their HIV status were referred to the counselors in the Anti-Retroviral Therapy (ART) clinic for counseling and possible enrolment into the ART programme if found positive.

The remaining serum was stored at -20^{o}C and transported once weekly to the Tropical Diseases Research Centre (TDRC) under cold chain where the serum was stored at -20^{o}C while awaiting analysis for *T. gondii* IgG.

The HIV testing was done using the Zambia National algorithm (Fig 8). Testing was first done using the Determine™ test (Abbott Diagnostic Division, Hoofddrop, Netherlands). This is an *in-vitro* qualitative immunochromatographic immunoassay for the determination of antibodies to HIV-1 and HIV-2.

If the test was positive for Determine, then Unigold™ (Trinity Biotech, Jamestown, Ireland), a follow-up test was used as a confirmatory test. This is a rapid immunoassay based on the immunochromatographic sandwich principle.

If there were discordant results between Determine and Unigold, then a third test, or tie breaker was to be used. This test is the SD Bioline (SD Biostandard Diagnostics Private Limited, Gurgaon, Haryana, India). The SD Bioline is an immunochromatographic test for the qualitative detection of antibodies of all isotypes (immunoglobulin G [IgG], IgM, and IgA) specific to HIV-1 and HIV-2 simultaneously, in human serum, plasma, or whole blood.

3.1.7 Laboratory Testing for HIV

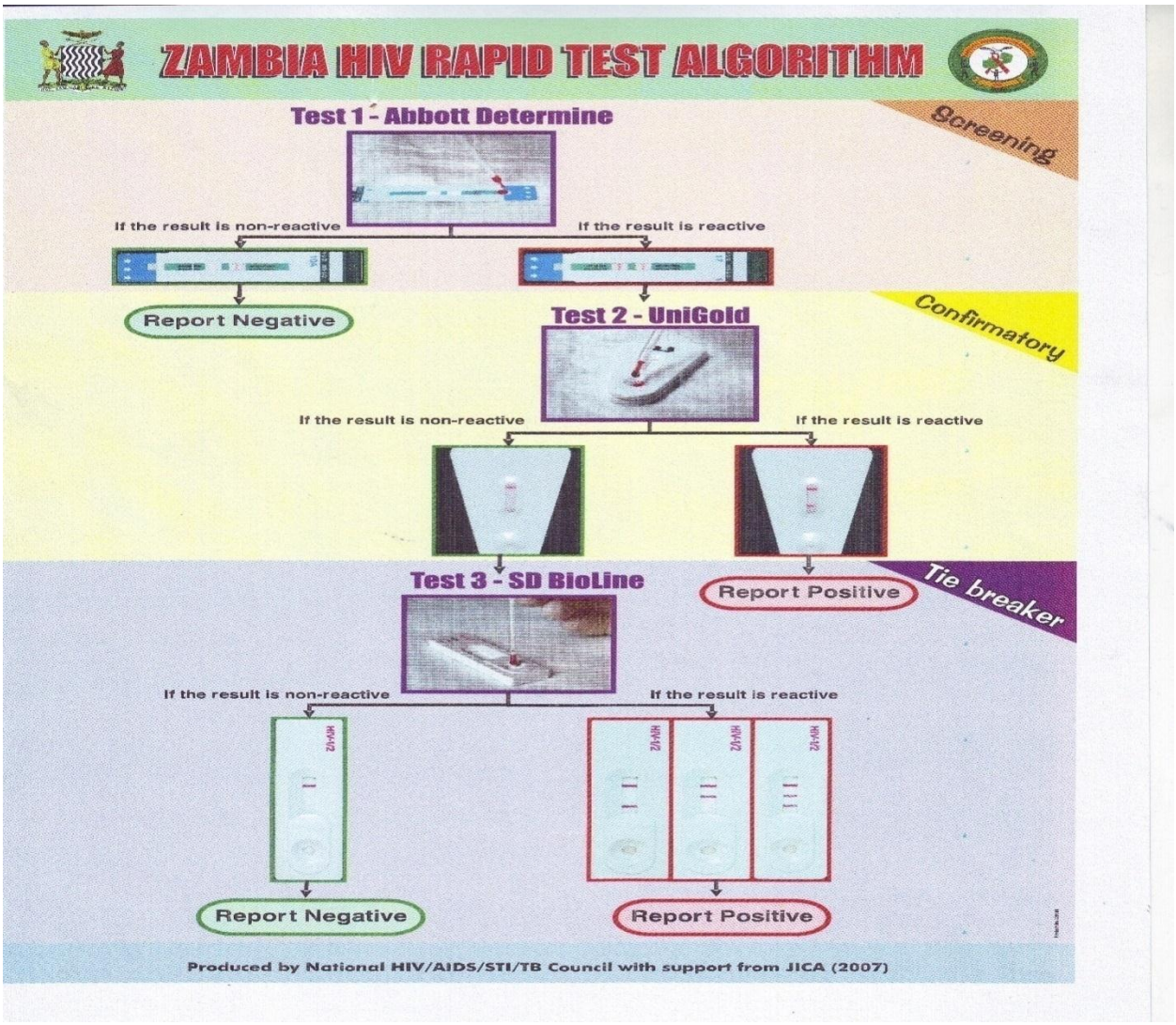

Figure 8: The national algorithm for HIV testing (Courtesy of NAC, 2007)

3.1.8 Laboratory Testing for *T. gondii*

Toxoplasmosis testing was done using the Human Toxo IgG ELISA (Human GmbH, Wiesbaden, Germany)). This test is based on the classical ELISA technique. The microtitre strip wells are coated with *Toxoplasma* antigen prepared from sonicated whole *T. gondii* parasites (Tachyzoites). The procedure was performed according to manufacturer's instructions. One hundred microlitres of pre-diluted samples were pipetted into the microtitre wells and incubated for 30 minutes at room temperature to allow corresponding specific antibodies present, if any, in

the patient's serum or controls to bind to the antigens in the wells. This was followed by a washing step, allowing all unbound antibodies to be removed and therefore not obscure any reaction. One hundred microlitres of the conjugate was then added followed by a second incubation step of thirty minutes at room temperature. The plate was then washed 5 times and 100µl of the substrate was added. An incubation period of 15 minutes at room temperature was then followed by addition of 100µl of the stop solution. The absorbance was then read using an ELISA plate reader at an absorbance of wavelength 450nm within a time frame of 30 minutes.

Test validation was evaluated using a strict criteria meeting the following requirements for the absorbances that were obtained:

1. Substrate blank in well A1 must be less than 0.150.
2. The mean negative control must be less than the mean cut off control.
3. The mean medium positive control must be less than 0.750
4. The ratio of mean positive medium control to the mean negative control were equal or greater than five.

The results for study participants were obtained by comparison with the cut off control given by;

1. Result was considered positive if the absorbance of the study participants serum at wavelength 450 nm (A_{450}) were equal or greater than the mean cut-off control (MCC) plus fifteen percent of the mean cut off control A_{450}=> MCC + 15%.
2. Result was considered negative when the absorbance of the participant's serum measured at 450 nm was less than the MCC minus fifteen percent of the MCC (A_{450}< MCC - 15%).

3.1.9 Data Management and Analysis

Results for toxoplasmosis and completed questionnaires were entered in an MS Access database of Epi Info™ 3.2.2, with in-built consistency and range checks. Double entry was used and the data was subsequently transferred to SPSS version 17 for recoding where necessary and final descriptive and statistical analyses.

The outcome (dependant) variable was sero-status to *Toxoplasma* IgG i.e. seropositive or seronegative while predictor variables were age, eating raw or undercooked meat, eating raw or undercooked vegetables, owning a cat and cleaning the cat litter box.

Descriptive statistics for each of the variables under study was presented in tables or graphs. Association between categorical variables was determined using the Chi-square test.The ages of participants were categorized according to methods used by Amuta, *et al* (2012), while women were classified into those of childbearing age using the benchmark of 15 to 44 years (Jones, *et al.,* 2001). The multiple logistic regression model was used to examine association of various predictors with the outcome. A p value less or equal to 0.05 was considered expressive of a statistically significant result for all inferential analyses.

CHAPTER 4

4.1 RESULTS

4.1.1 Seroprevalence of toxoplasmosis

A total of 408 individuals were investigated for toxoplasmosis using *Toxoplasma* IgG (Human GmbH, Wiesbaden Germany) ELISA. Of these, 44 (10.8%) individuals tested positive against *Toxoplasma* IgG. The seroprevalence by area of residence was 10.2% and 12.3% for Chifubu and areas other than Chifubu, respectively. There was no significant difference in prevalence between residences (χ^2 =0.37, p=0.54) (Table 4.1).

Table 1 Prevalence to *Toxoplasma* IgG based on residence

Residence	**n**	**Prevalence +ve (%)**	**95% C.I. for prevalence**	**P value**
Chifubu	294	30 (10.2)	6.7 – 13.7	0.54
Other	114	14 (12.3)	6.3 – 18.3	
Total	**408**	**44 (10.8)**	**7.8 –** 13.8	

n = sample size

Seroprevalence of toxoplasmosis was higher in males (12.1%) than in females (9.7%). There was no significant statistical difference in prevalence between the two sex categories (χ^2=0.61, p= 0.44) (Table 4.2).

Table 4.2 Seroprevalence of Toxoplasmosis by sex

Sex	n	Prevalence +ve (%)	95% C.I. for prevalence	P-value
Male	182	22(12.1)	7.4 – 17.0	0.44
Female	226	22(9.7)	5.8 – 13.6	
Total	**408**	**44 (10.8)**	**7.8 –** 13.8	

n = sample size

The age simple range of individuals who participated in this study was 3 years to 78 years with a mean age of 31.8 years. The age category with the greatest number of individuals enrolled into the study was the 24-33 years group, while the least was the 0-13 years age category (Fig 4.1). The seroprevalence of toxoplasmosis within the age categories of participants is shown in Table 4.3. There was no significant difference in prevalence among the age groups (χ^2=8.69, p= 0.12).

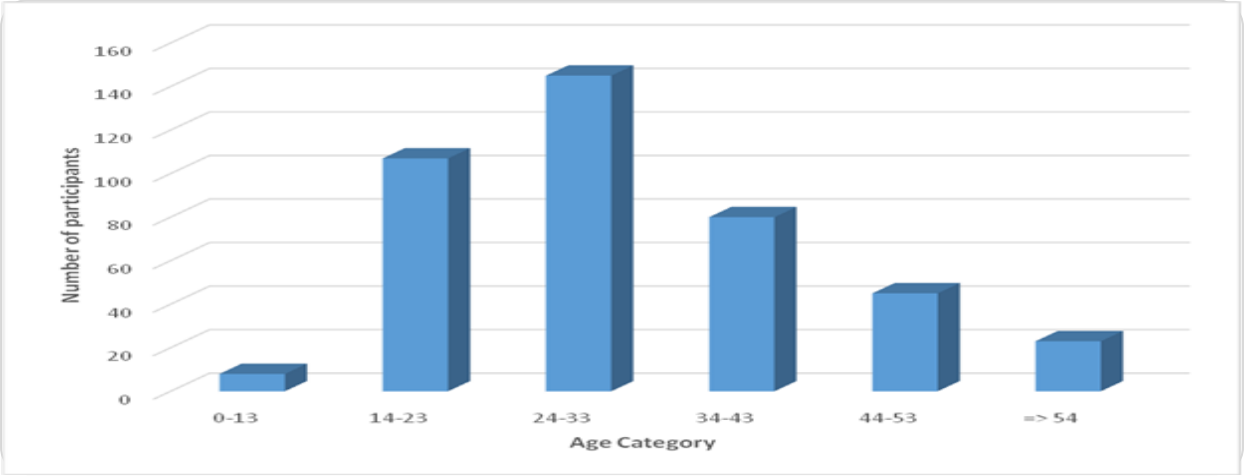

Figure 9 Frequency histogram of study participants by Age group showing the 24-33 age category to be the highest represented group

Table 4.3 Seroprevalence of toxoplasmosis by age category

Age Category	n	Prevalence +ve (%)	95% C.I. for prevalence	P-value
0-13	8	12.5	0.0 – 35.4	0.12
14-23	107	5.6	1.2 – 10.0	
24-33	144	13.2	7.7 – 18.7	
34-43	80	7.5	1.7 – 13.3	
44-53	46	15.2	5.0 – 26.2	
≥54	23	21.7	4.9 – 38.6	
Total	**408**	**10.8**	**7.8 – 13.8**	

n = sample size

The seroprevalence of toxoplasmosis within the HIV positive and HIV negative groups is summarized in Table 4.4. Toxoplasmosis seroprevalence was higher in the HIV positive (12.4%) compared to the HIV negative group (9.5%). There was no significant statistical difference in the prevalence between the two groups (χ^2=0.89, p= 0.12).

Table 4.4 Seroprevalence of Toxoplasmosis by HIV status

HIV status	n	Prevalence +ve (%)	95% C.I. for prevalence	P-value
Negative	222	21(9.5)	5.6 – 13.4	0.12
Positive	186	23(12.4)	7.7 – 17.1	
Total	**408**	**44 (10.8)**	**7.8 – 13.8**	

n = sample size

Respondents included the following occupations; housewives, business persons, students, drivers, bricklayers, those in formal employment and other occupations such as shopkeepers, bus conductors etc. The seroprevalence of toxoplasmosis by occupation is given by Table 4.5. The prevalence of toxoplasmosis was highest among farmers (22.2%) while a low seroprevalence was seen in housewives (8.3%), students (7.0%), and other (5.9%). There was no significance statistical difference in the prevalence among the different categories of occupation (χ^2=4.76, p= 0.58)

Table 4.5 Seroprevalence of toxoplasmosis by occupation

Occupation	**n**	**Prevalence +ve (%)**	**95% C.I. for prevalence**	**P-value**
Housewife	120	10(8.3)	3.4 – 13.2	0.58
Business person	63	8(12.7)	4.5 – 20.9	
Student	71	5(7.0)	1.1 – 12.9	
Farmer	36	8(22.2)	8.6 – 35.8	
Driver	34	4(11.8)	1.0 – 22.6	
Bricklayer	26	3(11.5)	0.0 – 23.8	
Formal employment	24	4(16.7)	1.8 – 31.3	
Other	34	2(5.9)	0.0 – 14.1	
Total	**408**	**44 (10.8)**	**7.8 – 13.8**	

The knowledge of respondents to toxoplasmosis is summarized in Table 4.6. Out of the 408 respondents, only 0.7% had heard of the disease toxoplasmosis. None of the 3 respondents with

knowledge of toxoplasmosis were seropositive to *Toxoplasma* IgG (0%). There was no statistically significant association between having knowledge of toxoplasmosis and being positive for the disease (χ^2=0.37, p=0.55).

Table 4.6 Association between awareness of toxoplasmosis and prevalence

Variable Response		Total respondents (%)	Prevalence (%)	95% C.I. for prevalence	P-value
Knowledge of toxoplasmosis	Yes	3(0.7)	0.00	0	0.55
	No	405(99.3)	10.9	7.8 – 13.9	

A total of 194 women were identified as being of childbearing age representing 85.1% [76.8 – 90.8] of the total women who participated in the study. The seroprevalence of *T. gondii* among women of childbearing age was found to be 9.3% [95% CI: 5.2 – 13.4].

Several risk factors for toxoplasmosis were investigated in this study and these included owning a cat, cleaning the cat litter box, eating raw or undercooked vegetables, eating raw or undercooked meat and being in regular contact with soil or in a soil related occupation. The results of the association between these factors and being positive for toxoplasmosis are shown in Table 4.7. Seroprevalence of toxoplasmosis was twice as much for individuals who owned cats (21.4%) than those who did not (10.4%). There was no significant difference in prevalence between those that owned a cat and those that did not (χ^2=1.71, p=0.19). There was no

significant difference in prevalence between respondents that cleaned the cat litter box and those that did not (χ^2=0.04, p=0.84). The seroprevalence to toxoplasmosis was comparable in individuals in regular contact with soil (11.3%) and those that reported not to (10.2%), respectively. There was no significant difference in prevalence between individuals that were in regular contact with soil and those that were not (χ^2=0.14, p=0.70. The prevalence for the two groups were 10.6% and 11.3% for those that reported regular eating of raw or undercooked vegetables and those that did not, respectively. There was no significant difference in prevalence between the two groups (χ^2=0.04, p=0.84. The seroprevalence was 11.2% and 10.5% for those that consumed raw or undercooked meat and those that did not, respectively. Similarly there was no significant difference in prevalence between those that ate raw or undercooked meat and those that did not (χ^2=0.05, p=0.82). Table 4.7 shows the seroprevalence by risk factor.

Table 4.7 Seroprevalence of toxoplasmosis and associated risk factors

Risk Factor	Response Level	No of respondent	Prevalence (%)	95% C.I. for prevalence	P-value
Owning a cat	yes	14	21.4	7.6 – 47.6	0.19
	no	394	10.4	7.8 – 13.8	
Cleaning the cat litter box	yes	5	20.0	3.6 – 62.4	0.84
	no	8	25.0	7.1 – 59.1	
Contact with soil/ soil occupation	Yes	211	11.4	7.8 – 16.4	0.70
	No	197	10.2	6.7 – 15.2	

Eating raw or undercooked vegetables	Yes	302	10.6	7.6 – 14.6	0.84
	No	106	11.3	6.6 – 18.8	
Eating raw or undercooked meat	yes	197	11.2	7.5 – 16.3	0.82
	no	210	10.5	7.0 – 15.4	

4.1.2 Predictors of Toxoplasmosis

Predictors for toxoplasmosis were investigated using a binary logistic regression model. The results indicated that none of the risk factors investigated in this study were significant predictors of being IgG positive for toxoplasmosis.

CHAPTER 5

5.1 DISCUSSION

Toxoplasmosis is an environmental disease as transmission of the infection has been shown to be promoted by poor environmental practices, poor eating habits, cat ownership, poverty, poor hygiene among others (Onadeko, *et al*., 1992).

This study was carried out at Chipokotamayamba clinic with the main objective being to determine the seroprevalence of toxoplasmosis and also to investigate any possible risk factors.

The overall seroprevalence for toxoplasmosis in individuals attending Chipokotamayamba clinic was found to be 10.8%. These results indicate that Chipokotamayamba clinic is a facility with low *T. gondii* seroprevalence in comparison to previously reported estimates of prevalence in some studies done in the region such as 20% in South Africa, 54% in Kenya and 35% in Tanzania (Kristiah, 2009). However, the results are comparable to those reported in Botswana with a seroprevalence of 11% (Kristiah, 2009). A similar study done in Zambia in 1991 found an overall seroprevalence of 7% (Zumla, *et al*., 1991). This shows a slight increase in prevalence although there may be limitations in drawing inference from this information as the populations from which the two sets of data were drawn from are different as well possible differences in research methods employed.

In this study the seroprevalence of toxoplasmosis at Chipokotamayamba clinic was comparable in HIV positive patients and HIV negative individuals (χ^2=0.89, p= 0.12). This could possible indicate equivalent exposure to the *Toxoplasma* parasite, since both study groups were selected from the same population pool. These results are supported by results from similar studies carried out from outside Zambia, including Nigeria (Ogoina, *et al*., 2013), Malaysia

(Nissapatorn, *et al*., 2002), Spain (Boto, *et al*., 1998) and Czechoslovakia (Sykora, *et al*., 1992) where there was no significant difference in seroprevalence to toxoplasmosis between HIV-infected and non-HIV infected individuals.

However contrasting findings have been reported in Nigeria (Akanmu, *et al*., 2010), Mali (Maija, *et al*., 2001) and Zambia (Zumla, *et al*., 1991) in which significant differences were found in prevalence between HIV positive and HIV negative individuals. These disparities may be due to differences in study designs, differences in study populations and environmental settings in which the different studies were carried out.

The relatively gradual increase in trend in the seroprevalence of toxoplasmosis with age suggests that adults may be more prone to infection with *T. gondii* as they would have had more possible encounters with potential sources of infection (Ertug, *et al*., 2005). The comparatively high prevalence in the age group 0 to 13 years of 12.5% is suggestive of infants engaging in risky behavior such as playing in soil environments (Jones, *et al.*, 2001). This behaviour is usually high in childhood years. The age group of 24 to 33 is however unexplained leading us to speculate that cohort effect may affect the distribution of toxoplasmosis across ages, with there being a higher risk in the past as an explanation for the age trends.

Seroprevalence did not vary significantly by sex (χ^2=0.210, p= 0.65). This is similar to a population-based study done in the United States which had the same findings (Jones, *et al*., 2001). Among women of childbearing age, the seroprevalence of toxoplasmosis was found to be 8.9%. This is less than the seroprevalence that was found in a study in the United States (Jones, *et al*., 2001). It also implies that 91.1% of women attending Chipokotamayamba clinic are susceptible to acute *toxoplasma* infection during their childbearing years, and therefore their infants are susceptible to congenital toxoplasmosis.

Of the 408 respondents, only 3 (0.7%) had knowledge on toxoplasmosis. This may lead to risky behavior as information on how to prevent infection from toxoplasmosis was lacking. The results from this study are similar to those observed in a study to investigate the knowledge of toxoplasmosis in pregnant women where it was found that knowledge levels were low (Jones, *et al*., 2003). This was further emphasized in another study in Poland where knowledge was found to be poor among health workers especially in pregnant women (Ziemba, *et al*., 2010). These results could be because of the fact that toxoplasmosis has not been factored into health educational programs for groups at risk such as pregnant mothers and HIV positive individuals. The paucity of information on the epidemiology of toxoplasmosis due, in part to an insufficient amount of research into the epidemiology of toxoplasmosis, is a major factor in prevailing inadequate knowledge levels among individuals at Chipokotamayamba clinic and possibly at many such facilities in Zambia.

The seroprevalence of toxoplasmosis was twice as high in individuals who own at least a cat (21.4%) compared to those that did not (10.4%) probably suggesting that contact with cats and cat litter is a known risk factor in transmission of *T. gondii*. However, there was no association between owning a cat and being seropositive for toxoplasmosis (p=0.19).

This study did not find any statistically significant risk factors for toxoplasmosis. In Multivariate logistic regression none of the risk factors investigated were significantly associated with the risk of toxoplasmosis. These findings were similar to results by Nissapatorn, *et al* (2003) in which no significant association was found between *Toxoplasma* seroprevalence and various possible risk factors. These results may be due to a low power of the study (80%) where only spurious associations may be elucidated (Nissapatorn, *et al* 2003; Danesh & Peto, 1998). However, other studies investigating risk factors for toxoplasmosis identified owning cats to be a risk factor for

toxoplasmosis (Baril, *et al*., 1999). Cleaning the cat litter box, eating raw or undercooked meat and vegetables and age were identified as risk factors for toxoplasmosis in Norway (Georg, *et al*., 1996). More recently a study in Zambia identified blood transfusion as a risk factor for *toxoplasma* infection (Sinyangwe, 2009). However, this factor was not investigated in this study as the study population did not include participants who had donated or received donated blood.

There are several possible limitations to this study. These are:

- Study participants from this study are drawn from individuals who were attending Chipokotamayamba clinic for health services. These individuals were seeking health services and could have an underlying ailment, possibly toxoplasmosis, hence the findings from this study could be biased towards overestimating the prevalence of toxoplasmosis and limiting the generalizability of the findings.
- There is limited information on the prevalence of toxoplasmosis in animals especially cats which are the definitive hosts. This information is important to understand the transmission dynamics of toxoplasmosis in the population under study.
- The use of serology as a marker for *Toxoplasma* infection may have several limitations: It may underestimate the prevalence of toxoplasmosis in HIV positive individuals with a deteriorated immune system (Schneider, *et al*., 1992). The choice of *Toxoplasma* IgG may also miss out infection in cases where there is a persistent IgM.
- Despite these limitations, serology remains the test of choice for evaluating exposure to *T. gondii*. Other tests such as PCR and histology depend on detecting bradyzoites in tissues and these may be missed as detection is subjected to proficiency of the technician doing the examination and targeted harvesting of bradyzoites from suspected tissues which may be missed.

CHAPTER 6

6.1 CONCLUSION AND RECOMMENDATIONS

6.1.1 Conclusion

The findings from this study have shown that toxoplasmosis is a public health concern at Chipokotamayamba clinic. Seroprevalence estimates indicate 10.8% of individuals attending Chipokotamayamba clinic have antibodies against *toxoplasma* and if pregnant or infected with HIV/ AIDS could be at risk of congenital and cerebral toxoplasmosis, respectively. Although generalization of these study findings is limited to the population attending Chipokotamayamba clinic, it provides vital information on the possible presence of toxoplasmosis in similar settings as Chipokotamayamba. No risk factors were identified in this present study and therefore no recommendations can be made on the prevention of toxoplasmosis as a direct result of these findings.

6.1.2 Recommendations

- This study was a facility-based study and therefore the results could not be generalised beyond the population attending Chipokotamayamba clinic. There is need for a population-based study in order to understand the distribution of *Toxoplasma* infection in Zambia.
- There is need for studies to be done in animals that provide a reservoir of infection of toxoplasmosis for humans. This will help in understanding the disease epidemiology of animals and their significance in the human infection of toxoplasmosis. Investigation into

the seroprevalence of *T. gondii* in vital hosts such as cats may provide critical information into the transmission dynamics of toxoplasmosis.

- The prevalence of toxoplasmosis in this population indicates that there is potential for infection in risk groups such as the HIV positive and pregnant mothers. Therefore, there is need for health education in pregnant mothers and HIV positive individuals to prevent possible transmission of toxoplasmosis and subsequent development of congenital and cerebral toxoplasmosis in the two groups, respectively.
- It is strongly recommended that future studies should be conducted over a longer period than four months. The seasonality in disease patterns may bias the findings of this study to a particular group of individuals seeking health services at Chipokotamayamba clinic within this period. In addition to a longer time period, serological testing should include testing for IgM which could provide vital information on new infections and temporal aspects of toxoplasmosis.
- Future studies may also benefit from investigating the spatial distribution of toxoplasmosis to investigate key landmarks such as a well for drinking, source of meat products or source of vegetables which could be potential sources of *Toxoplasma* infection.

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APPENDICES

Appendix 1 The Information Sheet

I am Victor Daka conducting a research in fulfilment of my project requirements for the Master of Science in One Health Analytical Epidemiology at the University of Zambia.

My study is looking at a disease called toxoplasmosis, its seroprevalence and associated risk factors. Transmission to humans may occur through ingestion of contaminated foods and soil. This disease is of particular concern as it affects individuals who may be immunosuppressed as is the case in HIV, children or pregnant women. This makes this disease of particular concern in people but has not been investigated extensively for its significance in Zambia. Therefore your participation in this study will help provide the much needed information on this disease that will be of much help to Zambia.

To do this, I will ask you a few questions using the form I have. If you are agreeable some small amount of blood, approximately 4ml, will be taken from the blood you will submit for other investigations for the purposes of this study. No other examinations that are not for the purposes of this study will be carried out on the blood.

There is minimal risk in this study and you will only feel some bit of discomfort during blood draw. This study has been reviewed by ERES ethics committee to determine its suitability to human participants.

This is entirely voluntary and be assured that the information you will provide and also the results of the test will be strictly confidential. This information will be restricted to the researchers and the principal supervisor for the duration of the study and after the thesis the blood collected will be retained for a further six months and then destroyed.

Nevertheless you have the right to seek further clarification or to withdraw. For further information you may contact the following;

Victor Daka:	Dr Careen Hankanga:	The Secretary
The researcher	Principal Supervisor	ERES Converge IRB
TEL; 0968-886424	TEL; 0978-375481	Lusaka. Tel:0955155633
Email; vmdaka@yahoo.co.uk	Email ; careen.hankanga@unza.zm	

Appendix 2 The Consent Form (for patients aged 16 and above)

I ……have agreed to take part in this research which is studying *The seroprevalence and risk factor analysis of toxoplasmosis in individuals attending chipokotamayamba clinic, Ndola, Zambia* in order to prevent or limit toxoplasmosis infection. I confirm that the study has been adequately explained to me and I understand the risks involved, if any.

I am participating voluntarily and I understand that I can withdraw at any time without repercussions

I understand that disguised extracts from my interview may be quoted in the thesis and any subsequent publications if I give permission.

I agree to provide necessary information and a small amount of blood.

Participant signature……Date……..

Participant thumbprint in the box above

Name of researcher: Victor Daka
Sign……

For further clarification you may contact the following:

Victor Daka:	Dr Careen Hankanga:	The Secretary
The researcher	Principle Supervisor	ERES Converge IRB

TEL; 0968-886424	TEL; 0978-375481	Lusaka. Tel:0955155633
Email; vmdaka@yahoo.co.uk	Email ; careen.hankanga@unza.zm	

Appendix 3 The Assent Form

PARENT/ GUARDIAN ASSENT (for patients below the age of 16.)

I ……have allowed my child/ dependant to take part in this research which is studying *The seroprevalence and risk factor analysis of toxoplasmosis in individuals attending chipokotamayamba clinic, Ndola, Zambia* in order to prevent or limit toxoplasmosis infection. I agree to provide necessary information and a small amount of blood.

I agree to provide necessary information and a small amount of blood.

Signature……Date……..

Participant Thumbprint in the box above

Name of researcher: Daka Victor
Sign……

For further clarification you may contact the following:

Victor Daka:	Dr Careen Hankanga:	The Secretary
The researcher	Principle Supervisor	ERES Converge IRB
TEL; 0968-886424	TEL; 0978-375481	Lusaka. Tel:0955155633
Email; vmdaka@yahoo.co.uk	Email ; careen.hankanga@unza.zm	

Appendix 4 The Questionnaire

THE UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
MSc. One Health Analytical Epidemiology- Questionnaire

Title:

A STUDY TO INVESTIGATE THE SEROPREVALENCE AND RISK FACTOR ANALYSIS OF TOXOPLASMOSIS IN INDIVIDUALS ATTENDING CHIPOKOTAMAYAMBA CLINIC, NDOLA, ZAMBIA.

Date……..

PATIENT ID……INITIALS……. SERIAL #.....

SEX…….. AGE…….....

DEMOGRAPHIC DATA

a) MARITAL STATUS……b) OCCUPATION……d) RESIDENCE……

RISK FACTORS

a) Have you ever heard of the disease called toxoplasmosis?.....
b) If so, what do you know about it?.....

c) Do you own a cat? YES ☐ NO ☐
d) If yes, do you ever clean the cat litter box YES ☐ NO ☐

e) Do you own a dog? YES ☐ NO ☐
f) Are you in regular contact with soil or in a soil related occupation?

YES ☐ NO ☐

g) Do you eat raw or undercooked meat? YES ☐ NO ☐
h) Do you eat raw or undercooked vegetables YES ☐ NO ☐

Appendix 5 Bemba Information Sheet

ICIPEPALA CE LYASHI

Nine Victor Daka ulecita ukufwailisha pa kufikilisha ifilefwaikwa mu ma sambililo ya Master mu Science mu One Health Analytical Epidemiology pa University yamu Zambia.

Isambililo lilelolesha pa bulwele ubwitwa toxoplasmosis, bwaseka mumulopa elyo na yambi amafya ayapalanako. Ukwambukila kubantu kuti kwa citika ukupitila mukulya ifya kulya ifya fiko ifyabipa na mu maloba. Ubu bulwele bwalisakamika pantu bulekata na Bantu abo amaka ya mumubili yacepa, ifyo ciba muli HIV, abana abanono nangu bana mayo abali pabukulu.

Paku cita ici, nala mwipushako amepusho aya nono uku bomfya icipepala ici nkwete. Nga cakuti mwasuminisha, utumulopa utu nono tulebulwa ukufuma mumulopa mulepela pa kufwailishamo ifintu fimbi pamulandu wa ili sambililo.

Ici cakuipeleshafye, elyo mwishibe ukuti ilyashi ilyo mulepela elyo nefikatumbuka mukupima fikaba ifya munkama. Lelo namukwata insambu iyaku fwaisha ukwipukisha nangu ukufumamo.

Pakwishibilapo ilyashi nalimbi kuti mwalanda na aba:

a) Ba Victor Daka
Bakafwailisha

Lamya: 0968-886424

Akeyala ka E-mail: vmdaka@yahoo.co.uk

b) Dr. Careen Hankanga
Bakangalila mukalamba

Lamya: 0978-375481

Akeyala ka E-mail: careen.hankanga@unza.zm

c) Ba kalemba
ERES converge IRB

Lusaka

Lamya: 0955155633

Appendix 6 Bemba Consent form

IPEPALA LYA KU SIMINISHANYA

IPEPALA LYA MULWELE ILYA KU SUMINISHANYA

(Abalwele abali ne myaka yakufyalwa16 no kuya pa mulu)

Ine……nasumina ukubulamo ulubali muli uku kufwailisha umo balesambilila ukuseeka/ukufula kwa toxoplasmosis elyo na mafya aya kumineko mu bantu abeleya pa Chipokotamayamba umo mumusumba wa Ndola, Zambia, pakucingilila nangu uku cefyako toxoplasmosis mu bantu. Nasumina ukupela ilyashi ililingile elyo no tumolopa utunono.

Ulebulamo ulubali:…….**.....**
Ukusaina:……

Ubushiku: ……./……./……../

Ukufwatika kwa ulebulamo ulubali, mukabokoshi pa mulu

Ishina lyakwa kafwailisha:……

Ukusaina:……
…….

Pakumfwilapo nafimbi kuti mwatumina aba:

d) Ba Victor Daka
Bakafwailisha

Lamya: 0968-886424

Akeyala ka E-mail: vmdaka@yahoo.co.uk

e) Dr. Careen Hankanga
Bakangalila mukalamba

Lamya: 0978-375481

Akeyala ka E-mail: careen.hankanga@unza.zm

f) Ba kalemba
ERES converge IRB

Lusaka

Lamya: 0955-155633

Appendix 7 Bemba Assent Form

IPEPALA LYA KUSUMINISHA

UKUSUMINISHA KWA MUFYASHI/KASUNGA

(Abalwele abashila fisha imyaka yakufyalwa 16)

Ine……. Nasuminisha umwana wandi/uwo nsunga ukubulamo ulubali muli uku kufwailisha umo balesambilila ukuseeka/ukufula kwa toxoplasmosis elyo na mafya aya kumineko mu bantu abeleya pa Chipokotamayamba umo mumusumba wa Ndola, Zambia, pakucingilila nangu uku cefyako toxoplasmosis mu bantu. Nasumina ukupela ilyashi ililingile elyo no tumolopa utunono.

Ukusaina:…… Ubushiku:……../……../……../

Ukufwatika kwa ulebulamo ulubali, mukabokoshi pa mulu

Ishina lyakwa kafwailisha……

Ukusaina:……..

Pakumfwilapo nafimbi kuti mwatumina aba:

a) Ba Victor Daka
Bakafwailisha

Lamya: 0968-886424

Akeyala ka E-mail: vmdaka@yahoo.co.uk

b) Dr. Careen Hankanga
Bakangalila mukalamba

Lamya: 0978-375481

Akeyala ka E-mail: careen.hankanga@unza.zm

g) Ba kalemba
ERES converge IRB

Lusaka

Lamya: 0955-155633

Appendix 8 Bemba Questionnaire

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETRINARY MEDICINE

MSc. One Health Analytical Epidemiology – Questionnaire

ISHINA LYE SAMBILILO

ISAMBILILO LYA KUFWAILISHA UKUSEEKA/UKUFULA KWA TOXOPLASMOSIS NA MAFYA AYAKUMINEKO MU BALWELE BA KASHISHI KA HIV MU CHIFUBU

UBUSHIKU:……/……/……../

ICISHIBILO CA MULWELE:…….. INAMBA:…….

UMWAUME/UMWANAKASHI:……. IMYAKA:……..

IFISHIBILO FIMO PA MUNTU

A) BALYUPA/BALYUPWA:…….

B) INCHITO
BABOMBA:……

C) INCENDE
BEKALAKO:……..

AMAFYA YAMO

a) Bushe mwaliteka pushi? Ee ☐ Iyo ☐

b) Bushe mwaliteka imbwa? Ee ☐ Iyo ☐

c) Bushe mulawamyamo mukabokoshi kakwa pushi? Ee ☐ Iyo ☐

d) Bushe mulekata amaloba libili libili nangu ukubomfya inchito iikumine ukwikata amaloba? Ee ☐ Iyo ☐

e) Bushe mulalya inama Iyibishi nangu iyabula Ukwipikisha? Ee ☐ Iyo ☐

f) Bushe mulalya umusalu Ee ☐ Iyo ☐

uubishi nangu uwabula

ukwipikisha?

Appendix 9 Letter of approval of research proposal

UNIVERSITY OF ZAMBIA
SCHOOL OF VETERINARY MEDICINE
OFFICE OF THE ASSISTANT DEAN POSTGRADUATE STUDIES

11 th November, 2013

Victor Daka
C/O Disease Control Department
Box 32379, Lusaka.

Dear Mr Daka,

RE: APPROVAL OF RESEARCH PROPOSAL

At the meeting of the School Board of Graduate Studies held on 18th October, 2013, your research proposal entitled '*Seroprevalence and Risk Factor Analysis of Toxoplasmosis in individuals attending Chipokotamayamba Clinic, Ndola, Zambia,* was tabled and discussed. I am therefore pleased to inform you that the research proposal was subsequently approved by the Board.

On behalf of the Board, I wish you success as you apply for ethical approval and carry on with your research activities.

UNIVERSITY OF ZAMBIA SAMORA MACHEL SCHOOL OF VET MED DEAN 13 NOV 2013

Dr. C. Hankanga Malombola
Assistant Dean (PG)

cc. Director - DRGS
Dean – School of Veterinary Medicine
Dr C. Hankanga
Course Co-ordinator – MSc OHAE

Appendix 10 Letter of ethical approval

ERES CONVERGE

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

23rd January, 2014

Ref. No. 2013-Dec-001

The Principal Investigator
Mr. Victor Daka
Tropical Diseases Research Center
P.O. Box 71769,
NDOLA.

Dear Mr. Daka,

RE: Sero prevalence and risk factor analysis of toxoplasmosis among individuals. attending Chipokotamayamba clinic, Ndola.

Reference is made to your corrections dated 20th January, 2014. The IRB members resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2013-Dec-001**
Approval and Expiry Date	Approval Date: 23rd January, 2014	Expiry Date: 22nd January, 2015
Protocol Version and Date	Version-Nil	22nd January, 2015
Information Sheet, Consent Forms and Dates	• English, Bemba.	22nd January, 2015
Consent form ID and Date	Version-Nil	22nd January, 2015
Recruitment Materials	Nil	22nd January, 2015
Other Study Documents	Questionnaires, Lab request form	22nd January, 2015
Number of participants approved for study	371	22nd January, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON

Appendix 11 Letter of approval from MCDMCH

Telephone: (260) 211 235341
Fax (260) 211 235342

In reply please quote:

No.:.....

REPUBLIC OF ZAMBIA

MINISTRY OF COMMUNITY DEVELOPMENT, MOTHER AND CHILD HEALTH

OFFICE OF THE PERMANENT SECRETARY
COMMUNITY HOUSE
SADZU ROAD
PRIVATE BAG W 252
LUSAKA

15th November 2013

Mr. Victor Daka
Tropical Diseases Research Centre
P.0 Box 71760
NDOLA

REF: REQUEST FOR PERMISSION TO CARRY OUT RESEARCH AT OUR FACILITY

I acknowledge receipt of the letter dated 1st November 20131th November 2013 in which you requested for permission to conduct a research at Chipokotamayamba Clinic in Ndola.

Permission to conduct the research has been granted but please get in touch with the District Medical Officer in Ndola to facilitate your research.

Prof. Elwyn Chomba
Permanent Secretary
MINISTRY OF COMMUNITY DEVELOPMENT, MOTHER AND CHILD HEALTH

cc Provincial Medical Officer – Copperbelt
cc District Medical Officer - Ndola

Appendix 12 Letter for permission to do research at Chipokotamayamba clinic

Republic of Zambia

MINISTRY OF COMMUNITY DEVELOPMENT, MOTHER AND CHILD HEALTH

Ndola District Community Health Office

Naidu Close Kanini
P.O.Box 70672
Ndola - Zambia

All communication to be addressed to
District Director of Health

Telephone: 612819
Telefax: 612819
Cell: 0977-835660

To: **The Health Centre In charge**

From: **PCCO**

Date: **28th January, 2014**

RE: **STUDY ON TOXOPLASMOSIS**

Refer to the above the bearer of this letter, Mr. Victor Daka has been granted permission to conduct the study at your facility.

Please avail him the support that he may require.

.....

Dr. L. S. Nyendwa