Seroprevalence and Risk Factors of *Toxoplasma gondii* Infection among Goats from Choma District, Zambia

By

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(COMPUTER NUMBER: 2019123517)

A Dissertation submitted to the University of Zambia in Partial Fulfillment of the requirements for the award for the degree of Master of Science in Tropical Infectious Diseases and Zoonosis

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Lusaka

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DECLARATION

I, Zimba Chiluba, do hereby declare to the senate of the University of Zambia that this dissertation is my original work and has not been submitted for a degree award in any other University.

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Signature   Date

APPROVAL

This dissertation by Chiluba Zimba has been approved as fulfilling the requirements for the award of the degree of the Masters of Science in Tropical Infectious Disease and Zoonosis by the University of Zambia.

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DEDICATION

I dedicate this report to my dearest parents, siblings, relatives, my family (my husband and children) and friends who love me and wished me well during my academic journey for their unending support, prayers and encouragement.

ACKNOWLEDGEMENTS

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ACEIHDA	African Centre for Infectious Disease in Humans and Animals
CDC	Centre of Disease Control and Prevention
CNS	Central Nervous System
CSF	Cerebral Spinal Fluid
DAT	Direct Agglutination Test
DC	Dendritic Cells
DH	Definitive Host
DNA	Deoxyribonucleic Acid
DVO	District Veterinary Officer
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food Agriculture Organisation
GDP	Gross Domestic Product
GI	Gastro-Intestinal
GIT	Gastro-Intestinal Tract
HIV	Human Immune Deficiency Virus
IFA	Indirect Fluorescent Test
IFN-y	Interferon-gamma
IgG	Immunoglobin G
IgM	Immunoglobin M
IH	Intermediate Host
IT	Indirect Haemagglutination Test
IL	Interleukin
ILA	Indirect Lathel Agglutination Test
IP	Intraperitoneal
ITSI	Internal Transcribed Spacer
LD	Lethal Dose
MAT	Modified Direct Agglutination Test

MO	Macrophages
NK	Natural Killer Cells
NO	Nitrogen Monoxide
OD	Optic Density
PBS	Phosphate Buffered Saline
PC	Positive Control
PCR	Polymerase Chain Reaction
PMNs	Polymorphonuclear Leucocytes
PV	Paraphistophorus Vacuole
PVO	Provincial Veterinary Officer
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase- Polymerase Chain Reaction
SNPs	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
TL	T-Lymphocytes
TNF	Tumour Necrosis Factor
USA	United States of America
VA	Veterinary Assistant

ABSTRACT

Toxoplasmosis is a disease of economic importance and of public health significance. Despite being a zoonotic disease, information on *Toxoplasma gondii (T. gondii)* infection in goats has not yet been reported in Zambia. A cross-sectional sero-epidemiological study of *T. gondii* infection in goats was carried out from 31st August to 5th September, 2021 in eight veterinary camps of Choma district, Southern Province, Zambia. A closed-ended questionnaire was also administered to forty one farmers whose goats were included in the study to identify potential risk factors for *T. gondii* infections in goats. Three hundred and twenty-four goat sera were examined for IgG anti- *T. gondii* antibodies using an ELISA kit. All factors attributing to *T. gondii* infection in goats were analyzed using Chi-square and logistic regression analysis. The serological results showed an overall seroprevalence of 3.11% (95% CI: 2.71-9.43). Female goats had a seroprevalence of 4.13% (95% CI: 3.55-13.61: p =0.001), while all the males were negative. According to age, adults had a seroprevalence of 4.00% (95% CI: 3.16-13.23: p =0.001) than the young 1.01% (95% CI: 0.09-10.63). The significant risk factors were presence of stray cats within the farm yards (aOR: 5.092, 95% CI: 2.442-16.376: p =0.033), cats access to water and feed troughs (aOR: 2.806, 95% CI: 1.002-11.528: p =0.041) and presence of cat feaces within the farm yards (aOR: 0.042: 95% CI: 0.010-0.761: p =0.029). The detection of *T. gondii* infection among goats indicates a potential risk presented to humans from food animals. This further highlighted the impact of *T. gondii* infection on the health, production and reproduction of goats and other ruminants.

Keywords: ELISA, Humans, Risk factors, Goats, Seroprevalence, *Toxoplasma gondii*, Choma

CHAPTER ONE

INTRODUCTION

1.0 Background

Livestock significantly contributes to food production and economic growth in most parts of the world. Despite the significant contribution of livestock to agricultural gross domestic product (GDP) (FAO, 2001), its productivity is affected by several factors including lack of pastures/folder and diseases. Previous studies indicate that most diseases that affect livestock are caused by parasites, which are a significant challenge in the development of the livestock industry in the developing world (Bartova *et al*., 2009). This results in a reduction of animal production that leads to a shortage of food supply of animal origin for human beings. One of the parasitic diseases includes toxoplasmosis, a zoonotic disease of great economic importance (Bartova *et al*., 2009)

Toxoplasmosis is an economically significant livestock disease since it causes major reproductive failures, including abortion and fetal malformation hence causing a significant drawback in the livestock industry (Bartova *et al*., 2009). Goats and sheep are the most susceptible to infection by *T. gondii,* with evident clinical signs of a brief episode of fever and anorexia for three to six days after infection and lasting about 4-10 days (Castano *et al*. 2016). The infection may lead to foetal death (with subsequent reabsorption), abortion, mummification, and/or the birth of weak kids (Estenban-Redondo *et al*., 1999). Several reports from other researchers have demonstrated a variation in seroprevalence of *T. gondii* infections in domestic animals across the world, and the ranges are between 3-92% in sheep (Buxton and Lesson, 2007), goats 5-77% (Sharif *et al*., 2007) and cattle 0-99% (Hall *et al*., 2001).

Toxoplasmosis is a disease of public health significance owing to its transmission to humans by ingestion of raw or undercooked meat, which contains tissue cysts and can be congenitally transmitted to the foetus during pregnancy (Alvarado *et al*., 2011). *Toxoplasma gondii* infection is harmful in immunosuppressed and immunocompromised individuals with Human Immune-Deficiency Virus (HIV) /Acquired Immune Deficiency Syndrome (AIDS) and congenitally infected children and may lead to serious health challenges (Tenter *et al*., 2000; Montoya and Liesenfeld, 2004). There is usually a high risk of developing severe infections in a population of women who are pregnant, those undergoing organ transplants, and HIV infected people or those with Hodgkin's disease (Saadatnia and Golkar, 2012). Most infections in humans are

usually asymptomatic, but serious complications can arise due to trans-placental infections. The infection may lead to abortions, mortality, and hydrocephalus in new-borns while in immunocompromised individuals with HIV infection symptoms are pneumonia, myocarditis, retinitis and encephalitis (Cook *et al*., 2000). Nearly one-third of the world's population is estimated to be infected with *the T. gondii* parasite, and about 7% of the people in China suffer from chronic infection of *T. gondii* (Zhou *et al*., 2011). The Centre for Disease Control and Prevention (CDC) reported that 11% of the USA population is infected with *T. gondii*, and infection affects young adults. In Zambia, the seroprevalence of *T. gondii* infection was estimated at 5.87% in pregnant women in Lusaka district (Frimpong *et al*., 2017).

Goat farming is an essential aspect of rural livelihoods in Zambia. Goat rearing serves as a source of milk, meat, hides, bones and manure, thus contributes to food security, employment and income generation (Ellis & Freeman, 2004). According to the 2018 national statistics data, Zambia's livestock population was 3.7 million cattle, 3.5 million goats, 170 thousand sheep, 1.1 million pigs, and 15 million chickens (CSO 2019). The rapid growth in the livestock sector in the past decade has largely been due to the increasing population, income and urbanization (Steinfied *et al*., 1997). Goat farming is therefore a key subsector which may significantly contribute to poverty reduction, livelihood security and economic growth.

In tropical Africa, including, Zambia, the benefits of goat rearing have not been fully realised due to constraining factors such as drought, lack of pastures, inefficient traditional rearing methods and disease outbreaks (Mitenga *et al*., 1994; Rege, 1994).

1.1 Statement of the Problem

Most of the causes of reproductive loss in goats remain uncertain in Zambia. According to Muma *et al*. (2006) most goats had a history of abortions characterized by arthritis in the final trimester but did not test positive to *Brucella* spp. Despite the global importance of the zoonotic parasite (*T. gondii*) little is known regarding its infection in Zambia. There is currently lack of information in Zambia regarding *T. gondii* infections in goats, highlighting the need for studies to determine the seroprevalence and risk factors for *T. gondii* infections.

1.2 Study Justification

Reduced reproductive efficiency contributes to low productivity which may lead to loss in herd milk, decreased kid crop, lost replacement or marketable stock of goats. This leads to poverty for small holder farmers hence indicating the need to investigate other possible causes of

abortions in goats in Zambia. The information from this study would help to raise farmer awareness about the disease and its impact. The data from this study will further help in planning and implementation of control measures in order to prevent the transmission of *T. gondii*.

1.3 Research questions
(i)   What is the seroprevalence of *T. gondii* in goats in the Choma district?

(ii)   What are the risk factors associated with *T. gondii* infections in goats in the Choma district, Zambia?

1.4 General objective
This study aimed to describe the epidemiology of toxoplasmosis among goats in Choma District, Zambia

1.4.1 Specific objectives
(i). To determine *T. gondii* seroprevalence in goats in Choma District, Zambia

(ii).To assess and identify the risk factors associated with *T. gondii* infections in goats in Choma District, Zambia.

CHAPTER TWO

LITERATURE REVIEW

2.0 History of Toxoplasmosis

Charles Nicolle and Louis Manceaux at the Institute of Pasteur in Tunis, Tunisia, discovered *Toxoplasma gondii* in a desert rodent known as *Ctenodactylus gundi* about 114 years ago (Ferguson, Henriuezet *et al.*, 2005; Ferguson, 2009). Later on Alfonso Splendore independently reported on *T. gondii* in rabbits at Sao Paulo (Dubey, 2010). The parasite is widely distributed and infects all warm-blooded animals globally. It is used extensively as a model for the cell biology of apicomplexan organisms (Ajioka and Soldati, 2007; Dubey, 2007). It has been reported that nearly one-third of the world's human population has been exposed to *T. gondii* infection (Pappas *et al.*, 2009)

2.1 Aetiology and lifecycle

Toxoplasma gondii belongs to the Kingdom Animalia, Phylum Apicomplexa, Class Conoidasida, Subclass Coccidiasina, Order Eucoccidiorida, Family Sarcocystida, Genus *Toxoplasma* and species *gondii*. The obligate intracellular protozoan parasite has a recognizable polarized cell structure and a complicated cytoskeletal system (Weiss and Kim, 2007). The conoid's apical ends of the parasites contains a variety of secretory organelles, such as granules, micronemes, and rhoptries (ROPs), which are involved in cell infiltration (Rorman *et al.*, 2006; Klevar, 2007; Dubey, 2010).

They are three infectious stages of *T. gondii* that are found in all hosts: -

(i). Tachyzoites
(ii). Bradyzoites
(iii). Sporozoites (oocysts)

Tachyzoites are highly active form of the parasite which invades host cells of an intermediate host and rapidly multiplies in a host. They are crescent-shaped, approximately 2×6 μm in size, having pointed anterior ends and round posterior ends (Dubey, 2010). Tachyzoites enter host cells by active penetration to the host cell membrane (Bohne *et al.*, 1993).

Bradyzoites are also called cystozoites, a stage that multiplies slowly in a host (Lekutis, 2000). They are enclosed in tissue cysts, and they are slender and crescent–shaped with a diameter of 7 μm to 15 μm. The bradyzoites are more prevalent in the central nervous system (CNS), the

eye, and skeletal and cardiac muscles (Dubey, 1988). In rare circumstances, they may be found in visceral organs, such as the lungs, liver, and kidneys.

Sporozoites, also referred to as unsporulated oocysts are subspherical to spherical in shape, with a diameter of 10 × 12 μm in size (Speer *et al*., 1998). *T. gondii* in the small intestine of the definitive host undergoes a typical coccidian development, resulting in the shedding of unsporulated and noninfectious oocysts in cat faeces. Oocysts containing sporozoites are infective when ingested by mammals, they later multiply in reticuloendothelial cells and give rise to the tachyzoites stage (Dubey *et al*., 2004).

Toxoplasma gondii has a complex-classical life cycle categorized into sexual reproduction, which occurs in the definitive host (cats and wild felids) and asexual reproduction in the intermediate host respectively (humans and warm-blooded animals) (Alfonso *et al*, 2006). The life cycle success is dependent on intermediate (IH) and definitive host (DH) on a level of prey-predator relationship and the environment (Deplazes *et al*., 2011).

The definitive hosts for *T. gondii* are members of the family Felidae (domestic and wild cats). A feline is infected with *T. gondii* after consuming a mouse carrying the parasite or by direct ingestion of sporulated oocysts in the environment and the parasite can survive in the GIT of the cat- small intestines for some weeks (Dubey *et al*., 1986). Unsporulated oocysts are shed in the cat's faeces and survive in the environment, then spread to the soil, water, food or anything that can potentially contaminate the environment (Remington *et al*.1990). Oocysts are usually shed for 1–3 weeks taking 1–5 days to sporulate in the environment and remain infective for many months in cold and dry climates (Remington *et al*., 1990).

Intermediate hosts, humans, and animals (including birds and rodents) become infected after ingesting soil, water or plant material contaminated with oocysts (Dubey *et al*., 1986; Remington *et al*., 1995). Oocysts transform into tachyzoites shortly after ingestion, in which the cyst's cell wall is damaged by proteolytic enzymes in the stomach and small intestines (Dubey, 1986; Ho-yen *et al*., 1992). The replication of tachyzoites is mostly intracytoplasmic in parasitophorous vacuoles (PV), which are formed by the parasite (Schluter *et al*., 2014). After more periods of rapid multiplication of tachyzoites, they localize in neural and muscle tissue, and the parasite establishes intracellular cysts, which transform into bradyzoites (Jerome *et al*., 1998; Radke *et al*., 2003). According to Schluter *et al*. (2014), tissue cysts formation preferential sites are brain tissue, skeletal and heart muscles, and the retina of infective

intermediates hosts. Tissue cysts in affected muscles form for about 7 to 10 days after the initial infection (Dubey, 1986)

Figure 2.1. The life cycle of *T. gondii* (http://www.dpd.cdcd.gov/dpdx/HTML/image Library/Toxoplasmosis_il.htm)

2.2 Transmission

All homoeothermic animals, including pigs, goats, livestock, and humans, are susceptible to infection by *T. gondii* (Dubey 2010). The prevalence of *T. gondii* disease varies among different species based on the geographic location, socio-cultural customs, and climatic conditions. The abundance of cats that excrete oocysts, which sporulate to become infectious to humans and animals, may also be related to prevalence rates (Ajzenberg *et al*., 2004; Dubey, 2004; Garcia *et al*., 2006). According to Edelhofer *et al*. (1996), the pathways of transmission are vertical which includes transplacental transmission of tachyzoite, blood transfusion, organ transfer, and consumption of unpasteurized milk. Horizontal transmission occurs through ingestion of infectious oocysts from the environment (Tenter *et al*., 2000; Remington *et al*., 1990).

2.2.1 Prevalence and transmission of *T. gondii* in animals and humans

There are different variations in the seroprevalence of *T. gondii* infection in domestic ruminants across the world, and have been reported to range from 3 –92 % in sheep (Tenter *et al*., 2000; Pinheiro *et al*., 2009), 5–77% in goats (Tenter *et al*., 2000; Sharif et al., 2007; Carneiro *et al*., 2009); and 0–99% in cattle (Hall *et al*., 2001). In Africa, *T. gondii* seroprevalence for livestock ranges from 3.6 –57.5 % (Sawadogo *et al*., 2005; Kamani *et al*., 2010; Schoonman *et al*., 2010; Khalil and Elrayah, 2011; Swai and Kaaya, 2013).

Humans are susceptible to toxoplasmosis in large numbers, and estimates of seroprevalence range by region, 16–40% in England and the USA, 50–80% in Europe, and 0–80% in South Africa (Dubey and Beattie, 1988). According to Tenter *et al*. (2000), the seroprevalence of *T. gondii* infection in women of childbearing age ranges from 4–77%, and among adult populations worldwide it ranges from 30-65%. (Flegr *et al*., 2002; Kijlstra and Jongert, 2008). Previous studies on human toxoplasmosis in Ethiopia have revealed variation among various populations, including children and adults, with a seroprevalence rate varying from 74.4 to 96.7%. (Gebre–Exabier *et al*., 1993; Woldemichael *et al*., 1998; Yimer *et al*., 2005; Shimelis *et al*., 2009).

The primary way that humans contract *T. gondii* is by consuming food or water that has been contaminated with oocysts. The majority of *T. gondii* infections in humans are caused by consumption of raw or undercooked meat contaminated with *T. gondii* tissue cysts, ingesting oocysts from the soil (through activities like gardening, handling/eating unwashed vegetables or changing a cat's litter box), or eating raw or undercooked meat that has been exposed to the soil (Jones *et al*., 2001a). The intake of undercooked or cured meat products has been linked to up to 63% of human *T. gondii* infections in Europe (Cook *et al*., 2000; Dubey, 2004; Dubey, 2008). Pregnant women may be at risk for congenital transmission if they consume meat that has *T. gondii* contamination (Baril *et al*., 1999). Freshly consumed homemade cheese made in small family farms from contaminated milk without prior pasteurization may pose a public health hazard (Fusco *et al*., 2007). Abortion, stillbirth, and a variety of morbidities have all been linked to vertical transmission in people (Tenter *et al*., 2000). Until recently, water-borne transmission of *T. gondii* was thought to be rare, but there was a human outbreak associated with wild felid causing contamination in municipal water reserves in Canada (Dubey, 2004).

Flies, cockroaches, dung beetles, and earthworms can mechanically disseminate oocysts in the soil which can also survive on fruits and vegetables for extended periods (Kniel *et al*., 2002).

2.2.2   Risk factors of *T. gondii* infection

According to epidemiological studies which have been conducted, the definitive host (Felids) and animal management (extensive and intensive) are the primary risk factors for *T. gondii* infection in cattle. Numerous seropositive cattle with *T. gondii* infections have been linked to the presence of resident cats and stray cats roaming freely on Brazilian farms (Fajardo *et al*., 2013). Further, increased infection rate has been found in small ruminants reared in densely populated agricultural regions compared to those in desert areas (Ahmad and Tasawar, 2015) and in females and older animals (Ahmad *et al*., 2015). A high prevalence of *T. gondii* was reported in females than in male sheep (van der Puije *et al*., 2000; Ramzan *et al*., 2009). Guimarães *et al*. (2013) and Ahmad *et al*. (2015) indicated that the production system/extensive farming practice, breed, age, poor hygienic conditions, presence of cats, and flock size larger than 50 individuals are the risk factors associated with *T. gondii* infection in sheep while poor hygienic conditions, usage of outdoor water sources, presence of cats, extensive farming practices, and flock sizes larger than 30 and 50 individuals are the risk factors for *T. gondii* infection in goats (Ahmad *et al*., 2015).

Age affects a person's vulnerability to *T. gondii* infection. *Toxoplasma gondii* infection was found to be more common in persons with lower educational levels, people who live in crowded areas, and people who work in jobs that involve been in contact with soil in the United States (Jones *et al*., 2001a). Owning cats, consuming raw or undercooked pork, mutton, lamb, beef, or minced meat products, or coming in contact with the soil are risk factors for human *T. gondii* infection (Kapperud *et al*., 1996; Baril *et al*., 1999; Weigel *et al*., 1999; Cook *et al*., 2000).

2.2.3   Pathogenesis and Clinical manifestation of *T. gondii* in Animals and Humans

Pathogenicity of *T. gondii* is influenced by many factors, including the susceptibility of the host species, the virulence of the parasitic strain and the stage. Oocyst-induced infections are the most severe clinically in intermediate hosts and are not dose-dependent (Dubey, 2004). *Toxoplasma gondii* has been adapted to an oocyst-oral cycle in herbivores (intermediate hosts) and a tissue cyst-oral cycle in carnivores, especially cats (Dubey, 2001). *Toxoplasma gondii*

oocysts are less infective and pathogenic for cats than for rats and mice. For example, one live oocyst is orally infective to mice and pigs, whereas 100 or more oocysts may be required to establish infection in a cat (Dubey, 2006). The reverse may be true for bradyzoites. Cats can shed millions of oocysts after ingesting as few as one bradyzoites. However, 100 bradyzoites may not be infective to mice by the oral route (Dubey, 1996a; Dubey, 1996b; Dubey, 1996c).

During the peak of the acute phase, organisms may appear in secretions and excretions, and tissue necrosis may be found in many body organs like the intestine, liver, spleen, pancreas, lung and heart (Dubey, 1996a). Whereas in chronic infection, lesions occur more often in muscles, eye and brain than in visceral tissue. The spread of toxoplasmosis is less from one animal to another in the acute phase, even when the animals are confined in a closed space (Soulsby, 1982). The clinical character of the disease varies with the organs attacked, which itself varies depending on whether the disease is congenital or acquired.

In goats and sheep, abortion, stillbirth and neonatal death are common sequels of the infection (Dubey and Beattie, 1988; Tenter *et al*., 2000). It causes embryonic death and resorption, fetal death and mummification inclusive cattle. Among companion animals, fatal toxoplasmosis may occur in immunosuppressed dogs following infection with concurrent distemper virus; the onset of illness is marked by fever with lassitude, anorexia and diarrhoea. Pneumonia and neurological manifestation are common (Dubey, 2004). Immunocompetence to *T. gondii* is not present before 60 days of gestation, and infection in early or mid-pregnancy results in fetal death with resorption or mummification. Some lambs infected in mid-pregnancy may survive to the near term and be stillborn or may survive to parturition but are weak and die shortly following birth (Buxton, 1990).

Immunocompromised persons, pregnant women and their fetuses are at risk of toxoplasmosis, with clinical manifestations such as encephalitis, brain abscess, hydrocephalus, hepatosplenomegaly and retinitis (Remington and Klein, 2001; Montaya and Linsenfield, 2004). Neonatal clinical manifestations of congenital toxoplasmosis vary widely and include hydrocephalus, microcephaly, intracranial calcifications, chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, petechial due to thrombocytopenia, and anaemia (Swisher *et al*., 1994). Hydrocephalus is unique to human congenitally acquired toxoplasmosis and has not been reported in animals (Remington *et al*., 2001; Jones *et al*., 2001b). Similarly, Montoya and Liesenfeld, (2004) indicated that toxoplasmosis in human causes retinal scarring, brain damage or abortion following primary maternal infection and a potentially fatal

encephalitic threat to immunocompromised individuals. In postnatal acquired toxoplasmosis, localized lymphadenitis is the most frequently observed clinical sign in humans (Montoya and Remington, 2000). Lymphadenopathy may be associated with fever, malaise, fatigue, muscle pain, sore throat and headache. Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the fetus. Encephalitis is the most clinically significant manifestation of toxoplasmosis in immunosuppressed patients. Toxoplasmosis is a major cause of death among patients with AIDS (Dubey, 2004).

2.3 Diagnosis

2.3.1   Diagnostic approach in *T. gondii*

Diagnosing *T. gondii* infection can be diagnosed by biological, serologic and histologic methods by directly detecting parasites or deoxyribonucleic acid (DNA) or indirectly by detecting antibodies of different isotypes. Many techniques are available, which may have strengths and shortcomings; hence the data obtained using these techniques should be interpreted with critical knowledge (Carme *et al*., 2002; Montoya, 2002).

2.3.2   Serological examination

Serological examination is used to indicate the presence of infection by detecting *T. gondii* specific antibodies or parasitic antigens in the blood of an infected individual. Among the different tests under serological tests are Sabin-Feldman dye test (DT), indirect haemaglutination test (IHT), indirect florescent antibody test (IFAT), indirect latex agglutination test (ILAT), direct agglutination test (DAT), modified direct agglutination test (MAT) which are used to detect different antibody classes and antigens (Shapaan *et al*., 2008; Rodrigues, 2009). Enzyme linked Immunosorbant Assay (ELISA), are used to detect the circulating antigens or antibodies for the diagnosis of toxoplasmosis in both animals and humans (Yang *et al*., 2000; Dubey, 2010). Although serological tests are useful techniques in diagnosing toxoplasmosis, there are limitations since serological dynamics in pregnant hosts may cause confusing or uncertain results. Diagnosis is delayed leading to undetected congenital toxoplasmosis or abortion (James *et al*., 1996; Montoya, 2002). Serology may be inadequate if the host is immunosuppressed, and maternal antibodies may interfere in testing neonates. Commercially available agglutination tests vary in their sensitivity and ability to detect chronic infections (Dubey and Carpenter, 1993).

2.3.3   Detection of the parasite

Directly visualizing of the parasite in the fluid or tissue allows for identification of the parasite, but this is a labor-intensive and low-yield procedure that requires the use of tissues obtained during post-mortem examination or biopsy samples from acute patients (Acha and Szyfres, 2003). Additionally, Sukthana (2006) discussed how difficult it is to make a conclusive diagnosis of toxoplasmosis through a clinic-pathological investigation because the disease frequently manifests as asymptomatic or subclinical case and its clinical manifestations are non-specific. Although tissue culture and mouse inoculation are very specific, they are time-consuming and may expose researchers to some zoonotic infections (Savva and Holliman, 1990). As a result, antigen detection is not always 100% accurate, interspecies antigen cross-reactivity has been observed.

2.3.4   Histologic or microscopic diagnostics

Histologic/Microscopic examination is most reliable when the organisms are present in encysted form, and even then, discrimination from related protozoa such as *Neospora caninum* may be difficult. Thus, microscopic observation of free toxoplasma tachyzoites in stained blood smears or CSF indicates an active infection and is a valuable diagnostic tool (Barratt *et al.*, 2010). The tachyzoite of *T. gondii* is morphologically identical to that of other apicomplexan parasites, including many zoonotic species. No pathognomonic histologic pattern occurs in animal tissues making differentiation by light microscopy between *T. gondii* and other cyst-forming protozoan parasites such as Sarcocystis, Besnoitia, Frenkelia, Balantidia, and Neospora difficult (Jennifer *et al.*, 1995).

2.3.5   Isolation of *T. gondii* (bioassay)

Toxoplasmosis can be diagnosed by isolation of *T. gondii* from cultures of body fluids (blood, CSF, bronchoalveolar lavage fluid) or tissue biopsy specimen in the appropriate clinical setting. This is the most convincing diagnostic methods and is obtained by inoculation of suspected materials into toxoplasma free mice by the intraperitonial or intracerebral route and subsequent demonstration of tachyzoites or bradyzoites in smears of organs or serous cavities (Dubey, 1998).

a) Bioassay in mice

It is used for isolation of *T. gondii* from body fluids, (blood, cerebrospinal fluid, aqueous humor, amniotic fluid, etc.) from a mouse. Isolation of *T. gondii* can also be conducted from muscle tissues by pepsin digestion procedure as described by Dubey, (1998).

b) Bioassay in cats

The quantity of tissue cysts in animals is frequently insufficient for bioassay in mice to identify them. Instead, cats are typically used to increase the likelihood of isolating the parasites because they can ingest ten or more times the volume of tissues that mice can during a bioassay, and they can be given 500 g or more of meat samples. Millions of oocysts can be shed by infected animals between days 3 and 14 after infection (Dubey and Beattie, 1988)

2.3.6   Molecular techniques

Molecular methods based on polymerase chain reaction (PCR) are simple, sensitive reproducible tests and can be applied to all samples (Contini *et al*, 2005). PCR is used to detect *T. gondii* from amniotic fluid, placental, brain tissues, whole blood, cerebrospinal fluid, urine, vitreous fluid, aqueous humor, broncho alveolar lavage fluid, and pleural and peritoneal fluids. PCR allows the selective amplification from DNA or RNA (in reverse transcription PCR, RT-PCR). PCR has revolutionized prenatal diagnosis of congenital toxoplasmosis by enabling early diagnosis, thereby avoiding use of more invasive procedures on the foetus (Montoya, 2002; Su and Dubey, 2010).

PCR should be considered a reliable, rapid, inexpensive, and definitive method of identification for *T. gondii* infection in formalin-fixed and paraffin-embedded material (Hyman *et al*., 1995). The sensitivity and specificity of the PCR depends on the DNA extraction protocol and the characteristics of the reaction optimization conditions (Edvinsson *et al*., 2006). The methods below consist of two groups. The first group of techniques for detection of *T. gondii* DNA in biological and clinical samples includes conventional PCR, Real time PCR, and genotyping. The second group consists of molecular methods which includes PCR-RFLP and Microsatelite.

a) Conventional PCR

The conventional PCR is carried out by denaturation of double stranded DNA to produce single stranded DNA. Short single stranded oligonucleotedes called primers bind to single stranded DNA molecules at specific locations. A polymerase enzyme recognizes the annealed primers and initiates elongations to synthesize double stranded DNA (Mullis, 1990). The process is repeated in cycles and the amount of DNA copies increases in each cycle. The specific DNA fragment is analyzed using an agarose gel, stained with ethidium bromide, and illuminated with ultraviolet light to make it visible (Edvinson, 2006)

b) Real-time PCR

The real-time PCR is performed using a video camera and inclusion of ethidium bromide in the PCR mixture. The emitted fluorescence was found to be proportional to the amount of double stranded products formed during the PCR. The advantage of this method is that it not only detects but also quantifies the pathogens. The detection threshold of real-time PCR in *T. gondii* is superior to nPCR assays (Bell and Ranford-Cartwright, 2002; Contini *et al*., 2005).

c) Genotyping of *T. gondii*

Genotyping plays a key role in epidemiological and population biology studies of *T. gondii* as well as identification of an infection source (Ajzenberg *et al*., 2002b). Various genotyping techniques have been used for classifying *T. gondii* into specific groups, including restriction fragment length polymorphism (RFLP)-PCR analysis (Bohne *et al*., 1993), random amplified polymorphic DNA analysis (Guo *et al*., 1997), and analysis based on sequence length polymorphism (Ajzenberg *et al*., 2002b).

d) Polymerase Chain Reaction-Fragment Length Polymorphism (PCR–RFLP)

The polymerase chain reaction-fragment length polymorphism (PCR-RFLP) method uses the polymorphism associated with the presence or absence of restriction sites for specific restriction enzymes. The markers of fragment length polymorphism are amenable to high throughout the analysis using PCR amplifications followed by restriction digestion and gel electrophoresis (Sibley *et al*., 2009). By using PCR–RFLP analysis, *T. gondii* strains can be classified into one of the three (I, II, or III) clonal types (Howe and Sibley, 1995; Howe *et al*., 1997).

e) Microsatellites

Microsatellites (MS) sequences are tanden repeats of short DNA (1 to 6) motifs appearing 2 to 20 times and tend to occur in non-coding DNA (Ajzenberg *et al*., 2002b; Sibley *et al*., 2009). Microsatellites exist everywhere in eukaryotes genomes and undergoes length changes due to insertion and deletion of one or multiple repeat units (Sibley *et al*., 2009). The mutation rate for microsatellites was 10-2 to 10-5 per locus per replication which is several orders of magnitude faster than that of single nucleotide polymorphisms (SNPs). Microsatellites are frequently used as genetic markers in a wide range of applications, their fast mutation rate makes them well suited for individual identification of *T. gondii* isolates (Ajzenberg *et al*., 2010).

2.4 Control and Prevention of Toxoplasmosis in humans and animals

The control of toxoplasmosis in animals and humans is a significant problem because of the difficulties in proper diagnosis at the early stage of the disease (Roser, *et al*, 1999). Different authors recommend various approaches to control *T. gondii* infection in animals and humans (Dubey, 1996a).

Management – felids are the definitive hosts that shade infective oocysts thereby, causing environmental contamination. Feed and water on the farm premises should be free from cat contact. To reduce contamination on the farm, keep a healthy and small population of cats that will minimize the shading of oocysts to the environment (Buxton, 1998).

Treatment – Cats should be given Sulfadiazine 15–60mg/kg divided into four doses. Pymerimethamine should also be given at 1mg/kg 24 hrs×3 day orally for several weeks, then later the dose should be reduced and the cats can be given 0.5–1.0 mg/kg 24 hrs until oocyst shedding stops. Ewes that are pregnant and are infected with *T. gondii,* should be given monensin and decoquinate to control abortion (Urquhart *et al*., 1996).

Vaccination-

Vaccine approaches include use of purified or recombinant *T. gondii* surface antigens, live attenuated or mutant strains of the parasite, or DNA with plasmids encoding colony-stimulating factors (Peterson *et al*., 1998; Ismael *et al*., 2003). Live Tachyzoites have been used to develop an effective vaccine to prevent toxoplasmosis in sheep in order to lessen losses from congenital toxoplasmosis. The vaccine (toxovacx1) is widely sold in the UK, France, and New Zealand (Buxton *et al*., 1991; Buxton and Innes, 1995). At initial dose the entire flock should be vaccinated, followed by yearly vaccinations for all replacements, according to the manufacturers recommendations. The vaccine produces a protective immunity for up to 18 months (Buxton & Innes, 1995). Currently, no vaccine prevents toxoplasmosis in humans (Innes *et al*., 2019).

Control in immunocompetent individuals -Immunocompetent adults and children with toxoplasmic lymphadenitis typically do not receive treatment unless symptoms are severe or chronic (Dunay *et al*, 2018). However, in patients with symptoms which includes myocarditis, encephalitis, a sepsis syndrome with shock, and hepatitis, the treatment to recommend should include pyrimethamine (100-mg loading dose and 25–50 mg/d), sulfadiazine or trisulfapyrimidines (4–8 g/d) for 4–6 weeks (Opravil *et al*., 1995) as well as folic acid (5–10 mg/d) for 4-6 weeks (Weiss and Kim, 2007). For immunocompromised individuals, John and

Petri (2006) suggested a combination of pyrimethamine to be given daily at doses of 25–100 mg and trisulfapyrimidines given daily at doses of 2–6 g for a month. This course of treatment inhibits dihydrofolate reductase in *T. gondii* (an enzyme), which prevents the synthesis of DNA and protein. He also mentioned the effectiveness of clindamycin in treating *T. gondii* infection in HIV/AIDS patients and spiramycin for acute treatment in pregnant women. For the treatment of infected individuals, Pal (2007) proposed combining pyrimethamine with the triple sulfonamides (sulfadiazine, sulfamerazine, and sulfamethazine) and folic acid.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study site

This study was conducted in Choma district, located in the Southern Province of Zambia. Choma is one of the largest districts in the Province (Figure 3.1). It has a latitude of 16.771111, a longitude of 26.992222 and is 1,337 meters above mean sea level (https://en.m.wikipedia.org). Samples were collected from veterinary camps within Choma District namely; Mapanza ($26^o 52$'E and $16^o 8$'S), Simaubi ($26^o 49$'E and $16^o 20$'S), Namoonza ($27^o 5$'E and $16^o 51$'S), Batoka ($27^o 14$'E and $16^o 46$'S), Choma central ($26^o 47$'E and $16^o 49$'S), Nakeempa ($26^o 54$'E and $17^o 0$'S), Simaluba ($27^o 2$'E and $17^o 4$'S) and Masuku ($27^o 5$'E and $17^o 15$'S). Farming is the primary source of income in the eight veterinary camps with major activities including crop production and rearing of animals under an extensive farming system for both meat and milk production.

Figure 3.1: Map of Choma district showing sampling sites – Veterinary Camps

About 84 % of farmers in Choma district are small holdings, while 16 % are large-scale growers (FAO, 2001). According to the national livestock statistics conducted in 2018, the Southern province had the highest population of goats, which is at 36.8% of the total national herd (Livestock census 2018). Most goats are kept extensively in homesteads; hence, little or no care is given to the animals. Most transmissions of infectious and parasitic diseases are acquired by constant contact with other animals while grazing and drinking water due to lack of good management, mixing of different species of animals and lack of veterinary services.

3.1 Study design, animal population and sample size

A cross-sectional sero-epidemiological study was conducted from 31st August 2021 to 5th September, 2021 to determine the sero-prevalence and risk factors of *T. gondii* infections in goats. The study areas were selected based on independent exposure factors in each veterinary camps which includes water source and veterinary camp boundaries. The study population was selected purposely and divided into eight veterinary camps as listed below in Table 1. Geographical factors were considered during the selection of the camps based on the location (north, south, east and west). The sample size was determined using Thrushfield (2005) for random sampling. The study population consisted of livestock farmers and their goats in the study areas. A simple random sampling method was used to select the household to participate. The total number of households visited in the eight veterinary camps were 41, of which 10 % of the flock from each household was sampled. The total number of goats sampled from each camp were calculated as a ratio of camp to total goat population for the study area.

Sample size was calculated by the following formula provided by Thrusfield (2005) for random sampling.

$$n = \frac{1.96^2 \text{ Pexp (1-Pexp)}}{d^2}$$

Where,

n = desired sample size,

Pexp = expected prevalence, and

d = 0.05

$$n = \underline{196^2 \text{ X } 0.5 \text{ (1- 0.5)}}$$

$(0.05)^2$

=384 goats

The actual samples collected were 324. The recommended and calculated sample size was supposed to be 384. The target was not achieved as highlighted in the study limitations.

Table 3.1: Location of each camp and their estimated goat population according to the livestock survey under the Ministry of Fisheries and Livestock- Veterinary department

Geographical location	Camps	Estimated number of flocks
East	Masuku	10,000
	Siamaluba	5000
West	Simaubi and Mapanza	13,000
North	Batoka and Namoonza	20,000
South	Choma central and Nakempa	11,000

3.2 Blood sampling and serological examination using ELISA

A total of 324 goat sera were collected during the study period. Approximately 3 ml of blood was drawn from the external jugular vein using plain vacutainers tubes. The blood was kept at room temperature to allow clotting to separate sera. Aliquots of sera were obtained following centrifuge at 3000 rpm for 10 minutes and later stored in the freezer. The samples were transported in a cooler box with ice to the University of Zambia, School of Veterinary Medicine. The samples were stored at -20°C until the time for analysis. Sera was removed from the freezer and left overnight at room temperature. The sera were tested for IgG antibodies against *T. gondii* using an ELISA diagnostic kit (IDVET Innovative Diagnostic kit, ID Screen, Montepelliar, France) following the manufacturer's instructions. The kit has a sensitivity which could reach up to 100 % and a specificity determined to be up to 96 % (according to the manufacturer's data) and has therefore been used by other researchers (Dubey and Beattie, 1988; Abdullah *et al.*, 2019; Hotea *et al.*, 2021).

About 90 µl of Diluted Buffer 2 was added to each microwell and then added 10 µl of the negative control was to A1 and B1 wells. Another 10 µl of positive control was added to C1

30

and D1 wells. Ten microliters (10 µl) of samples were added to the remaining wells. The plate was then incubated for 45 ± 4 min at $21\,^\circ\text{C}$ ($\pm 5\,^\circ\text{C}$). Each well was emptied and washed three times with approximately 300 µl of the wash solution. The conjugate 1x was diluted in the concentrated conjugate 10X to 1/10 in dilution buffer 3. Another 100 µl of the conjugate 1X was added to each well, and the plate was incubated for 30 ± 3 min at $21\,^\circ\text{C}$ ($\pm 5\,^\circ\text{C}$). The wells were emptied and washed three times with approximately 300 µl of the wash solution. About 100 µl of the substrate solution was added to each well, and the plate was incubated for 15 ± 2 min at $21\,^\circ\text{C}$ ($\pm 5\,^\circ\text{C}$) in the dark. Another 100 µl of the stop solution was added to each well to stop the reaction. Positive and negative controls were provided by the manufacturer and were used to validate each test. The results were read, and the OD was recorded at 450 nm using an ELISA plate reader. The samples were considered positive if the OD ≥ 50 %, doubtful for values between 40 and 50 %, and negative for values ≤ 40 %. The percentage was calculated as follows:

Percentage of positive $= 100$ x OD of the sample /OD of the PC

(Where, OD= Optic Density; PC= Positive Control).

Figure 3.2: Blood collection from the jugular vein in a goat

3.3 Questionnaire survey

A closed-ended questionnaire was administered to forty-one farmers whose goats were party of the study. The information gathered in the questionnaire included- age, sex, housing facility, feed storage facilities, water sources (borehole, river, water wagon), presence of cats, feral cats and cat feaces in the vicinity, presence of other animals on the farm (poultry, pig, dogs) as factors associated with the risk of *T. gondii*.

Figure 3.3: Recording information from farmers- questionnaire survey on risk factors

3.4 Data management and Statistical analysis

Questionnaires were sorted and checked for completeness and later coded and entered in a Microsoft Excel 2007 spreadsheet, and SPSS software (SPSS Inc., IBM Corporation, and version 21, Chicago, IL, USA). The seroprevalence was calculated by dividing the number of animals positive to anti-*T. gondii* antibodies by the total number of animals tested. The seroprevalence and the association of risk factors for *T. gondii* seropositivity assessed using Chi-square test. Logistic regression analysis was performed to estimate the multiple effect on categorical variables on *T. gondii* seropositivity. Further non-collinear variables and those presented $P < 0.025$ at 95 % CI level were entered into the multivariate logistic regression model. All statistical analyses were performed using the statistical software SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

3.5 Ethical Considerations

Clearance to conduct the study was obtained from the School of Veterinary Medicine, while ethical clearance was obtained from Excellence in Research Ethics and Science Converge (ERES) ethics committee. Written or thumb printed voluntary informed consent (Appendix 3)

was sought from participants. The participants' privacy and confidentiality were ensured with no names or national identity numbers used in the questionnaires.

CHAPTER FOUR

RESULTS

4   Socio Demographic Characteristics

4.1 Respondents demographics

Forty-one respondents were successfully interviewed from eight veterinary camps in Choma district. The respondent's demographics are summarised in Table 4.1. Females were in a proportion of 21.9 % (95 % CI: 11.11-38.03) and males 78.03 % (95 % CI; 61.96-88.89), who were the largest group to be interviewed. The study had different age groups >20-30 years at 19.51 % (95 % CI: 9.37-35.37), 31- 40 years at 29.27 % (95 % CI: 16.54-45.74), 41-50 years were at 31.71 % (95 % CI: 18.58-48.22), 51-60 years at 12.20 % (95 % CI: 4.58-27.00) and > 61 years at 7.32 % (95 % CI: 1.91-21.01). The marital status: was divorced at 14.63 % (95% CI: 6.09-29.86), Singles at 7.31 % (95% CI: 1.91-21.01) and the largest group were married at 78.05 % (95 % CI: 61.97-88.89). Levels of education: not educated at 7.31 % (95% CI: 1.901-21.01), Tertiary at 12.20 % (95% CI: 4.58-27.01), Primary at 39.02 % (95% CI: 24.62-55.46) and those up to the secondary level at 41.46 % (95% CI: 26.70-57.80). Their occupation: formal employment 7.32 % (95% CI: 1.9-21.01), business person 12.20% (95% CI: 4.58-27.01) and the largest group were farmers 80.49 % (95 % CI: 64.63-90.63). The number of dependents they kept was between > 61 at 2.44 % (95% CI: 0.13-14.41), 31-45 at 2.44 % (95% CI: 0.13-14.41), 16-30 at 7.32 % (95% CI: 1.91-21.01), and the largest group was < 15 at 87.80 % (95 % CI: 72.10- 95.4).

Table 4.1: **Respondents' demographic characteristics in Choma District**

Demographical variables	Category	n = 41	%	95 % CI
Sex	Female	9	21.9	11.11-38.03
	Male	32	78.03	61.96-88.89
Age group	>20- 30	8	19.51	9.37-35.37
	31- 40	12	29.27	16.64-45.74
	41-50	13	31.71	18.58-48.22
	51-60	5	12.20	4.58-27.00
	>61	3	7.32	1.91-21.01
Marital status	Divorced	6	14.63	6.09-29.86
	Married	32	78.05	61.97-88.89
	Single	3	7.31	1.91-21.01
Level of education	Not educated	3	7.31	1.901-21.01
	Primary	16	39.02	24.62-55.46
	Secondary	17	41.46	26.70-57.80
	Tertiary	5	12.20	4.58-27.01
Occupation	Businessperson	5	12.20	4.58-27.01
	Farmer	33	80.49	64.63-90.63
	Formal employment	3	7.32	1.9-21.01
Number of dependents	<15	36	87.80	72.10-95.42
	16-30 years	3	7.32	1.91-21.01
	31-45 years	1	2.44	0.13-14.41
	46-60 years	0	0.0	0.00-0.00
	>61 years	1	2.44	0.13-14.41

n= Number of respondents; CI= Confidence interval

4.2 Seroprevalence

In the current study, 324 samples were tested for the presence of *T. gondii* antibodies and the seroprevalence is summarised in Table 4.2. The overall seroprevalence of *T. gondii* was 3.11 % (95 % CI: 2.71-9.43). Females had a high prevalence of 4.13 % (95 % CI: 3.55-13.61: p = 0.001), 0.00 % for males. Regarding age, adults had a high seroprevalence of 4.00 % (95 % CI: 3.16-13.23: p = 0.001) and the young at 1.01 % (95 % CI: 0.09-10.63). For veterinary camps, Masuku recorded the highest seroprevalence at 5.97 % (95 % CI: 2.40-25.47), followed by Simaubi at 6.38 % (95% CI: 2.72-29.51), Batoka had 2.33 % (0.2-19.64), Namoonza with 2.22% (95 % CI: 0.23-18.01), Siamaluba with 1.92% (95 % CI: 0.34-26.94) and Mapanza, Nakeempa and Choma central recorded 0 %.

Table 4.2: **Seroprevalence of *T. gondii* in goats in Choma District**

Variables	Category	n	Positives	%	95% CI	*p*-value
Overall		**324**	**10**	**3.11%**	**2.71-9.43**	
Sex	Females	242	10	4.13	3.55-13.61	<0.001
	Males	82	0	0.00	0.00-8.73	
Age	Adult	225	9	4.00	3.16-13.23	<0.001
	young	99	1	1.01	0.09-10.63	
Vet camps	Masuku	67	4	5.97	2.40-25.47	
	Siamaluba	52	1	1.92	0.34-26.94	
	Batako	43	1	2.33	0.2-19.64	<0.86
	Namoonza	45	1	2.22	0.23-18.01	
	Simaubi	47	3	6.38	2.72-28.51	
	Mapanza	19	0	0	0.00-34.51	
	Nakeempa	25	0	0	0.00-30.11	
	Choma Central	26	0	0	0.00-32.44	

Significant; n= Number of respondents; CI = confidence interval; Significant level at 0.05

4.3 Measure of association

The results of Chi-square test of association between the outcome variable (seropositivity to *T. gondii*) and predictor variables are shown in Table 4.3. The only variable with significant association with sero-positivity was the presence of cats in the farm vicinity (p =0.006). All variables with p-value < 0.25 where taken for further screening in the binary logistic regression analysis.

Table 4.3: Univariate analysis of risk factors associated with *T. gondii* in goats in Choma District

Factors	Category	n	Reactors	Prevalence	95% CI	X^2value	*p*-value
Goat sex	Male	82	0	0.00	0.00-5.58	3.496	0.062
	Female	242	10	4.13	2.11-7.69		
Age	Adult	225	9	4.00	1.96-7.71	2.055	0.152
	young	99	1	1.01	0.05-6.30		
Gender	Male	244	7	2.87	1.26-6.07	0.156	0.693
	Female	80	3	3.75	0.97-11.32		
Cleaning abortion surface	Yes	75	4	5.33	1.72-13.81	1.647	0.1 99
	No	249	6	2.40	0.98-5.42		
Weak kids	Yes	221	9	4.07	2.00-7.84	2.259	0.133
	No	103	1	0.97	0.05-6.07		
Care of weak kids	Vet care	8	1	12.5	6.56-53.32	2.430	0.119
	Self-care	316	9	2.84	1.39-5.53		
Housing cats	Yes	145	7	4.83	2.13-10.07	2.660	0.103
	No	179	3	1.68	4.34-5.21		
General water source	Yes	74	4	5.41	1.74-13.98	1.724	0.189
	No	250	6	2.40	0.98-5.40		
Presence of Cat feaces	Yes	100	7	7.00	3.10-14.37	7.406	0.006
	No	224	3	1.34	0.35-4.18		

= significant level at 0.05%, P=prevalence, CI: confidence interval, X^2=Chi-square value, n =number of respondents

4.4 Risk factors for *T. gondii*

After adjustment for other variables in the stepwise binary logistic regression model, there were significant risk factors of *T. gondii* positivity ($p < 0.05$). Variables with ($p < 0.250$) in the univariate analysis were included in the model. The test had insignificant Hosmer-Lemeshow goodness-of-fit statistic ($p = 0.882$), and Omnibus Test of Model Coefficients values of ($p < 0.001$) were obtained, indicating the goodness of fit of the generated model. The significant risk factors were presence of cats within the farm yard, cat access to feed and water troughs for the goats and presence of cat feaces within the farm yard and the respectable odds ratio are presented in Table 4.4. Goats on farms with stray cats were (aOR: 5.092, 95 % CI: 2.442-16.376) more times likely to be *T. gondii* positive than goats in farms without stray cats ($p = 0.33$). Goats on farms with cat access to water and feed troughs were (aOR: 2.806, 95 % CI: 1.002-11.528) more times likely to be *T. gondii* positive than goats on farms that cats did not access water and feed troughs ($p = 0.014$). Goats on farms that had presence of cat feaces were (aOR: 0.042: 95 % CI: 0.010-0.761) more times likely to be *T. gondii* positive than goats on farms that had no cat feaces ($p = 0.029$).

Table 4.4: Multivariate analysis of risk factors related to *T. gondii* infections in goats in Choma District

Variable	Level	aOR	95% CI	*p*-value
Presence of stray cats (*n*=324)	No	Ref		
	Yes	5.092	2.442-16. 376	0.033***
Cats access feed and water troughs (*n*=324)	No	Ref		
	Yes	2.806	1.002-11.528	0.041***
Presence of cat feaces (*n*=324)	No	Ref		
	Yes	0.042	0.010-0.761	0.029***

*** = Significant at 0.05; aOR = adjusted Odds ratio; CI = Confidence interval; Significant level < 0.05; Ref = Reference category.

CHAPTER FIVE

DISCUSSION

The present study determined *T. gondii* infection in goats in eight veterinary camps of Choma district, Southern province of Zambia. The seroprevalence was found to be 3.11 %, which is lower than the prevalence in other regions of Africa, such as; 11.6 % in Central Africa (Zwedu, 2013), 8.5 % in Morocco (Benkirane *et al*., 2015), 4.3 % in South Africa (Samraa *et al*., 2007) and 3.2 % in Algeria (Dechicha *et al*., 2015). Outside Africa, for example, 11.2 % in Pakistan (Ramzan *et al*., 2009), 12.2 % in Northern America (Dubey and forey, 2000) and 3.8 % in India (Sharma *et al*., 2008) have been reported. The seroprevalence recorded in five veterinary camps in Choma district which includes Simaubi veterinary camp had 6.08%, Masuku veterinary camp had 5.79 %, Batoka veterinary camp 2.33 %, Namoonza veterinary camp had 2.22% and Siamaluba veterinary camp with 1.97 %. Mapanza, Choma central and Nakeempa veterinary camps recorded negatives respectively. The high seroprevalence recorded in the current study for the veterinary camps could be due to climatic conditions and widespread presence of cats (Zwedu *et al*., 2013; Gabremedhin *et al*., 2013). These conditions favour the sporulation and long-term survival of oocysts than the cold climatic conditions (Dubey and Beattie, 1988; Dubey, 2010).

Despite the low seroprevalence of *T. gondii* recorded, this study highlights the potential risk of the disease in Zambia to both humans and animals. According to Dubey, (2004) and Innes (2009) variations in seroprevalence of *T. gondii* infection is mainly influenced by different exposure factors such as source of water and feed given to the animals, climatic variation from one region to another, sample size collected, age of animals of interest, management variation of animals either intensive or extensive and the diagnostic techniques used if it serological, histological, bioassay or PCR.

The association of *T. gondii* seropositivity with biological characteristics of goats, shows that age and sex can influence the susceptibility of goats to toxoplasmosis. Age-related increase in seroprevalence were observed because adults had a four-fold higher chance of *T. gondii* infection than young goats. These results echo those of Jittapalapong (2005) and Teshale (2007), who recorded a low prevalence rate in young animals and a high frequency in adults. Older animals (2 years and above) have a longer life span hence they are exposed to various infections for a longer period of time than the young (Dubey, 2000; De Pereira, 2012; Gabremeidin *et al*., 2013; Rouatib *et al*., 2019). Seroprevalence increases with age, which is

attributable to an increased cumulative chance of exposure to environmental contamination (Lunden *et al*., 1994; Katzer *et al*., 2011; Dubey, 2016). In the case of sex, seroprevalence was high in females (4.13%) than in males (0%). This finding was in agreement with Clementino et al. (2007) and Zewdu *et al*. (2013). However, Silva *et al*. (2003) and Lashari and Tasawar (2011), observed the opposite trend, in which seroprevalence was higher in males than in females, and this was attributed to androgen production which occurs in males hence, lowering their immunity. Furthermore, other authors have reported no significant differences between the sexes (Gabremeidin *et al*., 2013). The increased seropostivity of females may be associated with a lower immunological resistance during specific periods of their lives (Guimaraes *et al*., 2013), such as the stress of lactation and pregnancy, which causes immunosuppression that renders them more liable to *T. gondii* infection (Dubey *et al*., 1998; Tilahun *et al*., 2019).

The major risk factors identified in the study were both direct and indirect; the significant risk factors were, presence of stray cats in the farm yards, cats access to goats feed and water troughs, and the presence of cat faeces within the farm yard. The risk of acquiring an infection for those with stray cats on the farm vicinity was 5.092 more times than those without stray cats. The risk of acquiring infection for those who had stray cats accessing the goat feed and water trough was 2.806 more times than those who did not. The risk of acquiring the infection for those with cat feaces in the farm was 0.042 than those that had no cat feaces. Other authors have also conducted extensive research and have categorized the presence of resident cats and stray cats close to goat farms as a significant risk factor for *T. gondii* infection in goats. Additionally, it has been discovered that small ruminants that have had direct or indirect contacts with cats, have a higher chance of acquiring the infection (Oncel and Vural, 2006; Vesco *et al*., 2007). *Toxoplasma gondii* oocysts sporulate and remain viable for months to years in moist earth which may later expose the goats to infection when they come in contact (Lukesova and Literak, 1998; Dubey and Odenning, 2001; Dubey, 2010). According to other research, there is no such association with the infection (Gebre–Exabier *et al*., 1993). However, it should not be disputed that feral cats contribute to environmental contamination, which then exposes humans and animals to *T. gondii* infection (Gebremedhin *et al*., 2013).

General water source exposes goats to *T. gondii* infection on the farm premises, since all animals on the farms access the same water source. In this current study general water source was not statistically significant. This is in agreement with Gabremedhin *et al* (2013) who reported a high infection rate in sheep and goats that accessed the same water source used by all animals within the farm premises. Sporulated oocysts in the soil can spread mechanically

by flies, cockroaches, dung beetles and earthworms and later can survive on fruits and vegetables for extended period of time (Kniel *et al*., 2002). They can also contaminate water bodies and become source of infection (Isaac- Renton et al. 1998). Watering troughs are also not risk-free due to the widespread practice of leaving the water in the troughs for several days to months and simply re-filling without cleaning or changing the water. Vesco *et al*., (2007) suggested that using surface water as a source of drinking water for small ruminants may be regarded as a risk factor for *T. gondii* infection.

Not cleaning abortion surfaces with disinfectants after goats have aborted can exposes goats to *T. gondii* infection than those that clean their surfaces in an event where *T. gondii* infection is present. Cats that consume placentas or any raw meat infected with *T. gondii*, possess a risk to themselves, hence can cause recurrent infections on farms. This is in agreement with Daragas *et al*. (2011) who reported a higher seroprevalence in cats following *T. gondii* infection at 80.6 % in Arad and 62.8 % in Timis countries. The presence of weak kids on farms was an indicator of the risk factors associated with *T. gondii* infection, although it was not statistically significant in this current study. Some lambs infected in mid-pregnancy may survive to almost near term and some may survive till full term but they may be born weak and die shortly due to *T. gondii* infection (Buxton, 1990).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

This study is the first report on the seroprevalence and risk factors of *T. gondii* in goats in Zambia. This study showed the presence of *T. gondii* in the eight veterinary camps of Choma district and the significant risk factors associated with the disease. The significant risk factors were, presence of stray cats within the farm yard, cat's access to feed and water troughs for goats as well as the presence of cat feaces within the farm yard. This information on *T. gondii* is essential for the affected rural communities and the country in general, as it may be starting point in raising farmer's awareness on the importance of the problem and the needs to implement control measures in order to prevent the transmission of *T. gondii*.

Based on the results of this study, the following are the recommendations:

a) More studies must be conducted to study the effects of different seasons on disease epidemiology.

b) Human epidemiological studies should be implemented in the veterinary camps of Choma district to see the occurrence of infections in humans.

c) The farmers should be sensitised on the disposal of materials from aborted goats in safe areas and later clean and disinfect the area in order to avoid the spread of infections.

d) Feeding and watering troughs for goats should be treated regularly and protected from being accessed by cats to avoid contamination by *T. gondii* oocysts.

e) The public health sector of the study areas should initiate a study on the health impact of toxoplasmosis to formulate guidelines for preventing and controlling the disease.

f) People in the study areas should be advised to boil or filter piped water before using it for drinking.

STUDY LIMITATIONS

Reasonably larger samples are often recommended for survey related studies. The recommended sample size should have been 384, due to resource constraints, however, only 324 samples were considered in this study. Increasing the sample size in future studies would be useful to enhance reliability of results. Given resource availability, it is suggested that a multi-district or province study should be considered in future to provide a more generalised picture of *T. gondii* prevalence in Zambia.

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APPENDICES

APPENDIX 1: **Ethical approval**

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23rd June, 2021.

Ref. No. 2021- Jun- 009

The Principal Investigator
Ms. Chiluba Zimba
C/o Department of Clinical Studies
School of Veterinary Medicine
University of Zambia
Lusaka, ZAMBIA.

Dear Ms. Zimba

**REF: SERO-PREVALENCE AND RISK FACTORS OF TOXOPLASMA GONDII
INFECTION AMONG SAMLL RUMINANTS IN CHOMA DISTRICT, ZAMBIA.**

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

Review Type	Fast Track	Approval No. 2021-June-009
Approval and Expiry Date	Approval Date: 23rd June, 2021	Expiry Date: 22nd June, 2022
Protocol Version and Date	Version - Nil.	22nd June, 2022
Information Sheet, Consent Forms and Dates	• English.	22nd June, 2022
Consent form ID arld Date	Version - Nil	22nd June, 2022
Recruitment Materials	Nil	22nd june.2022
Other Study Documents	Questionnaire.	22nd June, 2022
Number of participants approved for study	-	22nd June, 2022

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.Sc., PhD
CHAIRPERSON

APENDIX 2: Letter of introduction and informed oral consent

PI: Zimba Chiluba

Supervisors: Dr. Victor. C. Zulu, Prof Munyeme Musso and Prof John.B. Muma

Study title: <u>Seroprevalence and risk factors of *Toxoplasma gondii* in goats in Choma district</u>.

Purpose for the research:

This study will contribute to the knowledge regarding the epidemiology of toxoplasmosis in goats, to determine seroprevalence as well identify the risk factors associated with *T. gondii* infection in Choma district of Zambia. The study will help with the development of prevention and control strategies for toxoplasmosis to protect goats and humans in Zambia since it is a zoonotic disease.

Study procedure:

Participants in this study will be asked some questions, which I will record in the questionnaire

☐   The information required for this research in the questionnaire are all on risk factors associated with *Toxoplasma gondii* in goats.

☐   Blood samples will also be collected from your participant small ruminants (goats) and later be transported to University of Zambia, School of Veterinary Medicine laboratory to determine the seroprevalence of *Toxoplasma gondii* infections in goats.

Risks and discomforts

You may get tired when answering the questionnaire or may not want to give answers to specific questions, some questions which will be asked may appear to be sensitive but you are free to skip questions which you are not comfortable with. It is also possible that other neighbouring farmers may share what you talk about with them, but will ask everyone to respect each other's privacy.

Benefits

If you participate in this study, you will help us to determine and assess if *Toxoplasma gondii* exists in your area and the risk factors associated with *T. gondii* infections so that prevention and control programs on Toxoplasmosis shall be implemented in order to reduce the disease burden which affects both humans and animals.

Compensation

They are no rewards or incentives offered for participation in this study. Participation is purely voluntary.

Confidentiality:

Any information that will be collected during this study will be kept confidential and be kept in locked cabinets and computer only accessed by Dr. Chiluba Zimba and other team members. During data collection for the questionnaire no names shall be included and information collected will not be shared to the third part. The information gathered will be used for research purposes only.

Your alternatives to joining the study

You are entirely free not to participate in this study you agree to join as a participant and later change your mind, you are also free to leave the study and no longer be a participant of the study without any penalty. However, if you decide to participate, we will use the information collected from the point you left it. I therefore encourage you to participate for the betterment of your health and improve your livestock (goats) welfare.

Who do you call if you have questions or problems?

If you have any questions about this research, please contact Dr. Chiluba Zimba C/O School of Veterinary Medicine, the University of Zambia, and Great east road campus. P.O Box 32379, Lusaka, Zambia. Email:zimmbakc@gmail.com: cell 097711611/0953015790.

If you have questions about your rights as a study participant or if you feel you have not been treated fairly or if you have any concerns, you may contact the EREs Converge Private REB, 33 Joseph Mwila road, Lusaka, Zambia using the following contacts 0955155633/4.

Participant statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and have been provided with answers to my satisfaction. I will provide information on seroprevalence and risk factors associated with *Toxoplasma gondii* in small ruminants (goats) and blood can be collected from my goats. I have been assured of confidentiality on any information shared. My participation in this study is voluntary.

Signature   of   participant……………………………………..

Date……………………………..

Signature   of   witness   ……………………………………………

Date…………....................

APENDIX 3: Questionnaire

Questionnaire survey on risk factors of *Toxoplasma gondii* in Choma district, Zambia

Dear respondents,

I'm a Second Year Masters student at the University of Zambia- School of Veterinary Medicine. I am currently pursuing a master's degree in Tropical Infectious Diseases and Zoonosis. I'm carrying out a survey on risk factors of *Toxoplasma* gondii in Choma district, Zambia.

Interviewee: Adults of the age 18 years and above

You are being asked to participate in this research study to provide information on your knowledge of Toxoplasmosis

Kindly note that by completing this Questionnaire you are voluntarily agreeing to participate in this study.
You will remain anonymous and all information given will be treated as confidential.

Please tick with an (X) on your response or fill in the appropriate responses in the space provided.

Questionnaire code no: ....

Name of **interviewer**………………………………………………………………………………………………….

Date of interview (DD/MM/YY)

…………………………………………………………………………….

Start time…………… **End time**……………………

Thank you for accepting to take part in this study. The information you provide will be used in research to investigate the risk factors of *Toxoplasma gondii* in goats. Any information you provide will remain confidential and anonymous and only relevant to the research. You have the right to withdraw from this study at any time that is appropriate to you.

SECTION 1

A. QUESTIONNAIRE IDENTIFICATION INFORMATION

Questionnaire serial Number _____

District _____

Veterinary Camp _____

Village/Farm _____

Chief/Chiefdom _____

Name of Head of Household (HH) _____

Cell phone of interviewee or head of HH_____

Relationship of the interviewee to HH head _____

Response/Questionnaire filter Details codes :

> 1. Completed
>
> 2. Partially done
>
> 3. Not done at all

G. P. S Coordinates   North_____   South_____

B.   DEMOGRAPHICAL INFORMATION

SECTION C: GOAT MANAGEMENT AND CARE

No	Questions and filters	Responses	Codes
1. Goats information			
9	Do you have any housing facility for the goats ?	Yes …………..1 No ……………2 [ ]	
10	If yes to **Q 9**. Do you clean the housing facility for the goats?	Yes ……………1 No………………2 [ ]	
11	Who is primarily responsible for taking care for the goats when they are grazing?	Yourself……………1 Children…………….2 Workers…………….3 All the above………..4 None…………………5 [ ]	
12	What is the source of your water	Stream………………1 Borehole…………….2 Well…………….......3 Others……………….4 [ ]	
13	Have you ever experienced any abortions from the goats in the current/previous years?	Yes………………..1 No………………....2	

No	Questions and filters	Responses	For Codes
1. HOUSEHOLD CHARACTERISTICS			
1	Gender of respondent	Male………………….......1 Female…………………......2 [ ]	
2	What is your age as at last birth day?	1. .... Years 2. Refused to disclose [ ]	
3	What is your marital status?	Single………………...….…1 Married……………………..2 Divorced…………………....3 Widowed…………………...4 Separated…………………....5 [ ] Others (Specify)..	
4	What is your level of education?	Never went to school…...........1 Primary…………….............2 Junior secondary……...…......3 Senior Secondary……....…......4 Tertiary……………...........….…5 [ ]	
5	What is your occupation?	Farmer………..…...................1 Unemployed.............................2 Home maker.............................3 Business person........................4 Fishing …………………......5 [ ] Others Specify..	
6	Do you have any people living with you in this household?	Yes ………….................1 No. ………….....................…2 [ ]	
7	If yes in (6) above, how many?	……………………………	

8	What type of animals do you keep?	Cats ……………………….1 Dogs…………………………..2 Goats …………………………3 Pigs…………………………….4 Cattle …………………………5 Sheep …………………………6 All of the above ………………7 Others…………………………8 [ ]	
		[ ]	
14	If yes **Q 13**. What is the proportional of the abortions in your herd	High………………1 Medium……………2 Low………………..3 Others……………...4 [ ]	
15	Do you clean the surfaces where the female goats aborted from?	Yes…………….1 No……………...2 [ ]	
16	If yes to **Q 15**. What do you use for cleaning the surfaces where the animals aborted from?	Water……………..1 Disinfectant………2 Detergents…………3 Others………………4 [ ]	
17	Do you experience any neonatal mortalities?	Yes………………1 No………………...2 [ ]	

18	If yes to **Q 17**. How often do you experience neonatal mortalities?	Every 6 months……..1 Every year…………...2 Others……………… 3 [ ]	
19	Are they any weak goat kids that are born in your herd?	Yes………………...1 No…………………2 [ ]	
20	If yes to **Q 19**. How do handle the weak goat kids?		
21	Do you experience any health related problems on your farm?	Yes…………………..1 No……………………2 [ ]	
22	Which Sex of animals experience most health related problems on the farm?	Males……………1 Females…………...2 [ ]	
23	Do you seek veterinary services when the goats are sick?	Yes………………...1 No…………………..2 [ ]	
24	If yes to **Q 23**. Who are the providers for the animal health care?	Vet…………………1 Community livestock auxiliary……………..2 NGO…………………3 Others………………...4 [ ]	
2. Risk factors			

28	What are your reasons for keeping cats?	To get rid of rodents ……………………..1 Companionship……2 Others……………...3 [ ]	
29	Do you house the cats ?	Yes ………………1 No…………………2 [ ]	
30	Do your cats have access to the goat pens?	Yes………………..1 No…………………2 [ ]	
31	Are they any wild or stray cats that roam around the farm premises?	Yes………………1 No………………..2 [ ]	
32	Do your cats or wild cats have access to feed and water source which the goats use?	Yes………………1 No……………….2 [ ]	
33	Do all your animals have the same source to drinking water?	Yes…………………..1 No……………………2 [ ]	
34	Do your animals mix with other animals from neighboring farms during grazing?	Yes…………………1 No…………………...2 [ ]	
35	How do you dispose the remains for the abortions or stillbirth?	Bury……………..1 Burn………………2 Put in a pit…………3 Others………………4 [ ]	

36	Do you notice any feces in your farm vicinity (goat pens or house or farm surrounding)	Yes……………1 No……………..2 [ ]	
37	Do you have an idea of the dangers of the cat feces ?	Yes………………1 No…………………2 [ ]	
38	If yes to **Q 37**. What are some of the dangers?	……………………..	

3. Knowledge and practices about *T. gondii*

39	Have ever heard of the disease called Toxoplasmosis?	Yes………….1 No……………2 [ ]	
40	If yes to **Q 39**. What are the causes	…………………..	
41	How can it be transmitted?	……………………	
42	What are some of the signs/ symptoms?	………………………	
43	Do you have any ideas of the dangers it can pose to the humans and the animals?	Yes………….1 No……………2 [ ]	
44	If yes **Q 43**. what are some of the dangers?	………………………….	

Thank you for your time.

Principal Investigator

Name: Chiluba Zimba

Phone number: +260977116111

Address: The University of Zambia, School of Veterinary Medicine,

P.O Box 32379. Lusaka.

Email address: zimbakc@gmail.com

APENDIX 4: ELISA result

Table 1: Plate one

a. ELISA results based on ELISA reader at 450nm, the numbers in bold are the positives (4) and the rest were negatives which were calculates using OD

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.05	0.172	0.284	0.113	0.252	0.103	0.329	**0.538**	0.136	0.339	0.134	0.06
B	0.053	0.091	0.095	**0.442**	0.086	0.178	0.201	0.119	0.067	0.117	0.23	0.112
C	0.083	0.309	0.137	0.269	**0.454**	0.148	0.157	0.058	0.141	0.169	0.11	0.169
D	0.142	0.213	0.14	**0.466**	0.063	0.107	0.137	0.149	0.093	0.19	0.075	0.089
E	0.065	0.163	0.244	0.078	0.11	0.122	0.151	0.378	0.194	0.155	0.142	0.118
F	0.075	0.23	0.185	0.128	0.372	0.29	0.369	0.138	0.263	0.074	0.066	0.258
G	0.88	0.289	0.176	0.185	0.142	0.369	0.279	0.261	0.173	0.335	0.132	0.211
H	1.096	0.352	0.252	0.358	0.297	0.384	0.154	0.073	0.253	0.166	0.203	0.228

Table 2: Plate two

b. ELISA results based on ELISA reader at 450nm, the numbers in bold are the positives (5) and the rest were negatives which were calculates using OD

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.05	0.251	0.235	0.121	0.239	0.207	0.208	**0.841**	0.116	0.594	0.07	0.21
B	0.052	0.102	0.203	0.078	0.247	0.139	0.12	0.075	0.238	0.207	0.434	0.402
C	0.057	0.092	0.322	0.232	0.196	0.075	0.453	0.23	0.278	0.261	0.085	0.134
D	0.054	0.098	0.151	0.076	0.073	0.113	0.138	0.103	0.141	0.173	0.537	0.134
E	0.057	0.194	0.128	0.07	0.157	0.266	0.386	0.117	**1.145**	0.577	0.359	0.429
F	0.059	0.242	0.221	0.43	0.129	**2.141**	0.336	0.264	0.198	**2.345**	0.252	0.313
G	1.486	0.184	0.148	0.286	0.18	0.153	0.23	0.137	0.155	0.173	0.141	0.351
H	1.599	0.221	0.14	**2.106**	0.159	0.115	0.198	0.11	0.348	0.3	0.12	0.076

Table 3: Plate three

c. ELISA results based on ELISA reader at 450nm, the numbers in bold are the positives (1) and the rest were negatives which were calculates using OD

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.047	0.178	0.144	0.225	0.347	0.173	0.222	0.279	0.204	0.216	0.181	0.198
B	0.05	0.161	0.097	0.234	0.064	0.289	0.12	0.236	0.235	0.219	0.35	0.06
C	0.047	0.085	0.1	0.084	0.102	0.047	**0.722**	0.102	0.301	0.141	0.073	0.066
D	0.048	0.179	0.147	0.091	0.189	0.08	0.065	0.104	0.083	0.188	0.27	0.169
E	0.048	0.066	0.266	0.099	0.068	0.062	0.062	0.239	0.088	0.414	0.102	0.099
F	0.048	0.317	0.143	0.185	0.076	0.077	0.075	0.202	0.127	0.13	0.156	0.184
G	1.46	0.337	0.066	0.076	0.172	0.145	0.276	0.105	0.136	0.07	0.129	0.135
H	1.476	0.077	0.107	0.201	0.177	0.163	0.05	0.095	0.153	0.396	0.277	0.128

APPENDIX 5: **Regression results**

Table 1: Option one: Best fitting model of predictors for *T. gondii* risk factors associated with infection

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Goat_attendant			2.294	2	.318			
	Goat_attendant(1)	-.707	1.443	.240	1	.624	.493	.029	8.336
	Goat_attendant(2)	-1.873	1.479	1.604	1	.205	.154	.008	2.789
	Disposal_abortus			6.205	3	.102			
	Disposal_abortus(1)	-3.427	1.775	3.729	1	.053	.032	.001	1.053
	Disposal_abortus(2)	**-6.428**	**2.586**	**6.179**	**1**	**.013**	**.002**	**.000**	**.257**
	Disposal_abortus(3)	-22.115	6531.896	.000	1	.997	.000	.000	.
	Neonatal_mortality(1)	-1.187	.963	1.519	1	.218	.305	.046	2.016
	Cats_access_goathouse(1)	**-2.265**	**.962**	**5.543**	**1**	**.019**	**.104**	**.016**	**.684**
	Livestock_interaction(1)	-1.714	1.071	2.561	1	.110	.180	.022	1.470
	Presence_cat_feaces(1)	**-2.616**	**1.073**	**5.945**	**1**	**.015**	**.073**	**.009**	**.599**
	Constant	6.292	3.702	2.889	1	.089	540.158		

a. Variable(s) entered on step 1: Goat_attendant, Disposal_abortus, Neonatal_mortality, Cats_access_goathouse,

Livestock_interaction, Presence_cat_feaces.

Table 2: Option two: Best fitting model of predictors for *T. gondii* risk factors associated with infection

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
	Age(1)	1.917	1.119	2.934	1	.087	6.802	.758	61.012
	Goat_attendant			1.484	2	.476			
	Goat_attendant(1)	-1.056	1.650	.409	1	.522	.348	.014	8.833
	Goat_attendant(2)	-2.019	1.785	1.279	1	.258	.133	.004	4.395
	Disposal_abortus			5.527	3	.137			
	Disposal_abortus(1)	-3.326	1.873	3.155	1	.076	.036	.001	1.411
	Disposal_abortus(2)	**-6.959**	**2.968**	**5.497**	**1**	**.019**	**.001**	**.000**	**.319**
	Disposal_abortus(3)	-22.948	6213.685	.000	1	.997	.000	.000	.
Step 1[a]	Neonatal_mortality(1)	-1.734	1.121	2.393	1	.122	.177	.020	1.589
	Cats_access_goathouse(1)	**-2.694**	**1.074**	**6.290**	**1**	**.012**	**.068**	**.008**	**.555**
	Livestock_interaction(1)	-1.335	1.113	1.439	1	.230	.263	.030	2.331
	Presence_cat_feaces(1)	**-2.328**	**1.172**	**3.948**	**1**	**.047**	**.097**	**.010**	**.969**
	Dangers_humans_animals(1)	17.663	17179.052	.000	1	.999	46876264.392	.000	.
	Vet_Service_providers			.962	2	.618			
	Vet_Service_providers(1)	-1.556	1.653	.886	1	.347	.211	.008	5.390
	Vet_Service_providers(2)	-1.163	1.928	.364	1	.547	.313	.007	13.686
	Constant	-11.073	17179.053	.000	1	.999	.000		

a. Variable(s) entered on step 1: Age, Goat_attendant, Disposal_abortus, Neonatal_mortality, Cats_access_goathouse, Livestock_interaction, Presence_cat_feaces, Dangers_humans_animals, Vet_Service_providers.

Table 3: Option three: Best fitting model of predictors for *T. gondii* risk factors associated with infection

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Goat_attendant			1.670	2	.434			
	Goat_attendant(1)	-.819	1.535	.284	1	.594	.441	.022	8.942
	Goat_attendant(2)	-1.933	1.680	1.323	1	.250	.145	.005	3.897
	Disposal_abortus			5.627	3	.131			
	Disposal_abortus(1)	-3.727	1.909	3.813	1	.051	.024	.001	1.014
	Disposal_abortus(2)	**-7.480**	**3.154**	**5.626**	**1**	**.018**	**.001**	**.000**	**.273**
	Disposal_abortus(3)	-22.903	6426.877	.000	1	.997	.000	.000	.
	Neonatal_mortality(1)	-1.503	1.097	1.876	1	.171	.222	.026	1.911
	Cats_access_goathouse(1)	**-2.473**	**1.067**	**5.373**	**1**	**.020**	**.084**	**.010**	**.683**
	Livestock_interaction(1)	-1.589	1.131	1.973	1	.160	.204	.022	1.874
	Presence_cat_feaces(1)	**-2.702**	**1.207**	**5.011**	**1**	**.025**	**.067**	**.006**	**.715**
	Vet_Service_providers			.554	2	.758			
	Vet_Service_providers(1)	-.895	1.618	.306	1	.580	.408	.017	9.731
	Vet_Service_providers(2)	-.298	1.954	.023	1	.879	.743	.016	34.217
	Constant	7.641	4.430	2.975	1	.085	2080.953		

a. Variable(s) entered on step 1: Goat_attendant, Disposal_abortus, Neonatal_mortality, Cats_access_goathouse, Livestock_interaction, Presence_cat_feaces, Vet_Service_providers.