SEROPREVALENCE OF *TOXOPLASMA GONDII* AND ITS ASSOCIATED RISK FACTORS IN CATS IN LUSAKA DISTRICT, ZAMBIA

BY

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A Dissertation submitted to the University of Zambia in fulfillment of the requirements for the degree of Masters.

The University of Zambia

Lusaka

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DECLARATIONS

I, **Farai Phiri**, do hereby declare to the Senate of the University of Zambia that this thesis is my original work and has not been submitted anywhere else for a degree award.

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CERTIFICATE OF APPROVAL

This thesis submitted by **Farai Phiri** has been approved as fulfilling the requirements for the award of a degree of Masters in Veterinary Medicine by the University of Zambia

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1. Name……..Signature…….Date……

2. Name……..Signature…….Date……..…

3. Name……..Signature…….Date……

Chair of Board of Examiners

Name……..Signature…….Date……

DEDICATION

I dedicate this report to my husband, friends and family who have been nothing but supportive throughout this journey. This would have not been possible without their prayers and encouragement.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACEIDHA	African Centre for Infectious Disease in Human and Animals
CI	Confidence interval
DNA	Deoxyribonucleic acid
ELISA	Enzyme-Linked Immunosorbent Assay
Fig	Figure
IgG	Immunoglobulin G
IFAET	Fluorescent antibody test
IHAT	Indirect Haemagglutination Test
LAT	Latex agglutination test
MAT	Modified agglutination test
MLE	Multi-locus enzyme
MLST	Multi-locus sequence typing
PCR	Polymerase Chain Reaction

PV	Parasitophorus vacuole
nPCR	Nested polymerase chain reaction
SPSS	Statistical package for the social sciences
T. GONDII	*Toxoplasma gondii*
USA	United States of America

ABSTRACT

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*. It is of public health importance because of the economic loss caused in food animals and the severe disease it causes in fetuses through their pregnant mother and immune-compromised individuals. Despite this fact, there are still very few studies that have been done for *T. gondii* here in Zambia. This study was a cross section study carried out from February 2018 to September 2019 in different veterinary facilities as well as from stray cats trapped using cages in the surrounding areas. A questionnaire was also used to retrieve information from domestic cat owners in order to identify the potential risk factors of *T. gondii*. 178 sera samples were analyzed for Immunoglobulin G (IgG) anti bodies using an Enzyme-Linked Immunosorbent Assay (ELISA) kit for In vitro Diagnosis vet screen Toxoplasmosis multi-species Indirect kit (France). Results from the study on Toxoplasmosis infection in both domestic and stray cats were analyzed using Chi-square and binary logistic regression. The overall prevalence was calculated to be 16.85% while the seroprevalence of domestic cats was found to be 20.4% and that of stray cats was found to be 13.3%. It was established that sex and diet were both significantly associated with Seropositivity with IgG antibodies and these were considered as risk factors. When the stray and domestic cats were compared, there was a significant difference between the two groups. It was established that older cats were more likely to get *T. gondii* infection than younger cats. There was no significant association between the owners' awareness of the T. gondii and Seropositivity of their cat. There is need to have awareness campaigns to educate cat owners on hygiene practices when handling cat excreta as well as the need to confine cats and avoid them from straying.

CHAPTER ONE.

INTRODUCTION

1.0. Background

Toxoplasma gondii (T. gondii) is a nearly ubiquitous organism that infects humans, wildlife, birds, and domestic, as well as food animals. *T. gondii* has been reported in pigs, chickens, sheep, goats and cattle (Chikweto *et al*., 2011). However, only members of the cat family (*Felidae*) are the definitive host and shed the environmentally resistant oocyst form of the organism in their faeces. Transmission to humans usually occurs by ingestion of cysts in undercooked meat and exposure to soil and water contaminated by oocysts (Elmore *et al.*, 2010). Feline infections are typically subclinical; congenitally infected kittens are the most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (Vollaire *et al*., 2005).

It has been 100 years since the discovery and naming of *T. gondii*. The parasite was first found in laboratory animals (Dubey, 2007). Its medical importance remained unknown until 1939 when *T. gondii* was identified conclusively in tissues of a congenitally infected infant in New York City, USA (Wolf *et al*., 1939), and its veterinary importance became known when it was found to cause abortion storms in sheep in 1957 in Australia (Hartley and Marshall, 1957).

Toxoplasmosis is a disease of economic significance as it causes severe losses in livestock especially sheep through abortions, still births and death of newly born (Buxton and Henderson, 1999). Humans can also acquire the parasite from ingestion of tissue cysts in under cooked meat, uncooked/undercooked vegetables, transplantation, blood transfusion, laboratory accidents or congenitally (Dubey, 2008)

1.1 Statement of the problem

Toxoplasmosis is estimated to affect about 30% to 65% of the global population (Ayeh-Kumi *et al*., 2010). However, detailed information on Toxoplasmosis in Zambia is still missing.

A study of Toxoplasmosis in pregnant women in Zambia has been done by (Frimpong *et al*., 2017) but the actual source of the infection was not established hence this study to determine the existence of *T. gondii* in cats which are the definitive host and shed the environmentally resistant oocyst form of the organism in their faeces.

1.2 Justification of the study

Toxoplasmosis is zoonotic, therefore raises a public health concern that need to be addressed. It is expected that findings of this research will also influence pet owners and policy makers to find effective control, management and prevention strategies of this parasite/ disease. This study will not only provide insight into the prevalence of Toxoplasmosis and its associated risk factors in cats but also close the gap in information lapse.

There is a growing number of individuals owning cats as well as stray cats around therefore there is need to investigate the potential risk of these growing numbers to the public as a whole.

1.3 Study questions:

The study aimed to answer the following questions:

i. What is the seroprevalence of *Toxoplasma gondii* in cats from Lusaka province?
ii. What are the risk factors associated with *T. gondii* infection in cats from Lusaka province?

1.4. Purpose of the study

This study aimed at describing the epidemiology of *Toxoplasma gondii* among domestic and stray cats in Lusaka

1.5. Study objectives

i. To determine the Seroprevalence of *T. gondii* in domestic and stray cats in Lusaka, Zambia.
ii. To determine and identify the risk factors associated with *T. gondii* infection in cats found in Lusaka.

1.6. Significance of the study

The study bears significance because Toxoplasmosis is a zoonotic disease that may incidentally infect humans causing a variety of symptoms ranging from mild to severe. Despite the fact that humans are dead end hosts, pregnant mothers can transmit the infection to their unborn fetuses in utero, leading to abortions, stillbirths and mummification in affected humans.

Therefore, this study was important to determine the seroprevalence of Toxoplasmosis in cats, as well as delivering advice to cat owners on what Toxoplasmosis is and how to prevent it.

1.7. Scope of the study

The study encompassed all cats (strays) caught from the area surrounding the University of Zambia, as well as those (domestic) brought to health facilities (University Of Zambia Veterinary Clinic, Show grounds Vet and Pet-Vet Clinic).

1.9. Ethical considerations

No cats were harmed during the collection of samples, or restrained for more than was necessary. Questionnaire participants had their information kept anonymous for ethical and confidentiality.

CHAPTER TWO

LITERATURE REVIEW

2.1. Classification and ecology

Toxoplasma gondii belongs to the kingdom Protista, subkingdom protozoa, subphylum Apicomplexa, class Sporozoasida, order eucoccidiorida, family Sarcocystidae. It belongs to the genus *Toxoplasma* and species *gondii*. *Toxoplasma gondii* exists in three main forms: The first form is the oocyst which contains sporozoites. The Oocyst form is the infective form of the parasite. The second form is the trophozoites/Tachyzoites, which are the sexual form responsible for cell invasion and are crescent shaped. The third and last form is the tissue cyst or the bradyzoites. These are made up of intracellular trophozoites and develops within cytoplasm of the host cell (Dubey, 1998). The tachyzoites (in groups), the bradyzoites (in tissue cysts) and sporozoites (in oocysts). Sporozoites, bradyzoites and tachyzoites of *T. gondii* are ultra-structurally similar with all form having similar number of rhoptries, although they differ in appearance, all inclusion and organelles (Weiss and Kim, 2000, Dubey, 1998)

Toxoplasma gondii has adapted to different/various ecological systems, giving it high genetic variation and a very perculiar population structure (Sibley *et al*., 2009) (Beck *et al*., 2009). *Toxoplasma gondii* has been considered a single species of the genus *Toxoplasma*. Early studies on the parasite strains from Northern America and Europe identified limited genetic diversity which were classified into genetic types I, II and III (Howe and Sibley, 1995). The consequences of infection with *T. gondii* actually depend on parasite genotype and host species.

Type one (I) or type one variants are more likely to be associated with severe toxoplasmic retino-choroiditis (Grigg *et al*., 2001), and the atypical isolates often cause severe acute or disseminated Toxoplasmosis in immune-competent individuals (Bossi and Bricaire, 2004).

Type I isolates are uniformly lethal to out bred mice while type II and III isolates are significantly less virulent (Sibley and Boothroyd, 1992). It is highly suggestive that the origin of virulent strains of *T. gondii* is a single lineage and although this lineage has spread widely globally, it is still genetically homogeneous (Sibley and Boothroyd, 1992).

An unusual genotype (type X) of *T. gondii* has been identified to cause mortality in sea otters. Infection with this genotype has also been found in several coastal dwelling species, including marine bivalves and other filter feeding invertebrates.(Miller *et al*., 2008, Kapperud *et al*., 1996). Sea otters (Enhydralitrisnercis) in these contaminated habitants might ingest invertebrates that concentrates *T. gondii* oocysts as a consequence of their feeding habits and as a result, prevalence in the sea is high (Kapperud *et al*., 1996).

2.2. Life cycle

Toxoplasma gondii has a complex life cycle which involves development of asexual forms in different / multiple warm blooded hosts.The life cycle of *T. gondii* consist of three developmental stages; tachyzoite, bradyzoite, and sporozoite. The tachyzoite form is typically found in acute infections and multiplies rapidly. The bradyzoites form tissue cysts and multiplies slower than the tachyzoite hence found in chronic infections. The sporozoite is the only form that is produced in the definitive host during the sexual reproduction and is released in the oocysts through the cat faeces.

The life cycle starts from a domestic cat that eats a mouse that is infected with *T. gondii* in the muscle as a cyst (Dubey, 1995). The parasite persists and passes to the stomach where they infect the epithelial cells of the small intestines of the cat. The parasite undergoes sexual development and reproduces many zygotes that contain oocysts. The sexual replication only happens in the cats gastrointestinal tract (Webster, 2010) (Sibley and Ajioka, 2008). The epithelial cells of cats that are infected burst and they release the oocysts in their feces (Dubey, 2001). Intermediate hosts (other animal species like birds and rodents) acquire their infection from ingestion of oocysts-contaminated soil, water and plant materials (Alvarado-Esquivel *et al*., 2010).

The figure (figure 2.1) before shows the life cycle of *Toxoplasma gondii.*

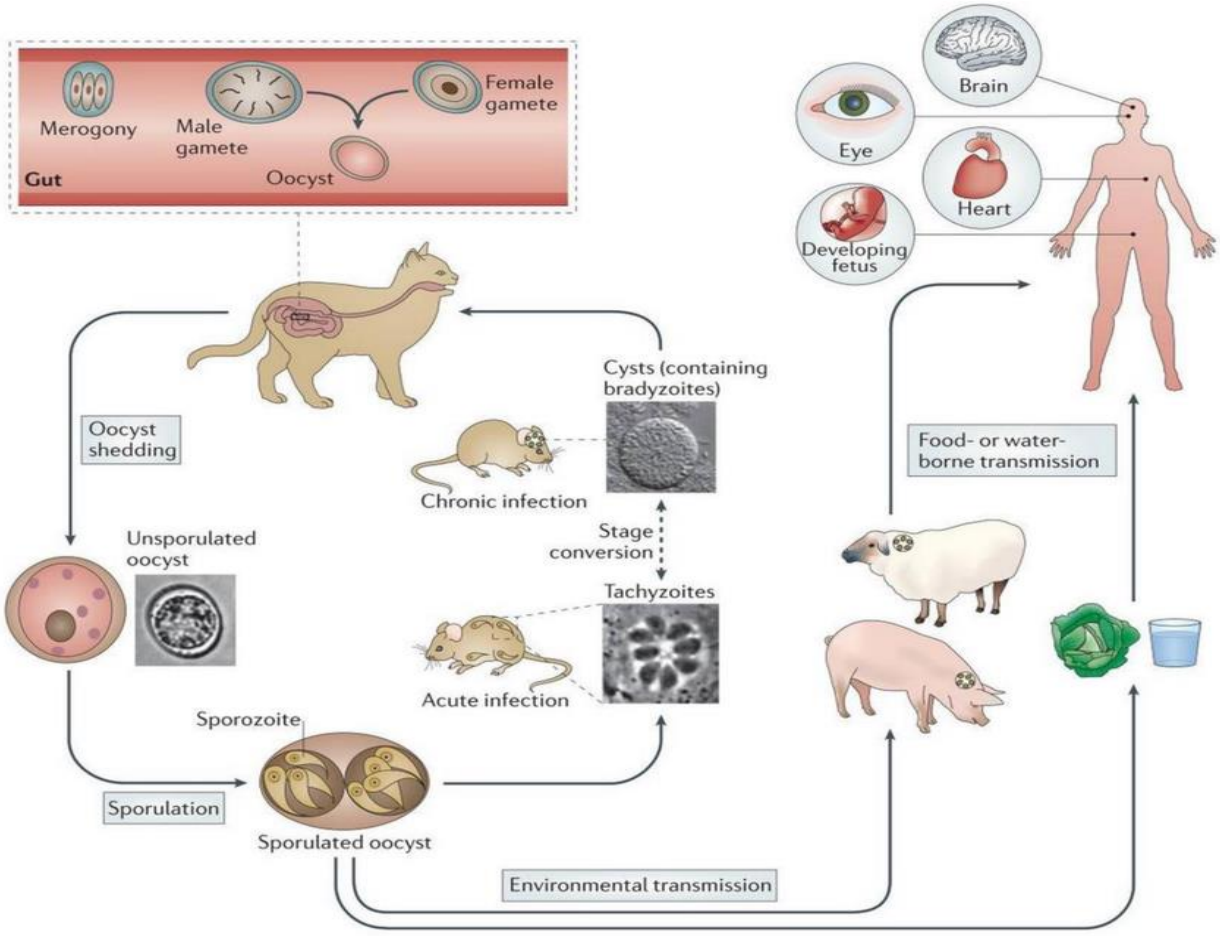

Figure 2.1: Life cycle of *Toxoplasma gondii*

Source: https://www.researchgate.net/profile/Valentin-Greigert/publication/343230474/figure/fig2 /AS:917841687629830 @1595841919228/Cycle-de-vie-de-T-gondii-Hunter-et-al-2011.ppm

2.3. Epidemiology

Humans can also acquire the parasite from ingestion of tissue cysts in under-cooked meat, uncooked or under-cooked vegetables, transplantation, blood transfusion, laboratory accidents or congenitally (Dubey, 2008). Most people infected after birth are asymptomatic, however some may develop, fever, malaise and lymphadenopathy (Remington *et al*., 2006). Post-natally infected humans especially those that are immuno-suppressed, can develop ocular complications like retinochorioditis, toxoplasma encephalitis, pulmonitis and other system diseases (Giannoulis et al., 2008) .

2.4. Pathogenesis.

Toxoplasma gondii is a widespread zoonotic protozoan that infects most, if not all species of birds and mammals.

As a definitive host for this organism, felines are the only animals that pass the oocyst in their feces, although intermediate hosts can harbor infective tissue cysts. The tissue cyst and oocysts are the main forms of the parasite that are involved in its transmission.

There has been change in the emergence, transmission and distribution of parasitic diseases, Toxoplasmosis inclusive. This is due to changes in the environmental conditions caused by global warming, urbanization and economic globalization. Infection of *T. gondii* is high in areas with hot, low altitudes and humid climatic conditions.(Patz et al., 2004, Yan et al., 2016, Dubey, 1998)

Most feline infections occur post-natally through ingestion of infected tissue cysts or rarely oocysts, although congenital infections can occur. Feline infections are typically subclinical; congenitally infected kittens are the most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (Vollaire *et al*., 2005). Cats are more likely to shed oocysts following ingestion of tissue cysts rather than tachyzoites or oocysts. The ingestion of only one bradyzoite will lead to feline infection as compared to 1000 oocysts that a cat has to ingest to develop an infection.

Human infections of *T. gondii* occur through ingestion of tissue cysts in poorly cooked meat, ingestion of food and water contaminated with mature oocysts fecal orally, trans-placental (vertical) transmission from mother to fetus in utero and finally although rare through injury, organ transplant and blood transfusion from seropositive donors.

It is thought that infection to one genotype of *T. gondii* confers immunity to all genotypes of *T. gondii*, but this phenomenon has not been explored in cats. In a mouse model, immunity was more effective using homologous rather than heterologous strains of the parasite (Dao *et al.*, 2001, Brandão *et al*., 2009).

In the past women with exposure to *T. gondii* prior to pregnancy through serology were considered safe from future infections as well as risk to fetus. However, a study showed that exposure to one strain does not mean protection from a different strain despite having residue antibodies post exposure. (Elbez-Rubinstein *et al*., 2009).

2.5. Survival Mechanism:

Toxoplasma gondii just like other apicomplexan parasites invade target cells through a mechanism that is more dynamic in relation to other intracellular microorganisms. *Toxoplasma gondii* depends on its myosin and actin to penetrate the host cell (Carruthers, 2002). Using the actin filaments it contains, *T. gondii* uses a form of gliding motility (Dobrowolski *et al*., 1997).

Toxoplasma gondii has a survival mechanism to escape damage by the host immune system. One of the ways it uses is by infecting the host cells using plasmids (Nielsen *et al.*, 1999). Being an obligate intracellular parasite means that *T. gondii's* survival depends on its successful and efficient invasion. In order to accomplish a successful invasion, a microneme and rhophy neck protein (RON) has to be secreted in a coordinated and sequential manner. This is meant to mediate the invasion. For a long term establishment and host modulation of the parasite in the host cell rhoptries derived potein (ROPS) and dense granule proteins (GRAS) which are the secretion set of *T. gondii* are involved. This perculiar method of invasion by the *T. gondii* which involves secretion regulated moving junction helps its firm attachment to itself (glideoosome) and the plasma membranes of the host. This parasite-host interface complex explains why the host range of *T. gondii* is widely diverse (Soldati *et al*., 2001, Wang and Yin, 2015, Opitz and Soldati, 2002, Opitz *et al*., 2002). After *T. gondii* invades successfully it detaches from the host membrane and stays in its parasitophorus vacuole (PV) which is non fusogenic and acts as its physical home (Håkansson *et al*., 2001).

In situations where the immune system puts pressure on the parasite (*T. gondii*), it changes into a persistant encysted form (which primarily reside in the muscle and brain tissues) which is resistant to current known medication for Toxoplasmosis and has the potential to reactivate giving rise to acute infection (Sanchez and Besteiro, 2021).

Toxoplasma gondii can also defend its survival by preventing autophagy (xenophagy) by the cell immunity. It does this by preventing the expression of the autophagy protein LC3 and vacuole

lysosome fusion through its micronemal proteins MIC3 and MIC 6 (Muniz-Feliciano *et al*., 2013) (Wang *et al*., 2009). This in itself enables to resist damage by the host immune system. *T. gondii* also uses anti-apoptotic mechanism. This is done by disrupting the pro-apoptosis effector proteins like bax and bax (Hippe *et al.*, 2009).

Disruption of these pro-apoptosis effector proteins changes the parasites' shape and structure, hence, enabling it to be transported to the host cells and in return, apoptosis is initiated. Another mechanism is through autophagy, thereby reducing the number of cells that can destroy *T. gondii*. Another mechanism which helps *T. gondii* survive the host immunity is through its anti-oxidant network which decomposes the superoxide anion radicals responsible for damaging the parasite as well as prevent its replication (Pino *et al*., 2007).

2.6. Clinical manifestations

Clinical signs of Toxoplasmosis are usually non pathognomonic and are not sufficiently for a definite diagnosis (Boothroyd and Grigg, 2002, Tenter *et al*., 2000). Toxoplasmosis in fact mimics several other infectious diseases (Hill and Dubey, 2002).

2.6.1 Toxoplasmosis in dogs and cats.

Feline infections are typically subclinical; congenitally infected kittens are the most likely to have clinical signs of infection, but previously clinically healthy adult cats may also be affected (Vollaire *et al*., 2005). Common symptoms of *T. gondii* infections in cats include fever, ocular inflammation, anorexia, lethargy, abdominal discomfort and neurological abnormalities. Hepatitis, cholangiohepatitis, pneumonia, encephalitis with concurrent signs of ascites, lethargy and dyspnoea have been seen in trans-placentally infected kittens as well (Dubey and Carpenter, 1993).

Clinical signs of adult cats are non-specific (Brennan *et al*., 2016), inflammatory bowel disease (Peterson *et al*., 1991) and regional lymphadenopathy (Cohen *et al*., 2016, McConnell *et al*., 2007) have also been recorded in cats with *T. gondii*. Hepatic disease has also been reported in cats with Toxoplasmosis (Cohen *et al*., 2016, De Tommasi *et al*., 2014).

There has been very little documentation of congenital Toxoplasmosis in dogs; *T. gondii* causes severe disease in dogs, with the first spontaneous case reported in 1910. There is serological evidence that dogs are frequently found infected in nature and may serve as reservoirs of human

infection (Jacobs *et al*., 1955). Dogs have recently been associated as a potential risk factor for *T. gondii* infection in humans due to their potential to transmit oocysts mechanically (Lindsay *et al*., 1997). Primary Toxoplasmosis in dogs is not common and most cases of acute Toxoplasmosis in the USA were observed in dogs not vaccinated against the immunosuppressive canine distemper virus (CDV), (Rhyan and Dubey, 1992, Capen and Cole, 1966).

Common clinical manifestations in dogs are pneumonia, hepatitis and encephalitis (Dubey and Lappin, 2006). Toxoplasmosis in dogs is also associated with some form of neurological disease manifested by seizures, ataxia, tremors as well as paresis and or paralysis (Patitucci *et al*., 1997). In some cases where there is double infection of *T. gondii* and sarcocystic neurona in dogs, signs of para-paresis and tetra-paresis which led to paralysis of the lower motor neuron has been reported (Gerhold *et al*., 2014).

Other signs in dogs include stiffness, muscle wasting, abnormal gait associated with myositis (Migliore *et al*., 2017). Sensitivity to noise was also seen in one case (Papini *et al*., 2009). Eye disease in dogs like uveitis, necrotizing conjunctivitis and chorioretinitis has also been recorded (Wolfer and Grahn, 1996).

2.6.2 Clinical symptoms in humans

Infection with *T. gondii* can cause severe neurological and debilitating ocular disease (retinochoroiditis and anterior uveitis) in fetus during human pregnancy. Visual impairments occur when infection involves or extends to the retina and the choroid. The lesions caused by infection leads to retina scarring hence impaired vision or blindness (Maenz *et al*., 2014). Four point two (4.2%) percentage of uveitis cases in Germany have been attributed to *T. gondii* infections (Maenz *et al.*, 2014).

Toxoplasma encephalitis has been reported as a cause of death in immune compromised individuals with Acquired Imuno-Deficiency Syndrome-AIDS (Luft *et al*., 1984). Once infected, humans are believed to remain infected for life. Infected individuals usually remain asymptomatic unless when the organism reactivates due to immuno-suppression.

Cerebral Toxoplasmosis has an incidence of three percent (3%) among receipts of allogeneic hematopoietic stem cells (Schmidt *et al.*, 2013, Hakko *et al.*, 2013).

However, there is an ongoing research on whether chronic *T. gondii* has an effect on reaction time (Havlíček et al., 2001, Flegr et al., 2000, Flegr et al., 1996, Lafferty, 2006, Lafferty, 2005) and mental illness (Yolken et al., 2001, Flegr et al., 2003, Brown et al., 2005). There also assumptions about how *T. gondii* infection is linked to mood disorders (Henriquez *et al*., 2009).

2.6.3 *Toxoplasma gondii* in meat and game animals

Toxoplasma gondii in meat animals is often regarded as an issue of medical importance and public health concern because of the major economic importance of the disease to meat animals and humans as consumers. Although the transmission of *T. gondii* by tissue cysts is high in carnivores, the transmission is also relatively high by herbivores and omnivorous meat animals (Fayer, 1981).

Generally speaking, today meat animals such as sheep, goats, cattle and pigs among others are consumed almost daily in Zambia. The average global sero-epidemiological infection rate of 25%, 31% and 29% have been reported for cattle, sheep and goats respectively for *T. gondii* (Dubey, 1986a, Dubey, 1986b). These meat animals and/or their products like unpasteurized milk can pose a health risk to consumers (particularly pregnant women and immuno-deficient individuals) if contaminated with the oocysts of *T. gondii*. Virtually all edible portions of an animal can harbor viable *T. gondii*. Infections in cattle is less prevalent as compared to infections in pigs and sheep. *T. gondii* may survive in food animals for years in tissue cysts.

Toxoplasmosis is also prevalent in wild game like water buffaloes (less prevalent), bears, raccoons and deer. Approximately 8% of black bears are infected in the USA (Dubey and Beattie, 1988) and about 60% of the raccoons have antibodies for *T. gondii* because raccoons and bears scavenge for their food. Infection in these animals is a good indicator of the prevalence of *T. gondii* in the environment. Sporadic cases of clinical Toxoplasmosis occur in rabbits (Leland et al., 1992, Rhyan and Dubey, 1992) Squirrels (Dubey, 2004, Bangari *et al.*, 2007), minks and pet birds (Frank, 2001), especially in canaries and finches (Dubey, 2002). Toxoplasmosis in Squirrels can stimulate signs of rabies (Soave and Lennette, 1959). Reported clinical signs in squirrels were anorexia, diarrhoea, lethargy, viciousness, labored breathing and in two cases, squirrels had bitten children. An unusual clinical presentation of Toxoplasmosis in canaries is blindness with almost complete destruction of eyes (Dubey, 2002).

2.7. Diagnosis in cats

Feline Toxoplasmosis has proven to be very difficult to diagnose and although there a number of changes that occur under radiography, hematology and biochemistry, these are not pathognomonic (Lappin *et al*., 1989). Several diagnostic methods have been used worldwide to determine *T. gondii* infection.

2.7.1 Serology

Bioassays and serological tests though traditionally trusted have limitations especially where sequencing is concerned. There are a number of serological tests available including the sabin-feldman dye test, indirect fluorescent antibody test (IFAT), modified agglutination test (MAT), Latex agglutination test (LAT), indirect hem agglutination test (IHAT) and the indirect enzyme-linked immune-absorbent assay (ELISA).

Among the ones mentioned the ELISA) is the most widely used serological test. However, the Modified agglutination test was identified as having the highest sensitivity among all serology tests with a sensitivity of 82.9%. In one study IFAT had the lowest sensitivity of 80.4% (Dubey *et al*., 1995). In Another study found the same information only at 96% with ELISA following at 90.1% (Shaapan *et al*., 2008). The indirect ELISA was used in this study because it is simple to perform and economical too.

Although a single positive IgG titer indicates exposure, clinical Toxoplasmosis is indicated by a positive IgM titer or a fourfold increase in the IgG levels in paired serum samples taken 2-4 weeks apart (Vollaire *et al*., 2005) because most cats sero-convert after they have finished shedding oocysts only at a single point in their lifetime. Antigen detection has also proven useful in detecting *T. gondii* in recently sub-clinically infected cats (Lappin *et al*., 1989).

2.7.2 Molecular methods

Molecular methods however have proven to be more reliable and efficient because of its high specificity and high sensitivity (Kotresha and Noordin, 2010, Bastien, 2002). These methods rely on polymerase chain reaction (PCR) for the specific detection or analysis of parasite DNA, *T. gondii* inclusive (Su *et al*., 2010).

These molecular methods have proved to be cost effective, simple, sensitive and reproducible in both animal and human clinical samples (Bell and Ranford-Cartwright, 2002).

These molecular methods are divided into two categories and the first category focuses on specific detection of *T. gondii* in biological samples while the second category focuses on a high resolution identification of *T. gondii* isolates. The first category comprises of Conventional PCR, nested PCR (n-PCR) and qualitative real time PCR (q PCR) of repetitive DNA sequences. The second category includes multi-locus PCR-RFLP, microsatellite and multi-locus sequence typing (MLST) of single copy DNA sequences (Su *et al*., 2010).

PCR is highly sensitive and specific and has proved very useful together with serological tests to differentiate the chronic, acute or reactivated infection. PCR is also very useful and important when the immune system of the patient is compromised or when anti-body titres have not reached the threshold levels of detection (Remington *et al*., 2004).

A variety of different methods exist to genotype *T. gondii* isolates, and they have distinct advantages and disadvantages. Early studies of strain typing were based on multi-locus enzyme electrophoresis (MLE). MLE is quite specific and it requires a large number of purified parasites to perform it (Dardé *et al*., 1992). Later, typing methods focused on microsatellite (MS) makers (Ajzenberg *et al*., 2002) which are short repeated segments of DNA that tend to occur in non-coding DNA. MS markers are sensitive, reliable and amenable to high- through-out analyses. Although MSs are highly polymorphic, they are prone to homoplasy as the number of repeats can expand and contract during replication.

The third type of genotyping and strain characterization of *T. gondii* involves randomly amplified polymorphic DNA (Guo and Johnson, 1995). The disadvantage of this technique is that it is highly influenced by contaminating host DNA, which is a significant source of variability. Restriction fragment length polymorphism (RFLP) analysis of specific genetic loci has been widely used for *T. gondii* genotyping. RFLP markers are also amenable to high-throughput analysis using PCR amplification, followed by restriction digestion and later gel electrophoresis.

Multi-locus RFLP has been used to characterize isolates into three major groups namely I, ii and iii in north America and Europe (Sibley and Boothroyd, 1992, Howe and Sibley, 1995, Howe *et al*., 1997).

The possibility of finding oocysts in the feces of a cat is low (Dubey, 1995). Complications in fecal detection is due to the fact that other coccidian morphologically resemble those of *T. gondii* (*Hammondia hammondi* and *Besnoitia spp*); Molecular and bioassay techniques can be used to distinguish between organisms and mouse bioassay and this is the only definitive confirmative method (Alvarado-Esquivel *et al*., 2010).

Diagnosis in Humans: Diagnosis in humans is carried out as mentioned for cats above.

2.8. Risks factors

2.8.1. Risk factors in cats

Risk factors of Toxoplasmosis in cats include sex, age, body condition score, diet, access to hunting (feeding raw meat) and number of cats in the house hold and these risks factors can be analyzed using multivariate methods which include Binary Logistic regression and Chi Square (Castillo-Morales et al., 2012, Besné-Mérida et al., 2008, Galván Ramírez et al., 1999). Cat owners can reduce the risk of exposure of their pets by keeping their cats indoors and avoiding feeding them raw or undercooked meat.

2.8.2: Risk factors in humans

Humans usually become infected by ingesting oocyst-contaminated soil and water, as well as tissue cysts embedded in undercooked meat. They can also become infected congenitally (mother to child transmission). (Elmore *et al*., 2010). In a study done in South Africa, risk factors associated with the increased Seropositivity in different species included age, location, climate, animal production system, rodent control, seropositive cat, cat-feed access and cat fecal disposal (Tagwireyi, 2016). Regions with high water outflow has also been recognized as a risk factor (Miller *et al*., 2002).

2.9. Prevention and Treatment

2.9.1 Prevention and treatment in humans

Prevention of *T. gondii* infection in humans includes wearing of gloves when gardening or changing cat litter as well as washing fresh vegetables thoroughly before eating (Foulon *et al*., 1999). Pregnant women and immune-compromised people should avoid contact with soil, cats and consumption of raw meat and products like unpasteurized milk (Kaye, 2011). Fruits and

other raw vegetables must be washed thoroughly before consumption (Hill and Dubey, 2002). Institution of education and public health programs, particularly during prenatal care and during times of immunodeficiency can help reduce *T. gondii* infections (Kaye, 2011). No vaccine is yet available to prevent either *T. gondii* infection or Toxoplasmosis in cats, humans, or other species. Cat owners can reduce the risk of exposure of their pets by keeping their cats indoors and avoiding feeding them raw or undercooked meat (Elmore *et al*., 2010).

The principal drug of choice used in the treatment of *T. gondii* in humans includes Spiramycin, pyrimethamine and sulfadiazine. Spiramycin is used as a prophylaxis from vertical transmission of *Toxoplasma gondii* to the fetus in a cases where there is maternal infection but the fetus is uninfected (Montoya and Rosso, 2005). Pyrimethamine and sulfadiazine are used in a case where the fetal infection has taken place. However these two drugs should not be administered in the first trimester of the pregnancy as it has potential teratogenicity (Montoya and Rosso, 2005) and causes thrombocytopenia. Thrombocytopenia can however be reduced by folic acid therapy.

2.9.2 Prevention and treatment in cats and dogs

Most cats and dogs that have Toxoplasmosis can recover with treatment (Migliore *et al*., 2017). Treatment usually involves a course of antibiotics. Clindamycin has been widely used world-wide because it has fewer side effects than other drugs used to treat Toxoplasmosis like Pyrimethamine and sulfadiazine. The two drugs act synergistically to inhibit *T. gondii* reproduction. (Petersen and Schmidt, 2003) (Nath and Sinai, 2003). Treatment must be started as soon as possible after diagnosis and continued for several days after signs have disappeared.

In acute illness, treatment is sometimes started on the basis of a high antibody titer in the first test. If clinical improvement is not seen within two to three days, the diagnosis of Toxoplasmosis should be questioned (Dubey, 2004).

2.10. Public health

Many owners allow their cats to defecate in the surrounding environment, there is a possible likelihood for the *T. gondii* oocyst to enter the environment and later be transmitted to humans (Dabritz and Conrad, 2010). Other than the domestic cats responsible for infecting the environment, there are large populations of unowned cats world-wide (Dabritz and Conrad, 2010). This in itself is a public nuisance especially if the numbers of strays are too many.

Pregnant woman infected with *T. gondii* transfer the infection to their fetuses and they suffer from severe neurological and debilitating ocular disease (retinochorioditis and anterior uveitis). Visual impairments come in when infection reaches the ocular sites that is the retina and the choroid. The lesions caused by infection leads to retina scarring hence impaired vision or blindness (Maenz *et al*., 2014). For instance four point two (4.2%) percentage of uveitis cases in Germany have been attributed to *T. gondii* infections (Maenz *et al*., 2014) which brings a serious public health concern.

The social and economic impact Toxoplasmosis has on the public is huge and this is through human suffering and the financial cost of caring for the sick children with blindness and mental retardation and illness (Roberts *et al*., 1994, Roberts and Frenkel, 1990).

Toxoplasma gondii in meat animals (goat, sheep) also poses a huge medical importance and public health concern because of the severe diseases it causes to animals and humans as consumers. The economic losses occur through animal product loss and also the expenses of medicine used to treat the disease. Although the transmission of *T. gondii* by tissue cysts is high in carnivores, the transmission is also relatively high by herbivores and omnivorous meat animals (Fayer, 1981).

CHAPTER 3.

MATERIALS AND METHODS

3.1 Study area

This study was conducted in Lusaka district of Lusaka province of Zambia. Lusaka district has a total area of 418km² with an altitude/ elevation of 1,279m and located 15° 24'59.99''S and 29° 00'0.00''E. Lusaka, the capital of Zambia has an approximate human population of three million (REF, 2022). The study was conducted from 2018 to 2019. The area in which the samples were collected has the same climate patterns as they are in the same district with of course few minor differences in the environmental patterns. Lusaka is 1281metres above sea level and is considered to be temperate and warm. It has an average annual temperature of 20.4 ^{o}C and hottest times are around October with temperatures as high as 24.7^{o}C. July has the lowest average temperature of 16^{o}C. The recorded average annual rainfall is 970mm. (http.//en.climate-data.org/Africa/Zambia/Lusaka-province/Lusaka-510/#climate-table).

3.2 Study population

The study population was both domestic and stray cats. The study areas for domestic cats were purposively and strategically selected from within the city namely, Pet vet clinic, University clinic and Lusaka show grounds clinic where questionnaires were administered to the cat owners bringing their pets in for check-ups and samples (blood) collected from the pets. The stray cats were captured all across the city, some of which were caught within the University of Zambia premises. The inclusion criteria used for domesticated cats was to include all cats that were neither too small nor too sick, and must have been from within Lusaka district. The cats were excluded if they did not meet the criteria.

Stray cats were caught using trap cages as shown in Figure 3.1 below:

Figure 3.1: Showing traps and trapped stray cats.

3.1.3 Cat management system in the study area

Most domestic cats in Lusaka are both indoors and out door, which means that they are allowed to roam outside the house and some have access to the outside of the yard. There are a number of stray cats that roam around the district with very little or no control and management.

3.2. Study design and sample size calculation

A cross-section study design was used in this study. The total population of cats in Lusaka district is unknown. However, the 2022 Zambian livestock survey reported 24,735 of cats raised within Lusaka province in that year. Sample size for detection of antibodies of *Toxoplasma gondii* was therefore calculated using the prevalence threshold of 50% with the confidence of 95%. The sampling method was therefore a convenient one because the population of cats in Lusaka was unknown at the time of sampling. The formula used for sample determination was:

$N=Z^2P\ (1-P)\ /d^2$ (Cochran, 1977)

Where N is the sample size, Z is statistic value (1.96), P is the expected prevalence in the population based on previous prevalence (50%, since previous prevalence is unknown) and d is the absolute error (0.05).

The sample size calculated was 385 and the inclusion criteria was cats within Lusaka district visiting the selected veterinary clinics and stray cats trapped from within Lusaka district.

3.3. Data and sample collection

Data and sample collection occurred between the year 2018 and 2019. A questionnaire survey was carried out during sample collection from domestic cats. Owners were interviewed through a questionnaire to assess the risk factors for Toxoplasmosis in cats. The questionnaire was meant to assess the risk factors like age, sex, whether the cat was indoor or outdoor, contact with other cats, straying and diet. The age of the strays was estimated using the number of teeth and size. For the stray subjects, all cats that were trapped were included in the study apart from those not meeting the inclusion criteria.

3.4. Blood collection and serum preparation

About 2ml of blood was collected into a plain tube from each cat from the Cephalic, Jugular or Saphenous vein (depending on the size of the cat) as seen in figure 3.2. The cephalic was the preferred vein for small cats while the jugular was used for small cats. The blood was then stored at room temperature to facilitate clotting. Then it was centrifuged for 5 minutes at 3000rpm to separate the serum from other blood components. The serum was then stored at -20^{o}C until analysis.

Figure 3.2: Showing blood collection

3.5. Detection of Toxoplasma gondii using antigen-antibody ELISA Kit.

Indirect multispecies ELISA kit (indirect ELISA assay (ID screen® Toxoplasmosis-indirect Multispecies, IDVET, France) was used to detect the antibodies of *T. gondii* in blood (serum) against *Toxoplasma gondii* p30 protein. The procedure was followed according to manufacturer's instructions. The serum was thawed at room temperature. Ninety microliters of diluted buffer 2 was added to each micro well. Thereafter ten microliters of the serum sample were added to the remaining micro wells. The plate was then incubated for 45 minutes at 21ºC. Each well was then emptied and washed with 300µl of wash solution. Then 100µl of substrate solution was added to each well and the plate was incubated for 15 minutes at 21ºC in the dark. After this, 100µl of the stop solution was then added to each well to stop the reaction. The results were then read and validated using the negative and positive control provided for by the manufacturer at 450 nm. The samples were considered positive if the Optic Density (OD) was greater than or equal to 50%, doubtful if between 40 and 50% and considered negative if below 40%. The formula used for determination of positivity was;

% = 100*OD of sample/OD of PC (where OD is the Optic Density and PC is Positive Control)

3.6. **Data Analysis**

The data collected was organized in excel and analysed using Chi-square and Binary logistic regression using SPSS. For further interpretation for the binary logistic regression, the omnibus test of model coefficients had to be significant with a P value of less than 0.05 and an overall percentage of 85.3% (above the recommended 80% by researcher) on the classification table. Additionally the Hosmer's value had to be above 0.05.

CHAPTER FOUR.

RESULTS

4.1. Seroprevalence:

A total of 178 samples were tested. The overall seroprevalence in both domestic and stray cats was found to be 16.85%. Of the 178 samples tested, 88 were from domestic cats and 90 from stray cats. Of the 178 samples, 30 (16.85%) were seropositive for *Toxoplasma* Immunoglobulin G. From the 30 seropositive samples, 60% (18) originated from domestic cats and 40% (12) originated from stray cats. The seroprevalence by source of sample was 20.4% for domestic cats and 13.3% for stray cats.

The results from the ELISA were as shown in figure 4.1 below:

Figure 4.1: showing the ELISA plate results

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4.2. Proportion of Seropositivity:

Table 4.1: The table below shows proportions of Seropositivity according to variables investigated, the information displayed were obtained using chi square where only 149 samples were considered valid.

Variables	Negatives	Positives	Total
Sample Origin			
Stray cats	73(48.9%)	12 (8.10%)	85
Domestic cats	46(30.8%)	18 (12.1%)	64
			N=149
Sex			
Male	49(32.9%)	18 (12.1%)	67
Female	70 (46.98)	12(8.1%)	82
			N=149
Contact with others			
Yes	95(63.8%)	24 (16.1%)	119
No	21(14.1)	6 (4.0%)	27
Missing	3 (2%)	0	3
			N=149
Passing of stool			
Missing	3(2%)	0	3
Litterbox	11(7.4)	4 (2.6%)	15
Outside	105(70.5%)	26(17.4%)	131
			N=149
Habitation			
Missing	3(2%)	0	3
Indoor	15(10.1%)	2 (1.3%)	17
Outdoor	86(57.7%)	20(13.4%)	106
Both indoor and outdoor	15(10.1%)	8(5.4%)	23
			N=149
Straying behavior			
Missing	9(6.0%)	1 (0.7%)	10
Yes	90(60.4%)	22(14.8%)	112
No	20(13.4%)	7(4.7%)	27
			N=149
Age			
Missing	5(3.4%)	3(2.0%)	8
0-6months	21(14.1%)	3(2.0%)	24
>6m-12months	29(19.5%)	2(1.3%)	31
>1-3yrs	58(38.9%)	19(12.8%)	77
>3yrs	6(4.0)	3(2.0%)	9
			N=149

4.3. Risk factors of *Toxoplasma gondii* infection in cats.

To measure association between the outcome and the different predictor variables, the chi-square was used. Two variables were found to be significantly associated with Seropositivity. The variables included eating other foods (p value of 0.007) and sex with a p value of 0.038.
This is shown in Table 4.2 below:

Table 4.2: Measure of Association between variables and outcome (Seropositivity)

Variable	Odds Ratio	DF	P VALUE	95% C,I (0.05)
Contact with others	0.411	2	0.530	0.422-5.338
Excretion (where they pass stool)	0.375	1	0.214	0.103-1.696
Habitation (Indoor/outdoor)		1	0.205	0.079-2.007
Straying behavior (leaving the yard or not)	0.923	1	0.252	0.434-31.387
Consumption of raw meat	0.511	1	0.810	0.072-7.864
Consumption of kitchen food	0.64	1	0.530	0.187-2.372
Consumption of commercial food	0.7	1	0.649	0.221-2.567
Consumption of other diet/foods	**11.52**	**1**	**0.007**	**1.395-95.418**

Sex	**0.264**	**1**	**0.038**	**0.071-0.976**
Age	1.30	4	0.094	
Presence of other cats	2.9	1	0.107	0.768-10.949
Presence of other animal species		1	0.290	
Neutered		1	0.290	0.148-1.785
Vaccination		1	0.221	0.118-1.664
Disposal of fecal material		1	0.573	0.207-2.390

When the stray cats were compared with the domestic cats, sample source was significantly associated with Seropositivity with a p value of 0.035 and there was a significant difference between the two groups as shown by Table 4.3 below:

Table 4.3: Measure of Association between sample source and Seropositivity

	DF	P-value	CI,95%	Comment
Sample source (domestic, stray)	1	0.035		significant

4.3.2. MEASURE OF STRENGTH OF ASSOCIATION

From the regression analysis of the following variables: sex, age, diet, living environment, place of defecation, contact with other animals and vaccination status, only eating other diets was found to be a predictor variable and significant strength of Association. This variable had a p value of 0.025 and a confidence interval of 1.550-616.35 at 95%. It was therefore established in the binary logistic regression analysis that older cats were more likely of getting *T. gondii* infection than younger cats. As the cat got older, the chances of getting infected became higher and this was observed by noting the increase in odds ratio over time.

The findings were detailed in table 4.4 below:

Table 4.4: Binary logistic regression

	B(estimated logit coefficient)	S.E.(standard error of coefficient)	Wald	df	Sig.(significant level of coefficient)	Exp(B),odds ratio	95% C.I.for EXP(B) Lower	Upper
Sex(1)	-.929	.938	.982	1	.322	.395	.063	2.480
Age			1.858	4	.762			
Age(1)	-.465	41497.106	.000	1	1.000	.628	.000	.
Age(2)	16.122	40192.979	.000	1	1.000	10037337.447	.000	.
Age(3)	19.336	40192.979	.000	1	1.000	249789355.695	.000	.
Age(4)	18.313	40192.979	.000	1	1.000	897716242.99	.000	.
Diet_raw_meat	-3.254	2.307	1.989	1	.158	.039	.000	3.553
Diet_other	3.431	1.527	5.048	1	**.025***	30.905	1.550	616.353
Do_they_live_indoor_outdoor	.643	.708	.825	1	.364	1.902	.475	7.618
Pass_stool	-1.819	1.406	1.675	1	.196	.162	.010	2.549
Contact_with_others	-.045	.913	.002	1	.960	.956	.160	5.719
Vaccinated	.942	1.097	.737	1	.390	2.566	.299	22.040
Constant	-18.575	40192.980	.000	1	1.000	.000		

Variable(s) entered: Sex, Age, Diet_raw_meat, Diet_other, do_they_live_indoor_outdoor, Pass_stool, Contact_with_others, Vaccinated

- Statistically significant

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4.3.3. AWARENESS OF *TOXOPLASMOSIS*

From the 86 questionnaires administered only 22/86 (25.6%) of the respondents had some knowledge about *Toxoplasma gondii*, while 35/86 (40.7%) had no knowledge. The rest 29/86 (33.7%) did not respond to the awareness question. From the valid respondents of whom were 57, 61.4% (35/57) of them had no knowledge of the disease while 38.6 % (22/56) had little information. Using Chi Square, it was established that there was no association between awareness and IgG Seropositivity.

Table 4.5 below shows the Seropositivity representation according to the awareness of the valid respondents. Using Chisquare, we found no association between awareness of *T.gondii* and Seropositivity with a pearson 2-sided value of 0.542.

Table 4.5: Seropositivity according to cat owner awareness of Toxoplasmosis:

		Negative	Positive	Total
Awareness	Yes	15(26.3%)	7(12.3%)	22
	No	27(47.4%)	8(14.0%)	35
Total		42(73.7%)	15(26.3%)	57

4.3.4. RESIDENTIAL INFORMATION FOR THE DOMESTIC CATS

Roma had the highest representation of cats with a total of 10 coming from the area. From the 10, only 1 (10%) was positive. However, Kamwala had the highest number of positives (three) with a representation of 60% from the 5 collected in the area. Note that there were a total of 86 questionnaires administered to domestic cat owners (from the 88 domestic cats), meaning and only 54 are presented in the table above. This means that there were 34 cats that did not have the demographic information. See table 4.6 below:

Table 4.6 Seropositivity according to source of sample/ residential area

Residential area	negative	positive	Total number
Kabulonga	2	1	3
Mandahill	1	0	1
Lilayi	2	0	2
Levy area	0	1	1
Roma	9	1	10
Makeni	1	0	1
Chudleigh	5	0	5
woodlands	1	0	1
Kamwala	2	3	5
Salama Park	1	0	1
Kalingalinga	0	1	1
Kalundu	1	2	3
Emmasdale	2	0	2
Meanwood	0	1	1
Olympia	2	1	3
Mtendere	1	0	1
Roma Extention	1	0	1
Ng'ombe	1	0	1
Rhodes Park	2	2	4
Chilenje	1	0	1
Longacres	1	0	1
Helen Kaunda	1	0	1
Leopards Hill	1	0	1
Northmead	2	0	2
Other	1	0	1
Total	41	13	54

CHAPTER FIVE

DISCUSSION

The overall seroprevalence of both domestic and stray cats was found to be 16.85%. This study indicated that the seroprevalence (IgG) of Toxoplasmosis in cats Lusaka was much lower than the one found by Hammond-Aryee et al (2015) in South Africa which was 37.1%. Other *T. gondii* serological studies conducted in Thailand and Egypt found a prevalence of 15.4% and 97.4% respectively (Thiangtum *et al* (2006) and Al-kapanny *et al* (2010). It could be seen that the results of this study were similar to that of Thailand. This could be because of similarities in climate in the two regions (tropical climate). The difference in Seropositivity with other regions could be attributed to different environmental conditions. A study conducted by Tagwireyi *et al* (2017) established that *T. gondii* is most prevalent in hot humid regions.

This study therefore established that the source of the cat (being a domestic cat) was associated to *Toxoplasma* Seropositivity. This finding could be due to the fact that the majority of cats were kept partly or entirely outdoor (as shown in table 4.1), thereby increasing the exposure time in the environment and/or exposure load. This suggested that most domestic cats are in constant contact with the environment just like strays or feral cats. Similar to findings by Rochlitz *et.al* (2000), who estimated that 14 to 36% of cats are acquired as strays, a good number of stray cats in Lusaka were previously-owned cats that are later re-homed after being rescued by animal welfare societies in Lusaka. It was therefore safe to assume that there was a huge overlap between the domestic and stray cats in Lusaka. The environment was contaminated with *T. gondii* oocysts thereby exposing the domestic cats to the disease agent when they went outside.

The contaminated environment could also partly explain the detected antibodies in humans in a study conducted by Frimpong *et al* (2017) in Lusaka. The hypothesized source of infection to humans could either be the people came in contact with the infected cats or they came in contact with a contaminated environment (ingestion of food contaminated with infected cat feces).

Must *et al* (2015) established that contamination with *T. gondii* oocyst poses a long term risk to other hosts including humans.

This study showed that the *Toxoplasma* IgG seroprevalence of domestic cats is higher than that of stray cats. These results are contrary to other studies like that done in Seoul, Korea which showed that domestic cats were not exposed, while strays had a very high prevalence (Lee *et.al*., 2010). The reason for these results could be because the domestic cats in Zambia are not purely

indoors but also have access to the outside almost on a daily basis. This then makes them not very different from the strays that also have access to the environment.

This study established that sex was associated with Seropositivity. The male cats were more likely to be infected with Toxoplasmosis than females. There was a significant difference between the males and females with a p value of 0.038. This is similar to a study done by Lee *et al* (2010), in Korea, where they found that the Seropositivity in male strays was higher than that of the females. The difference in the males and females in our study could be attributed to the different behavior of male and female cats with males known to stray a lot more than female cats thereby exposing themselves to *T. gondii. (*Lee *et al., 2010).* This finding in our study is different from a study done by Lopez *et al* (2008), who established no difference in Seropositivity between the males and females

From the linear regression analysis, it was established that older cats were at a higher risk of infection with *T. gondii* infection than younger cats. As the cat got older, the chances of getting infected became higher. This is similar to a study done by Must *et al* (2015), which found that cats older than one year old were 8.7 times more likely to test seropositive than younger cats, a clear indication that there is an increasing exposure to the infectious agent with increasing age. This supports a number of authors like Opsteegh *et al (*2012) who found that infection in older cats is due to the continuous exposure to *T. gondii* in the environment with time.

It has also been found in several hosts, domestic cats inclusive that the Seropositivity of *T. gondii* infections increases with age, indicating that most of these infections are postnatally acquired , Dubey *et al*. (2010), Castillo-Morales *et al* (2012), however ,had different findings from those in this study, whose study showed that infection by *T. gondii* was high in younger cats and this was attributed to the weak immunity that young stray undernourished kittens have.

It was also established in this study that those cats that were fed on other foods (other than raw meat, kitchen left overs and commercial food), were associated with Seropositivity. One study showed that those that ate raw meat had a high risk of getting Toxoplasmosis infection. This is different from our study which indicates a high risk in cats fed on other foods. Because these foods were not specific it is possible the cats hunted thereby exposing them to *T. gondii* through eating of other foods such as infected rats.

The adjusted odds ratio of cats that fed on other diets, and cats that were kept both indoor and outdoor were all above one meaning they had an increased chance of (increased odds) of being seropositive with *T. gondii*. Only 'other diets' was a predictor variable. The other very high odds ratio from the age groups could be due to the low numbers of cats in the said groups and not due to an increased risk.

The risk factors associated with Seropositivity of *Toxoplasma* were found to be sex and diet which is different from that found by Tagwireyi *et al* (2016) who found the risk factor to be age in cats. Besné-Mérida *et al*. (2008) however found risk factors to be sex and the frequent consumption of raw meat. There was also no significant association between sex or diet with Toxoplasmosis infection in a study done by Castillo-Morales *et al* 2012. Other factors like age and outdoor access were found to be associated with toxoplasma infection Deske *et al* (2013). Most studies found eating raw meat to be associated with Toxoplasmosis IgG infection. However, this study found that those cats that were fed on other diets were associated with Seropositivity. Other diets could be anything from hunting to feeding from rubbish cans.

This study also established that there was no association between awareness of the protozoa by owners of the cats to Seropositivity of Toxoplasmosis in the cats. It is logical that those clients that were enlightened with information would care more about their cats and the prevention practices but that's not the case in this study. There is limited information on the knowledge and attitudes of cat owners towards the prevention of Toxoplasmosis (Nurseha *et al*., 2023).

A study was carried out in Nigeria to establish the knowledge levels of Toxoplasmosis among medical doctors and there were serious knowledge gaps that need to be addressed (Efunshile *et al*., 2017).

Most studies correlate knowledge of cat owners with infection and /or related practices in humans and not in cats like in our study. Studies that established association between cat owner's knowledge of Toxoplasmosis and their potential of getting infected and their practices include those done by Eroglu *et al* (2021) and Uddin *et al* (2023).

CHAPTER SIX

6.1. CONCLUSION

To the best of my knowledge, this study is the first report on *T. gondii* in cats in Lusaka, Zambia and it showed presence of *T. gondii* antibodies (both in stray and domestic cats with a seroprevalence of 16.85%. Of these 60% was from domestic cats and 40% were stray cats and there was a significant difference between the two groups. The risk factors found were sex and diet. This information is vital not only for communities that harbor cats but also for policy makers on the control of cat populations and strays. There was little information from cat owners on Toxoplasmosis and there was no association between awareness of Toxoplasmosis and Seropositivity. This information is important to ensure public awareness on the importance of hygiene practices when handling cats.

6.2. RECOMMENDATIONS

Based on the results the following are the recommendations

a) Future studies carried out must include PCR in efforts to try and isolate and classify the prevalent strain of *T. gondii* in Lusaka and Zambia at large.
b) Sensitization to the public must be carried out to educate them on the importance and dangers of *T. gondii* in immune-compromised individuals.
c) Campaigns should be carried out to educate the communities about measures that can be put in place to reduce or prevent transmission. The communities can also be educated about hygiene practices that are required when handling and disposing cat excrement.
d) There is also need to do a campaign to educate pet owners about the importance of keeping the cats confined to the resident premises rather than letting them freely roam around the compounds and streets.
e) It is recommended that future studies involve a wider coverage of Zambia (different districts) in order to assess the overall seroprevalence of Toxoplasmosis is cats here in Zambia.

6.3. STUDY LIMITATION

Generally, a large sample size would have been more ideal but due to the limited number of cats that came to the veterinary facilities as well as those trapped; the calculated sample size was not achieved.

CHAPTER SEVEN

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APPENDICES

APPENDIX 1: CONSENT FROM CAT OWNERS

CONSENT FORM

The objective of this study is to find out whether cats in Lusaka have Toxoplasma gondii and the risk factors associated with the parasite. The information you will give in this questionnaire is confidential and will not be revealed to anyone apart from the primary investigators and to you the owner on request. The blood that is collected will be analysed in the laboratory and the findings will be correlated with the questionnaire findings and risk factors of toxoplasma gondii established. In the best of my knowledge, there are no known risks associated with participating in this study. However the benefit is that you will know whether or not your cat is carrying the parasite and necessary measures will be put in place to treat the pet and avoid contracting the disease. If you agree to take part in the study, you will be required to respond to the questionnaire in the best of your knowledge and it will take about 12 minutes to complete. If you feel uncomfortable with some of the questions in the survey, you are allowed to withdraw from participating in the study without any consequences. If you have any further questions please contact Dr Farai Phiri, The University of Zambia, Faculty of Veterinary Medicine on +260-979-779-241 (faraiphiri08@gmail.com) or contact the ERES Converge (ethics committee) who were responsible for reviewing this research protocol on eresconvergeltd@gmail.com or call on +260977493220, +260955155634 and +260955155633. To show that you have given consent for us to work with you in this study please sign below

Name/Signature..... Date.....

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APPENDIX 2: QUESTIONNAIRE OF RISK FACTORS

CODE / NUMBER:

QUESTIONNAIRE

A STUDY TO DETERMINE THE SERO-PREVALENCE AND MOLECULAR CHARACTERISATION OF TOXOPLASMA GONDII IN CATS AND ITS ASSOCIATED RISK FACTORS IN LUSAKA, ZAMBIA.

I, Farai Phiri, a student of master's in public health at the University of Zambia, Faculty of Veterinary Medicine, will be conducting a study to determine the prevalence of Toxoplasma Gondii in Lusaka.

You have been selected as one of our respondents to kindly answer the questions based on the best of your knowledge. Any information provided will be kept strictly confidential and will be used for research and planning purposes only. . If you agree to take part in the study, you will be required to respond to the questionnaire in the best of your knowledge and opinion. The questionnaire should take about 12 minutes to complete. If you feel uncomfortable with some of the questions in the survey, you are allowed to withdraw from participating in the study without any consequences. For any clarifications, contact the researcher on +260 979779241. Please sign on the consent form below.

Name of participant (Optional)..... Signature.....

Contact details.....

Name of researcher..... Signature.....

Kindly Tick √ in the box or fill where appropriate.

SECTION A

1. How old is your pet?

   ……..

2. What is the sex of your pet?

   MALE ☐ FEMALE ☐

3. What is the breed of your pet?

   ……

4. What is the weight of your pet?

   ……

5. What is the residential area of the pet?

   ……

6. Is your pet neutered (spayed/ castrated) or not?

   YES ☐ NO ☐

7. Is your pet vaccinated?

   YES ☐ NO ☐

8. If the answer to 6 is yes, what vaccines?

   ……

9. Do you de-worm your pet?

   YES ☐ NO ☐

10. What do you feed your PET?

Commercial food☐ kitchen food ☐raw meat ☐ others (specify) .....

11. How long have you had your pet?

.....

12. Are you the first owner or the pet belonged to someone else before you?

1ST ☐ 2ND ☐ 3RD ☐

13. Do you have other cats?

YES ☐ NO ☐

14. How many other cats do you have?

CATS.....

15. Do you keep any other animal SPECIES?

YES ☐ NO ☐

16. If the answer to question 15 is YES, which other animals do you keep?

SHEEP ☐ GOATS ☐ PIGS ☐ CATTLE ☐

OTHERS..... (PLEASE SPECIFY)

17. Is your pet indoor or outdoor?

INDOOR ☐ OUTDOOR ☐ BOTH ☐

18. Does your pet go out of the yard?

YES ☐ NO ☐

19. Are there any stray animals around your yard/house?

YES ☐ NO ☐

20. If the answer to 19 is YES, which animals exactly are stray?

.....

APPROVED
03 SEP 2019
ERES CONVERGE
P/BAG 125. LUSAKA.

21. Where does your pet pass urine/ stool?

Litter Box ☐ around the yard ☐

22. How do you can rid of the excreta?

.....

SECTION B

In this section, I am going to ask you questions about the disease in question.

23. Are you familiar with the disease called toxoplasmosis?

YES ☐ NO ☐

24. If the answer to question 23 is **YES**, how strong is your familiarity with the disease?

Weak ☐ Strong ☐ Very strong ☐

25. If the answer to question 22 is yes, how did you come to know about it?

Family ☐ Friend ☐ Internet ☐ School ☐

26. How would you like feedback about the results of your pet? (TICK)

PHONE CALL ☐ WHATSUP ☐

EMAIL ☐ TEXT MESSAGE ☐

THANKYOU SO MUCH FOR YOUR COOPERATION

APPENDIX 3: ETHICS APPROVAL

Plot No. 1, Cnr Joseph Mwilwa & Great East Road
Rhodes Park, Lusaka - Zambia
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 977 493220
Email: eresconvergeltd@gmail.com

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

3rd September, 2019

Ref. No. 2019-Jan-008

The Principal Investigator
Dr. Farai Phiri
University of Zambia
School of Veterinary Medicine
Dept. of Disease Control
P.O. Box 32379,
LUSAKA.

Dear Dr. Farai,

RE: SERO-PREVALENCE AND MOLECULAR CHARACTERIZATION OF *TOXOPLASMA GONDII* AND ITS ASSOCIATED RISK FACTORS IN CATS IN LUSAKA DISTRICT, ZAMBIA.

Reference is made to your protocol. The IRB resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	**Ordinary**	Approval No. **2019-Jan-008**
Approval and Expiry Date	Approval Date: 3rd September, 2019	Expiry Date: 2nd September, 2020
Protocol Version and Date	Version - Nil.	2nd September, 2020
Information Sheet, Consent Forms and Dates	• English	2nd September, 2020
Consent form ID and Date	Version - Nil	2nd September, 2020
Recruitment Materials	Nil	2nd September, 2020
Other Study Documents	Questionnaires.	2nd September, 2020
Number of participants approved for study	-	2nd September, 2020

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.
- A reprint of this letter shall be done at a fee.

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. Jason Mwanza
Dip. Clin. Med. Sc., BA., M.Soc., PhD
CHAIRPERSON

APPENDIX 4: PICTURE OF SERO RESULTS AFTER STOP SOLUTION WAS ADDED