Farmer's risk perception, and seroprevalence and associated risk factors of *Toxoplasma gondii* in small ruminants and backyard chickens in selected districts of West Gojjam Zone, Northwest Ethiopia

by

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Submitted in partial fulfilment of the requirements for the degree MSc Veterinary Tropical Diseases in Global One Health

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DECLARATION

I hereby declare that this mini dissertation, which I hereby submit for the Master of Science (Global One Health) degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and that this work has not been previously submitted by me for degree purposes at another tertiary institution.

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LIST OF ABBREVIATIONS

AEC	Animal ethics committee
AIDS	Acquired Immuno-Deficiency Syndrome
CNS	Central nervous system
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
HIV	human immunodeficiency virus
IFAT	Indirect fluorescent antibody test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect hemagglutination assay
LAT	Latex agglutination test
MAT	Modified agglutination test
OR	Odds ratio
PCR	Polymerase chain reaction
PV	Parasitophorous vacuole
RBCs	Red blood cells
REC	Research ethics committee
ROPs	Rhoptry proteins
T. gondii	*Toxoplasma gondii*
UP	University of Pretoria
VIF	Variance inflation factor

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DISSERTATION SUMMARY

Farmer's risk perception, and seroprevalence and associated risk factors of *Toxoplasma gondii* in small ruminants and backyard chickens in selected districts of West Gojjam Zone, Northwest Ethiopia

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Toxoplasmosis, caused by an intracellular protozoan parasite *T. gondii,* is a widespread and neglected zoonotic disease with significant health and economic impacts that affects nearly one-third of the global human population. A cross-sectional survey and questionnaire study was conducted from May 2023 to December 2023 to estimate the seroprevalence of *T. gondii* infection in small ruminants and backyard chickens in Bahir Dar Zuria and Sekella Districts, Northwest Ethiopia. The study aimed to identify risk factors and assess risk perceptions of the farmers' towards the disease. Furthermore, animal owners were provided with a structured questionnaire to collect data on household demographics and animal management practices, and checklists were employed to record individual animal level factors along with the collection of serum samples. Simple random sampling technique was employed to select small ruminants and backyard chickens from the study population. A total of 541 serum samples from small ruminants and backyard chickens were tested for antibodies against *T. gondii* using a commercially available latex agglutination test kit. One hundred and ninety one samples tested positive for the presence of *T. gondii* antibodies, resulting in an overall animal-level seroprevalence of 35.31% (95% CI= 0.314 - 0.395). The prevalence of *T. gondii* varied significantly between the two districts (OR = 2.78, 95%CI = 1.893 - 4.072 and P = 0.000). The prevalence of *T. gondii* infection was found to be significantly influenced by management practices (OR=2.85; 95%CI: 1.532- 5.308, P= 0.001), with extensively managed small ruminants being three times more likely to be affected by *T. gondii* compared to semi-intensively

managed small ruminants. Compared to exotic chicken breeds, the local breed of backyard chickens were four times more likely to acquire a *T. gondii* infection (OR= 4.61, 95%CI: 1.951 -10.869, P= 0.000). Backyard chickens were two times more likely at risk of acquiring a *T. gondii* infection (OR= 2.035; 95%CI: 1.389 - 2.979, P = 0.000) when compared to small ruminants. Small ruminants and backyard chickens in close contact with cats were found to have significantly higher risks of acquiring *a T. gondii* infection compared to those with limited or no cat exposure.

In conclusion, the findings of this study indicate a high prevalence of *T. gondii* in the animals sampled in the current study sites. Furthermore, multiple risk factors influencing the prevalence of *T. gondii* infection were investigated. The increased prevalence and poor awareness of the disease among the participants could have a significant impact on the health of people and other animals in the study areas, highlighting the necessity for evidence-based integrated strategies and promotion of behaviour change to control and prevent *T. gondii* infection in both humans and other animals in the West Gojjam Zone, Ethiopia.

Keywords: sero-prevalence, *Toxoplasma gondii*, latex agglutination test, small ruminants, backyard chickens, risk factors, Northwest Ethiopia

CHAPTER 1: INTRODUCTION

1.1. Background and Justification of the Study

Chickens, and small ruminants, such as sheep and goats are important animal protein sources and play a crucial role in the food and nutrition security in Ethiopia (Tiao et al., 2013a, Wodajo et al., 2020). Nevertheless, products derived from these animals can serve as main sources of zoonotic pathogens if not treated properly. *Toxoplasma gondii*, an intracellular parasite, causes Toxoplasmosis, which is a major zoonotic disease, infecting approximately one-third of the global human population (Tenter et al., 2000, Torgerson and Mastroiacovo, 2013). This disease is recognized as an emerging foodborne protozoan parasitic disease and is ranked fourth among foodborne parasites initiating serious concerns globally, due to the considerable impact it can have on the health of people and animals and thus the economics of a country (Dorny et al., 2009, World Health, 2014).

The first description of this parasite happened in 1908 by Nicolle and Manceaux when it was discovered from the North African rodent *Ctenodactylus gundi* at the Pasteur Institute of Tunis and was widely recognized as a common infection in various warm-blood animals, including rodents and other mammals (Dubey, 2009, Wojcik-Fatla et al., 2015). Currently, this protozoan parasite s widely distributed and exhibits notable variations in seroprevalence depending on geographic difference. Different species have varying levels of disease prevalence, influenced by geographic, climatic, sociocultural, and local factors. According to S. Al-Malki (2021) there have been reports of high seroprevalence in African countries, portions of south-east Asia, the Middle East, sections of Central and Eastern Europe, and Latin America. Conversely, seroprevalence estimates for North America, Northern Europe, and Southern Europe) (Ajzenberg et al., 2004) range from moderate (30 to 50%) to low (10 to 30%).

Limited studies conducted on the seroprevalence of *T. gondii* infection in animals in Ethiopia, revealed the presence of the disease in domestic animal species in different parts of the country ranging from; 22.9% to 54.7% in sheep, 11.6% to 74.8% in goats, 38.4% to 72.4% in chicken and 85.4% to 91.7% in cats (Dubey et al., 2013b, Tiao et al., 2013b, Endrias Zewdu and Getachew, 2015, Tilahun et al., 2018, Chaklu et al., 2020, Tarekegn et al., 2020). These studies may show the widespread distribution of

T. gondii with potential variations in prevalence between regions and within specific locations in Ethiopia.

Toxoplasma gondii infects all species of warm blooded animals including humans, through various routes like ingesting contaminated feed and water, undercooked or raw meat containing tissue cysts, organ transplantation or blood transfusion, congenital transmission and accidental inoculation of tachyzoites (Tenter et al., 2000, Dorny et al., 2009, World Health, 2014, Omonijo et al., 2022). While most animals and humans act as the intermediate hosts; domestic cats and wild felids are the definitive hosts responsible for transmitting *T. gondii* by shedding environmentally resistant oocysts in their feaces (Webster, 2010, Alobaidii et al., 2020). The parasite's prevalence could be linked to cats that expel oocysts, which are infectious to humans and other intermediate animals, upon sporulation (García-Bocanegra et al., 2012).

In humans and other animals, toxoplasmosis is asymptomatic but it could be severe and life-threatening in immune-compromised patients including causing encephalitis, chorioretinitis, congenital infection and neonatal mortality (Weiss and Dubey, 2009). In the livestock farm, *T. gondii* infection can lead to financial setbacks and is often associated with abortion, stillbirth, foetal malformations, premature deliveries, and new-born mortality, particularly in sheep and goats, posing a significant threat to the global small ruminant industry (Gebremedhin et al., 2013b, Tagwireyi et al., 2019).

1.1.1. Justification of the study

Despite the high prevalence of *T. gondii* reported in domestic animals worldwide, limited studies have been done in Ethiopia on the seroprevalence of *Toxoplasma* in small ruminants and chickens. The close proximity of people and livestock, sociocultural customs, unhygienic environmental conditions, shared water sources, poor livestock management practices, the presence of felids in the study areas and the poor living condition of a community could contribute to the persistence of the parasite in the livestock population in these communities. Despite these circumstances, there is no information available on the prevalence of *T. gondii* infection in the West Gojjam zone.

Knowing the prevalence and identifying relevant risk factors for the maintenance of *T. gondii* infection in small ruminants and backyard chickens in a communal area is

important for developing effective control and prevention strategies in Ethiopia and specifically in the selected districts of the West Gojjam Zone. Therefore, this research project aims to estimate the seroprevalence and identify putative risk factors for *T. gondii* infection in small ruminants and backyard chickens in West Gojjam Zone, Northwest Ethiopia.

Based on the background and justification mentioned above, this study was initiated to meet the following aim and objectives;

1.2. Aim and objectives

The aim of this project was to investigate the seroprevalence of *T. gondii* infections in small ruminants and backyard chickens in West Gojjam zone, Northwest Ethiopia.

To achieve this aim the following objectives had to be met.

- To estimate the sero-prevalence of *T. gondii* infection in small ruminants and backyard chickens in West Gojjam Zone, Northwest Ethiopia
- To identify the associated risk factors affecting the occurrence of *T. gondii* infection in the study area
- To assess risk perception and awareness of the farmers' towards the disease

1.3. Research questions

1. What is the sero-prevalence of *T. gondii* infection in small ruminants and backyard chickens in selected districts of West Gojjam Zone, Ethiopia?
2. What are the potential risks factors influencing the occurrence and maintenance of *T. gondii* infection in small ruminants and backyard chickens?
3. Is the consumption of raw animal products and contact with cats a risk for human health in the study area?

CHAPTER 2: LITERATURE REVIEW

2.1. History of Toxoplasmosis

The first report of *T. gondii* infection was believed to have occurred in 1908 by Nicolle and Manceaux, who identified the parasite from a North African rodent, called *Ctenodactylus gundi,* in the Pasteur Institute of Tunis. This infection was later recognized as a common one among various warm-blooded animals, including rodents and other mammals (Dubey, 2009, Wojcik-Fatla et al., 2015). The term "*Toxoplasma gondii*" (*T. gondii*) was derived from its morphology, with the Greek words "toxon" meaning arc or bow, and "plasma" indicating shape, and its host, the rodent *Ctenodactylus gundi* (Innes, 2010a, Rouatbi et al., 2019). The medical significance of toxoplasmosis in humans was first noted in the early 1921s when cases of encephalitis, retinochoroiditis, and hydrocephalus were observed in children. In the 1980s, this parasitic infection was recognized as a prominent opportunistic parasitic infection among patients with weakened immune systems due to conditions like HIV/AIDS (Lim and Othman, 2014).

In Italy, Mello (1910) documented fatal cases of toxoplasmosis in domestic animals, particularly in dogs suffering from acute visceral toxoplasmosis. In the 1960's; *T. gondii* was reported as the cause of abortion in sheep, in New Zealand. The discovery that felines serve as the definitive host for toxoplasmosis opened up significant opportunities to understand the nature of the parasites, their life cycle, and the sources of infection (Innes, 2010b). Domestic cats and wild felids play a crucial role in transmitting *T. gondii* infection by shedding environmentally resistant oocysts in their feaces (Webster, 2010). Toxoplasmosis affects all warm-blooded animal species, with most animals and humans serving as intermediate hosts (Robert-Gangneux et al., 2023).

2.2. Aetiology of Toxoplasmosis

Toxoplasmosis is caused by the protozoan parasite *T. gondii* which is an obligate intracellular protozoal parasite, able to infect humans, domestic animals, wildlife and is present in different ecosystems, including water, soil and food (Dubey, 2016, de Barros et al., 2022). *Toxoplasma gondii* belongs to the Kingdom Animalia, Phylum

Apicomplexa, Class Protozoa, Subclass Coccidian, Order Eucoccidia, Family Sarcocystidae and Genus *Toxoplasma* (Dubey and Beattie, 1988).

The parasites morphology and genome structure exhibited a polarized cell structure with a complex organelle arrangement at its apical end, known as the conoid, that plays a role in cell invasion. Numerous secretory organelles include rhoptries (ROPs), dense granules, and micronemes used for invading and manipulating of the host cell by the parasite (Dubey et al., 1998, Paredes-Santos et al., 2012).

Toxoplasma gondii harbours a haploid genome comprising of 14 chromosomes, totalling *approximately 63* mega bases. Within its genetic makeup, there are roughly 8,000 protein-coding genes, which play key roles in host-cell invasion, immune evasion, and metabolic functions. Additionally, the genome encodes a variety of surface antigens crucial for interactions between the parasite and its host (Kissinger et al., 2003).

Toxoplasma gondii exhibits genetic diversity with different strains related to three clonal lineages; Type I, Type II, and Type III, which differ in virulence and epidemiological pattern of its occurrence. These lineages are further subdivided into multiple subtypes based on genetic markers. Studies on *T. gondii* strains in South America showed a higher genetic variability that further complicates the knowledge of genetic diversity of this parasite (Sibley et al., 2009, Khan et al., 2014, Galal et al., 2019).

2.3. Life cycle of *Toxoplasma gondii*

The life cycle of *T. gondii* is complex and involves multiple stages as sexual and asexual reproduction phases, varying between definitive and intermediate hosts. The sexual phase known as gametogony, occurs within felids such as domestic and wild cats, leading to the release of oocysts into the environment. These oocysts sporulate and become infective. Additionally, an asexual phase can occur within almost all warm-blooded animals (Fux et al., 2007). *Toxoplasma gondii* can sexually reproduce only within the Felidae family, making them the definitive hosts, while other hosts are considered as intermediate hosts (Dubey, 2020). In intermediate hosts, the cycle is outside of the intestine, resulting in tachyzoites and bradyzoites. The invasive tachyzoites are the proliferative stage of the parasite, which multiplies asexually by

endodyogeny upon entering a cell. Bradyzoites are found within tissue cysts that are the latent form of the parasite (Vismarra et al., 2022).

The three infectious stages of *T. gondii* are the oocysts, the rapidly dividing tachyzoites, and the slow dividing bradyzoites in tissue cysts *(S. Al-Malki, 2021)*. The conversion of these parasite structures are biologically important in the life cycle of *T. gondii* (Dubey, 2005) (Figure 1).

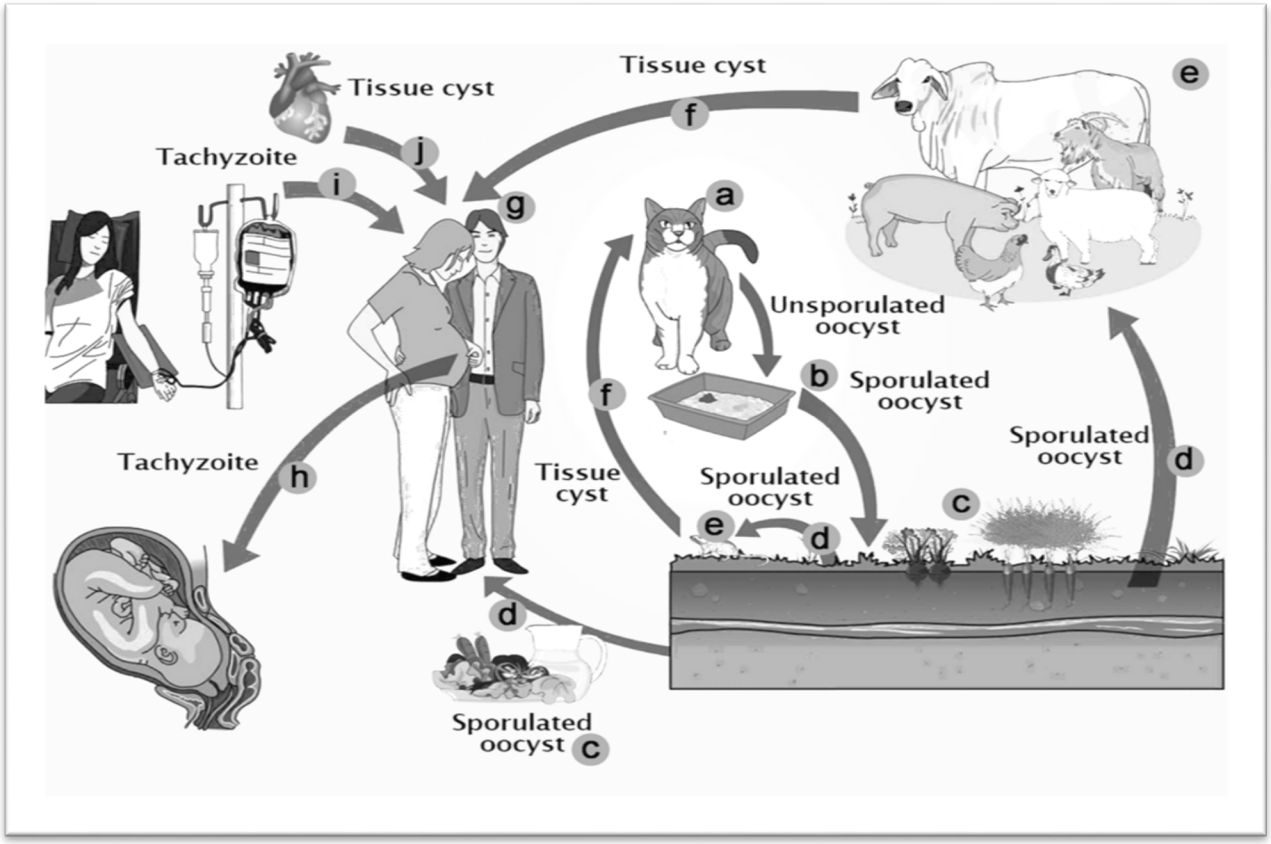


Figure 1: A diagrammatic representation of the life cycle of *T. gondii* (Attias et al., 2020).

A. Feline definitive host (cat). **B**. Unsporulated oocysts in cat feaces **C**. Food contaminated with sporulated oocysts. **D**. Oocysts may be ingested by intermediate hosts via water or raw vegetables. **E**. Intermediate hosts become infected (e.g. cattle, sheep, poultry and swine). **F**. Ingestion of tissue cysts in uncooked meat. **G**. Intermediate hosts (humans) become infected. **H**. Tachyzoites transmitted through the placenta to the foetus. **I**. Transmission by blood transfusion and **J**. organ transplant.

Toxoplasma gondii in the small intestine of the definitive host undergoes sexual development, resulting in the shedding of unsporulated and non-infectious oocysts in felids faeces (Lobetti and Lappin, 2012, Ahn et al., 2019). Oocysts (sporozoites) are

spherical in shape and 10 - 12 µm in diameter. The oocysts become sporulated within 1 – 5 days after shedding in felids faeces and become highly infectious to humans and other animals. Oocysts containing sporozoites are transmitted when ingested by intermediate hosts and multiply in the reticuloendothelial cells and give rise to four nuclei, which are located at the periphery of the zygote and finally, four sporozoites are formed in each sporocyst (Dubey et al., 1998) .

Once oocysts are ingested, tachyzoites are released from the oocytes and enters the host cells by active penetration of the cell membrane, and become enclosed by a parasitophorous vacuole (PV). In the PV, the asexually replication of the tachyzoite occurs by repeated binary division (endodyogeny) until the host cells burst, then the parasite develops into bradyzoites (tissue cysts). Tachyzoites are often rapidly multiplying and the motile stage of *T. gondii*, which has crescent shape and measures 2-6 µm in length (Elmore et al., 2010).

Bradyzoites enclosed in tissue cysts (pseudo cysts) in different tissues of the intermediate host like brain, eye and muscle. Tissue cysts contain numerous bradyzoites, which vary in size from 30 µm to 70 µm and have a high resistance to environmental factors and can survive for long periods. Each bradyzoite has a slender crescent–shape, measuring 1.5 µm to 7 µm. They are more prevalent in the central nervous system (CNS), the eye, as well as the skeletal and cardiac muscles. They can also establish themselves in visceral organs, such as the liver, lungs, and kidneys (Dubey et al., 1998) .

2.4. Host range, susceptibility and transmission of *Toxoplasma gondii*

It is essential to understand the host range and the susceptibility of different animal species to *T. gondii* infection, in order to know the epidemiology of the parasite. Apart from humans, many warm-blooded animals, such as mammals and different bird species, can become infected with *T. gondii*. There are reports that this parasite has been found in more than 350 species of vertebrates worldwide (Dubey, 2016, Niehaus et al., 2020). While mammals such as humans, livestock, birds, rodents, and marine mammals can contract the parasite by consuming *T. gondii* oocysts from the environment, they are regarded as intermediate hosts. Felines are regarded as the

only natural definitive host in which the parasite can reproduce sexually (Black and Boothroyd, 2000, Paula et al., 2020, Sinha et al., 2022).

The host susceptibility to *T. gondii* might differ significantly between species, with some being more resistant or tolerant to infection than others. Domestic animals including cat, small ruminants, pigs and chickens are highly susceptible, often acquiring the parasite through ingestion of oocysts from contaminated feed or water. In contrast, cattle and equines are less susceptible to *T. gondii* infection compared to other species (Tenter et al., 2000, Aguirre et al., 2019).

There are various transmission routes in which animals and humans might get infected with *T. gondii.* The most common route of transmission is via the ingestion of oocysts, which are excreted in the faeces of infected cats. These oocysts can contaminate soil, water, fruits, vegetables, and other feed sources for animals and humans. Domestic cats and wild felids play a crucial role in transmitting the parasite to mammals and birds, as the feline intestinal tract is the sole site for the multiplication of *T. gondii* oocysts (Dubey and Petersen, 2001). Cats infected with *T. gondii* can excrete oocysts, in their faeces, that are resistant forms of the parasite, and can survive for up to five to twelve days in the environment. These oocysts can live in the environment and continue to be infectious in soil for several years under specific situations (Kniel et al., 2002, Jones and Dubey, 2010).

The transmission of the parasite to the definitive hosts could be by ingestion of oocysts from the environment or infected tissue cysts from the intermediate hosts (Almeria and Dubey, 2021). Livestock can be infected by ingesting contaminated feed and drinking water containing sporulated oocysts (Chemoh et al., 2016). The transmission to humans usually occurs horizontally through the consumption of sporulated oocysts, which can be found in soil, cat litter, garden vegetables and water. Additionally, humans can be exposed to the parasite by ingesting tissue cysts (bradyzoites) from raw or undercooked meat, through blood transfusion, or via vertical transmission such as transplacental transmission or organ transplant involving tachyzoites (Tenter et al., 2000, Dubey, 2010). Furthermore, it can spread mechanically by flies, cockroaches, dung beetles and earthworms in the soil (Shapiro et al., 2019).

2.5. Epidemiology of Toxoplasmosis

2.5.1. Global distribution of *Toxoplasma gondii*

Toxoplasma gondii is a widespread parasite with varying seroprevalence rates across different regions (García-Bocanegra et al., 2012). The prevalence of *T. gondii* infections in humans globally is estimated to be around 25.7%, with a range of 0.5% to 87.7%. African countries had the highest average seroprevalence rate of 61.4%, followed by Oceania with 38.5%, South America with 31.2%, and Europe with 29.6%, and Canada with 17.5%, and Asia with 16.4% (Molan et al., 2019, S. Al-Malki, 2021).

Meta analytical studies conducted from 1969 to 2016 across 24 African countries revealed varying prevalence rates of *T. gondii* infection in different species of animals, using diagnostic methods and across different regions. Studies obtained on the seroprevalence of *T. gondii* in African countries ranges from 29.2 to 72.4% in chickens, 18.0 to 56.0% in camels, 8.0 to 17.0% in cattle, 17.0 to 57.0% in sheep, 12.3 to 36.0% in goats and 20 to 32.0% in pigs (Tonouhewa et al., 2017, Gebremedhin, 2019). These *T. gondii* infection rate variations highlight the significance of understanding *T. gondii* infections in domestic animals and implementing effective control measures to mitigate economic losses and prevent zoonotic transmission. Globally, the seroprevalence of *T. gondii* infection varies among chickens and small ruminants (Stelzer et al., 2019) (Table 1).

Table 1: Seroprevalence of *T. gondii* infection from different parts of the world in different livestock species

Author(s)	Year	Country	Species	Test	Number tested	Prevalence
El-Massry et al. (2000)	2000	Egypt	Chicken	MAT	108	47.20%
More et al. (2012)	2012	Argentina	Chicken	IFAT	32	53.00%
Chumpolbanchorn et al. (2013)	2013	Australia	Chicken	IFAT	20	90.00%
Awais et al. (2014)	2014	Pakistan	Chicken	LAT	300	36.33%
Vismarra et al. (2016)	2016	Italy	Chicken	ELISA	66	36.40%
Mose et al. (2016)	2016	Kenya	Chicken	PCR	105	79.00%
Schares et al. (2017)	2017	Germany	Chicken	ELISA	86	47.70%
Ying et al. (2017)	2017	USA	Chicken	MAT	1185	19.40%
Tagwireyi et al. (2019)	2019	South Africa	Chicken	LAT	137	33.58%
Camillo et al. (2020)	2020	Brazil	Chicken	IFAT	597	49.20%

dos Santos Silva et al. (2020)	2020	Brazil	Chicken	IFAT	200	36.00%
Wang et al. (2020)	2020	China	Chicken	ELISA	96	10.70%
Sarr et al. (2020)	2020	Senegal	Chicken	MAT	665	7.60%
Bachan et al. (2018)	2018	India	Goats	ELISA	445	42.47%
Batista et al. (2022)	2022	Brazil	Goats	IFAT	229	21.39%
Ouchene et al. (2023)	2023	Algeria	Sheep	MAT	220	35.90%
Paștiu et al. (2023)	2023	Romania	Sheep	ELISA	2650	53.50%
Hove et al. (2005)	2005	Zimbabwe	Shoats	IFAT	335	67.9 %)
Elfadaly et al. (2017)	2017	Egypt	Shoats	LAT	692	47.5 %
Tonouhewa et al. (2019)	2019	Benin	Shoats	ELISA	368	23.37%
Bentum et al. (2019)	2019	Ghana	Shoats	ELISA	347	29.68%
Sun et al. (2020)	2020	China	Shoats	MAT	481	36.80%
Martínez-Rodriguez et al. (2020)	2020	Colombia	Shoats	ELISA	1038	23.50%
Fadiel et al. (2021)	2021	Libya	Shoats	IFAT	470	55.30%
Condoleo et al. (2023)	2023	Italy	Shoats	ELISA	405	53.80%
Khattak et al. (2024)	2024	Pakistan	Shoats	LAT	3505	20.08%

Shoats- Sheep and goats; No.-Number sampled; ELISA, enzyme linked immunosorbent assay; MAT; modified agglutination test; LAT-, latex agglutination test; IFAT, indirect fluorescent antibody test; PCR, polymerase chain reaction,

2.5.2. Prevalence of *Toxoplasma gondii* infection in Ethiopia

In Ethiopia the close association of humans with their livestock is a high-risk country for the transmission of *T. gondii*. Other factors that contribute to the risk of acquiring *T. gondii* in humans and other animals are related to the sociocultural practices, poor environmental hygiene, sharing water points, house dwelling cats and poor living standards of the communities. These factors can contribute to the maintenance of parasite transmission. In Ethiopia, the seroprevalence of the parasite varied in different studies of the country. In domestic animals it ranges from 17.7% to 74.9% in sheep and goats, 30.5% to 72.4% in chickens, 85.4% to 91.7% in cats, 32.1% in pigs and 82.9% in dogs (Dubey et al., 2013b, Tiao et al., 2013b, Tilahun et al., 2013, Endrias Zewdu and Getachew, 2015, Tilahun et al., 2018, Chaklu et al., 2020, Esubalew et al., 2020, Tarekegn et al., 2020) (Table 2).

In central Ethiopia, Tilahun et al. (2018) had reported a seroprevalence of *T. gondii* infection as 33.7% and 27.6% in sheep and goats respectively. A recent study from the southern part of Ethiopia indicates high seroprevalence of *T. gondii* infection in sheep (57.8%) and goats (47.8%) (Jilo et al., 2021). However, a lower seroprevalence

of *T. gondii* infection has been reported with 15.48% and 31.6% in goats and sheep respectively, from the central parts of Ethiopia (Gebremedhin et al., 2014c). There are not many reported studies on the seroprevalence *T. gondii* infection in chickens in western Ethiopia, but they are high (30.5%- 72.4%) in the central and northern part of Ethiopia (Table 2).

Table 2: Seroprevalence *T. gondii* in domestic animals, Ethiopia

Author(s)	Year	Study location	Species	Test	No.	Prevalence
Dubey et al. (2013a)	2013a	Central Ethiopia	Cat	MAT	36	91.70%
Tiao et al. (2013a)	2013a	Central Ethiopia	Cat	MAT	48	85.40%
Chaklu et al. (2020)	2020	Northern Ethiopia	Chicken	LAT	384	72.40%
Gebremedhin et al. (2015a)	2015a	Central Ethiopia	Chicken	MAT	601	30.50%
(Tegegne et al., 2016b)	2016b	Central Ethiopia	Chicken	MAT	125	38.40%
Gebremedhin et al. (2021)	2021	Central Ethiopia	Dogs	DAT	385	82.90%
Zewdu et al. (2013)	2013	Central Ethiopia	Goat	ELISA	927	19.70%
Gebremedhin et al. (2015b)	2015b	Central Ethiopia	Pigs	DAT	402	32.10%
Negash et al. (2004)	2004	Central Ethiopia	Shoats	MAT	174	45.40%
Teshale et al. (2007)	2007	Central and Southern Ethiopia	Shoats	MAT	641	74.90%
(Tilahun et al., 2013)	2013	Eastern Ethiopia	Shoats	ELISA	1360	30.32%
Gebremedhin and Gizaw (2014)	2014	Southern Ethiopia	Shoats	ELISA	184	26.10%
Esubalew et al. (2020)	2020	Northern Ethiopia	Shoats	LAT	576	70.48
Jilo et al. (2021)	2021	Southern Ethiopia	Shoats	LAT	400	52.80%
Gebremedhin et al. (2013c)	2013c	Central Ethiopia	Shoats	ELISA	1372	31.80%
Gebremedhin et al. (2014b)	2014b	Central Ethiopia	Shoats	DAT	628	17.70%
Tegegne et al. (2016a)	2016a	Southern Ethiopia	Shoats	LAT	368	57.60%

Shoats- sheep and goats; No.-Number sampled; ELISA, enzyme linked immunosorbent assay; MAT, modified agglutination test; LAT-, latex agglutination test; CSE, Central & Southern Ethiopia; LAT , Latex agglutination test

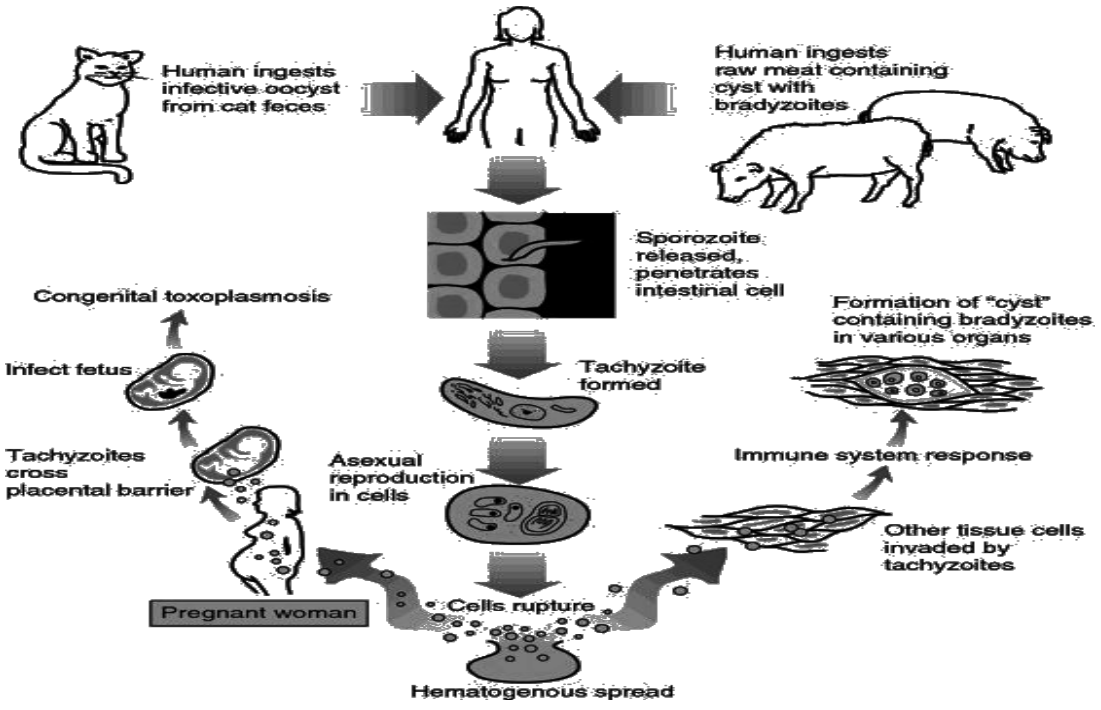
2.6. Pathogenesis and clinical features of *Toxoplasma gondii* in animals

The pathogenesis and clinical presentation of *T. gondii* infection in animals are influenced by species-specific and individual host factors, including immune status, age, concurrent diseases, and parasite virulence, leading to varying severity and manifestations of the infection (Sanchez and Besteiro, 2021). After the parasite is ingested, they enter the host's cells, multiply, and then disseminate throughout the

body, leading to infection in animals. *Toxoplasma gondii* is thought to be a major cause of reproductive losses in small ruminants, including sheep and goats (Stelzer et al., 2019).

During the acute phase, animals may exhibit non-specific signs such as fever, lethargy, anorexia, and lymphadenopathy. In some cases, severe systemic illness, including pneumonia, hepatitis, and neurologic signs, can occur. *Toxoplasma gondii* can establish a chronic infection characterized by the formation of tissue cysts in various organs. In pregnant animals, *T. gondii* can cross the placenta and infect developing foetuses, leading to congenital toxoplasmosis. In immunocompromised animals, *T. gondii* can reactivate from tissue cysts, leading to severe clinical manifestations, particularly neurological signs. Many infected animals may not show clinical signs (Mose et al., 2020). Tissue necrosis may be found in many organs of the body like intestine, liver, spleen, pancreas, lung and heart in the acute phase. Whereas, tissue lesions occur more often in ocular muscles and brain, than in visceral tissues during chronic infections (Robert-Gangneux and Dardé, 2012).

In humans, retinochoroiditis is a common clinical presentation of *T. gondii* that occurs when the parasite invades the retina, causing inflammation and tissue damage resulting in blurred vision, eye pain, redness, and light sensitivity. Without any treatment, ocular toxoplasmosis can lead to permanent blindness. Congenital toxoplasmosis poses high risks to foetal health if a pregnant woman contracts *T. gondii*



for the first time when pregnant. The parasite can pass the placenta and infect the foetus, resulting in neurologic disorders like hydrocephalus, microcephaly, intracranial calcifications, seizures, cognitive impairments, and developmental delays (Elsheikha Hany et al., 2020).

Figure 2: Pathogenesis of toxoplasmosis in human and animals **(**Mahon and Manuselis, 2003)

2.7. Risk factors of *Toxoplasma gondii* transmission

The risk factors for *T. gondii* transmission in domestic animals can vary depending on their management system, environment, behaviour and exposure to potential sources of the parasite. Understanding these factors is crucial for safeguarding both human and animal health from *T. gondii* transmission. In particular, it is important to have knowledge about the risk factors associated with *T. gondii* transmission in small ruminants, such as in sheep and goats, as well as in chickens. Identifying the main route of transmission in animals can help to recognize specific risk factors that need to be addressed (Stelzer et al., 2019).

Many reports on the risk factors were based on the seropositivity of small ruminant's (sheep and goats) and chickens. Researchers have identified major key risk factors for *T. gondii* infection including agroecology, species of animals, sex, age, flock size, farming practice, and close contact with pets (Tonouhewa et al., 2017).

The occurrence of *T. gondii* infection is mainly associated with geography and climate. In midland areas, the occurrence of the disease is reported to be more common in lowland than in highland areas in Ethiopia. Some studies have been conducted specifically on the factors which lead to the difference in the infection rates associated with the variation of temperatures, rainfall, humidity and altitude. Studies in Benin showed that humidity is one of the influential factors in the transmission rates among small ruminants (Tonouhewa et al., 2019). Most of them found a statistically significant influence on the seropositivity of the infection in domestic animals (Yang et al., 2012, Gebremedhin et al., 2014a)

On the other hand, serological studies in Ethiopia from backyard chickens showed that altitude had a significant association with *Toxoplasma* infection in which highland

chickens are less likely to be infected than the midland chickens (Chaklu et al., 2020). The probable factors which affect the variation of the infection could be that the midland areas have moderate environmental conditions with sufficient aeration, moist and warm macroclimate supporting the survival and sporulation rates of oocysts. While the infection rate in highland areas support a colder climate which could potentially decrease the sporulation rates of *T. gondii* (Tenter et al., 2000, Yang et al., 2012).

2.7.1. Species

There have been many studies done on the seroprevalence of *T. gondii* infection in sheep and goats globally. According to the findings from systematic reviews and meta-analyses from 70 countries, the total seroprevalence of *T. gondii* infection was 33.86% in sheep and 31.78% in goats. The prevalence estimates of the parasite in sheep and goats varied significantly by countries (Ahaduzzaman and Hasan, 2022). Esubalew et al. (2020) reported higher prevalence of *T. gondii* in sheep than in goats while Gebremedhin et al. (2013b) reported that the prevalence of *T. gondii* is higher in goats than in sheep in different regions of Ethiopia.

2.7.2. Sex

In different animal species, there could be variance levels of susceptibility to *T. gondii* infection due to differences in reproduction or hormonal effects on the immune system. It has been reported as a risk factor for the likelihood of *T. gondii* infection in small ruminants. A higher prevalence of *T. gondii* was reported in female sheep than in male sheep (van der Puije et al., 2000, Ramzan et al., 2008). The sero-prevalence of *T. gondii* infection in Ethiopia revealed that the likelihood of acquiring infection was higher in in female sheep and goats compared to males (Tegegne et al., 2016b, Tilahun et al., 2018, Jilo et al., 2021). Some studies have shown that male sheep are significantly more prone to the infection than females (Yan et al., 2020). However, studies conducted in China and Benin has shown that there is no significant difference in susceptibility between males and females (Zhang et al., 2016, Tonouhewa et al., 2019). This difference could be arising due to the difference in physiological, behavioural, and hormonal variables.

2.7.3. Age groups

Studies in animals have indicated that, the prevalence of *T. gondii* infection tends to rise with age. Older animals had higher rates of *T. gondii* infection when compared to younger animals. This is due to cumulative exposure to the parasite over the animal's lifetime. Furthermore, older animals might have had more opportunities for contact with infected intermediate hosts or contaminated environments with oocysts (Dámek et al., 2023). According to Ahmad and Tasawar (2015), an increased rate of infection has been found in older and small ruminants due to the immune system being compromised with old age.

2.7.4. Farm management

Epidemiological studies have shown that small ruminants and backyard chickens husbandry and management practices, such as grazing in contaminated pastures, feeding practices, housing conditions, watering point, and biosecurity measures can be a risk for *T. gondii* exposure and transmission to domestic animals. Small ruminants under intensive management systems have a lower prevalence of *T. gondii* infection than those under extensive management systems due to their degree of confinement. In extensive production systems, animals have access to outdoor facilities in which the level of exposure to infectious stages of the parasite is comparatively higher than in intensive production systems (Stelzer et al., 2019). The association of seropositivity in small ruminants due to management are often linked to different risk factors under which the animals are reared, fed, shelter, and water sources. These conditions may influence the likelihood that feed and or water or the farmland may be contaminated with *T. gondii* oocysts (Dubey et al., 2020).

2.7.5. Flock size

The size of flocks is closely related to the management and production systems used. Typically, larger flocks are managed more intensively, while smaller flocks may indicate less specialized approaches and more open environments. Farms that emphasize on animal welfare production systems require more space for animal raising, which can limit the size of the flocks. Moreover, the flock size may be associated with factors affecting the likelihood of livestock exposure to *T. gondii*, such as contamination of feed, water, or farmland with oocysts and contact with other infected intermediate hosts like rodents. There is a noticeable pattern showing that

smaller flocks are linked to a higher likelihood of seropositivity (Stelzer et al., 2019). Ahmad et al. (2015) and Mungai (2021) indicated that having a flock size larger than 50 animals increases the chances of *T. gondii* infection in sheep.

2.7.6. Presence of definitive hosts

The presence of felidae family especially domestic cats nearby animal rearing areas is playing an important role in the transmission of *T. gondii*. Epidemiological studies have shown that the main risk factors of *T. gondii* infection in small ruminants and backyard chickens are associated with the presence of definitive hosts. In Brazil, there was a relationship between the number of seropositive small ruminants and the presence of resident cats, stray cats, free roaming cats, rat control by using cats and feed storage practices (Fajardo et al., 2013). According to research done in Ethiopia by Chaklu et al. (2020), chickens lived closely with cats are four times more likely acquire T.gondii infection than those living without cat.

2.8. Diagnostic tests and diagnosis of Toxoplasmosis

Diagnosing *T. gondii* infections is crucial for preventing and controlling toxoplasmosis in both animals and humans, and it can also contribute to surveillance programs. A combination of clinical observations and laboratory testing are usually used to diagnose toxoplasmosis. Laboratory diagnosis can be accomplished through a variety of techniques, including molecular techniques like polymerase chain reaction (PCR), biological approaches like inoculating laboratory animals or tissue cultures, histological techniques such as examining smears from body fluids or biopsies and using immuno-histochemical staining, and serological techniques like the indirect agglutination test or enzyme linked immunosorbent assays and latex agglutination test (LAT) (Montoya et al., 2010, Liu et al., 2015a).These diverse approaches help in correctly identifying and confirming cases of toxoplasmosis, enabling the necessary management and treatment.

2.8.1. Serological tests

Serological tests are the most common primary methods for diagnosing toxoplasmosis because they can help determine the presence of specific antibodies to *T. gondii* and usually show high sensitivity, and are simple to perform and a convenient means of

diagnosis (Tenter et al., 2000). These tests detect antibodies produced by the body in response to the *T. gondii* infection. Serological tests such as, the indirect fluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA), latex agglutination test (LAT) and indirect hemagglutination assay (IHA) are commonly used for diagnosis of *T. gondii* infection in animals and humans (Reuben et al., 2008, Dong et al., 2018).

The main serological tests used for diagnosing *T. gondii* infection are the IgM and IgG antibody tests. IgM antibodies are produced early upon infection, while IgG antibodies indicate a previous infection. It is important to note that each testing method has its own advantages and limitations, therefore, a combination of tests and thorough clinical observations are essential for reaching a confirmatory diagnosis (Remington et al., 2004)

Indirect fluorescent antibody test (IFAT)

This test detects both IgG and IgM antibodies. IFAT is used in the detection of *T. gondii* antibodies in humans and animals. This test involves the use of killed *T. gondii* tachyzoites, incubated with the test serum, the fluorescent anti-species antibodies are added. The results are analysed with a fluorescence microscope and has been reported to show sensitivities of 80.4–100% and specificities of 91.4–95.8% (Shaapan et al., 2008, dos Santos et al., 2010).

Enzyme Linked Immuno-Sorbent Assay (ELISA)

This assay usually can be used to detect either the presence of an antigen or antibodies. To detect *T. gondii* antibodies or antigens, different types of ELISA tests have been developed, including indirect, and capture ELISAs. The tests are almost all used to detect anti-*T. gondii* IgG, IgM, and IgA antibodies rather than antigens (Velmurugan et al., 2008).

Latex agglutination test (LAT)

The LAT is easy to use and detects IgG antibodies against *T. gondii*. This test involves coating latex particles with soluble antigen, and when the positive serum is added agglutination is observed. The latex agglutination test is quick and a relatively simple technique to perform for detecting anti-*T. gondii* IgG antibodies in animals and

humans. In humans, LAT has a sensitivity of 86–94 % and specificity of 100 %; in sheep, it has a low sensitivity of 78.6 % and specificity of 61.9 % (Oncel *et al.*, 2005).

Species-specific conjugates are not required and has been used on samples from different animal species. LAT is primarily used as a screening test in epidemiologic surveys, especially in developing countries, because of its high specificity and sensitivity in humans, and due to the fact that it is simple and convenient to use (Shahiduzzaman et al., 2011, Kyan et al., 2012).

Indirect hemagglutination test (IHA)

The principle of IHA is that red blood cells (RBCs) are sensitized with a soluble antigen derived from *T. gondii.* When an infected animals serum containing antibodies against *T. gondii* is added to these sensitized RBCs, it can cause agglutination (clumping) of the RBCs (Liu et al., 2015a).

2.8.2. Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a precise and sensitive molecular method utilized to identify *T. gondii* DNA in clinical specimens. The PCR tests are employed to detect the genetic material of *T. gondii* in bodily fluids such as cerebrospinal fluid. This diagnostic tool is especially valuable for identifying active infections, particularly in individuals and animals with weakened immune systems (Savva et al., 1990, Bin Dajem and Almushait, 2012, Liu et al., 2015a).

2.9. Control, prevention and treatment of Toxoplasmosis

The control and prevention of toxoplasmosis in all species is a challenge due to obtaining a proper diagnosis at the early stage of the disease, especially in countries such as Ethiopia, where extensive management of domestic ruminants is widely practiced. The situation is exacerbated by frequent exposure to sources of infection and on the scarcity of information about the disease. Raw or undercooked meat used for human consumption can be important sources of *T. gondii* infection in humans which has necessitated epidemiological investigation of the parasite in production animals to provide effective prevention of toxoplasmosis and control measures in animals and humans (Cenci-Goga et al., 2013).

2.9.1. Control of Toxoplasmosis in animals

Biosecurity and hygiene are essential in preventing the transmission of *T. gondii* infection. In order to prevent contamination, it is important to maintain proper sanitation in animal housing and feeding equipment by regularly cleaning and disinfecting. Proper disposal of animal waste is also crucial to decrease environmental contamination. Regular follow-up of animals for clinical signs of infection and sero-monitoring against *T. gondii* infection are also important measures to mitigate the infection.

A live vaccination comprised of tachyzoites attenuated by repeated passage in mice is available to prevent toxoplasmosis in sheep. The vaccine consists of a strain (S48) of *T. gondii* originally isolated from an aborted lamb in New Zealand (Reddy, *et al*., 2006). The vaccine is given in as a single dose (2 ml) subcutaneously at least three weeks prior to mating. The vaccine provides protective immunity for at least 18 months (Buxton et al., 2007). Monensin and decoquinate have also been administered to ewes in mid-pregnancy in trials to control abortion, due to infection with *T. gondii* (Ibrahim, 2017).

2.9.2. Control of Toxoplasmosis in humans

Avoid contamination of the environments with felids faeces, wear personal protective equipment when gardening or handling soil, and wash hands thoroughly after outdoor activities related to soil. Safe food handling practices, such as cooking meat at 67ºC, freezing meat at -20ºC, and washing fruits and vegetables thoroughly before consumption can help to reduce the risk of infection. Avoid drinking contaminated water and ensuring water safety through proper filtration and treatment methods is also paramount (Elmore et al., 2010, Hill and Dubey, 2018). At present, there are no vaccines to prevent toxoplasmosis in humans (Webster, 2010). While most healthy adults recover from the disease without any treatment, severe cases or immunocompromised individuals may require medications like sulfadiazine or pyrimethamine or spiramycin (Smith et al., 2021).

CHAPTER 3: MATERIAL AND METHODS

3.1. General description of the study site

The study was conducted in two selected districts (Bahir Dar Zuria and Sekella) in West Gojjam Zone, Northwest Ethiopia from March to December 2023 (Figure 1). Bahir Dar Zuria is characterised as a midland, located s560 km north-west of the capital city of the country, Addis Ababa. It is situated at an average altitude ranging from 1750-2300 m. above sea level. The area obtains an average annual rainfall ranging from about 820 to 1035 mm. The minimum and maximum daily temperatures of the area are 10˚C and 32°C, respectively. According to West Gojjam Zone Livestock and Fisheries resource Development Agency (WGZLFRDA, 2021), small ruminant and chicken population in this district is estimated to be about 15,805 sheep, 10,000 goats and 280,309 chickens. The Sekella district is one of the highland areas located 459 km away northwest from Addis Ababa, 160 km away southwest from Bahir Dar, the capital of Amhara national regional state, and 74 km away to the northeast of Finote Selam, the capital town of West Gojjam Zone. The area lies within elevations ranging from 1800 to 3,535 m above sea level. The average temperature of the Woreda is 18°C and the annual rainfall ranges from 1600 mm to 1800 mm. The total population of sheep, goats and chickens in the district is estimated to be 106,667, 19,889 and 255,077 respectively (WGZLFRDA, 2021). Small ruminants (sheep and goat) and chickens are the backbone of the household economy through live animal markets in both districts and as a source of animal protein, which plays an important role in food and nutrition security.

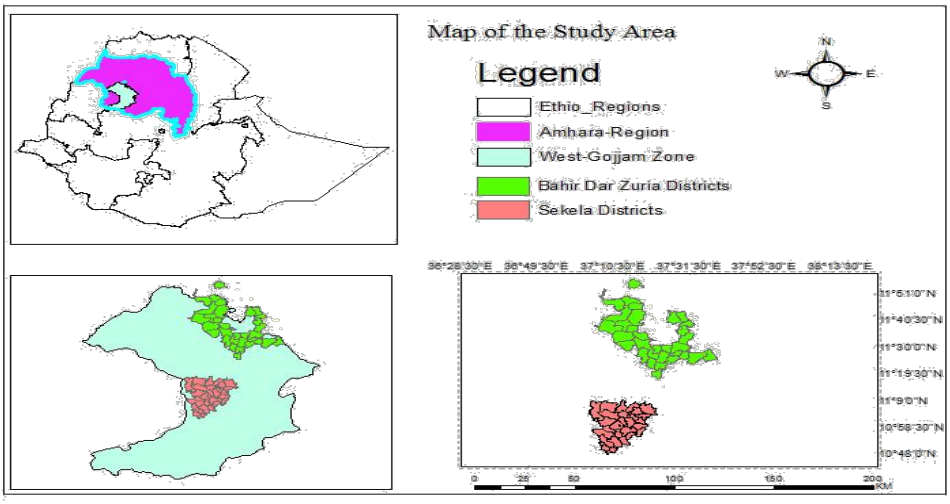


Figure 3: Map of the study areas (Geographical information system, 2023)

3.2. Study population, inclusion and exclusion criteria

Indigenous small ruminants and backyard chickens kept under traditional management systems were considered as the study population. The ages of small ruminants were categorized into two groups as young (six months ≤ three years old) and adults (> three years old), based on information provided by farmers and observation of dental eruption. Flock size was also grouped into small (less than ten small ruminants) and large (greater than ten small ruminants) by considering the number of animals in the household level. Similarly, for chickens, age groups were classified as adult and young, and flock sizes were divided as small (≤ fifteen chickens) and large (> fifteen chickens). For animals younger than six months of age and the owners who refused the collection of blood samples from their animals or an animal that exhibited signs of illness at the time of sample collection were excluded from the study. Furthermore, the study also involved farmers from both districts to assess the risk factors, perceptions and awareness of *T. gondii* infection in the study areas. The study participants were individuals aged 18 and above residing in rural areas with livestock-raising experience.

3.3. Study design and period

Between May and December 2023, a cross-sectional study was conducted to estimate the seroprevalence of *T. gondii* and to identify potential risk factors associated with seropositivity in small ruminants and backyard chickens in selected districts of the West Gojjam Zone, Northwest Ethiopia. Additionally, a survey involving key informants was conducted to assess risk factors, risk perception and awareness of Toxoplasmosis in the study locations.

3.4. Sampling strategy and sample size determination

Prior to starting the actual study, a list of kebeles (smallest administrative unit of districts), was taken from Bahir Dar Zuria and the Sekella district agricultural office. The selection of study districts, kebeles, villages (subdivision of kebele), and individual animals per household was carried out using a multistage sampling technique. The districts were selected purposively based on their ecology (midland and highland). Kebeles known for small ruminant and backyard chicken production were purposively selected based on the availability of these animal flocks. However, a simple random

sampling technique was employed to select small ruminant and backyard chicken producing households. Additionally, individual animals were also sampled randomly for serum sample collection. A total of nine kebeles, proportionally from each district (five from Bahir Dar Zuria and four from Sekella district) were chosen for the study. All relevant epidemiological data for individual animals were recorded using data collection formats to capture potential risk factors associated with *T. gondii* infection sero-positivity.

The sample size required for the study was calculated according to (Thrusfield, 2007) based on the following formula:

$$\frac{Z^2 \mathrm{P}_{\mathrm{exp}}\left(1-\mathrm{P}_{\mathrm{exp}}\right)}{d^2}$$

Where: Z = 1.96, Pexp = expected prevalence and d = 0.07 (the desired level of precision or accuracy). The commonly used values of precision in human studies are ± 3%, ± 5%, ± 7%, or ± 10%; which was the range of accuracy in estimating the true value of the parameters (Al-Subaihi, 2003).

Considering the expected prevalence of 58.73% for sheep, 55.18% for goats, and 72.4% for chicken (Tegegne et al., 2016b, Chaklu et al., 2020) at 7% absolute precision and 95% confidence level, the required sample size was estimated to be 541 (sheep = 190, goats = 194 and backyard chicken = 157) individual animals. The estimated sample size was proportionally drawn from the two study districts (Bahir Dar Zuria and Sekella) based on their sheep, goats and chicken population size. Consequently, 314 animals were sampled from Bahir Dar Zuria kebeles while, 227 animals were selected from Sekella district kebeles.

The total sample size of respondents for the questionnaires was determined by using the formula ($n = 0.25/SE^2$) given by Arsham (2002) at the Standard Error (SE) of 0.05 with 95% confidence interval. $n = 0.25/SE^2 = 0.25/ (0.05)^2 = 100$
Where: n = the required interviewed sample size SE = Standard Error

3.5. Data collection methods

3.5.1. Blood sample collection, transportation and serum separation

After proper restraining of animals, whole blood samples (approximately 2 – 5 ml) were collected aseptically from the jugular vein of sheep and goats, and the wing vein in chicken using sterile disposable syringes with 21 gauge needles and plain sterile tubes. The tubes, in which the blood was collected, was properly labelled and transported to the College of Agriculture and Environmental Science, Bahir Dar University, biotechnology laboratory in cooled containers with ice packs. The blood was then kept at 4°C overnight and centrifuged at 4000 rpm for 5 min to separate serum. Afterwards, the serum was decanted into cryovials or Eppendorf tubes of 1.8 ml volume, labelled for each animal and stored at -20°C until the laboratory analysis was done.

3.5.2. Laboratory analysis

Each serum sample was tested for antibodies against *T. gondii* using a commercially available Latex agglutination (LAT) test kit (PASTOREXTM TOXO, Bio-Rad 3, Boulevard Raymond Poincaré, and F-92430 Marnes-la-Coquette, France) following the manufacturer's protocol and recommendations. A volume of 15 µl of blood sera and a drop of each positive and negative control sera (to validate the test quality) were added to each field of the test card. A drop of diluent was added beside the first drop on each field, followed by vigorously shaking the latex reagents and adding a drop of latex suspension to each field. The three drops in each circle were mixed thoroughly using a stirring rod. The test cards were then placed on a mechanical agitator and rotated for 5 minutes. After removing the test card from the agitator, readings were taken between 5 and 7 minutes to decide the presence or absence of agglutination, indicating the positivity or negativity of *anti-Toxoplasma* antibodies, respectively. However, it was not possible to differentiate between a current infection and a prior infection, as the assay does not allow differentiation between IgG and IgM antibodies against the parasite.

3.5.3. Questionnaire survey

During the blood sample collections from the animals, a pre-tested structured questionnaire was administered to 100 animal owners in order to assess the risk factors and perceptions associated with Toxoplasmosis. The questionnaire included closed-ended questions about individual animal details including agro-ecology, species, age, sex, breed, flock size, management system, location, breed types,

source of water, and the presence or absence of pets. Moreover, questions assessing owners' awareness of Toxoplasmosis, including socio-demographic characteristics, knowledge, and preventing practices were included. Prior to participation, the purpose of the study was explained to the participants, and those who agreed to take part were provided with a written consent form translated into the local language (Amharic) for commencement and approval by signature (Annex 1).

3.6. Study variables

The seroprevalence of *T. gondii* was the dependent variable. species (goat, sheep, and chickens), breed (for chickens; local vs exotic), study site (districts), pets (presence or absence), age (adult and young), sex (male and female), flock size (small and large), management (extensive and semi intensive) and water source (drain and pipe/well) were considered as independent variables, which may affect the exposure of animals and prevalence of *T. gondii* infection. The present study used a methodology similar to that employed by Fekadu et al. (2018) , using the Health Belief Model (HBM) as a theoretical framework, to determine whether participants consumed raw meat or not. Those who did were considered as "high risk," while those who did not were considered as "low risk." The independent variables included socio-demographic factors, knowledge indicators related to *T. gondii,* and other behaviours related to managing cats that may pose potential risks.

3.7. Quality control

Before statistical analysis, the daily collected data was reviewed and verified for completeness; the data were also carefully screened for errors and coded appropriately. Standardized operating procedures and manufacturer's instructions were strictly followed for laboratory testing. Positive and negative controls were used to verify the quality of LAT kits for *anti-T. gondii* test. Agglutination will be expected with positive control and but not agglutination with negative control. If the latex suspension does not react with positive control, it should not be used, while if it reacts with negative control; these reactions might be due to inappropriate storage environments or contamination of the latex.

3.8. Data management and analysis

The data collected using a questionnaire and laboratory findings were entered into Microsoft Excel, version 2010 spread sheet and analysed by STATA/ version 13.0 for window (Statacorp. college station, 2013). Descriptive and analytical statistics were employed. The prevalence of seropositivity was estimated by dividing the number of seropositive animals for *T. gondii* antibodies to the overall number of serum samples tested. In addition, an inferential statistics using univariable and multivariable logistic regression, analysis were conducted to identify and compute the association between the prevalence of *T. gondii* and its putative risk factors. After checking the multi-collinearity between predictor variables using variance inflation factor (VIF), univariable logistic regression for proportion was used to screen the non-important hypothesized risk factors with ($P \leq 0.25$). This was further tested by multiple logistic regression for final conclusion with probability predictive limit less than 5% ($P < 0.05$). Those significant predictor variables in the multivariable logistic regression model were selected as the final risk factors of *T. gondii* infection. Odds ratio was used to assess the strength of association between exposure variables associated with seropositivity of the disease. The statistically significant association between variables and the disease was considered when the P-value was less than 0.05 at 95% confidence level.

3.9. Ethical considerations

The study protocol was reviewed and approved under AEC Reference number REC102-22 by the Faculty of Veterinary Science, Research and Animal ethics committees at University of Pretoria, South Africa. Additionally, approval was also obtained from the Faculty of Humanities for the questionnaire study under ethical clearance reference number HUM041/0822. Local ethical approval was also obtained from Bahir Dar University, School of Animal Science and Veterinary Science Research Ethics Committee, with Reference number 1/367-1-1-3. Momentary description and discussion about the study was made to animal owners and written informed consents were obtained from all of the animal owners before collecting blood samples from the animals. Serum samples were collected in accordance with the guidelines on research and testing animals (Annex 3, 4, 5 and 6).

CHAPTER 4: RESULTS

4.1. Overall seroprevalence

A total of 541 serum samples were collected from the two districts of West Gojjam Zone and were screened for specific antibodies against *T. gondii*. A 191 sera tested positive, making the overall apparent seroprevalence of *T. gondii* in small ruminants (sheep and goats) and backyard chickens 35.31 % (95%CI: 0.314 - 0.395). District wise, the highest *T. gondii* seroprevalence was observed in Bahir Dar Zurai district 44.59% (140/314, 95%CI: 0 538 - 0.621) and the lowest seroprevalence was recorded in Sekella district 22.47% (51/227, 95%CI: 0.379 - 0.462). Small ruminants and backyard chickens found in Bahir Dar Zuria district were 2.8 times more likely at risk of acquiring *T. gondii* infection when compared to those found in Sekella district (OR = 2.78, 95% CI: = 1.893 - 4.072 and P = 0.000). Moreover, backyard chickens were two times more likely at risk of acquiring *T. gondii* infection (OR = 2.035; 95%CI: 1.389-2.979, P = 0.000) when compared to small ruminants.

4.2. Risk factors analysis of *T. gondii* infection seropositivity in small ruminants

The overall seroprevalence in small ruminants (sheep and goats) was 30.47% (117/384, 95%CI: 0.261 - 0.353). From all the serum samples tested, 38.42% (73/190, 95%CI: 0.301 - 0.734) from sheep and 22.68% (44/194, 95%CI: 1.363 - 3.321) from goats were found seropositive for *T. gondii* infection. Higher seropositivity was also recorded in midland area (35.83%), male (31.76%), adult (33.91%), small flock size (35.19%), extensively managed small ruminants (34.53%), dog presence (37.50%) and cat presence (46.15%) when compared to their counterparts.

The univariable logistic analysis result showed that eight (08) predictor variables (agro-ecology, species, age groups, flock size, management system, dog presence, cat presence, and study site) were found significantly (P ≤ 0.25) associated with the prevalence of *T. gondii* infection. However, there was no significant association in seropositivity between sex groups at P<0.25 (Table 3). While in multivariable logistic regression model, only five (05) predictor variables (species, flock size, management system, dog presence and cat presence) were found to be significantly associated with the seropositivity of *T. gondii* infection (Table 3).

Table 3: Univariable and multivariable logistic regression analysis of risk factors associated with the seropositivity of *T. gondii* infection in small ruminants

Risk factor	Category	Number sampled	Positive	Prevalence (%)	Univariable		Multivariable	
					COR (95%CI)	P-value	AOR (95% CI)	P-value
Agroecology	Highland	130	26	20.00	-	-	-	-
	Midland	254	91	35.83	2.23 (1.354 - 3.684)	0.002	1.76 (0.691 - 4.464)	0.236
Species	Goats	194	44	22.68	-	-	-	-
	Sheep	190	73	38.42	2.13 (1.363 - 3.3216)	0.001	4.27 (2.462 - 7.401)	0.000*
Sex	Female	214	63	29.44	-	-	-	-
	Male	170	54	31.76	1.12 (0.721 - 1.727)	0.623	-	-
Age groups	Young	154	39	25.25	-	-	-	-
	Adult	230	78	33.91	1.51 (0.961 - 2.383)	0.074	1.46 (0.862 - 2.462)	0.160
Flock size	Large	222	60	27.03	-	-	-	-
	Small	162	57	35.19	1.47 (0.946-2.271)	0.087	2.17 (1.282-3.677)	0.004*
Management system	SI	106	21	19.81	-	-	-	-
	E	278	96	34.53	2 .14 (1.247- 3.656)	0.006	2.85 (1.532- 5.308)	0.001*
Dog present	No	152	30	19.74	-	-		
	Yes	232	87	37.50	2.44 (1.510-3.942)	0.000	2.45 (1.427-4.193)	0.001*
Cat present	No	215	39	18.14	-	-	-	-
	Yes	169	78	46.15	3.87 (2.441-6.129)	0.000	4.85 (2.811-8.374)	0.000*
Study site	Sekella	175	36	20.57	-	-	-	-
	B/Zuria	209	81	38.76	2.44 (1.542 - 3.871)	0.000	1.79 (0.768- 4.171)	0.177

SI = Semi-intensive, E = Extensive, B/Zuria = Bahir Dar Zuria district, COR= crude odds ratio, AOR= adjusted odds ratio, * = significant variables

Accordingly, sheep were four times more likely at risk of acquiring *T. gondii* (OR= 4.27; 95%CI: 2.462 - 7.401, P= 0.000) when compared to goats. Small ruminant flock sizes below ten (<10 animals) were two times (OR= 2.17, 95%CI: 1.282 - 3.677, P= 0. 0.004) more likely to be affected by *T. gondii* when compared to small ruminants with a flock size greater than 10 animals (>10 animals). The prevalence of *T. gondii* infection was significantly influenced by the management system (OR=2.85; 95%CI: 1.532- 5.308, P= 0.001) in which extensively managed small ruminants were three times more likely affected by *T. gondii* when compared to semi-intensively managed small ruminants (Table 3).

The occurrence of *T. gondii* infection was significantly associated with those small ruminants that lived closely with pet animals (P < 0.05). The odds of small ruminants having close contact with cats were five times (OR = 4.85, 95% CI: 2.811- 8.374, P =0.000) more at risk for infection with *toxoplasma* than those living in the absence of cats. Likewise, the probability of acquiring a *T. gondii* infection among small ruminants when dogs are present was two times (OR = 2. 45, 1.427 - 4.193, P = 0.001) higher than those animals living in the absence of dogs around the vicinity (Table 3).

4.3. Risk factor analysis of *T. gondii* infection seropositivity in backyard chicken

From the total number of backyard chicken serum samples tested (n=157), 47.13% (74/157, 95%CI 0.394 - 0.550) were seropositive for *T. gondii* infection. Higher seropositivity was recorded in Bahir Dar Zurai district (56.19%), female (48.70%), adult (48.15%), and local breed (60.61%), small flock size (51.19%), midland (52.27%), dog presence (57.47%), cat presence at home (64.18%) and drain water (57.66%) when compared to their counterparts. The risk factor study site, agroecology, breed, pet animal (dog and cat) presence at home and source of water were significantly associated with *T. gondii* infection seropositivity; While, sex, age and flock size were found to be insignificant by univariable logistic regression analysis (P ≤ 0.25) (Table 4).

Table 4: Risk factors associated with *T. gondii* infection seropositivity using univariable and multivariable logistic logistic regression analysis in backyard chicken West Gojjam Zone

Risk factors	Category	Number sampled	Positive	Prevalence (%)	Univariable COR (95%CI)	P-value	Multivariable COR (95%CI)	P-value
Study site	Sekella	52	15	28.85	-	-	-	-
	B/Zuria	105	59	56.19	3.16 (1.551 - 6.455)	0.002	3.58 (1.300 - 9.835)	0.014*
Sex	Male	42	18	42.86	-	-	-	-
	Female	115	56	48.70	1.27 (0.621 -2.579)	0.517	-	-
Age group	Young	49	22	44.90	-	-	-	-
	Adult	108	52	48.15	1.14 (0.579 – 2.444)	0.705	-	-
Breed	Exotic	58	14	24.14	-	-		
	Local	99	60	60.61	4.84 (2.344 -9.974)	0.000	4.61 (1.951 -10.869)	0.000*
Flock size	Large	73	31	42.47	-	-	-	-
	Small	84	43	51.19	1.42 (0.756 - 2.671)	0.275	-	-
Dog presence	No	70	24	34.29	-	-	-	-
	Yes	87	50	57.47	2.59 (1.350 -4.968)	0.004	1.51 (0.652 - 3.494)	0.336
Cat presence	No	90	31	34.44	-	-	-	-
	Yes	67	43	64.18	3.41 (1.759-6.612)	0.000	2.84 (1.233 - 6.556)	0.014*
Water source	PU	46	10	21.74	-	-	-	-
	Drain	111	64	57.66	4.90 (2.213 - 10.859)	0.000	2.99 (1.168 - 7.665)	0.022*
Agro-ecology	Highland	25	5	20.00	-	-	-	-
	Midland	132	69	52.27	4.38 (1.552 -12.367)	0.005	1.80 (0.429 - 7.579)	0.421

PU = Pipe/underground, B/Zuria = Bahir Dar Zuria district, COR= crude odds ratio, AOR= adjusted odds ratio, * = significant variables

After conducting univariable analysis, any variables with a P-value of 0.25 or less in the univariable logistic regression analysis were subsequently fitted with multivariable analysis using a logistic regression model. The results of the multivariable logistic regression analysis revealed that four independent variables (study site, breed, presence of cats, and water source) were significantly associated with *T. gondii* seropositivity.

The odds ratio of backyard chickens found in Bahirdar Zuria district, were found to be 3.58 times more likely at risk of acquiring a *T. gondii* infection (OR = 3.58; 95%CI: 1.300 - 9.835, P= 0.014) compared to Sekella district chickens. Breed type was found to be one of the significant risk factors affecting the prevalence of *T. gondii* infection (P<0.001). Specifically, local breeds of backyard chickens were four times more likely to be affected (OR= 4.61, 95%CI: 1.951 - 10.869, P= 0.000) by *T. gondii* than exotic breed chickens. The prevalence of *T. gondii* infection in chickens that have close contact with cats was three times (OR = 2.84, 95% CI: 1.233 - 6.556, P = 0.014) more than those living in the absence of a cat. Additionally, the odds of having a *T. gondii* infection among chickens that were drinking from drain water source were three times higher than the odds of drinking from a pipe/underground water source (OR = 2.99, 95%CI: (0.168 - 7.665, P = 0.022) (Table 4).

4.4. Questionnaire Survey

In a questionnaire survey conducted with 100 respondents, it was found that 67% (67/100, 95%CI: 0.571 - 0.756) reported consuming raw meat, which can be considered as ‘‘high risk’’ behaviour of acquiring *T. gondii.* Conversely, 33% (33/100, 95%CI: 0.244 - 0.429) of respondents who did not consume raw meat were categorized as the ‘‘low risk’’ of acquiring *T. gondii*.

According to the survey, 89% of participants resided in rural areas; 76% of them were male; 42% of them were older than forty; and 80% have no formal education. In comparison to other demographic categories, there was a higher risk of *T. gondii* infection among males (78.95%), individuals above forty years old (78.56%), and residents of rural regions (69.66%) who consumed raw meat. The univariable logistic regression analysis result showed that gender and age were significant risk factors

associated with *T. gondii* risk behaviour due to the practice of consuming raw meat (Table 5).

Table 5: Univariable logistic regression analysis of risk perception of *T.gondii* associated with eating habit of raw meat by risk groups, West Gojjam Zone

Risk factors	Category	Number =100	High risk (%)	Low risk (%)	OR (95%CI)	P-value
Gender	Male	76	60(78.95)	16 (21.05)	-	-
	Female	24	7 (29.17)	17 (70.83)	0.12 (0.039 - 0.310)	0.000*
Age	20-30	18	8 (44.44)	10 (55.56)	-	-
	31-40	40	26(65.00)	14 (35.00)	2.32 (0.745 - 7.217)	0.146
	Above 40	42	33(78.57)	9 (21.43)	2.77 (1.399 - 15.012)	0.012*
Edu. level	Illiterate	80	52(65.00)	28(35.00)	-	-
	Students	17	13(76.47)	4(23.53)	1.75 (0.521- 5.875)	0.365
	Graduate	3	2(66.67)	1(33.33)	1.08 (0.093 - 12.405)	0.953
Residence	Rural	89	62(69.66)	27(30.34)	-	-
	Urban	11	5(45.45)	6(54.55)	0.36 (2 0.102- 1.292)	0.118

* = significant variables

From the study participants, 96% of them lacked knowledge about toxoplasmosis, while 84% of the participants practiced hand washing after gardening. Surprisingly, 73% of respondents had a cat at home, and of this 85% were unaware of the potential role of cats in the transmission of the pathogen to humans. Conversely, 72% were aware that eating of raw meat could aid in the transmission of the pathogen to humans. Based on univariable analysis, the risk factors related to the knowledge of the pathogen (*T. gondii*), included not washing hands after gardening, consumption of raw meat and being unaware of cat faeces contact as a source of infection, which were found statistically associated with high risk behaviour in contracting the pathogen due to consumption of raw meat (Table 7).

Table 6: Univariable logistic regression analysis of Knowledge of Pathogen (*T. gondii)* associated with eating habit of raw meat by risk groups, West Gojjam Zone

Knowledge related risk factors	Category	Number =100	High risk (%)	Low risk (%)	OR (95%CI)	P-value
Have you ever heard of toxoplasmosis?	No	96	65(67.71)	31 (32.29)	-	-
	Yes	4	2(50.00)	2 (50.00)	0.48 (0.064-3.546)	0.469
Do you wash your hands after gardening with soil?	No	16	15 (93.75)	1 (6.67)	-	-
	Yes	84	51 (60.71)	33 (39.29)	0.12 (0.014- 0.859)	0.035*
Can consumption of raw meat a risk of contracting a pathogen (*T. gondii)*?	No	15	14(93.33)	1(6.65)	-	
	Yes	72	42(58.33)	30(41.67)	0.10 (0.012- 0.802)	0.030*
	DK	13	11(84.62)	2(15.38)	0.39 (0.031- 4.917)	0.469
Can contact with cat faeces leads to source of pathogen (*T. gondii)*?	No	85	58(68.24)	27(31.76)	-	
	Yes	6	1(16.67)	5(83.33)	0.09 (0.010 - 0.836)	0.034*
	DK	9	8(88.89)	1(11.11)	3.7 (0.443 - 31.288)	0.226

DK- Don't know OR- Odds ratio, * = significant variables

The potential barriers identified to prevent toxoplasmosis in this study were, limited access to safe water sources, with only 15% (95%CI: 0.092 - 0.236) having piped water, the consumption of raw small ruminants meat (67%, 95%CI: 0.571 - 0.756) (Table 6), presence of high numbers of domestic (73%, 95%CI:0.633 - 0.809) and free-roaming (81%, 95%CI: 0.719 - 0.876) cats, improper disposal of cat faeces (62%), and improper feeding of cats, including feeding them raw animal products and scavenging rodents (78%). According to the results of the univariable analysis, the risk factors associated with perceived barriers to preventing toxoplasmosis include limited access to clean water, and disposing of cat faeces anywhere in the immediate environment and these were found to be statistically associated with high risk behaviour (Table 7).

Table 7: Univariable logistic regression analysis of perceived barriers to prevent toxoplasmosis among livestock owners, West Gojjam Zone

Perceived barriers related variables	Category	Number =100	High risk (%)	Low risk (%)	OR (95%CI)	P-value
Do you have domestic cat?	No	27	16(59.25)	11 (40.74)	-	-
	Yes	73	51(69.67)	22 (30.14)	1.59 (0.638 - 3.984)	0.319
Is there any wild cat in your surroundings?	No	19	10 (52.63)	9 (47.37)	-	-
	Yes	81	57 (70.37)	24 (29.63)	2.12 (0.772 - 5.922	0.144
How do you feed your cat?	HCF	12	4(33.33)	8(66.67)	-	-
	RAP	10	7(70.00)	3(30.00)	4.67(0.765- 28.466)	0.095
	S	10	7(70.00)	3(30.00)0	4.67(0.765- 28.466)	0.095
	Both	68	49 (72.06)	19 (27.94)	1.52 (0.236 - 9.715)	0.661
How do you dispose cat faces?	AW	62	54 (87.10)	8(12.90)	-	-
	CWD	10	1 (10.00)	9(90.00)	0.02 (0.002 - 0.148)	0.000
	OC	28	12 (42.86)	16(57.14)	0.11 (0.039 -0.319)	0.000
Where do you get water?	Pipe	15	6(40.00)	9(60.00)	-	-
	Well	33	19(57.56)	14(42.43)	2.04 (0.588 - 7.052)	0.262
	Stream	52	42(80.77)	10(19.23)	6.3 (1.819 - 21.814	0.004

DK- Don't know; HCF- homemade cooked feed; RAP- raw animal products; S- scavenging, Both -(raw animal product + scavenging), AW- Anywhere, CWD- Common waste disposal, OC- Outside the compound, OR- odds ratio

CHAPTER 5: DISCUSSION AND CONCLUSION

5.1. Discussion

The current research findings revealed that the animal level seroprevalence of *T. gondii* infection in small ruminants and backyard chickens was 35.31% (95%CI: 0.314 - 0.395), which directs the widespread distribution of the parasite within Western Gojjam Zone, Ethiopia. Results from this study show that an overall seroprevalence of *T. gondii* found with in ranges from 30.5% to 72.4% in backyard chickens and 17.7% to 74.9% in small ruminants reported in different parts of Ethiopia, each with different farming systems and climatic conditions (Teshale et al., 2007, Dubey et al., 2013b, Tiao et al., 2013b, Endrias Zewdu and Getachew, 2015, Tilahun et al., 2018, Chaklu et al., 2020, Esubalew et al., 2020, Tarekegn et al., 2020). These results emphasize the notable occurrence of *T. gondii* infection in both backyard chickens and small ruminants in Ethiopia, indicating further consideration and actions to minimize its prevalence on domestic animals.

The findings of this study on small ruminants (30.47%, 95%CI: 0.261 - 0.353) including goats and sheep) was found to be consistent with previous reports from Ethiopia; 31.8% by Gebremedhin et al. (2013b) , 31.8% by (Tilahun et al., 2018) and 34.59 % by Gebremedhin and Tadesse (2015a) . This study is also comparable to other reports of *T. gondii*, from different countries, such as China, 36.80% (Sun et al., 2020). On the contrary, the results found in this study are relatively lower when compared to other reports in different regions of Ethiopia; where 74.9% (Teshale et al., 2007), 70.5% (Esubalew et al., 2020), 57.6% (Tegegne et al., 2016b) , 52.8% (Jilo et al., 2021), and 45.4% (Negash et al., 2004), and in other African countries like 67.9 % in Zimbabwe (Hove et al., 2005), 55.3% in Libya (Fadiel et al., 2021), 47.5 % in Egypt (Elfadaly et al., 2017). However, it was relatively higher sero-prevalence compared to other reports in Ethiopia, such as 17.7% in East Shewa and West Shewa zone (Gebremedhin et al., 2014b), and in other countries like 20.08% in Pakistan (Khattak et al., 2024), 23.5% in Colombia (Martínez-Rodriguez et al., 2020) and 23.37% in Benin (Tonouhewa et al., 2019). The variations in the prevalence *T. gondii* within Ethiopia and globally might be due to the complexity of its epidemiology, difference in socioeconomic and cultural practice, variant in animal husbandry practices and the public health interventions.

The species-specific prevalence results of this study shown the widespread seroprevalence of *T. gondii* among small ruminants and backyard chickens reared in selected districts of West Gojjam Zone of Ethiopia, with 38.42% (95%CI: 0.301 - 0.734) of sheep, 22.68% (95%CI: 1.363 - 3.321) of goats, and 47.13% (95%CI 0.394 - 0.550) of backyard chickens testing positive for *T. gondii* infection. Specifically, there was a significant difference in the infection rate among the three animal species in which backyard chickens were twice more likely to be at risk of contracting *T. gondii* compared to sheep and goats. In addition, sheep were found to have a significantly higher percentage of seropositivity compared to goats ($p < 0.05$). This might be due to the fact that, sheep which tend to graze closer to the ground, are more susceptible to ingesting sporulated oocysts from the pastures compared to goats, which prefer browsing (Gebremedhin et al., 2014b). Similarly, backyard chickens, due to their ground-feeding nature, may be at a higher risk to consume *T. gondii* oocysts from contaminated feed and water sources (Dubey, 2010).

The current estimated seroprevalence of *T. gondii* in sheep in this study is consistent with those previously reported in Ethiopia, 33.73% from East Hararghe Zone (Tilahun et al., 2018), 37.0% in selected districts of Oromia Regional State, (Gebremedhin et al., 2013b) and when compared to other Africa countries such as Egypt (38.7%) (Fereig et al., 2016), Ghana (35.9%) (Bentum et al., 2019) and Algeria (35.9%) (Ouchene et al., 2023). Conversely, it is comparatively lower than previous studies from various parts of Ethiopia; 57.8% in Yabello District, Borana Zone (Jilo et al., 2021), 52.6% in Nazareth (Negash et al., 2004), and 53.8% in Italy (Condoleo et al., 2023), 47% in Poland (Moskwa et al., 2018) and higher than 21.8% in Pakistan (Khattak et al., 2024), 21.33% in China (Liu et al., 2015b), 1.4% in Benin (Tonouhewa et al., 2019), 6.7% in Nigeria (Kamani et al., 2010).

In goats, the prevalence of *T. gondii* infection was in agreement with the previous reported prevalence of 24.8% (Gebremedhin et al., 2013b),and 24% (Negash et al., 2004) in central Ethiopia, as well as similar prevalence reported elsewhere in other countries like 23.7% in Ghana (Bentum et al., 2019) ,21.39% in Brazil (Batista et al., 2022), 25.4% in Pakistan (Ramzan et al., 2009), 21% in Poland (Moskwa et al., 2018) and higher than 4.4% in Iran (Rasti et al., 2018) and 4.6% in Nigeria (Kamani et al., 2010) . However, on the contrary, it was lower than the reported prevalence of 47.8%

in Yabello District, Borana Zone, Southern Ethiopia (Jilo et al., 2021), 27.56% from East Hararghe Zone of Oromia Region (Tilahun et al., 2018) , 42.47% in India (Bachan et al., 2018), 53% in Benin (Tonouhewa et al., 2019) and 35.64% in Pakistan.

The current serological study conducted on backyard chickens revealed higher seroprevalence of *T. gondii* which is relatively comparable to other findings like 49.2% in Brazil (Camillo et al., 2020), 53.0% in Argentina (More et al., 2012), 47.7% in Germany (Schares et al., 2017) and 47.2% in Egypt (El-Massry et al., 2000) but higher than 38.4% (Tilahun et al., 2013), 30.5% (Gebremedhin et al., 2015a) in central Ethiopia and other parts of the world as 19.4% in USA (Ying et al., 2017), 36.33% in Pakistan (Awais et al., 2014), 36.0% in Brazil (dos Santos Silva et al., 2020) , 9.4% in Greece (Andreopoulou et al., 2023), 10.7% in China (Wang et al., 2020) , 36.4% in Italy (Vismarra et al., 2016), 33.58% in South Africa (Tagwireyi et al., 2019) and in 7.6% Senegal (Sarr et al., 2020). However, the prevalence observed in backyard chickens is lower than that reported in Northern Ethiopia and elsewhere globally, such as 72.4% in Northern Ethiopia (Chaklu et al., 2020), 90.0% in Australia (Chumpolbanchorn et al., 2013) and 79.0% in Kenya in (Mose et al., 2016).

A variety of attributes, including differences in hygienic conditions, husbandry systems, climatic variability, feline densities and management practice, sample sizes, breed variation, types of diagnostic methods used, and their corresponding cut-off values, could be the possible contributor for the variation in the animal level seroprevalence observed in this study compared to the aforementioned studies (Innes et al., 2009, Dubey, 2010, Beltrame et al., 2012, Gebremedhin et al., 2014a, Gebremedhin and Tadesse, 2015b, Tegegne et al., 2016a).

In addition, investigations were done to explore the possible risk factors associated with toxoplasmosis in small ruminants and backyard domestic chickens. The odds of having small ruminant flock sizes exceeding 10 sheep and goats were significantly acquire *T. gondii* infection compared to those with fewer than 10 animals ($p < 0.05$). This finding is in agreement with previous findings by Gebremedhin et al. (2013a) and Stelzer et al. (2019), who stated that a significantly higher seroprevalence of *T. gondii* infection in small flocks sizes occurred compared to larger flock sizes. The probable justification for this result might be because of smaller flocks are often allowed to graze

in close proximity to human dwellings leads to frequent contact with cats, and thus source of infection by oocysts. However, large flocks are often kept far from the homestead, where cats are not usually roaming and less likely to contaminate the grasslands with a sporulated oocyts. This finding contradicts previous findings sating that large flocks, managed under extensive management systems, are at higher risk of contracting sporulated oocyst compared to small flocks (Anderlini et al., 2011, Ahmad et al., 2015). Conversely, different flock sizes of sheep and goats indicated no significant association with *T. gondii* seropositivity, probably due to an equal chance of oocyte exposure (Jilo et al., 2021).The aforementioned result variations might be because of the difference in oocyst exposure among animals together with definitive host management.

The prevalence of *T. gondii* infection was significantly influenced by the management system in which extensively managed small ruminants were three times more likely affected by *T. gondii* when compared to semi-intensively managed small ruminants (P= 0.001). This finding was supported by (Dubey, 2010, Gebremedhin et al., 2015c) stating that small ruminants managed extensively are more exposed to sporulated *T. gondii* oocysts shed by felines in the environment, as they graze on pasture directly in contact with the soil. This could be related to the fact that small ruminants housed in semi-intensive management systems are more restricted, which improves their hygiene and limits their exposure to contaminated environments with sporulated oocysts.

The prevalence of *T. gondii* infection in both small ruminants and backyard chickens in this survey was significantly associated with the presence of cats (P<0.05). The odds of small ruminants having close contact with cats were four times more likely to be at risk of *T. gondii* infection compared to those living without cats at home. Similarly, chickens that had close contact with cats at home were twice as likely to acquire *T. gondii* when compared to those without such contact in the study areas. This result supported by different studies conducted in different countries demonstrating a significant association between presence of cats at the homestead and livestock animals seropositivity for *T. gondii* infection (Beltrame et al., 2012, Clementino Andrade et al., 2013, Liu et al., 2015b, Bawm et al., 2016, Deng et al., 2016, Zhang et al., 2016, Chaklu et al., 2020, Condoleo et al., 2023). This is because of the fact that not just the presence of cats but due to the likelihood of their potential to contaminate

the pasture, animal feed and water sources with oocysts. As definitive hosts of *T. gondii*, cats can excrete oocysts in their faeces, which become infectious and resistant to environmental changes following sporulation (Stelzer et al., 2019).

Likewise, this study confirmed that the odds of having *T. gondii* infection among small ruminants in the presence of dogs were found to be two times higher compared to those who living without dogs around the vicinity. Studies have stated that dogs can mechanically transfer *T. gondii* oocysts through their hair or feet after coming into contact with contaminated environments such as from the contaminated soil or cat faeces potentially leading to the contamination of animal feed and water sources (Lindsay et al., 1997). Even if the dogs are not definitive hosts for *T. gondii*, their role in mechanical spreading of oocysts highlights proper dog management to minimize the risks of transmission.

Like small ruminants, the odds ratio of backyard chickens from Bahirdar Zuria district were found to 3.58 times more at risk of acquiring T. *gondii* infection compared to chickens from Sekella district. This result is supported by the findings of Chaklu et al. (2020) in Ethiopia, stating that chickens from midland are significantly more affected by *T. gondii* compared to highland chickens. Yan et al. (2016), also described that, environmental factors play an important role in the prevalence of toxoplasmosis. This finding probably confirmed that geographical difference of the two districts; being Bahirdar Zuria situated in a midland area and Sekella in a highland area contributed to this variation in toxoplasmosis prevalence.

Breed type was found to be one of the significant risk factors affecting the prevalence of *T. gondii* infection ($P<0.001$) in this study. The odds of being local breed chickens were found to be four times more likely to be affected by *T. gondii* compared to exotic breed chickens. The current study was supported by the findings of Chaklu et al. (2020) in southern Ethiopia who stated that local breed of chickens were more likely to be infected by *T. gondii* compared to exotic or crossbred chickens. On the contrary, other studies have confirmed that that local chicken breeds are better disease resistance than exotic breeds (Mpenda et al., 2019).The probable justification for this variation might be due to the difference in feeding behaviour of the local breeds compared to exotic breeds. Local breeds of chicken prefer digging the soil when

feeding compared to the exotic breeds, increasing the likelihood of exposure to oocysts from buried cat faeces in the soil.

In this study, it was confirmed that the odds of having a *T. gondii* infection among chickens that were drinking from drain water source were three times higher than the odds of drinking from underground or pipe water source. This finding is consistent with different reports demonstrating high risk of *T. gondii* transmission through unprotected water sources (Dahmane et al., 2024). Other researchers also indicates that drain water can be considered a significant risk factor for exposing backyard chickens and other animals to *T. gondii* infection (Jones and Dubey, 2010, Krueger et al., 2014, Sah et al., 2019, Stelzer et al., 2019).The observed low risk of infection in backyard chickens that drank pipe/underground water might be because of a low probability of infected definitive hosts contaminating the water source. Conversely, drain water was found to be more contaminated with *T. gondii* oocytes that originated from different sources like the soil with oocytes and cat faces. This result indicates that keeping the hygiene of water sources is crucial to minimize the exposure of animals by the parasite.

According to the surveys carried out in the study area, majority of respondents 67% (95%CI: 0.571 - 0.756) consume raw meat, demonstrating a high-risk behaviour for contracting *T. gondii*. This result was supported by findings conducted in Ethiopia, where 79.3% respondents in and around Sululta District (Biru et al., 2014), and 75% of respondents in central Ethiopia (Fekadu et al., 2018) has consumed raw meat. This parasite is mainly acquired through consuming undercooked or raw meat containing viable tissue cysts, or by ingesting food or drinking water contaminated with oocysts (Liu et al., 2015a). Undercooked or raw meat is regarded as the main source of *T. gondii* infection in humans (Stelzer et al., 2019). This result indicates the lack of awareness of the risk of parasite transmission through consuming undercooked or raw meat together with the cultural practice of eating raw meat in Ethiopia.

This study also confirmed that limited access to safe drinking water, close relationships between cats and humans and disposing of cat faeces anywhere in the immediate environment increases the odds acquiring oocytes from infected cats, and poor management of felids. These findings supported by researches conducted in the United States, stating that *T. gondii* infection was found higher among persons with a

lower educational level, and those who worked in soil-related jobs (Jones et al., 2009). Hussain et al. (2017) also indicated that the risk factors for human *T. gondii* infection include eating raw or undercooked animal meat products, having contact with soil and owning cats. This indicates the importance of public health interventions to address the major causes of this high-risk behaviour in the study areas and to aware the community about the importance of proper food and water handling and consumption or drinking practices.

In this study, respondents who were interviewed did not show awareness of the possible risk factors associated with toxoplasmosis, which likely contributes to a high risk of contracting the disease. The perception of toxoplasmosis severity was not evaluated in the study because the participants' were unaware of the likely risk factors for the disease on the health of humans and animals. Although self-efficacy in preventing toxoplasmosis was not particularly addressed in the study, it is likely that participant self-efficacy in preventing the disease was low due to lack of knowledge and perceived barriers.

5.2. Conclusion and Recommendations

In conclusion, the study found a high prevalence of toxoplasmosis (35.31%) in backyard chickens and small ruminants. The presence of pet animals around the homestead, type of chicken breed, species, flock size, management practices, and water sources were among the risk factors significantly associated with the seropositivity of toxoplasmosis. The surveys that were carried out as part of this study also indicated livestock owners were at a high risk of acquiring *T. gondii* through the consumption of raw or undercooked meats. Moreover, the high prevalence of toxoplasmosis in the study area could hinder the region's livestock development initiative. Therefore, it is an indicator to implement evidence-based disease control and prevention strategies in West Gojjam Zone, Ethiopia. Based on these findings, the following recommendations are proposed:

- Implement interventions targeted at mitigating the identified risk factors associated with toxoplasmosis
- Conduct regular screening of small ruminants and backyard chickens to facilitate necessary measures for parasite control

- Take precautionary measures when handling cats, engaging in gardening, and consuming animal source products.
- Conduct public education and awareness campaigns to a wear people about the risks of toxoplasmosis and motivate them to take the appropriate safety measures
- Conduct further comprehensive studies using molecular techniques across the region to assess the overall magnitude of the disease and its implications

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7. ANNEXES

Annex 1: Farmer interview protocol for *T. gondii* infection its risk factors

I. Consent:

I, _____hereby give permission for blood collection from my animal/s and thereby give my consent to take part in the research project entitled:

"*Toxoplasma gondii* infection in small ruminants and backyard chicken in West Gojjam Zone, Northwest Ethiopia: seroprevalence, sssociated risk factors and risk perceptions"

Signature of the owner**:** _____

II. General Information

2. Name of the respondent _____ Sex _____Age _____ Educational status_____

2) Region_____Zone_____District_____PA_____

3) Domestic animal owned by the interviewee (species and number):

Species	Number/ Flock size	Sex	Age	Breed	Body condition	Remark
Ovine						
Caprine						
Chicken						
Cat						

III. Domestic animal production practices

Species	Production practices			
	Intensive	Extensive	Free-roaming	Indoors
Ovine				
Caprine				
Chicken				
Cat				

IV. Domestic animal management practices

1. Which grazing system do you commonly used for your animal?

a. Communal b. Private c. Zero grazing/stall feeding

2. From where your animal get water most of the time?

   A. Pipe (tap water) B. Spring water C. River

3. Do you see cats/rodents on your house?

A. No B. Yes, cats only

C. Yes, rodents only D. Yes, both cats and rodents

4. Do cats/ rodents have access to feed storage or troughs?

   A Yes B. No

5. Is there any cat contact of your animal in the house or surroundings? A. Yes B. No
6. Is there any feral cat in your surroundings? A. Yes B. No
7. Do you have your own cats? A. Yes B. No
8. What do you feed your cats? A. left over B. Raw offal C. Commercial pet feed D. Not feed
9. How do you dispose cat faeces?

A. Left exposed in the environment B covered up with soil

10. Do your animals have any contact with wildlife? A. Yes B. No

    If yes (Specify)

---

11. Have you face or know about T.gondii infection or Toxoplasmosis disease?

    A.Yes B. No

12. Have you knowing about food borne disease? A. Yes B. No
13. Do you have raw meat (uncooked meat) eating habit? A. Yes B. No
14. Do you have raw vegetables and or poorly washed fruits eating habit? A. Yes B. No
15. Do you wash your hand after handling raw meat, soil and vegetable A. Yes B. No
16. Do you know cat as a pathogen source A. Yes B. No

Annex 2: Latex Agglutination testing Protocol and procedure

The Toxo-latex is a slide agglutination test for the qualitative and semi-quantitative detection of anti-toxoplasma antibodies. Latex particles coated with soluble *Toxoplasma gondii* antigen are agglutinated when mixed with samples containing antibodies anti-Toxoplasma.

Materials required:

- PASTOREXTM TOXO latex agglutination test kit (Bio-Rad, 3 Boulevard Raymond Poincaré, F-92430 Marnes-la-Coquette, France)

- Blood serum samples
- Positive and negative control sera
- Diluent
- Stirring rod

Procedure:

1. Prepare the workspace and gather all necessary materials.
2. Label each test card appropriately for identification.
3. Take 15 µl of blood sera sample using a micropipette and add it to the designated fields on the test card.
4. Add a drop of both positive and negative control sera to their respective fields on the test card to validate test quality.
5. Next to the first drop in each field, add a drop of diluent.
6. Vigorously shake the latex reagents provided in the kit.
7. Add a drop of latex suspension to each field on the test card.
8. Using a stirring rod, thoroughly mix the three drops in each circle on the test card.
9. Place the test cards on a mechanical agitator and rotate for 5 minutes to ensure proper mixing and reaction.
10. After 5 minutes, remove the test cards from the agitator.
11. Take readings between 5 and 7 minutes to determine the presence or absence of agglutination.
12. Presence of agglutination indicates the presence of anti-Toxoplasma antibodies.
    - **Note:** this test does not differentiate between IgG and IgM antibodies.

Annex 3: Letter of ethical approval from research ethics committee, facility of veterinary science

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Research Ethics Committee

07 October 2022

LETTER OF APPROVAL

Ethics Reference No	**REC102-22**
Protocol Title	**Seroprevalence and risk factors of Toxoplasma gondii infection among selected domestic animals in selected districts of West Gojjam Zone, Northwest Ethiopia**
Principal Investigator	**Dr YT Mebratie**
Supervisors	**Dr D Morar-Leather**

Dear Dr YT Mebratie,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Veterinary Sciences Research Ethics committee.

Please note the following about your ethics approval:

1. Please use your reference number (REC102-22) on any documents or correspondence with the Research Ethics Committee regarding your research.
2. Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
3. Please note that ethical approval is granted for the duration of the research as stipulated in the original application (for Post graduate studies e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
4. The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

1. The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
2. **Applications using Animals**: FVS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

We wish you the best with your research.

Yours sincerely

Mrs. MR Watson-Kriek
Chairperson (acting): Research Ethics Committee

Annex 4: Certificate of ethical approval from animal ethics committee, facility of veterinary science

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

1 December 2022

Approval Certificate
New Application

AEC Reference No.: REC102-22
Title: Seroprevalence and risk factors of Toxoplasma gondii infection among selected domestic animals in selected districts of West Gojjam Zone, Northwest Ethiopia
Researcher: Dr YT Mebratie
Student's Supervisor: Dr D Morar-Leather

Dear Dr YT Mebratie,

The **New Application** as supported by documents received between 2022-08-12 and 2022-11-22 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2022-11-22.

Please note the following about your ethics approval:

1. The use of species is approved:

Species	Number
Cats (domestic) - Feline	117
Goats - Caprine	380
Poultry - Birds	307
Sheep - Ovine	373
Samples	Number
Cat - blood (Samples from live animals)	117
Chickens - blood (Samples from live animals)	307
Goat - blood (Samples from live animals)	380
Sheep - blood (Samples from live animals)	373

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2023-12-01.
3. Please remember to use your protocol number (REC102-22) on any documents or correspondence with the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days, and must be subsequently submitted electronically on the application system within 14 days.
6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all

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Fakulteit Veeartsenykunde
Lefapha la Disense tša Bongakadinawa

documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof A Tordiffe

DEPUTY CHAIRMAN: UP-Animal Ethics Committee

Annex 5: Ethical approval from human ethics committee, facility of humanities

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomotho

Humanities 100. Since 1919

10 November 2022

Dear Dr YT Mebratie

Project Title:	Seroprevalence and risk factors of Toxoplasma gondii infection among selected domestic animals in selected districts of West Gojjam Zone, Northwest Ethiopia
Researcher:	Dr YT Mebratie
Supervisor(s):	Dr D Morar-Leather
Department:	Veterinary Tropical Diseases
Reference number:	22917609 (HUM041/0822)
Degree:	Masters

I have pleasure in informing you that the above application was **approved** by the Research Ethics Committee on 10 November 2022. Please note that before research can commence all other approvals must have been received.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely,

Prof Karen Harris
Chair: Research Ethics Committee
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

Annex 6: Ethical approval paper from Bahir Dar University, school of animal science and veterinary medicine, ethics committee

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BAHIR DAR UNIVERSITY
SCHOOL OF ANIMAL SCIENCE AND
VETERINARY MEDICINE
Bahir Dar - Ethiopia

ቁጥር/Ref. No.: 1367-1-1-3
ቀን/date: 29/07/2022

Yechale Teshome

(Principal investigator)

Subject: Ethical clearance paper

Recently you requesting the school of animal science and veterinary medicine research ethics review committee to get ethical clearance for your proposal entitled ''**Seroprevalence and risk factors of *Toxoplasma gondii* infection among selected domestic animals in selected districts of West Gojjam Zone, Northwest Ethiopia**''. Accordingly, the school research ethics committee reviewed your proposal in the context of research ethics. Finally the committee confirmed that you have included the ethical consent issue in your serological study, so that there is no ethical problem on the objective and methodology of the proposal. Therefore, you are authorised to implement the research project in the proposed study areas.

We are looking forward to see the output of the research project.

With regards,

Shewatatek Melaku (DVM, MSc, Assistant professor)
Department head of Veterinary Science