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# SEROEPIDEMIOLOGY OF TOXOPLASMOSIS IN DOMESTIC RUMINANTS, PUBLIC HEALTH SIGNIFICANCE AND ISOLATION OF *TOXOPLASMA GONDII* FROM ANIMAL TISSUES IN SELECTED DISTRICTS OF EAST HARARGHE ZONE OF OROMIA REGION, ETHIOPIA

PhD dissertation

BY

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October, 2015

Bishoftu, Ethiopia

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A Dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Veterinary Parasitology

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October, 2015

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Department of Pathology and Parasitology

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As memebers of the Examining Board of the final PhD open defense, we certify that we have read and evaluated the Dissertation prepared by: **Berhanu Tilahun Chefek** entitled **Seroepidemiology of toxoplasmosis in domestic ruminants, public health significance and isolation of *Toxoplasma gondii* from animal tissues in selected districts of east Hararghe zone of Oromia region, Ethiopia** and recommended that it be accepted as fulfilling the Dissertation requirement for the degree of: **Doctor of Philosophy in Parasitology.**

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## DEDICATION

In memory of W/ro Ayelech Gebremichael Defaru, who passed away accidentally during my study. May she rest in peace!

## STATEMENT OF AUTHOR

First, I declare that this thesis/dissertation is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for a PhD degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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## BIBLIOGRAPHICAL SKETCH

I the author of this dissertation was born on November 29, 1958 in Harar town. I attended my elementary education at Harar 2nd model and Harar Haileselasie 1st elementary schools and completed high school education at Harar secondary school. In 1980, I got my diploma at Debrezeit Assistant Veterinarian Training College. I hold degree of Doctor of Veterinary Medicine (DVM) at Kishinov Agricultural Institute in the former USSR/MSSR. Thenafter, I joined Addis Ababa University, College of Veterinary Medicine and received MSc degree in Tropical veterinary Epidemiology. Again, I joined Addis Ababa University, College of Veterinary Medicine and Agriculture and received the degree of Doctor of Philosophy (PhD) in Veterinary Parasitology. In my career, I have served in various governmental offices as field Veterinary Officer, and now I am working as an instructor at Haramaya University, College of Veterinary Medicine at a position of Assistant professor.

## ACKNOWLEDGMENTS

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| CNS | Central Nervous System |
| CSA | Central Statistics Agency |
| CSF | Cerebro Spinal Fluid |
| DA | Direct Agglutinattion |
| DAT | Direct Agglutination Test |
| DNA | Deoxyribonucleic Acid |
| DT | Dye Test |
| ELISA | Enzyme-Linked Immunosorbant Assay |
| FAO | Food and Agricultural Organization |
| GDP | Gross Domestic Product |
| IFAT | Immuno Flouresence Agglutination Test |
| IFN | Interferon |
| IFNγ | Interferon gamma |
| IHT | Indirect Heamagglutination Test |
| IL | Interleukin |
| ILAT | Indirect Latex Agglutination Test |
| IP | Intraperitoneal |
| ISAGA | Immunosorbant Agglutination Assay |
| MAT | Modified Agglutination Test |
| MoAEH | Ministry of Agriculture East Harerghe |
| NAHDIC | National Animal Health Diagnostic and Investigation Center |
| NF | Necrotic Factor |
| NO | Nitric Oxide |
| OD | Optic Density |
| PCR | Polymeraze Chain Reaction |
| RNA | Ribonucleic Acid |
| RT-PCR | Real Time-Polymeraze Chain Reaction |
| SNP | Single Nucleotide Polymorphism |

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SEROEPIDEMIOLOGY OF TOXOPLASMOSIS IN DOMESTIC RUMINANTS, PUBLIC HEALTH SIGNIFICANCE AND ISOLATION OF *TOXOPLASMA GONDII* FROM ANIMAL TISSUES IN SELECTED DISTRICTS OF EAST HARARGHE ZONE OF OROMIA REGION, ETHIOPIA

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## ABSTRACT

Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii* capable of infecting all warm-blooded animals including man. It is unevenly spread across the world at varied prevalences in different host species. In Ethiopia, as there was no nation-wide survey, the status of *T. gondii* infection in several areas remains unknown. Therefore, a cross-sectional sero-epidemiological study of *T. gondii* infection was conducted from July 2011 to September 2013 in three selected districts of East Hararghe Zone of Oromia Region, Ethiopia, in order to determine the seroprevalence and identify the risk factors of *T. gondii* infection in animals and humans as well as to isolate *T. gondii* from infected animal tissues. A structured questionnaire was administered to one hundred randomly selected individuals whose animals were included in the study to collect information on variables such as sex, age, herd/flock size, cats contact, presence of feral cats, and source of water, type of housing and geographical location in an attempt to identify the risk factors attributing to *T. gondii* infection in animals. Animals sera (n = 1360) were tested for the presence of anti-*T. gondii* antibodies by ELISA using the ID vet Screen Toxoplasmosis multi-species indirect kit (IDVET, Montpellier, France). Sera were collected from humans and a questionnaire was administered to collect data on potential risk factors. Toxoscreen DA and Toxo-ISAGA diagnostic kits were employed for the detection of Anti-*T. gondii* IgG and IgM antibodies in human sera, respectively. All factors attributing to *T. gondii* infection in human and animals were analyzed using chi-square test and logistic regression analysis. Accordingly, serologic evidence of *T. gondii* infection was detected in 32 (84.2%) out of 38 farm areas. The seroprevalence of *T. gondii* infection in sheep, goats, cattle and camels was 33.7%, 27.6%, 10.7%

and 14.4% at animal level and 60.8%, 55.8%, 23.2%, and 53.2% at flock/herd level, respectively. On multivariable logistic regression analysis the risk factors significantly associated with *T. gondii* seropositivity were female gender [odds ratio (OR) 2.63, 95% confidence interval (CI) 1.18–5.88, $P = 0.019$], use of pond (OR: 4.25, 95% CI: 2.15–8.38, $P < 0.001$) and pipe water in sheep (OR: 9.57, 95% CI: 5.00–18.33, $P < 0.001$); adult age (OR: 3.45, 95% CI: 1.34–8.90, $P = 0.010$), use of pond (OR: 6.03, 95% CI: 2.42–15.05, $P < 0.001$) and pipe water (OR: 11.61, 95% CI: 4.35–30.95, $P < 0.001$) in goats; use of pond (OR: 5.60, 95% CI: 2.12–14.78, $P < 0.001$) and pipe water (OR: 10.68, 95% CI: 2.23–51.22, P = 0.003) in cattle; adult age (OR: 2.49, 95% CI: 1.14–5.42, P = 0.22) in camels. In human, the *T. gondii* IgG (n = 354) and IgM (n = 167) seroprevalences were 65.8% (95% CI: 60.62–70.75) and 8.9% (95% CI: 5.11–14.38) respectively. Gender difference in IgG seroprevalence was not statistically significant ($P > 0.05$), but 69.5% of adults exhibited an IgG seroresponse to *T. gondii*. Pregnant women showed 76.4% and 9.3% anti-*T. gondii* IgG and IgM antibodies, respectively. The risk factors identified significantly associated with *T. gondii* seropositivity in human on multivariable logistic regression analysis were district (OR = 2.24, 95% CI: 1.25–4.01, $P = 0.007$), pipe water source (OR = 6.70, 95% CI: 2.70–16.64, $P < 0.001$), adult age (OR = 4.32, 95% CI: 1.91–9.75, $P < 0.001$), and keeping cats in the home (OR = 2.01, 95% CI: 1.11–3.65, $P = 0.021$). Viable *T. gondii* tissue cysts were isolated by bioassay in mice from the hearts of 23 (67.6%) sheep and goats. In conclusion, the detection of *T. gondii* infection among domestic ruminants indicates the potential risk presented to humans from food animals. The IgM seropositivity detected among pregnant women warrants the potential risk of congenital transmission. The principal source for acquisition of *T. gondii* infection both in animals and humans was the water source. Thus, inlight of this the impact of *T. gondii* infection on the health, production and reproduction of domestic ruminants, the extent of congenital transmission and water contamination requires further future investigation..

***Key words:*** DAT, District, East Harerge Zone, ELISA, Humans, ISAGA, Oromia, Risk factors, Ruminants, Seroprevalence, *Toxoplasma gondii,*

# 1. INTRODUCTION

Livestock contribute significantly to food production and economic output in all regions. The relative contribution of livestock to agricultural gross domestic product (GDP) is higher in the developed and developing regions (FAO, 2001) even though their production is constrained by several factors including diseases. Previous studies indicated that, diseases caused by parasites are among major challenges in the development of the livestock industry in the world causing reduction of animal production that leads to shortage of food supply of animal origin to human beings. Among these diseases, toxoplasmosis is recognized as zoonotic and disease of great economic importance. It is caused by an obligatory intracellular, protozoan parasite *T. gondii*, often found in humid, warm regions which allow the oocysts survival in the environment (Sawadogo *et al*., 2005). Domestic and wild cats are among major reservoirs of infection because they are the only hosts capable of shedding oocysts whereby play a key role in the transmission of *T. gondii* to mammals and birds (Dubey, 2010).

Toxoplasmosis is widely distributed in the world and prevalent among animals and humans with varied infection rate. It is an important cause of abortion, stillbirth and neonatal mortality in sheep and goats (Tenter *et al*., 2000; Dubey, 2010). Usually it causes embryonic death and resorption, fetal death and mummification in animals. In goats and sheep abortion, stillbirth and neonatal death are common sequels of the lnfection (Underwood and Rook, 1992; Tenter *et al*., 2000). It causes heavy losses through abortion, stillbirth, neonatal mortality, encephalitis and pneumonia particularly in sheep and goats (Tenter *et al*., 2000; Radostitis *et al*., 2007). The annual economic impact of toxoplasmosis in the United States is estimated to be $7.7 billion (Buzby and Roberts, 1996). In Uruguay, 1.4 to 4.7 million US dollar lose per year has been reported due to sheep toxoplasmosis (Freyre *et al*., 1997). Masala *et al.* (2007) in Italy accounted 10 million Euros economic loss per year due to lamb mortality and missed lactation caused by toxoplasmosis. The cost of toxoplasmosis to the UK sheep industry based on lost output (abortions/stillbirths), input costs, and the cost of control measures was £11 million (Bennet and Ijpelaar, 2003).

Toxoplasmosis affects upto one-third of the world's population. It can produce a wide range of clinical manifestations or, in most cases, progress asymptomatically (Remington *et al*., 2001). Humans acquire the infection by the oral route through the consumption of undercooked meat contaminated with cysts, food products or water contaminated with oocysts (Tenter *et al*., 2000). Infection of pregnant women may cause serious health problems if the parasite is transmitted to the foetus to cause congenital toxoplasmosis. The congenital form results in a severe systemic disease because if the mother is infected for the first time during gestation, she can present a temporary parasitemia that will infect the foetus. Congenital toxoplasmosis may cause abortion, neonatal death, or foetal abnormalities with detrimental consequences for the foetus (Ebbesen, 2000; Koneman *et al*., 2004). *T. gondii* tachyzoites have been detected in milk of ewes and goats and some occurrences of human toxoplasmosis have been attributed to the consumption of non-pasteurized goat milk (Sacks *et al*., 1982; Skinner *et al*., 1990).

Livestock are an important and integral part of the farming system in Ethiopia apart from being a source of high quality protein (meat, milk and eggs). They contribute to the economic welfare of the people by providing hides, skins, traction power and fertilizer for increasing the productivity of small holdings (Minjauw and McLeod, 2003). Ethiopia is known as the leading African country in livestock population and ranks 9$^{th}$ in the world (FAO, 2005). The countrys' livestock population is estimated in millions, sheep 27.35, goat 28.16, cattle 55.03 and camel 1.10 (CSA, 2014). The livestock sub-sector accounts for about 40% of the agricultural GDP and 20% of the total GDP (Aklilu, 2002) without considering the contribution of livestock in terms of draught power, manure and transport services. However, the full exploitation of animal resources is hindered in the tropical environment and particularly in Africa due to a combination of factors such as draught, poor genetic potential of the animals, traditional system of husbandry and the presence of numerous prevalent diseases (Mitenga *et al*., 1994; Rege, 1994). In Ethiopia, it is reported that abortions, stillbirth, prenatal lamb or kid mortality are the most common reproductive problems of small ruminants, where the overall prenatal lamb and kid mortality ranges from 12% to 68% (Kasali *et al*., 1993). However, in the country the cause of this lamb and kid mortality yet hasn't been studied in detail.

In Ethiopia, the epidemiological status of *T. gondii* among domestic ruminants that are used as a source of milk and meat for human consumption is inadequate. To date, no nation-wide survey

had been conducted on the prevalence, risk factors, circulating genotypes of *T. gondii* and its role on reproductive problems among domestic ruminants including camels. The available data are limited to the central part of the country mainly focusing to small ruminants, where the seroprevalence range from 22.9%–56% in sheep and 11.6%–74.8% in goats (Bekele and Kasali, 1989; Demissie and Tilahun, 2002; Negash *et al*., 2004; Teshale *et al.,* 2007; Gebremedihin *et al.,* 2013a; Zewdu *et al.,* 2013). Similarly, in human few studies were conducted in Ethiopia. Seroprevalence of 60%–96.7% have been documented in different groups of people (Gebre-Exabier *et al*., 1993; Woldemichael *et al*., 1998; Yimer *et al*., 2005; Negash *et al*., 2008; Shimelis *et al*., 2009; Gebremedihin *et al*., 2013b). There is paucity of information on the epidemiology of *T. gondii* infection among animals and human in the study area. However, domestic cats and other feline species capable of contaminating the environment by shedding infective oocysts of *T. gondii* are abundant in the area. Besides, abortion and still births are common amongst sheep and goats. Hydrocephalus among newly born child and encephalitis in HIV–AIDS patients are also observed. Consuming raw and under cooked meat and drinking raw milk is a common practice. This study attempted to fill the information gap on *T. gondii* infection, which could provide information to develop a sound control strategy Thus, the following objectives were designed to study toxoplasmosis in domestic ruminants and humans in the study area.

**General objective**

The general objective of the study focused on seroepidemiology of *T. gondii* infection in domestic ruminants and humans, also assess the role of food animals in the transmission of infection, hence produce base line data used to improve the health of animals and humans in the study area

**Specific objectives**

- to determine the seroprevalence and identify risk factors of *T. gondii* infection in domestic ruminants of East Haraghe Zone.
- to isolate *T. gondii* from the tissues of infected domestic ruminants.
- to assess the public health significance and identify the risk factors of *T. gondii* infection in human.

# 2. LITERATURE REVIEW ON TOXOPLASMOSIS

## 2.1. History of Toxoplasmosis

*Toxoplasma* was first discovered in the desert rodent *Ctenodactylus gundi* by Charles Nicolle and Louis Manceaux at the Institute of Pasteur in Tunis in 1908. At about the same time, Alfonso Splendore independently discovered *Toxoplasma* in a rabbit at Sao Paulo (Dubey, 2010). The name *Toxoplasma* (toxon = arc, plasma = form, in Greek) was derived from its crescent shape. The discovery of a *T. gondii* specific antibody test, Sabin-Feldman dye test, in 1948 led to the recognition that *T. gondii* is a common parasite of warm blooded hosts with a worldwide distribution. Also the *T. gondii* life cycle was completed by the discovery of the sexual phase of the parasite in the small intestine of the cat. Its medical importance remained unknown until 1939 when *T. gondii* was identified conclusively in tissues of a congenitally-infected infant in New York City. Likewise, the veterinary importance of *T. gondii* became known when it was found to cause abortion storms in sheep in 1957 (Dubey, 2008).

*Toxoplasma gondii* is one of the most well studied parasites because of its medical and veterinary importance. It is used extensively as a model for cell biology of apicomplexan organisms (Ajioka and Soldati, 2007; Dubey, 2007). *T. gondii* is ubiquitous parasite found in all classes of warm blooded vertebrates. Nearly one-third of humans have been exposed to this parasite (Dubey, 2004). In immunocompetent adults, acute infection normally results in transient influenza-like symptoms, but in immunocompromised persons retinochoroiditis and encephalitis are more common.

## 2.2. Aetiology

*Toxoplasma gondii* belongs to the Kingdom Animalia, Phylum Apicomplexa, Class Protozoa, Subclass Coccidian, Order Eucoccidia, Family Sarcocystidae and Genus *Toxoplasma*. It is an obligate intracellular protozoan parasite that has a characteristically polarized cell structure and a complex cytoskeletal and organellar arrangement at their apical end, the conoid, involved in cell invasion and numerous secretory organelles rhoptries (ROPs), dense granules, and micronemes

(Rorman *et al*., 2006; Klevar, 2007; Weiss and Kim, 2007; Dubey, 2010). *T. gondii* was previously considered that it consists of various strains related to three clonal lineage: type I, type II, and type III, which differ in virulence and epidemiological pattern of occurrence (Howe and Sibley, 1995). Recent studies on *T. gondii* strains in South America revealed the presence of a higher genetic variability (Khan *et al*., 2006; Pena *et al*., 2008). In spite of the fact that many protozoan parasites have a zoonotic transmission, *T. gondii* considered to be the most successful parasite due to its efficient transmission through the ingestion of infective oocysts in contaminated food and water or the ingession of tissue cysts in undercooked meat (Smith, 2009; Ajzenberg, 2011).

Three infectious stages of *T. gondii* are found in all hosts:-

- **Tachyzoites** are often crescent shaped and 2×6 μm in size, their anterior ends are pointed and the posteriors are round (Dubey, 2010). Tachyzoites enter host cells by active penetration of host cell membrane and become surrounded by a parasitophorous vacuole (PV). In PV, tachyzoite replicates asexually by repeated binary division (endodyogeny) until the rupture of the host cells, then quickly changes to a slow growing phase and give rises to bradyzoite (tissue cysts).

- **Bradyzoites** enclosed in tissue cyst have slender and crescent–shaped each measuring 7 μm to 1.5 μm. Tissue cysts contain hundreds and thousands of bradyzoites vary in size from 5 μm to 70 μm, and have a high affinity for neural and muscular tissues. Hence, they are more prevalent in the central nervous system (CNS), the eye as well as skeletal and cardiac muscles. However, they may also be found in visceral organs, such as lungs, liver, and kidneys.

- **Oocysts (sporozoites)** are spherical in shape, 10 × 12 μm in size. *T. gondii* in the small intestine of the definitive host undergoes a typical coccidian development, resulting in the shedding of unsporulated and noninfectious oocysts in cat feces. The oocysts sporulated within 1–5 days after shedding in cat feces and produced eight sporozoites that are highly infectious to animals and humans. Oocysts containing sporozoites are infective when ingested by mammals (including man), multiply in reticuloendothelial cells and give rise

to the tachyzoite stage (Dubey *et al*., 2004). The oocyst wall is an extremely robust multilayer structure protecting the parasite from mechanical and chemical damages. It enables the parasite to survive for long periods, up to more than a year, in a moist environment (Mai *et al*., 2009).

### 2.3. Life cycle

The life cycle involves two developmental stages, an enteroepithelial, in cats and other felines and the extraintestinal stages both in cats and other intermediate hosts (Figure 1). The sexual reproduction occurs only in the intestine of cats. After the ingestion of tissue cysts by cats, the tissue cyst wall is dissolved by proteolytic enzymes in the stomach and small intestine. The released bradyzoites penetrate the epithelial cells of the small intestine and initiate the development of numerous generations of *T. gondii*. Five morphologically distinct types (A to E) of *T. gondii* develop in intestinal epithelial cells before gametogony begins. The sexual cycle starts two days after ingestion of tissue cysts by the cat. The origin of gamonts has not been determined, but the merozoites released from schizont types D and E probably initiate gamete formation. Gamonts occur throughout the small intestine but most commonly in the ileum, 3 to 15 days after inoculation. Microgametes use their flagella to swim to, penetrate, and fertilize mature macrogametes to form zygotes. After fertilization, an oocyst wall is formed around the parasite. Infected epithelial cells rupture and discharge oocysts into the intestinal lumen subsequently shedding with cat feces to the environment. Infected cats can shed more than 100 million oocysts in their feces (Dubey, 2010).

The asexual reproduction of *T. gondii* undergoes two phases of development in various tissues of intermediate hosts. In the first phase, tachyzoites (or endozoites) multiply rapidly by repeated endodyogeny. The last generation of tachyzoites give rise to the second phase of development which results in the formation of tissue cysts. They found predominantly in the central nervous system (CNS), the eye as well as skeletal and cardiac muscles. Bradizoites (or cystozoites) multiplies slowly by endodyogeny within the tissue cyst (Dubey *et al.,* 1998a; Dubey, 2010).

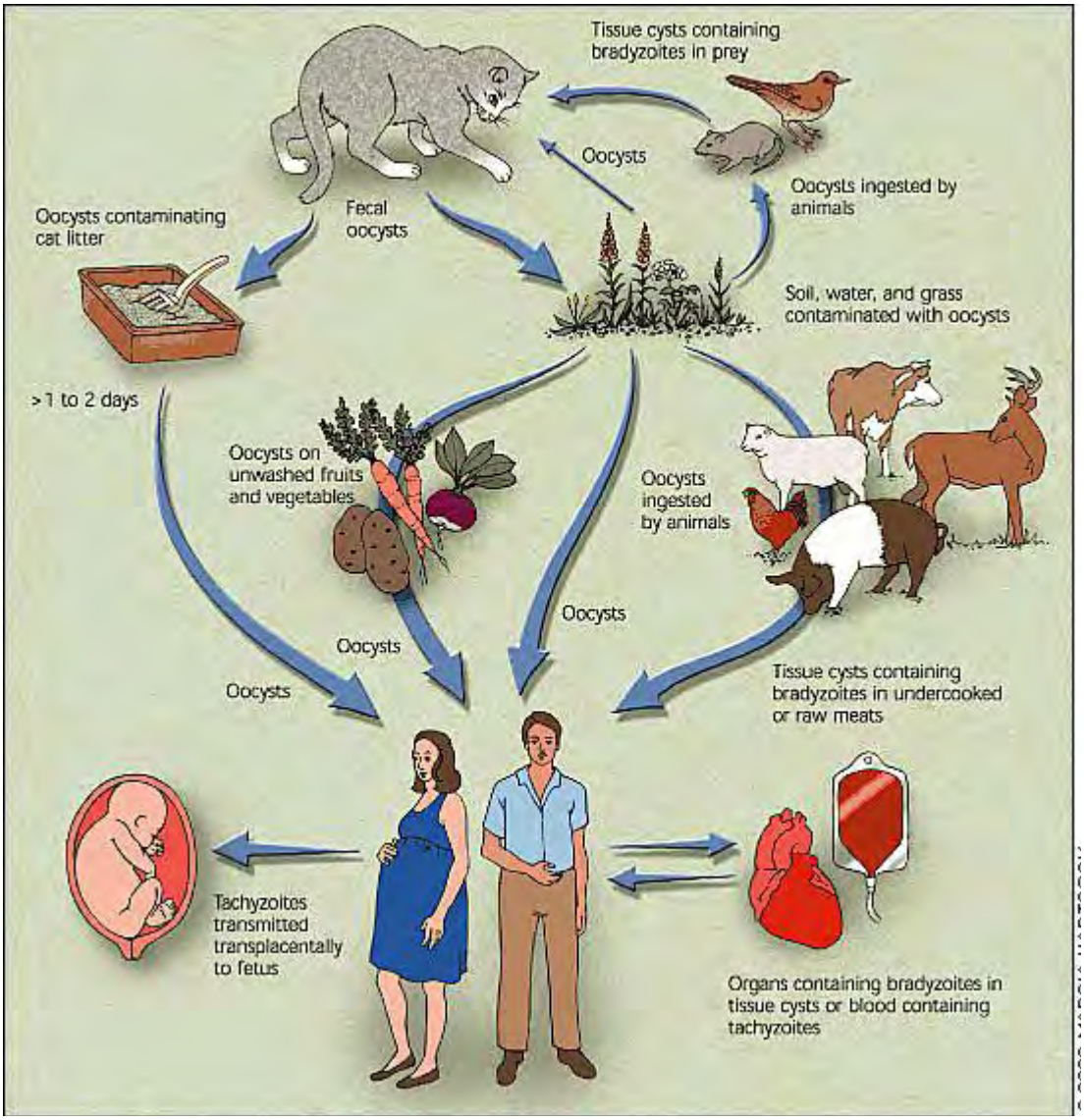


Figure 1: The life cycle of *T. gondii*

Source: Adopted from Jones *et al.* (2003).

### 2.4. Immune response on *T. gondii*

*Toxoplasma gondii* is able to triggering the nonspecific activation of macrophage, natural killer (NK) cells and the other cells such as fibroblasts, epithelial or endothelial cells during the earliest stages of infection. This activation is for limiting parasite proliferation because of its direct or indirect cytotoxic action and to activate a specific immune response in order to the presentation of *Toxoplasma* antigens. This non-specific immune response reacts immediately after the first

contact between the parasite and the host. In mice, the activation of macrophages by cytokine interferon gamma (IFN-γ) in the presence of co-signals, such as tumor necrosis factor-α (TNF-α), is necessary to trigger the cytotoxic activity of the macrophages against *T. gondii* (Filisetti and Candolfi, 2004). The innate immune response is occurred in the early induction of proinflammatory cytokines during infection of naive animals. Dendritic cells, neutrophils and macrophages respond directly to parasite antigens by producing inter leukin (IL)-12, IFN-γ and TNF-α in acute stage of toxoplasmosis (Scharton-Kersten *et al*., 1996; Denkers and Gazzinelli, 1998).

The specific protective immunity is predominantly a cell-mediated immune response. This is because the *T. gondii* is an intracellular parasite. The cytokine, IFN-γ is mainly secreted by T-cells (Joynson and Wreghitt, 2001; Lang *et al.,* 2006). In addition; many studies showed that normally avirulent strains of *T. gondii* become highly virulent in T-lymphocyte-deficient animals. The macrophages and NK cells are primary cells of defense against the parasite during the early infectious stage (Sher and Sousa, 1998). Interleukin12 (IL-12), activates the production of IFN-γ by NK cells and T-lymphocyte (CD4+ and CD8+) cells. The CD4+ and CD8+ T-lymphocyte (TL) are the main players involved in resistance of the host to Toxoplasma infection. The IL-4, IL-5 and IL- 10 are associated with down regulation of protective cell mediated immune response (Filisetti and Candolfi, 2004). Both the proliferation of CD8+ cell and the maturation of NK cells also induce the processing of IFN-γ production (Khan and Kasper, 1996). TNF-α is produced by monocyte, macrophage and T-lymphocyte. It is essential to activate macrophage and to inhibit the parasite replication (Bhopal, 2003) Based on information from various studies, the cytokines involve in the immune process against *T. gondii* infection include IL-2, IL-12, IFN-γ, TNF-α, and possibly IL-6. Both IFN-γ and TNF-α are the critical mediators in the cell mediated immune response against *T. gondii* infection (Fung and Kirschenbaum, 1996). Nonoxidative mechanisms, represented mainly by the production of nitrogen monoxide (NO) by macrophages activated by IFN-γ, with NO also involved during the chronic phase in inhibition of intracerebral parasite proliferation (Schluter *et al*., 1991). IFN-γ also increases the activity of indoleamine 2, 3-dioxygenase, resulting in the breakdown of tryptophan, required for growth of the parasite (Waree, 2008). The GI mucosal immune response to *T. gondii* is presented in Figure 2.

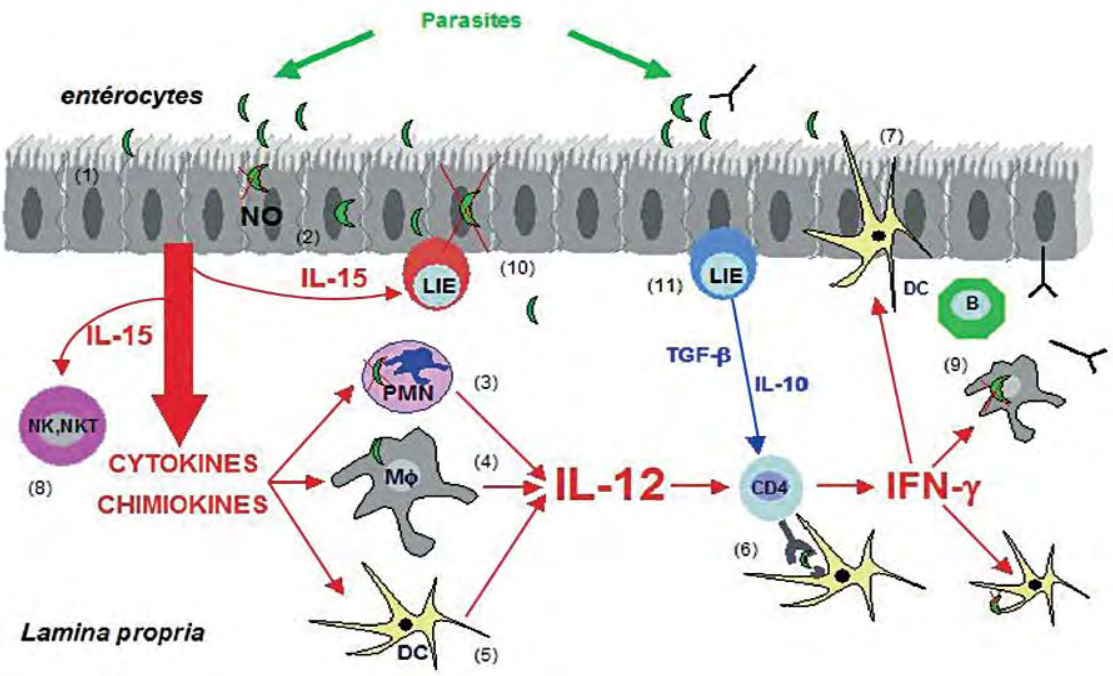


Figure 2: A model of GI mucosal immune response to *T. gondii*

Source: Schulthess *et al.* (2008)

When parasites invade the mucosal intestinal epithelium, they first face a physical barrier brought by the enterocytes bound together by tight junctions (1). Parasites have developed multiple strategies to adhere, sometimes to invade the enterocytes and to spread beyond the epithelium. When enterocytes are infected by the parasites, physiological and morphological disturbances occur, and enterocytes might secrete cytotoxic molecules such as nitric oxyde (NO) (2). In addition, enterocytes respond to the infection by secretion of chemokines and cytokines that attract polymorphonuclear leukocytes (PMNs) (3), macrophages MΦ (4) and dendritic cells (DCs) (5). When stimulated, these cells from the innate immune system can be directly microbicidal. They are also source of cytokines such as IL-12 that triggers the adaptive CD4 immune response (6). To be elicited specific immune response needs antigen presentation, mainly through DCs. DCs sample the antigen by different pathways, one of them, is direct antigen capture into the lumen by elongation of the dendrites through the tight junctions (7). Activated T cells, in addition to NK and NKT cells (8) stimulated by cytokines produced by infected enterocytes such as IL-15, secrete IFN-γ that activates MΦ, DCs, enterocytes for parasite clearance. B cells (9) are also triggered to secrete antibodies that can cross the epithelial barrier by active transcytose and reach parasite into the lumen. Besides microbicidal activities, IFN-γ if not controlled, might damage the intestinal integrity. Intraepithelial lymphocytes (IEL) (10) are cytotoxic for infected enterocytes and might produce TGF-β that limits IFN-γ production (11).

*Toxoplasma gondii* infection is not silent but induces rapid activation of transcription factors such as STAT-1 and NFkB. The parasite blocks nuclear translocation of both factors and macrophages cannot produce IL-12 or TNF-α and the parasite is able to evade or subvert the immune response of its host (Denkers, 2003; Buzoni-Gatel and Werts, 2008). One distinct characteristic of *T. gondii* in immunocompetent hosts, the tissue cysts are able to persist for several years (life of the host) after infection and the immunity does not eliminate an established infection. The antibodies in humoral immune response act as a minor role but still important for diagnosis toxoplasmosis in human. In addition, these antibodies limit multiplication of the Toxoplasma, by involve in lysing the parasites in the presence of the complement. They are also stimulated via opsonization or via an increasing in phagocytosis by the macrophages (Bhopal, 2003; Filisetti and Candolfi, 2004).

## 2.5. Epidemiology of Toxoplasmosis

*Toxoplasma gondii*, can infect almost all the homeothermic animals, including human beings throughout the world, the prevalence of the disease in different species varies depending on the epidemic area, socio–cultural habits, geographical and climatic factors. Prevalence rate may also be associated with the presence of cats that excrete oocysts, which after sporulation become infectious to man and animals (Ajzenberg *et al*., 2004; Dubey, 2004; Garcia *et al*., 2006). *Toxoplasma gondii* oocyst are shed by domestic cats and other felines resulting in wide spread contamination of the environments, where the sporulated oocysts survive in moist soil for months to years (Lukesova and Literak, 1998; Ocholi *et al*., 1989; Dubey and Odenning, 2001; Dubey, 2010).

### *2.5.1. Prevalence and transmission of T. gondii in animals and humans*

Several reports indicating a great variation on the seroprevalence of *T. gondii* infection in domestic ruminants across the world have been reported and range from 3%–92% in sheep (Tenter *et al*., 2000; Pinheiro *et al*., 2009); 5%–77% in goats (Tenter *et al*., 2000; Sharif *et al*., 2007; Carneiro *et al*., 2009); and 0%–99% in cattle (Hall *et al*., 2001). On the other hand, in Africa, *T. gondii* seroprevalence range from 3.6%–57.5% (Sawadogo *et al*., 2005; Kamani *et al*., 2010; Schoonman *et al*., 2010; Khalil and Elrayah, 2011; Swai and Kaaya, 2013).

*Toxoplasma gondii* infection is widespread among humans and estimates of seroprevalence varied in different geographical areas. Accordingly, it has been determined as 16%–40% in England and USA, 50–80% in Europe and central and South Africa (Dubey and Beattie, 1988). Seroprevalence of *T. gondii* infection has been reported as 4%–77% in women at child-bearing age (Tenter *et al*., 2000) and 30%–65% among adult population elsewhere in the world (Fleger *et al*., 2002; Kijlstra and Jongert, 2008). In Ethiopia, previous studies conducted on human toxoplasmosis have showed variation among different groups of people,where the seroprevalence rate range 74.4%–96.7% (Gebre–Exabier *et al*., 1993; Woldemichael *et al*., 1998; Yimer *et al*., 2005; Shimelis *et al*., 2009; Gebremedihin *et al*., 2013b).

Humans become infected with *T. gondii* mainly by ingesting food or water contaminated with oocysts. Most *T. gondii* infections among humans occur by eating raw or undercooked meat containing *T. gondii* tissue cysts, ingesting oocysts from soil (for example, through gardening, handling/eating unwashed vegetables, or changing a cat litter box, or by acquiring congenital infection through the placenta (Jones *et al*., 2001a). Up to 63% human *T. gondii* infections in Europe is attributed to the consumption of undercooked or cured meat products (Cook *et al*., 2000; Dubey, 2004; Dubey, 2008). Consumption of cattle and sheep meat infected by *T. gondii* could be a risk for congenital transmission in pregnant woman (Baril *et al*., 1999). Fresh consumed home-made cheeses produced in small family-based farms from contaminated milk without previous milk pasteurization can represent a risk factor for public health (Fusco *et al*., 2007). In humans, vertical transmission has been associated to abortions, stillborns and variable morbidity (Tenter *et al*., 2000). Recently water-borne transmission of *T. gondii* was considered uncommon but a large human outbreak linked to contamination of a municipal water reservoir in Canada by wild felids has been reported (Dubey, 2004). Oocysts in soil can be spread mechanically by flies, cockroaches, dung beetles and earthworms. They are known to survive on fruits and vegetables for long periods (Kniel *et al*., 2002).

### *2.5.2. Factors influencing T. gondii transmission*

*Toxoplasma gondii* oocysts are shed by domestic cats and other felines which are extremely resistant to external influences and can survive in the environment for years causing a wide spread of contamination (Dubey and Odenning, 2001; Sawadogo *et al*., 2005). Tissue cysts

survive storage at 4–6$^{o}$C for up to 2 months. Some tissue cysts survive for several days after the death of an infected animal, even though its tissues have begun decomposing, but cooking at 60$^{o}$C or higher and freezing at -12$^{o}$C may kill them (Dubey, 2010). Higher prevalence rates of toxoplasmosis in warm and moist areas compared to those which are cold and dry is attributed to the longer viability of *T. gondii* oocysts in moist or humid environments (Vander Puije *et al*., 2000). In extensive management systems, cats can be attracted to pen where animals are kept, and cats allowed free roaming on pastures lands where animals grazing may increase the chance of environment, food and water contamination (Cavalcante *et al*., 2008). Oocysts can remain viable for long periods of time in water and can resist freezing and moderately high water temperatures. They are not killed by chemical and physical treatments currently applied in water treatment plants, including chlorination and ozone treatment (Dumetre *et al.*, 2008). Cultural habits of consuming raw or undercooked meat in a population may facilitates the acquisition of *T. gondii* infection from ingesting of tissue cysts (Gebre-Exiabier *et al*., 1993).

*2.5.3 Risk factors of T. gondii infection*

In animals, geographical differences in the prevalence of infection have been observed (Khamesipour *et al*., 2014). In cattle, a significantly higher prevalence of *T. gondii* antibodies were reported in males than in females and in the age group less than one year old than in other age groups (Elfahal *et al*., 2013). Epidemiological investigations have shown that the main risk factors of *T. gondii* infection in cattle are related to animal management and to the definitive host. There was a relationship between the number of seropositive cattle and the presence and number of resident cats, presence and number of stray cats, presence of cats walking freely, rat control by using cats and feed storage in Brazil (Fajardo *et al*., 2013).

An increased rate of infection have been found in small ruminants reared in densely populated agricultural region compared to those in desert areas (Ahmad and Tasawar, 2015) and, in female and older animals (Ahmad *et al*., 2015). A higher prevalence of *T. gondii* was reported in female than in male sheep (van der Puije *et al*., 2000; Ramzan *et al*., 2009). Guimarães *et al*. (2013) and Ahmad *et al*. (2015) indicated that the production system/extensive farming practice, breed, age, poor hygienic conditions, presence of cats, and flock size larger than 50 individuals are the risk factors associated with *T. gondii* infection in sheep. Whereas, poor hygienic condition, usage of

outdoor water source, presence of cats, extensive farming practice and flock sized larger than 30 and 50 individuals are the risk factors of *T. gondii* infection in goats Ahmad *et al*. (2015).

In human, *T. gondii* infection increased with age. In the United States, *T. gondii* infection was found higher among persons who were foreign-born, persons with a lower educational level, those who lived in crowded conditions, and those who worked in soil-related occupations (Jones *et al*., 2001a). The risk factors for human *T. gondii* infection include owning cats, eating raw or undercooked pork, mutton, lamb, beef, or minced meat products and having contact with soil (Kapperud *et al*., 1996; Baril *et al*., 1999; Weigel *et al*., 1999; Cook *et al*., 2000).

### 2.6. Pathogenesis and Clinical features of *T. gondii* in Animals and Humans

Pathogenecity of *T. gondii* is influenced by many factors including the susceptibility of the host species, virulence of the parasitic strain and the stage. Oocyst induced infections are the most severe clinically in intermediate hosts, and this is not dose dependent (Dubey, 2004). *Toxoplasma gondii* has been adapted to an oocyst-oral cycle in herbivores (intermediate hosts) and tissue cyst-oral cycle in carnivores, especially in the cat. *T. gondii* oocysts are less infective and less pathogenic for the cat than for rats and mice. For example, one live oocyst is orally infective to mice and pigs, whereas, 100 or more oocyst may be required to establish infection in a cat. The reverse may be true for bradyzoites. Cats can shed millions of oocysts after ingesting as few as one bradyzoite. However, 100 bradyzoites may not be infective to mice by the oral route (Dubey, 1996a; Dubey, 1996b; Dubey, 1996c; Dubey, 2001).

During the peack of the acute phase, organisms may appear in secretions and excretions and tissue necrosis may be found in many organs of the body like intestine, liver, spleen, pancreas, lung and heart (Dubey, 1996a). Whereas in chronic infection, lesion occurs more often in muscle eye and brain than in visceral tissue. The spread of toxoplasmosis is less from one animal to another in the acute phase, even when the animals are confined in a close space (Soulsby, 1982). If a women is pregnant and infected for the first time, the tachyzoites associated with the acute phase can cross the placenta and infect the foetus, which can result in severe birth defects, including hydrocephaly, calcification, neurological defects and chorioretinitis, which may be recurrent (Montoya and Remington, 2008). However, with the onset of the host immune response, a subpopulation of tachyzoites in the brain, undergo stage conversion in bradyzoites

which multiply slowly to form large tissue cysts with numerous of bradyzoites that are capable of persisting for the life of the host (Montoya and Liesenfeld, 2004).

### *2.6.1. Clinical manifestation of toxoplasmosis in animals*

The clinical character of the disease varies with the organs attacked, which itself varies depending on whether the disease is congenital or acquired. The principal manifestations are encephalitis when infection is congenital and febrile exanthema with pneumonitis and enterocolitis when very heavy infections occur postnatally (Radostitis *et al*., 2007). It causes embryonic death and resorption, fetal death and mummification in animals. In goats and sheep abortion, stillbirth and neonatal death are common sequels of the lnfection (Dubey and Beattie, 1988; Tenter *et al*., 2000). Among companion animals, fatal toxoplasmosis may occur in dogs that are immunosuppresed following infection with concurrent distemper virus, the onset of illness is marked by fever with lassitude, anorexia and diarrhea. Pneumonia and neurological manifestation are common (Dubey, 2004). Immunocompetence to *T. gondii* is not present before 60 days of gestation and infection in early or mid pregnancy results in fetal death with resorption or mummification. Some lambs infected in mid pregnancy may survive to near term and be stillborn or may survive to parturition, but are weak and die shortly following birth (Buxton, 1990). Despite the fact that cats are frequently infected, clinical disease is rare, although enteritis, enlarged mesenteric lymphnodes, pneumonia, degenerative changes in the central nervous system (CNS) and encephalitis have been recorded in experimental infection.

### *2.6.2. Clinical manifestations of toxoplasmosis in human*

Congenitally infected children in mild disease may consist of slightly diminished vision only, whereas severely diseased children may have the full tetrad of signs including retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcification. Of these, hydrocephalus is the least common but most dramatic lesion of toxoplasmosis. This condition is unique to congenitally acquired toxoplasmosis in humans and hasn't been reported in other animals (Remington *et al*., 2001; Jones *et al*., 2001b). Similarly, Montoya and Liesenfeld (2004) indicated that *T. gondii* is a major cause of human disease as it can lead to retinal scarring, brain damage or abortion following primary maternal infection, and a potentially fatal encephalitic threat to immunocompromised individuals. In postnatally acquired toxoplasmosis localized lymphadenitis

is most frequently observed clinical sign in humans. Lymphadenopathy may be associated with fever, malaise, fatigue, muscle pain, sore throat and headache. Although the condition may be benign, its diagnosis is vital in pregnant women because of the risk to the foetus. Encephalitis is the most clinically important manifestation of toxoplasmosis in immunesuppresed patients. Toxoplasmosis is a major cause of death among patients with AIDS (Dubey, 2004).

Immunocompromised persons, pregnant women and their foetuses are population at risk of toxoplasmosis, in which the clinical manifestations such as encephalitis, brain abscess, hydrocephalus, hepatosplenomegaly and retinitis are apparent. Conversely, in the immunocompetent host, clinical signs and acute toxoplasmosis thus usually passes unnoticed and it followed by a chronic quiescent stage. Recently, available evidence suggests that the host tissue pathology associated with *T. gondii* infection may play an important role in latent infection and the reactivation process (Herion and Saavedra, 1993). Neonatal clinical manifestations of congenital toxoplasmosis vary widely and include hydrocephalus, microcephaly, intracranial calcifications, chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, petechia due to thrombocytopenia, and anaemia (Swisher *et al.*, 1994).

### 2.7. Diagnostic approach in *T. gondii*

Diagnosis of *T. gondii* infection is made by biologic, serologic and histologic methods using either directly, by detecting parasites or deoxyribonucleic acid (DNA), or, indirectly by detecting antibodies of different isotypes. Many techniques are available, which all have strengths and shortcomings, and data obtained by use of these techniques should be interpreted with critical knowledge. Parasite detection or isolation is far less common in immunocompetent patients, except for severe cases of acquired toxoplasmosis with multiorgan failure, where the parasite may be found in the blood or other pathological products several weeks and up to 2 months after contamination (Carme *et al*., 2002; Montoya, 2002).

#### *2.7.1. Serological examination*

Serological examination is used to indicate the presence of infection by detecting toxoplasma specific antibodies or parasitic antigens in body fluid of infected individuals. Different serological tests such as Sabin-Feldman dye test (DT), indirect haemaglutination test (IHT),

indirect florescent antibody test (IFAT), indirect latex agglutination test (ILAT), direct agglutination test (DAT), modified direct agglutination test (MAT). Enzyme linked immunosorbant Assay (ELISA), are popularly used to detect the circulating antigens or antibodies for the diagnosis of toxoplasmosis in animals and humans (Yang *et al*., 2000; Dubey, 2010). More recently, an ELISA test has been developed which is capable of detecting a recent infection by the estimation of IgM, as compared to IgG antibody (Andrews *et al*., 1992). In the ELISA, soluble antigen is coated to microtiter plates and sample serum is added to form an antigen antibody complex. A secondary enzyme linked antibody specific to the host species is added to detect the antigen antibody complex. This diagnostic test requires an ELISA reader (Dubey and Beattie, 1988). The Sabin-Feldman dye test, which is still considered as the `gold standard' for detection of antibodies to *T. gondii* in humans, it is highly sensitive and specific in diagnosis of human toxoplasmosis. However, labour-intensive and has the disadvantage that it requires a continuous supply of live tachyzoites (Su and Dubey, 2010). In the IFAT, inactivated tachyzoites are incubated with sample serum and then fluorescent labeled anti species IgG is added. Detection of *T. gondii* specific IgG is achieved using a fluorescent microscope. The disadvantages of IFAT are the need of a fluorescent microscope and species specific labeling of anti species IgG. IHT relies on the ability of the antibody to cross link red blood cells by the antigen on their surface as a result the red blood cells will agglutinate and give a positive read out. In MAT, antigen (formalin killed tachyzoites) is mixed with 2-mercaptoethanol and then added to serum samples that are placed into microtiter plates. The 2-mercaptoethanol destroys natural IgM antibodies that cause false positive of agglutination test. This significantly improves the test specificity. The sensitivity and specificity of MAT has been validated by comparing serologic data and isolation of the parasite from naturally and experimentally infected pigs. Due to its ease of use and high specificity, the MAT test has been the preferred method for the diagnosis of toxoplasma infection in animals(Dubey, 2010).

Although serological tests are usefull techniques in diagnosing toxoplasmosis, there are limitations since serological dynamics in pregnant hosts may cause confusing or uncertain results. Diagnosis is delayed leading to undetected congenital toxoplasmosis or abortion (James *et al*., 1996; Montoya, 2002). Serology may be inadequate if the host is immunosuppressed, and maternal antibodies may interfere in testing neonates. Commercially available agglutination tests vary in their sensitivity and ability to detect chronic infections (Dubey and Carpenter, 1993).

*2.7.2. Detection of the parasite*

A definitive diagnosis rests on demonstration of the active form of the organism in tissues taken at post mortem examination or in biopsy samples from acute patients by directly visualizing the parasite in the fluid or tissue, but this is difficult and low yield process (Acha and Szyfres, 2003). In addition, Sukthana (2006) described the difficulties in achieving definitive diagnosis of toxoplasmosis through clinic-pathological examination because the clinical manifestations are nonspecific to the disease and often the disease run asymptomatic or subclinical course. Antigen detection is not 100% accurate because interspecies antigen cross-reactivity has been observed, and tissue culture and mouse inoculation, although specific, are time consuming and potentially expose researchers to a Zoonotic (Savva and Holliman, 1990; Tsai and O'Leary, 1993). However, used PCR based approaches hybridization, histologic and isolation of *T. gondii* can be made by mouse inoculation; it removes confusions and misinterpretations of detection of similar parasites like *Sarcocystis* and *Neospora* though it is considered as the reference method for studies, because of its high sensitivity and specificity (Hove *et al*., 2005).

**Histologic or microscopic**

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

*2.7.3. Isolation of T. gondii* (*bioassay*)

Toxoplasmosis can be diagnosed by isolation of *T. gondii* from cultures of body fluids (blood, CSF, bronchoalveolar lavage fluid) or tissue biopsy specimen in the appropriate clinical setting. This is the most convincing diagnostic methods and is obtained by inoculation of suspected materials into toxoplasma free mice by the intraperitonial or intracerebral route and subsequent demonstration of tachyzoites or bradyzoites in smears of organs or serous cavities (Dubey, 1998b). A highly virulent strain produces acute and generalized fatal infection one to fourteen days after the intra peritoneal route of infection and few days earlier if the intracerebral route has been used. Isolation of toxoplasma organisms from acute infection is complicated; therefore, failure to identify the parasite does not necessarily reflect lack of causality. Unfortunately, isolation studies may not be helpful for a rapid diagnosis of toxoplasmosis since up to six weeks of culture may be required (Urquhart *et al*., 1996).

**a) Bioassay in mice**

For the isolation of *T. gondii* from body fluids, (blood, cerebrospinal fluid, aqueous humor, amniotic fluid, etc.), the samples can be centrifuged at 1000 × g for 10 min, the pellet (may be invisible) is resuspended in phosphate buffered saline (PBS), and up to 0.5-1 ml of suspension can be inoculated to mice by intraperitoneal (IP) or subcutaneous injection (Liu, 2010). Recently, isolation of *T. gondii* conducted from muscle tissues by pepsin digestion procedure as described by (Dubey, 1998b)

**b) Bioassay in cats**

Often the number of tissue cysts in animals may be too low to be detectable by bioassay in mice. To increase the possibility of isolating the parasites, cats are usually used instead because they can consume much larger volume of tissues ten times or more to bioassay in cats, 500 g or more meat samples can be fed to specific pathogen free cats. Infected cats can shed millions of oocysts between days 3 and 14 post infection (Dubey and Beattie, 1988).

*2.7.4. Molecular techniques*

The advent of the polymerase chain reaction (PCR) has revolutionized parasitological research and has found broad applicability mainly because its sensitivity permits the amplification of genes or gene fragments from minute amounts of parasite material. DNA technology has had a major impact in many areas of parasitology, including the identification and systematics of parasites, the diagnosis of infections, the epidemiology of parasites, the analysis of population genetic structures, gene expression and organisation, the study of drug resistance and vaccine development. The method of PCR allows the selective amplification from DNA or RNA (in reverse transcription PCR, RT-PCR). PCR has revolutionised prenatal diagnosis of congenital toxoplasmosis by enabling early diagnosis, thereby avoiding use of more invasive procedures on the foetus (Montoya, 2002; Su and Dubey, 2010). PCR with amniotic fluid, placental and brain tissues, whole blood, cerebrospinal fluid, urine, vitreous fluid, aqueous humor, broncho alveolar lavage fluid, and pleural and peritoneal fluids has proved of value. Diagnosis is particularly useful in immune compromised patients or patients with AIDS in whom antibody synthesis may be delayed and low, or where it cannot be made by finding *T. gondii* in host tissue removed by biopsy or at necropsy (Bastien, 2002).

PCR should be considered a reliable, rapid, inexpensive, and definitive method of identification for *T. gondii* infection in formalin-fixed, paraffin-embedded material (Hyman *et al*., 1995). PCR has clearly improved the diagnosis of toxoplasmosis and is today an inescapable technique for revealing the presence of the parasite in clinical specimens (Cassaing *et al*., 2006). Most PCR-based techniques make use of the B1 gene, and less commonly the SAG-1 (P-30) single-copy sequence, which has been shown to be a satisfactory PCR target for the detection of *T. gondii*.

Among many different markers, the 35-copy B1 gene and the 300-copy 529-bp element are most frequently used. The 110-copy internal transcribed spacer (ITS1) sequence was also successfully but less frequently applied for diagnosis but the B1 gene is absent in mammalian cells (Burg *et al*., 1989; Brézin *et al.,* 1990). SAG-1 (the so-called P-30 antigen) is the major stage-specific surface antigen of the *T. gondii* tachyzoite, but not of bradyzoites or tissue cysts. Other single-copy sequences, including the SAG-2, SAG-3, GRA-4 and ROP-1 genes, have been used as PCR targets in research laboratories (Contini *et al*., 2006). The sensitivity and specificity of the PCR

depend on the DNA extraction protocol, the characteristics of the optimization of the reaction conditions. The main problem with PCR used to detect *T. gondii* is the lack of a standardized protocol (Bastein, 2002).

**a) Conventional PCR**

The PCR conventional is performed by denaturation of double stranded DNA to generate single stranded DNA. Primers, which are short single stranded oligonucleotedes anneal to the single stranded DNA molecules at specific locations, apolymerase enzymes recognizes annealed primers and iniates elongations to synthesize double stranded DNA. The process is repeated in cycles and the amount of DNA copies increases exponentially for each cycle. The specific DNA fragment analyzed using an agarose gel, and visualized by staining with ethidium bromide and illuminations with ultraviolet light (Edvinson, 2006).

**b) PCR Oligochromatography**

A commercially available kit for detection of *T. gondii* is based on PCR followed by oligochromatography. The principle of oligochromatography of PCR products is that a specific probe recognizes the PCR product, which gives to rise to a red band on oligochromatography strip (Edvinson *et al*., 2004).

**c) Real-time PCR**

The real-time PCR is performed using a video camera and inclusion of ethidium bromide in the PCR mixture. The emitted fluorescence was found tobe proportional to the amount of double stranded products formed during the PCR. The advantage of this method is that it not only detects but also quantifies the pathogens. Because of that, real-time PCR is gaining popularity in detection of *T. gondii.* The real-time PCR is extremely useful when standard methods (usually microscopical or serological) for quantification are difficult or are less sensitive for detection of clinically relevant parasite burdens. The ability to quantify low-level parasite burdens allows the monitoring of the progression of infections under drug treatment and diagnosis of low-level infections or carrier states. The detection threshold of real-time PCR in *T. gondii* is superior to nPCR assays (Bell and Ranford-Cartwright, 2002; Contini *et al*., 2005).

**d) Genotyping of *T. gondii***

Genotyping has a key role to play in studies of the population biology of *T. gondii*, in epidemiological studies, and in identification of an infection source (Switaj *et al*., 2005). Various genotyping techniques have been used for classifying *T. gondii* into specific groups, including restriction fragment length polymorphism (RFLP)-PCR analysis (Bohne *et al*., 1993), random amplified polymorphic DNA (Guo *et al*., 1997) analysis, and analysis based on sequence length polymorphism (Ajzenberg *et al*., 2002b).

*Toxoplasma gondii* was initially considered to be clonal with very little genetic variability and the isolates from Europe and North America have been classified into three closely related clonal types named I, II and III (Howe and Sibley, 1995; Ajzenberg *et al*., 2002a; Ajzenberg *et al*., 2002b). Type I is highly virulent in murine infections and shows a 100% lethal dose ($LD_{100}$) of just one parasite, whereas type II and III strains are relatively less virulent, showing an $LD_{100}$ of >103 parasites (Howe *et al*., 1997). However recent studies of *T. gondii* strains from Brazil and French Guiana revealed a higher genetic variability of the parasite than previously reported (Ajzenberg *et al*., 2004; Khan *et al*., 2006; Pena *et al*., 2006; Pena *et al*., 2008). The *GRA6* gene was used as a marker because it can clearly differentiate between the three *T. gondii* genotypes, as well as between some atypical genotypes (Fazaeli *et al*., 2000). However, typing of *T. gondii* using one marker may not identify non-clonal strains. To discriminate closely related isolates and achieve high resolution within a clonal lineage, multilocus, PCR-RFLP and microsatellite analysis should be used (Su *et al*., 2006).

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

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

**f) Microsatellites**

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

**g) Sequencing technique**

*Toxoplasma gondii* genotypes identified by analysis of single nucleotide polymorphisms (SNPs) using suitable sequencing technique. Sequencing of genomic regions reveals the complete genetic diversity including single nucleotide polymorphisms and deletions. Direct sequencing generally detects much greater genetic diversity than other methods. Thus, the method provide the best approach for detecting polymorphisms in new isolates or from previously unsampled populations (Sibley *et al*., 2009).

## 2.8. Status of *T. gondii* infection in Ethiopia

In Ethiopia, the limited studies conducted on animals indicated that *T. gondii* seroprevalence range from 6.6% to 74.8% (Table 1, Demssie and Tilahun, 2002; Teshale *et al*., 2007). Regarding the feral cats at Addis Ababa, *T. gondii* seroprevalence was reported as 85.4% and 23.9% for IgG and IgM specific anti-*T. gondii* antibodies, respectively. Additionally, few studies conducted on oocysts shed by cats revealed a prevalence of 12.5% in Debrebirhan and 13.5% in Bahirdar (Negash, 2000; Yihinew, 2012) respectively. Recently, Gebremedhin *et al.* (2014b) reported the isolation of 47 viable *T. gondii* cysts from the hearts of sheep and goats. They described that 93.6% (44/47) circulating *T. gondii* strains are non-virulent to mice. The prevalence of *T. gondii* infection in food animals, the habit of consuming raw or undercooked meat, keeping of domestic cats at home and the presence of feral cats can predispose the people to *T. gondii* infection in the

country. Particularly those infected with HIV/AIDS persons exposed to serious complication due to the resulting opportunistic infections (Gebre-Exabier *et al*., 1993; Gebremedihin *et al*., 2013b).

A wide range of *T. gondii* infection has been reported among different groups of people in the country (Table 2), where the seroprevalence range from 8.2% to 96.7% (Negash, 2008; Gebremedhin *et al*., 2013b). Nevertheless, there is no information on congenital toxoplasmosis in childrens. Higher seropositivity to *T. gondii* antibody in Ethiopia is commonly associated with the consumption of raw or undercooked meat that facilitates higher transmission rate of the parasite as indicated by different authors (Mengesha *et al*., 1984; Gebre-Exabier *et al*., 1993).

Table 1: Summary of selected *T. gondii* seroprevalence in animals from some parts of Ethiopia

| Study sites | Study year | Species | Tests used | Number tested | Prevalence (%) | Reference |
|---|---|---|---|---|---|---|
| Addis Ababa | 1985-1987 | Sheep | IHA | 899 | 22.9 | Bekele and Kasali, |
| and Debrebirhan | 1985-1987 | Goat | IHA | 753 | 11.6 | 1989 |
| | 1985-1987 | Cattle | IHA | 785 | 6.6 | |
| NG* | NG* | Sheep | IHAT | 94 | 25.6 | Deconinick *et al*., 1996 |
| Debrebirhan | 2000-2001 | Sheep | MDAT | 375 | 34 | Demissie and Tilahun, |
| | | Goat | IHA | 133 | 35 | 2002 |
| Nazareth | 1999 | Sheep | MDAT | 116 | 52.6 | Negash *et al*., 2004 |
| | | Goat | MAT | 58 | 24 | |
| South Omo and East Shewa zone | 2005-2006 | Goat | MAT | 641 | 74.8 | Teshale *et al*., 2007 |
| Central Ethiopia | 2010-2011 | Goat | ELISA | 927 | 19.7 | Zewdu *et al*., 2013 |
| | 2010-2011 | Sheep | ELISA | 1130 | 31.59 | Gebremedhin *et al*., 2013a |
| Central Ethiopia | 2011-2012 | Goat | DAT | 323 | 15.48 | Gebremedhin *et al*., 2014a |
| | | Sheep | DAT | 305 | 17.68 | |

(Compiled from published articles) NG*- Not Given

Table 2: Summary of selected *T. gondii* seroprevalence in humans from some parts of Ethiopia

| Study Year | Population examined | Test used | Number Tested | Prevalence (%) | Reference |
|---|---|---|---|---|---|
| | Filariasis patients | DT | 52 | 50.0 | De Roever-Bonner, 1980 |
| | Lymphadenopathy patients | IHAT | 61 | 8.2 | Tsega and Belehu, 1980 |
| 1981–1982 | General population | ELISA | 614 | 42.0 | Mengesha *et al*., 1984 |
| | Males aged 13–16 years | ELISA | 20 | 95.0 | Lopez *et al*., 1992 |
| | Pregnant women aged 17–32 | ELISA | 94 | 20.2 | Eshete *et al*., 1993 |
| 1990–1991 | Six geographic area | ELISA | 1016 | 74.4 | Gebre-Exabier *et al*., 1993 |
| 1995–1996 | Factory workers, aged 18–45 years, | DT | 170 | 80.0 | Woldemichaiel *et al*., 1998 |
| | HIV study, Addis Ababa | LAT | | 77.6 | |
| | Patients aged 15–49 years | ELISA | 456 | 95.1 | Tedla *et al*., 2011 |
| | People aged 15 days–65 years adama hospital | MAT | 65 | 60.0 | Negash *et al*., 2008 |
| 2007 | Hospitalized patients Addis Ababa | ELISA | 330 | 93.3 | Shimellis *et al*., 2009 |
| 2011 | Pregnant women | ELISA | 201 | 83.6 | Zemene *et al*., 2012 |
| 2011 | Pregnant women | ELISA | 213 | 81.4 | Gebremedhin *et al*., 2013b |
| | HIV infected and non infected people | IgG ELISA | 103 | 87.4 | Walle *et al*., 2013 |
| | | IgM ELISA | | 10.7 | |
| NG* | Adis Ababa abattoir workers | ELISA | 279 | 96.77 | Yimer *et al*., 2005 |

Adopted from (Dubey *et al*., 2012)

## 2.9. Control and Prevention of Toxoplasmosis

The Control of toxoplasmosis in animals, including human being is a great problem because of the difficulties in proper diagnosis at the early stage of the disease. Particularly in countries like Ethiopia where extensive management of domestic ruminants widely practiced is more complicated by the frequent exposure to source of infection and lack of the information about the disease. However, different authors recommending various approaches to control *T. gondii* infection in animals and humans (Dubey, 1996a).

### *2.9.1. Control of toxoplasmosis in animals*

**Management** – felines are definitive hosts shading infective oocyst with their feces and caused environmental contamination. Thus feeds and water should be kept free from cats contact. On the other hand reduce environmental contamination by keeping healthy and small population of cats will minimize shading of oocyst to the environment (Buxton, 1998).

**Treatment -** Sulfadiazine 15–60mg/kg divided in to 4 doses and pyrimethamine 1mg/kg.q. 24 hrs×3 day, then 0.5–1.0 mg/kg 24 hrs until oocyst shedding stops in cats or pyrimethamine (1 mg/kg) and sulfadiazine (100 mg/kg) is given daily orally for several weeks. On farms control is more difficult but was possible, animal feed stuffs should be covered to exclude access by cats and insects. Monensin and decoquinate have also been administered to ewes in mid-pregnancy in attempts to control abortion due to toxoplasmosis (Urquhart *et al*., 1996).

**Vaccination-** For prevention of toxoplasmosis in sheep a live vaccine available this is consisting of tachizoites attenuated by repeated passage in mice. The vaccine consists of a strain (S48) of *T. gondii* originally isolated from an aborted lamb in New Zealand (Buxton, 1993; Reddy, 2006). The strain used has lost the capacity to form tissue cysts and therefore the potential to form oocysts in cats. Currently used a live vaccine (toxovacx1) is commercially marketed in UK, France and New Zealand for reducing losses to the sheep industry from congenital toxoplasmosis (Buxton and Innes, 1995). It is usually recommended to vaccine the whole flock initially and there after only annual vaccination of replacements. The vaccine consists of $10^4$–$10^6$ tachizoites and it is given 2ml as a single dose subcutaneously at least three weeks prior to tupping, it produces a protective immunity for at least 18 months (Buxton and Innes, 1995). At present,

there is no vaccine to prevent toxoplasmosis in humans (Dubey, 2010). Currently most of the research works focused on vaccine candidates that can induce protective Th1 and humoral responses, both systemic and at the intestinal mucosa level with the hope to mimic the lifelong immunity conferred by natural infection. Vaccine approaches have included use of purified or recombinant *T gondii* surface antigens, live attenuated or mutant strains of the parasite, 11 or DNA with plasmids encoding colony-stimulating factors (Peterson *et al*., 1998; Ismael *et al*., 2003).

*2.9.2. Control of toxoplasmsis in humans*

**Control in Immunocompetent people -**Immunocompetent adults and children with toxoplasmic lymphadenitis are usually not treated unless symptoms are severe or persistent. However, in rare patients with symptoms like myocarditis, encephalitis, a sepsis syndrome with shock, and hepatitis, treatment should be given with pyrimethamine (100-mg loading dose and 25–50 mg/d) and sulfadiazine or trisulfapyrimidines (4–8 g/d) for 4–6 weeks. Folinic acid (5–10 mg/d) should also be given (Weiss and Kim, 2007).

**Control in Immunocompromised patients -** John and Petri (2006) recommended for immunocompromised patients a treatment with combination of pyrimethamine given at 25-100 mg daily and trisulfapyrimidines given at 2-6 g daily for a month. This course of treatment inhibits dihydrofolate reductase in *T. gondii,* which prevents synthesis of DNA and protein. He also indicated the effectiveness of clindamycin in treating *T. gondii* infection in HIV AIDS patients and spiramycine in acutely infected pregnant woman. Pal (2007) suggested a treatment with pyrimethamine combined with triple sulfonamides (sulfadiazine, sulfamerazine, sulfamethazine) and folic acid is used in chemotherapy of man. Trimethoprim/sulfamethoxazole appears to be equivalent to pyrimethamine/sulfadiazine to treat patients with HIV AIDS (Torre *et al*., 1998). Immunocompromised patients need lifetime therapy with pyrimethamine plus sulfadiazine and leucovorine (Plaut *et al*., 1996). Flucozanole is used as alternative to patients intolerant to sulfanomides and suffered from hematological toxicisity caused by pyrimethamine (Martins-Duarte *et al*., 2010).

# 3. MATERIALS AND METHODS

## 3.1 Study Area

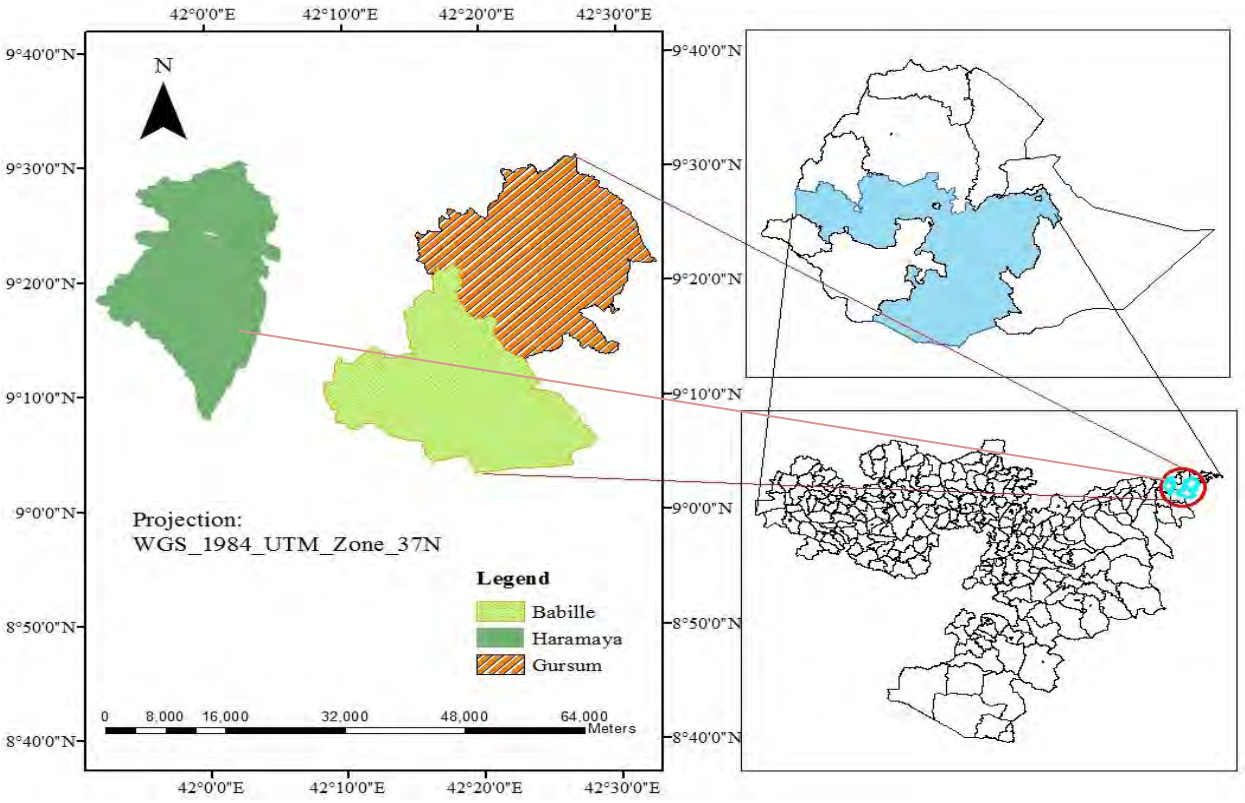


Figure 3: Map of study areas

The study was conducted in three purposively selected districts of East Hararghe Zone of Oromia Region, Ethiopia. The districts were found at altitude range of 950 to 2950 meter above sea level (masl). Accordingly, Gursum district is located 9°7′–32°N latitude to 42°17– 42°38′E longitude with an altitude ranging from 1200 to 2950 masl; Babile district is located 8°9′– 9°23′N latitude to 41°16′– 41°46′E longitude and an altitude ranging from 950 to 2000 masl; and Haramaya district is found at 9°9′– 9°32′N latitude to 41°50′– 42°05′E longitude with an altitude ranging from 1600 to 2140 masl. Even though the districts are predominantly inhabited by the Oromo communities, people belonging to other tribes are also living in the area. Crop production and livestock rearing are the main economic activities in rural areas, whereas in urban areas the major activity practiced is small scale trading. Sorghum, maize, groundnut, chat and wheat are the main crops produced in the area (MoAEH, 2014).

### 3.2 Study Design, Animal Population and Sample size

A cross-sectional seroepidemiological study was conducted from July 2011 to September 2013 to determine the seroprevalence of *T. gondii* and risk factors for the infection in domestic ruminants (sheeps, goats, cattle and camels) of both sexes. Animals included in this study were zebu breeds allowed to graze in grazing fields during day time. The required sample size was determined using the formula given by Thrusfield (2005) for random sampling. The sample size was calculated based on an expected prevalence rate of 56% and 25.9% for sheep and goats, respectively (Negash *et al*., 2004). Regarding cattle and camel, the sample size was determined at an expected prevalence of 50%, as there were no data concerning the occurrence of this infection in the country, with 95% confidence interval and 5% absolute precision. Subsequently, the measured sample size allocated proportionally to purposively selected districts, whereas, 38 peasant associations (villages) were randomly selected from the districts including the urban areas. Sheep, goats, cattle and camels found in these settlements were used as the study population. Out of which individual animals/ sample units were identified systematically at their night rest places, grazing areas and water points. Sheep, goats and cattle aged six months and above were sampled, while camels two years and above were included for this study.

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

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where,

n = desired sample size,

$P_{exp}$ = expected prevalence, and

d = 0.05

The distribution of domestic ruminant population and the required sample size across the selected districts are presented in Table 3.

Table 3: The distribution of animal population and sample size across the study districts.

| District | Animal Population | | | | Sample size | | | |
|---|---|---|---|---|---|---|---|---|
| | Cattle | Sheep | Goat | Camel | Cattle | Sheep | Goats | Camel |
| Gursum | 79129 | 22624 | 6400 | 5449 | 159 | 112 | 118 | 131 |
| Babile | 46161 | 16470 | 21641 | 1043 | 92 | 84 | 40 | 252 |
| Haramaya | 65336 | 37083 | 74846 | - | 131 | 184 | 138 | - |
| Total | 190626 | 78177 | 160457 | 15879 | 382 | 380 | 296 | 383 |

*3.2.1 Blood samples collection and ELISA*

A total of 1360 (sheep = 332; goats = 410; cattle = 326; camel = 292) animals sera were collected during the study period. Blood (5–10 ml) was drawn from the jugular vein using plain vacutainer tubes and kept at room temperature to allow clotting for sera separation. Aliquots of sera were obtained by centrifugation at 3000 rpm for 10 min and transported under cold chain using an ice box to Bishoftu, College of Veterinary Medicine and Agriculture and stored at -20°C until tested. Then after, all sera were tested for the presence of IgG antibodies against *T. gondii* using ELISA diagnostic kit (ID VET Innovative Diagnostic, ID Screen®, and Montpellier, France) following the manufacturers rcommendation. Antigen and sera required for serological testing in each day were taken out from the cold storage and brought to room temperature before testing was undertaken. Preparation of the reagents and the ELISA test were conducted according to the protocol described by the manufacturer [AppendixII(c)] at the National Animal Health Diagnostic and Investigation Center (NAHDIC) Sebeta, Ethiopia. Positive and negative controls were included in each test and an animal was considered infected if the serum was presented an OD% $\geq$ 50% with ELISA.

*3.2.2 Questionnaire survey*

A closed-ended questionnaire was administered to hundred consenting animal owners whose herds and flocks of animals (n = 351) were included in the study. The sample size was determined using the formula given by Arsham (2002). It was designed to collect information on management practices and factors attributed *T. gondii* infection in ruminants, including, cat ownership at home, the presence of feral cats in the vicinity, animal houses, feed storage, grazing

types, source of water etc. Additionally, data on animal characteristics (age, sex, breed, herd size, status and stage of pregnancy) to all species were recorded at the time of blood collection.

### 3.3. Bioassay in mice

Sera of animals presented for slaughter (n = 110) were tested for seropositivity of *T. gondii* using Toxo-latex test (Toxo-Latex SA, Montgal-Barcelona, Spain) following the procedure indicated by the manufacturer [Appendix II(d)]. Then, the hearts of 34 latex positive sheep (n = 18) and goats (n = 12) brought from East Hararghe Zone and slaughtered at Hashim abattoir at Bishoftu, also cattle (n = 3) and camel (n = 1) slaughtered at Gursum and Babile district slabs were processed and tested in bioassay for isolation and quantification of *T. gondii* cysts in mice, following the methods described by Dubey (1998b). Bioassays were performed within 1–3 days after slaughter in case of sheep and goats and within 5–6 days in cattle and camels. To avoid contamination among samples, the samples were processed separately. In brief, approximately 50 g of heart tissue was cut in small pieces and homogenized in a blender for 30 sec. Followed by suspension in 125 ml of saline solution. After homogenization digested by 250 ml of a pepsin solution (i.e. porcine stomach pepsin, biological activity 5.2 g, 10.0 g NaCl, 14 ml HCl and distilled water to make 1000 ml, PH 1.10-1.20). Each digested sample was inoculated intraperitoneally (1 ml/mouse) into a group of five female *T. gondii* seronegative Swiss albino mice (weighing 20–25 g) of about thirty days of age [Appendix II (e)]. The inoculated mice were observed for illness daily for 60 days and information on number of survivors, dead, day of death, symptoms, and weight (initial and final) were recorded. The mice were fed with pelleted feed and municipal chlorinated water was provided ad-libitum. *T. gondii* isolate was considered as virulent when 100% mortality of mouse was observed within four weeks of infection (Pena *et al*., 2006; Pena *et al*., 2008).

Blood samples were taken from surviving mice after anesthetizing with di-ethyl ether (Biolab laboratories ltd, Israel)., and the mice were killed by atlantoaxial dislocation to remove the brain, after 47–60 days post inoculation. To demonstrate *T. gondii*, the brain of the mice were homogenized separately with mortar and pestle and mixed with 1ml PBS. Brain smears were made from each mouse and examined for tissue cysts by microscopy (Dubey and Beattie, 1988). The numbers of cysts in three aliquots of 10 µl each were counted under microscope with a

10×and40x objective lens, summed and converted to a count per mouse brain (Fritz *et al*., 2012). A bioassay was considered positive if at least one *T. gondii* cyst was detected in any of the five inoculated mice or any of the mice sera reacting positive by latex.

### 3.4. Study Design, Human Population and sample size

The study was also conducted in Haramaya, Babile and Gursum districts of East Hararghe zone of Oromia region, Ethiopia from April 2013 to September 2013 alongside the study on animals. The study population consisted of individuals from rural and urban areas, visiting the selected health centers, during the study period. The health centers were, well organized to give different health services to many people coming for medical support. Thus, the active performance of the health services enables us to obtain the required sample size for our study. The study subjects comprised both genders, whose age is ranging from 5 to 70 years.

Sample size was determined using the method recommended by Thrusfield (2005) for random sampling. The requird sample size on the expected prevalence rate of 74.4% (Gebre-Exabier *et al,* 1993), desired absolute precision ±0.05 and 95% confiedence level gave the desired sample size of 296. This was inflated by 20% and increased the sample size to 354. The study population consisted of people from rural and urban areas, visiting the selected health centers during the study period. The study subjects were identified by systematic random method among different demographic groups comprised both genders, whose age is ranging from 5 to 70 years. Thus, the calculated sample size was allocated proportionally to the selected health centers at district bases.

#### *3.4.1. Questionnaire survey*

A questionnaire was prepared to collect data from the study participants, whose blood was drawn for serological test. Data were collected on socio-demographic characteristics (age, sex, residence etc) and factors attributing human *T. gondii* infection, which comprised variables including, consumption of raw meat, raw vegetables, raw milk, cat holding at home, presence of feral cats, residence area, source of drinking water, status of pregnancy and stage of pregnancy, etc. to identified the possible risk factors and the association with *T. gondii* infection in humans.

*3.4.2. Serological survey*

Approximately 4 ml of venous blood was collected aseptically from 354 individuals consented to participate in the study of human toxoplasmosis. Blood samples were kept under room temperature to allow clotting, then the serum separated by centrifugation at 3000 rpm for 10 minutes. Separated serum kept under -20$^{o}$C until test for *T. gondii* conducted.

**a) Direct Agglutination Test (DAT)**

Direct agglutination test (DAT) employs intact tachizoites as antigen for detecting the IgG antibodies developed against *T. gondii*. Hence, the antibodies are determined by using a commercially available kit (Toxo-Screen DA. Biomerieux SA, Leon, France) with a relative sensitivity and specificity of 96.22% and 98.80%, respectively. The test result measured as follows: the serum considered free of IgG antibodies, if the test is negative at 1/40 and 1/4000 dilution (or border line at 1/40 and negative at 1/4000), the serum contains IgG antibodies, if the test is positive at the 1/40 dilution and negative, borderline or positive at the 1/4000 dilution [Appendix II (b)].

**b) Immunosorbant Agglutination Assay (ISAGA)**

The test conducted to detect toxoplasma IgM antibodies using a commercially available kit (Toxo-screen ISAGA. Biomerieux SA, Leon, France). The test principle lies on the human IgM antibodies in the serum sample bind with the anti-human IgM monoclonal antibodies in the strip wells. Specific *Toxoplasma* IgM is then revealed by addition of Toxoplasma organism. The test result considered negative, when sedimentation of *Toxoplasma* occurs, if the reaction is positive, the Toxoplasma are agglutinated in a MAT. The ISAGA index was interpreted as follows: 0 to 5, negative reaction; 6 to 8, borderline; 9 to 12, positive reaction [Appendix II(a)].

**3.5 Ethical consideration**

Ethical issues were addressed as per the international guidelines for biomedical research.The research work activities related to animals was approved by the ethical clearance committee for experimental animals of the College of Veterinary Medicine and Agriculture, Addis Ababa University (Ref.No.VM/ERC/004/02/04/2013). For human related research work activities,

Ethical approval was obtained from Ethical Review Board of Oromia Regional State Health Bureau (Ref.No. BEFO/HBTFH/1-8/2297). Subsquently, study subjects were informed about the aim of the study. Then, written informed consent was obtained from study subjects. Individuals who were not capable of doing so proxy consent from the guardians were obtained.

### 3.6. Data Management and Analysis

The data generated were stored in Microsoft excel spreadsheet 2007 (Microsoft Corporation) and analyzed using STATA version 11.0 for MA Windows (STATA Corp. College Station, TX. USA 2009). The collected data were categorized to ease the analysis. Accordingly, sheep and goats were classified by age as young ($\leq$ 1 year) and adults (> 1 year), cattle as calf ($\leq$ 1 year), young (> 1-5 years), adults (> 5 years) and camels as young ($\leq$ 4 years) and adults (> 4 years). Flock/herd size for sheep, goats and cattle was considered as small (< 10 animals) or large ($\geq$ 10 animals), for camels as small ($\leq$ 34 animals) or large (> 34 animals). Type of housing for sheep, goats and cattle categorized as pen with total confinement, fence/enclosure with access to outside. Water is classified for all species as mixed (river, stream, pond, well), pond (stagnant water) and piped water (except camels). Likewise, human data were classified by age as kids and teenagers (5–18 years) youth (> 18–35 years) and adult (> 35–70 years), residence (urban, rural), cats holding at home (yes, no), meat and vegetable consumption habit (raw, cooked) meat source (beef, shoats meat and mixed), water source (stream, tap water and mixed), women pregnancy status (yes, no).

Animals and human seroprevalence and the association of risk factors with *T. gondii* sero-positivity as independent categorical variables were analyzed by the chi-square test. Logistic regression analysis was performed to measure the strength of association between risk factors and *T. gondii* seropositivity. Further non collinear variables and those presented $P \leq 0.20$ at 95% CI level were entered to multivariate logistic regression model. To ease the analysis the first category was taken as a reference group. For statistical inference, the level of significance was set as $P < 0.05$.

# 4. RESULTS

## 4.1. Seroprevalence and Risk factor Analysis in Animals

### *4.1.1. Animal level Seropevalence and associated risk factors*

In the present study a total of 1360 animal sera were examined by ELISA. Thus, the overall seropositivity of *T. gondii* infection of domestic ruminants was 22.2% (302/1360; 95% CI: 20.0 – 24.5). Accordingly, at animal level 33.7% (112/332; 95% CI: 28.66–39.10), 27.6% (113/410; 95% CI: 23.29–32.16), 10.7% (35/326; 95% CI: 7.59–14.61) and 14.4% (42/292; 95% CI: 10.57–18.94) in sheep, goats, cattle and camels, respectively. The highest seroprevalence was observed in sheep (33.73%) and the lowest in cattle (10.74%). Figure 4 displays the distribution of *T. gondii* seropositivity among domestic ruminants in the three districts surveyed. Among the 38 farm areas ("kebeles") where the study animals were sampled, *T. gondii* infection was detected in 32 (84.21%).

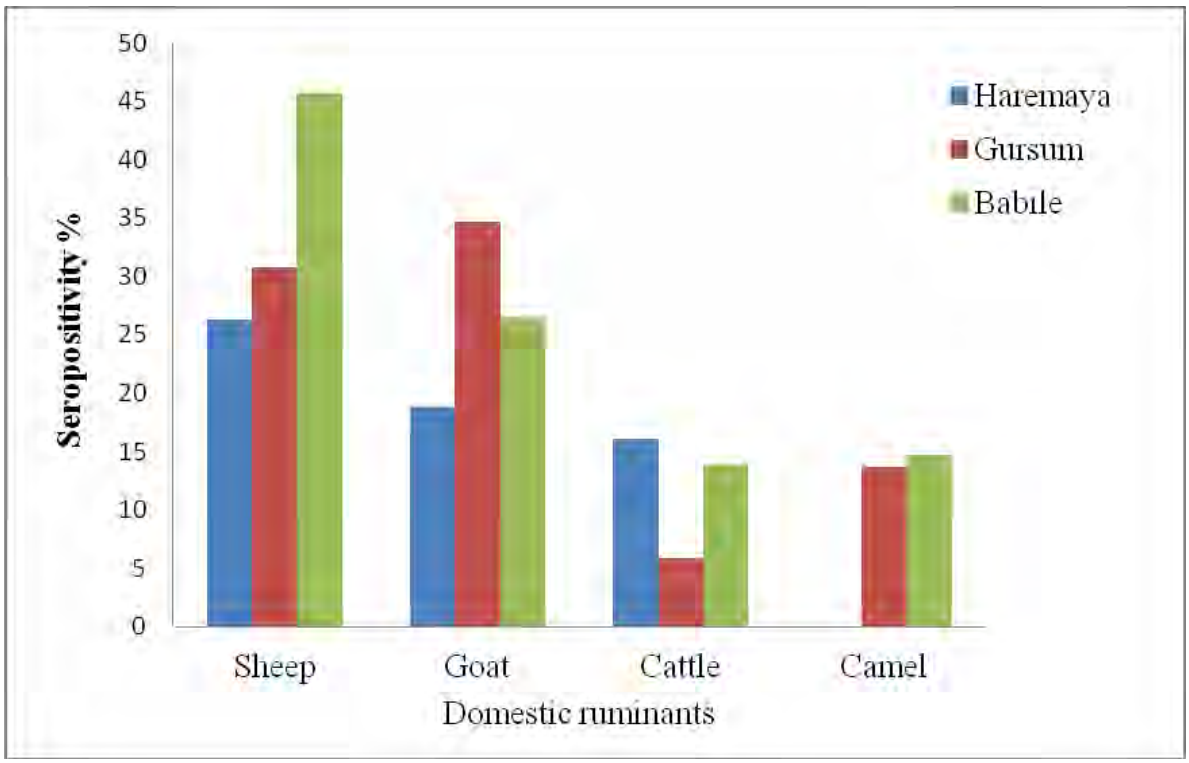


Figure 4: Distribution of *T. gondii* among domestic ruminants in the selected study districts

Univariable logistic regression analysis for domestic ruminant species have shown that goats (OR = 0.75, 95% CI: 0.55–1.02, $P = 0.069$) had the same risk level as that of sheep whereas cattle (OR = 0.24, 95% CI: 0.16–0.36, $P < 0.001$) and camels (OR = 0.33, 95% CI: 0.22–0.49, $P < 0.001$) were at low risk of infection.

Multivariable logistic regression analysis showed that sex of the animal and water source were risk factors for increased seroprevalence in sheep. A higher risk of *T. gondii* infection occured in females (OR = 2.63, 95% CI: 1.18–5.88, P = 0.019) than in males, in those given pipe water (OR = 9.57, 95% CI: 5.00–18.33, $P < 0.001$) and pond water (OR = 4.25, 95% CI: 2.15–8.38, $P < 0.001$) compared to those drunk from other water sources (Table 4). But, the variables district, age and cats contact were insignificant ($P > 0.05$). Nonetheless, a relatively higher seroprevalence was observed in sheep from Babile district (45.7%) than in Gursum (30.8%) and Haramaya (26.3%), in adults (35.2%) than in young (23.1%), and in those having contact with cats (40.0%) than in those not in contact (30.0%).

District, age and water source were found as risk factors for *T. gondii* infection in goats on multivariable logistic analysis. An increased risk of infection occurred in adult goats (OR = 3.45, 95% CI: 1.34–8.90, P = 0.010) compared to young, in those given pipe water (OR = 11.61, 95% CI: 4.35–30.95, $P < 0.001$) and pond water (OR = 6.03, 95% CI: 2.42–15.05, $P < 0.001$) than those that drunk from water sources other than pond and pipe water. In contrast, goats in Babile district (OR = 0.15, 95% CI = 0.05–0.48; $P <0.001$) were found to be a lower risk of infection (Table 5). Variables sex, breed and flock size were not significant ($P > 0.05$). However, a raised seroprevalence was obtained in females (30.6%) compared to males (17.2%) and in smaller flocks (29.3%) compared to larger (20.9%).

Multivariable logistic regression analysis indicated that district, herd size of cattle and water source were risk factors for *T. gondii* infection. Using pond water (OR = 5.60, 95% CI: 2.12–14.78, $P < 0.001$) and pipe water (OR = 10.68, 95% CI: 2.23–51.22, P = 0.003) had significantly increased the risk of acquiring *T. gondii* infection compared to using water from other sources, while living in Gursum district (OR = 0.19, 95% CI: 0.06–0.59, P = 0.004) and belonging to large herd size (OR = 0.35, 95% CI: 0.13–0.97, P = 0.044) were associated with lower risk (Table

6). Among the variables included in the statistical analysis, cats contact and house type were not significant ($P > 0.05$).

The sole risk factor identified in camels using the multivariable logistic regression analysis was age. Adult camels (OR = 2.49; 95% CI: 1.14–5.45, P = 0.022) showed an increased risk for *T. gondii* infection than young camels (Table 7). Although not significant ($P > 0.05$), *T. gondii* infection in camels having contact with cats (21.7%) is higher than those not having contact (13.0%).

Table 4: Analysis of risk factors related to *T. gondii* seropositivity in sheep at animal level

| Variables | Category | Number Tested | Positive (%) | Crude OR (95%CI) | Adjusted OR (95%CI) | *P*-value |
|---|---|---|---|---|---|---|
| District | Haremaya | 95 | 25 (26.3) | 1.00 (ref.) | 1.00 (ref.) | |
| | Gursum | 143 | 44 (30.8) | 1.24 (0.70–2.22) | 0.69 (0.35–1.1.34) | 0.270 |
| | Babile | 94 | 43 (45.7) | 2.36 (1.28–4.35) | 0.95 (0.46–1.94) | 0.881 |
| Environment | Rural | 253 | 58 (22.9) | 1.00 (ref.) | - | |
| | Urban | 79 | 54 (68.4) | 7.26 (4.16–12.68) | - | - |
| Sex | Male | 68 | 11 (16.2) | 1.00 (ref.) | 1.00 (ref.) | - |
| | Female | 264 | 101 (38.3) | 3.21 (1.61–6.41) | 2.63 (1.18–5.88) | 0.019* |
| Age | Lambs and Yearlings | 39 | 9 (23.1) | 1.00 (ref.) | 1.00 (ref.) | |
| | Adults | 293 | 103 (35.2) | 1.81 (0.83–3.95) | 1.15 (0.45–2.91) | 0.772 |
| Flock size | Small | 270 | 89 (33.0) | 1.00 (ref.) | - | |
| | Large | 62 | 23 (37.1) | 1.20 (0.68–2.13) | - | - |
| Cats contact | No | 207 | 62 (30.0) | 1.00 (ref.) | 1.00 (ref.) | |
| | Yes | 125 | 50 (40.0) | 1.56 (0.98–2.48) | 1.28 (0.74–2.20) | 0.371 |
| Water source | Others† | 198 | 34 (17.2) | 1.00 (ref.) | 1.00 (ref.) | |
| | Pond‡ | 55 | 24 (43.6) | 3.73 (1.95–7.14) | 4.25 (2.15–8.38) | <0.001* |
| | Pipe water | 79 | 54 (68.4) | 10.42 (5.71–19.00) | 9.57 (5.00–18.33) | <0.001* |
| House type | Barn | 24 | 3 (12.5) | 1.00 (ref.) | - | |
| | Both | 207 | 48 (23.2) | 2.11 (0.60–7.39) | - | - |
| | Fence | 101 | 61 (60.4) | 10.68 (2.99–38.15) | - | - |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

Table 5**:** Analysis of risk factors related to *T. gondii* seropositivity in goats at animal level

| Variables | Category | Number Tested | Positive (%) | Crude OR (95%CI) | Adjusted OR (95%CI) | *P*-value |
|---|---|---|---|---|---|---|
| District | Haremaya | 121 | 22 (18.2) | 1.00 (ref.) | 1.00 (ref.) | |
| | Gursum | 176 | 61 (34.7) | 2.39 (1.37–4.16) | 0.52 (0.20–1.36) | 0.185 |
| | Babile | 113 | 30 (26.6) | 1.63 (0.87–3.03) | 0.15 (0.05–0.48) | 0.001* |
| Environment | Rural | 340 | 79 (23.2) | 1.00 (ref.) | - | |
| | Urban | 70 | 34 (48.6) | 3.12 (1.83–5.31) | - | - |
| Sex | Male | 93 | 16(17.2) | 1.00 (ref.) | 1.00 (ref.) | - |
| | Female | 317 | 97 (30.6) | 2.12 (1.18–3.82) | 1.65 (0.86–3.18) | 0.134 |
| Age | Kids and yearlings | 63 | 6 (9.5) | 1.00 (ref.) | 1.00 (ref.) | |
| | Adults | 347 | 107 (30.8) | 4.24 (1.77–10.12) | 3.45 (1.34–8.90) | 0.010* |
| Breed | Hararghe highland | 29 | 5 (17.2) | 1.00 (ref.) | 1.00 (ref.) | |
| | Long ear Somali | 349 | 95 (27.2) | 1.80 (0.67–4.84) | 1.10 (0.36–3.39) | 0.863 |
| | Undetermined | 32 | 13 (40.6) | 3.28 (0.99–10.84) | 2.77 (0.68–11.31) | 0.155 |
| Flock size | Small | 324 | 95 (29.3) | 1.00 (ref.) | 1.00 (ref.) | |
| | Large | 86 | 18 (20.9) | 0.64 (0.36–1.13) | 0.85 (0.45–1.60) | 0.607 |
| Cats contact | No | 270 | 70 (25.9) | 1.00 (ref.) | - | |
| | Yes | 140 | 43 (30.7) | 1.27 (0.81–1.99) | - | - |
| Water source | Others† | 188 | 29 (15.4) | 1.00 (ref.) | 1.00 (ref.) | |
| | Pond‡ | 152 | 50 (32.9) | 2.69 (1.60–4.52) | 6.03 (2.42–15.05) | <0.001* |
| | Pipe water | 70 | 34 (48.6) | 5.18 (2.80–9.56) | 11.61 (4.35–30.95) | <0.001* |
| House type | Barn | 27 | 5 (18.5) | 1.00 (ref.) | - | |
| | Both | 292 | 73 (25.0) | 1.47 (0.54–4.01) | - | - |
| | Fence | 91 | 35 (38.5) | 2.75 (0.95–7.93) | - | - |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

Table 6: Analysis of risk factors related to *T. gondii* seropositivity in cattle at animal level

| Variable | Category | Number Tested | Prevalence (%) | Crude OR (95% CI) | Adjusted OR (95% CI) | *P*-value |
|---|---|---|---|---|---|---|
| District | Haremaya | 87 | 14 (16.1) | 1.00 (ref.) | 1.00 (ref.) | |
| | Gursum | 153 | 9 (5.9) | 0.33 (0.13–0.79) | 0.19 (0.06–0.59) | 0.004* |
| | Babile | 86 | 12 (14.0) | 0.85 (0.37–1.95) | 1.60 (0.49–5.16) | 0.434 |
| Environment | Rural | 303 | 30 (9.9) | 1.00 (ref.) | - | |
| | Urban | 23 | 5 (21.7) | 2.53 (0.88–7.30) | - | - |
| Sex | Male | 97 | 11 (11.3) | 1.00 (ref.) | - | |
| | Female | 229 | 24 (10.5) | 0.92 (0.43–1.95) | - | - |
| Age | Calves and Yearlings | 34 | 3 (8.8) | 1.00 (ref.) | - | |
| | Young | 186 | 19 (10.2) | 1.18 (0.33–4.21) | - | - |
| | Adults | 106 | 13 (12.3) | 1.44 (0.39–5.40) | - | - |
| Herd size | Small | 218 | 27 (12.4) | 1.00 (ref.) | 1.00 (ref.) | |
| | Large | 108 | 8 (7.4) | 0.57 (0.25–1.29) | 0.35 (0.13–0.97) | 0.044* |
| Cats contact | No | 223 | 19 (8.5) | 1.00 (ref.) | 1.00 (ref.) | |
| | Yes | 103 | 16 (15.5) | 1.97 (0.97–4.02) | 2.23 (0.86–5.77) | 0.097 |
| Water source | Others† | 234 | 14 (6.0) | 1.00 (ref.) | 1.00 (ref.) | |
| | Pond‡ | 69 | 16 (23.2) | 4.75 (2.18–10.32) | 5.60 (2.12–14.78) | <0.001* |
| | Pipe water | 23 | 5 (21.7) | 4.37 (1.41–13.49) | 10.68 (2.23–51.22) | 0.003* |
| House type | Barn | 29 | 5 (17.2) | 1.00 (ref.) | 1.00 (ref.) | |
| | Both | 246 | 23 (9.4) | 0.50 (0.17–1.42) | 0.23 (0.05–0.99) | 0.049 |
| | Fence | 51 | 7 (13.7) | 0.76 (0.22–2.67) | 0.42 (0.08–2.32) | 0.318 |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

Table 7: Analysis of risk factors related to *T. gondii* seropositivity in camels at animal level

| Variables | Category | Number of herds | Positive (%) | Crude OR (95%CI) | Adjusted OR (95%CI) | *P*-value |
|---|---|---|---|---|---|---|
| District | Gursum | 95 | 13 (13.7) | 1.00 (ref.) | - | |
| | Babile | 197 | 29 (14.7) | 1.09 (0.54–2.20) | - | - |
| Sex | Male | 73 | 11 (15.1) | 1.00 (ref.) | - | |
| | Female | 219 | 31 (14.2) | 0.93 (0.44–1.96) | - | - |
| Age | Young | 110 | 9 (8.2) | 1.00 (ref.) | 1.00 (ref.) | |
| | Adults | 182 | 33 (18.1) | 2.49 (1.14–5.42) | 2.49 (1.14–5.45) | 0.022* |
| Herd size | Small | 203 | 30 (14.8) | 1.00 (ref.) | - | |
| | Large | 89 | 12 (13.5) | 0.90 (0.44–1.85) | - | - |
| Cat contact | No | 246 | 32 (13.0) | 1.00 (ref.) | 1.00 (ref.) | |
| | Yes | 46 | 10 (21.7) | 1.86 (0.84–4.10) | 1.87 (0.84–4.18) | 0.127 |
| Water source | Others† | 215 | 30 (14.0) | 1.00 (ref.) | - | |
| | Pond‡ | 77 | 12 (15.6) | 1.14 (0.55–2.35) | - | - |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

*4.1.2. Herd/Flock level Seropevalence and associated risk factors*

The overall seropositivity of *T. gondii* infection among domestic ruminants at herd and flock level was 49.3% (173/351; 95% CI: 43.7–54.7). Accordingly, the respective seroprevalence was 60.8% (62/102; 95% CI: 50.62–70.31), 55.8% (67/120; 95% CI: 46.48–64.89), 23.2% (19/82; 95% CI: 14.56–33.80) and53.2% (25/47; 95% CI: 38.08%–67.89%) for sheep, goats, cattle and camels.

At herd/flock level and water source were found to be the mere risk factors for *T. gondii* infection in sheep, goats and cattle on multivariable logistic regression analysis. A higher risk of *T. gondii* infection was observed in sheep flocks (OR = 5.32, 95% CI: 1.27–22.31, P = 0.022) and goat flocks (OR = 4.13, 95% CI: 1.33–12.88, P = 0.014) that drunk pipe water compared with those that used other water sources (Table 8 and 9). Unlike, in cattle high risk of infection was detected in herds that drunk pond water (OR = 8.20, 95% CI: 1.71–17.77, P = 0.004) than those that drink

from other water sources (Table 10). However, in camels none of the variables showed association with *T. gondii* seropositivity at herd level (Table 11).

Table 8: Analysis of risk factors related to *T. gondii* seropositivity in sheep at flock level

| Variables | Category | Flocks Tested | Positive (%) | Crude OR (95%CI) | Adjusted OR (95%CI) | *P*-value |
|---|---|---|---|---|---|---|
| District | Haremaya | 32 | 18 (56.3) | 1.00 (ref.) | 1.00 (ref.) | |
| | Gursum | 39 | 19 (48.7) | 0.74 (0.29–1.89) | 0.51 (0.19–1.42) | 0.202 |
| | Babile | 31 | 25 (80.7) | 3.24 (1.04–10.05) | 2.00 (0.58–6.94) | 0.272 |
| Environment | Rural | 79 | 42 (53.2) | 1.00 (ref.) | - | |
| | Urban | 23 | 20 (87.0) | 5.87 (1.61–21.37) | - | - |
| Flock size | Small | 92 | 55 (59.8) | 1.00 (ref.) | - | |
| | Large | 10 | 7 (70.0) | 1.57 (0.38–6.46) | - | - |
| Cats contact | No | 67 | 37 (55.2) | 1.00 (ref.) | 1.00 (ref.) | |
| | Yes | 35 | 25 (71.4) | 2.03 (0.84–4.87) | 1.89 (0.72–4.98) | 0.197 |
| Water source | Others† | 55 | 26 (47.3) | 1.00 (ref.) | 1.00 (ref.) | |
| | Pond‡ | 24 | 16 (66.7) | 2.23 (0.82–6.07) | 2.80 (0.97–8.09) | 0.057 |
| | Pipe water | 23 | 20 (87.0) | 7.44 (1.98–27.94) | 5.32 (1.27–22.31) | 0.022* |
| House type | Barn | 7 | 3 (42.9) | 1.00 (ref.) | - | |
| | Both | 62 | 33 (53.2) | 1.52 (0.31-7.35) | - | - |
| | Fence | 33 | 26 (78.8) | 4.95 (0.89-27.49) | - | - |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

Table 9: Analysis of risk factors related to *T. gondii* seropositivit in goats at flock level

| Risk factors | Category | Flocks Tested | Positive (%) | Crude OR (95%CI) | *P*-value |
|---|---|---|---|---|---|
| District | Haremaya | 34 | 17 (50.0) | 1.00 (ref.) | - |
| | Gursum | 50 | 30 (60.0) | 1.5 (0.62–3.61) | 0.366 |
| | Babile | 36 | 20 (55.6) | 1.25 (0.49–3.20) | 0.642 |
| Environment | Rural | 99 | 51 (51.5) | 1.00 (ref.) | |
| | Urban | 21 | 16 (76.2) | 3.01 (1.02-8.86) | 0.045 |
| Flock size | Small | 104 | 56 (53.9) | 1.00 (ref.) | |
| | Large | 16 | 11 (68.8) | 1.89 (0.61-5.81) | 0.269 |
| Breed | Hararghe hiland | 12 | 5 (41.7) | 1.00 (ref.) | |
| | Long ear somali | 94 | 53 (56.4) | 1.81 (0.54-6.12) | 0.340 |
| | Undetermined | 14 | 9 (64.3) | 2.52 (0.52-12.30) | 0.253 |
| Cats contact | No | 79 | 46 (58.2) | 1.00 (ref.) | |
| | Yes | 41 | 21 (50.2) | 0.75 (0.35–1.61) | 0.464 |
| Water source | Others† | 55 | 24 (43.6) | 1.00 (ref.) | |
| | Pond‡ | 44 | 27 (61.4) | 2.05 (0.91–4.60) | 0.081 |
| | Pipe water | 21 | 16 (76.2) | 4.13 (1.33–12.88) | 0.014* |
| House type | Barn | 10 | 5 (50.0) | 1.00 (ref.) | |
| | Both | 84 | 45 (53.6) | 1.15 (0.31–4.28) | 0.831 |
| | Fence | 26 | 17 (65.4) | 1.89 (0.43–8.29) | 0.400 |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

Table 10: Analysis of risk factors related to *T. gondii* seropositivityin cattle at herd level

| Variables | Category | Number of herds Tested | Positive (%) | Crude OR (95% CI) | Adjusted OR (95% CI) | *P*-value |
|---|---|---|---|---|---|---|
| District | Haremaya | 22 | 7 (31.8) | 1.00 (ref.) | 1.00 (ref.) | - |
| | Gursum | 37 | 6 (16.2) | 0.41 (0.12–1.45) | 0.28 (0.06–1.24) | 0.094 |
| | Babile | 23 | 6 (26.1) | 0.76 (0.21–2.75) | 1.91 (0.40–9.11) | 0.417 |
| Environment | Rural | 76 | 17 (22.4) | 1.00 (ref.) | - | - |
| | Urban | 6 | 2 (33.3) | 1.74 (0.29–10.30) | - | - |
| Herd size | Small | 63 | 16 (25.4) | 1.00 (ref.) | - | - |
| | Large | 19 | 3 (15.8) | 0.55 (0.14–2.14) | - | - |
| Cats contact | No | 56 | 12 (21.4) | 1.00 (ref.) | - | - |
| | Yes | 26 | 7 (26.9) | 1.35 (0.46–3.96) | - | - |
| Water source | Others† | 57 | 8 (14.0) | 1.00 (ref.) | 1.00 (ref.) | - |
| | Pond‡ | 19 | 9 (47.4) | 5.51 (1.71–17.77) | 8.20 (1.86–36.07) | 0.005* |
| | Pipe water | 6 | 2 (33.3) | 3.06 (0.48–19.57) | 3.33 (38.21–29.06) | 0.276 |
| House type | Barn | 7 | 3 (42.9) | 1.00 (ref.) | 1.00 (ref.) | - |
| | Both | 65 | 13 (20.0) | 0.33 (0.07–1.68) | 0.41 (0.05–3.27) | 0.404 |
| | Fence | 10 | 3 (30.0) | 0.57 (0.08–4.30) | 1.55 (0.12–20.35) | 0.738 |

†Others (river, stream water and well), ‡Pond (stagnant water), *Significant, ref.: reference

Table 11: Analysis of risk factors related to *T. gondii* seropositivity in camels at herd level

| Variables | Category | Herds Tested | Prevalence (%) | Crude OR 95%CI | *P*-value |
|---|---|---|---|---|---|
| District | Gursum | 12 | 6 (50.0) | 1.00 (ref.) | - |
| | Babile | 35 | 19 (54.3) | 1.19 (0.32-4.41) | 0.797 |
| Herd size | Small | 37 | 19 (51.4) | 1.00 (ref.) | - |
| | Large | 10 | 6 (60.0) | 1.42 (0.34-5.83) | 0.628 |
| Cat contact | No | 37 | 19 (51.4) | 1.00 (ref.) | - |
| | Yes | 10 | 6 (60.0) | 1.42 (0.34-5.88) | 0.628 |
| Water source | Others† | 30 | 17 (56.7) | 1.00 (ref.) | - |
| | Pond‡ | 17 | 8 (47.1) | 0.68 (0.21-2.25) | 0.527 |

†Others (river, stream water and well), ‡Pond (stagnant water), ref.: reference

### 4.1.3 Questionnaire Survey of Livestock Owners

The questionnaire survey revealed that domestic ruminants were kept mainly for meat and milk production, while few were used as a source of draught power. The majority of livestock owners (80.0%) graze their cattle, sheep and goats on communal pasture, while 20% of them use privately owned marginal pasture around their crop farm. On the contrary, camel herds browse solely farther from the farm in valleys and in hilly areas covered with bushes. Half of the respondents (50.0%) kept cats at home usually for heeding rats. The study showed that, livestock owners use river, well and stream (59.0%) as well as ponds (41.0%) as the main sources of water for animals. They also described the use of various types of houses such as fence (57.0%), barn and fence (27.0%) and barn (16.0%) for animals to rest. Furthermore, 60% of them described the occurrence of abortion in their herds and/or flocks. Nevertheless, all the respondents leave aborted material either on the field or throw to dogs and cats as well as other flesh eating scavengers.

### 4.2. Bioassay in Animals

In the current study,over all viable *T. gondii* tissue cysts were isolated from hearts of latex seropositive domestic ruminants 67.6% (23/34). Of which, 75.0% (9/12) and 77.8% (14/18) were goat and sheep respectively (Table 12). Viable *T. gondii* cyst was not isolated from seronegative hearts.Most of the *T. gondii* isolates 63% (21/34) were avirulant to mice, out of the 170 mice inoculated with tissue homogenates of seropositive sheep (n = 90), goats (n = 60), cattle (n = 15) and camels ( n = 5). However, two of the 12 *T. gondii* isolates from sheep samples (no. TgSp53 and no. TgSp63) killed 3 mice after two days post inoculation, while no mice inoculated with *T. gondii* isolates from goat died [Appendix I(e)].

Table 12: *T. gondii* Tissue cysts isolated from goats and sheep (all male) in study areas

| ID No of animals | Age in months | Organ for bioassay | No. of mice cyst positive/inoculated | No of dead | Day of death | Remark |
|---|---|---|---|---|---|---|
| Goat | | | | | | |
| Tg Gt1 | 6 | Heart | 4/5 | 0 | Survived | |
| TgGt2 | 6 | ″ | 4/5 | 0 | ″ | |
| TgGt7 | 12 | ″ | 5/5 | 0 | ″ | |
| TgGt41 | 13 | ″ | 3/5 | 0 | ″ | |
| TgGt16 | 12 | ″ | 3/5 | 0 | ″ | |
| TgGt26 | 13 | ″ | 1/5 | 0 | ″ | |
| TgGt21 | 14 | ″ | 4/5 | 0 | ″ | |
| TgGt36 | 13 | ″ | 3/5 | 0 | ″ | |
| TgGt42 | 14 | ″ | 1/5 | 0 | ″ | |
| Sheep | | | | | | |
| TgSp53 | 15 | Heart | 1/5 | 2 | 2 | |
| TgSp55 | 15 | ″ | 4/5 | 0 | Survived | |
| TgSp61 | 15 | ″ | 2/5 | 0 | ″ | |
| TgSp64 | 15 | ″ | 2/5 | 0 | ″ | |
| TgSp73 | 13 | ″ | 5/5 | 0 | ″ | |
| TgSp65 | 14 | ″ | 5/5 | 0 | ″ | |
| TgSp63 | 13 | ″ | 2/5 | 1 | 2 | |
| TgSp72 | 13 | ″ | 2/5 | 0 | Survived | |
| TgSp86 | 16 | ″ | 1/5 | 0 | ″ | |
| TgSp76 | 16 | ″ | 2/5 | 0 | ″ | |
| TgSp83 | 17 | ″ | 4/5 | 0 | ″ | |
| TgSp90 | 15 | ″ | 2/5 | 0 | ″ | |
| TgSp75 | 14 | ″ | 3/5 | 0 | ″ | |
| TgSp78 | 14 | ″ | 2/5 | 0 | ″ | |

### 4.3. Seroprevalence and Risk Factor Analysis in Humans

#### *4.3.1. Serprevalence*

A total of 354 human subjects participated in the study (age range 5–70 years, mean 33.04±13.36 years and median 30 years), with a higher proportion of female (53.7%) than male participants (46.3%). Approximately one-quarter (27·1%) study participants kept cats at home, Among the study subjects (26.8%) were consumed raw meat. IgG *T. gondii* seropositivity in all participants was 65.82% (233/354; 95% CI: 60.62–70.75), with 66.3% in females and 65.2% males. The over all seroprevalence of women chiled bearing age (n=167) was 74.8%. Out of which 8.9% (15/167; 95% CI: 0.05–0.14) and 65.9% (110/167; 95% CI: 0.58–0.73) were seropositive for *T. gondii* IgM and IgG, respectively, with varied distribution across the districts (Figure 5). Likewise, 33 (76.4%) and 4 (9.3%) out of the 43 pregnant women were seropositive for anti-*T. gondii*-specific IgG and IgM antibodies respectively (Table 13) with a higher IgM seropositivity in the second trimester of pregnancy (figure 6). By $\chi^2$ analysis residence, age, source of meat, cat holding at home, presence of feral cats and water source showed an association with IgG seropositivity. None of the investigated risk factors showed association with IgM seropositivity (Table 12).

Table 13: Seroprevalence of anti-*T. gondii* antibodies to host related and other variables in study districts

| | | IgG (n = 354) | | | IgM (n =167) | | |
|---|---|---|---|---|---|---|---|
| Variables | Category | Frequency (%) | Number positive (%) | *P*-Value | Frequency (%) | Number positive (%) | *P*-Value |
| Districts | Haremaya | 120 (33.9) | 70 (58.3) | 0.071 | 49 (29.3) | 4 (8.2) | 0.813 |
| | Gursum | 105 (29.7) | 70 (66.7) | | 44 (26.4) | 5 (11.4) | |
| | Babile | 129 (36.4) | 93 (65.8) | | 74 (44.3) | 6 (8.1) | |
| Residence | Rural | 284 (80.2) | 174 (61.3) | <0.001* | 133 (79.6) | 12 (9.0) | 0.971 |
| | Urban | 70 (19.8) | 59 (84.3) | | 34 (20.4) | 3 (8.8) | |
| Sex | Male | 164 (46.3) | 107 (65.2) | 0.832 | - | - | - |
| | Female | 190 (53.7) | 126 (66.3) | | - | - | - |
| Age | Kid and teenagers | 36 (10.2) | 14 (38.9) | 0.002* | 10 (6.0) | 2 (20.0) | 0.189 |
| | Youth | 206 (58.2) | 141 (68.5) | | 118 (70.7) | 12 (10.2) | |
| | Adults | 112 (31.6) | 78 (69.5) | | 39 (23.4) | 1 (2.6) | |
| Meat consumption | Cooked | 259 (73.2) | 167 (64.5) | 0.380 | 140 (83.8) | 12 (8.6) | 0.673 |
| | Raw | 95 (26.8) | 66 (69.5) | | 27 (16.2) | 3 (11.1) | |
| Source of meat | Shoats meat | 29 (8.2) | 16 (55.2) | 0.010* | 8 (4.8) | 1 (12.5) | 0.629 |
| | Mixed‡ | 300 (84.7) | 194 (64.7) | | 151 (90.4) | 14 (9.3) | |
| | Beef | 25 (7.1) | 23 (92.0) | | 8 (4.8) | 0 (0.0) | |

(*Continued*…)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Milk consumption | Boiled | 105 (29.7) | 66 (62.9) | 0.453 | 65 (38.9) | 6 (9.1) | 0.965 |
| | Both§ | 178 (50.3) | 116 (65.2) | | 64 (38.3) | 6 (9.5) | |
| | Raw | 71 (20.1) | 51 (71.8) | | 38 (22.8) | 3 (7.9) | |
| Vegetable consumption | Cooked | 146 (41.2) | 94 (64.4) | 0.633 | 83 (49.7) | 5 (6.0) | 0.18 |
| | Raw | 208 (58.8) | 139 (66.8) | | 84 (50.3) | 10 (11.9) | |
| Cat holding at home | No | 258 (72.9) | 160 (62.0) | 0.013* | 131 (78.4) | 9 (6.9) | 0.069 |
| | Yes | 96 (27.1) | 73 (76.0) | | 36 (21.6) | 6 (16.7) | |
| Presence of feral cats | No | 160 (45.2) | 95 (59.4) | 0.020* | 76 (45.5) | 5 (6.6) | 0.321 |
| | Yes | 194 (54.8) | 138 (71.1) | | 91 (54.5) | 10 (11.9) | |
| Water source | Stream | 63 (17.8) | 33 (52.4) | <0.001* | 27 (16.2) | 2 (7.4) | 0.947 |
| | Mixed‖ | 221 (62.4) | 141 (63.8) | | 106 (63.5) | 10 (9.4) | |
| | Pipe water | 70 (19.8) | 59 (84.3) | | 34 (20.4) | 3 (8.8) | |
| Status of pregnancy | No | 124 (77.0) | 77 (62.1) | 0.081 | 124 (73.1) | 11 (8.9) | 0.932 |
| | Yes | 43 (23.0) | 33 (76.4) | | 43 (25.8) | 4 (9.3) | |
| Stage of pregnancy (n=43) | $2^{nd}$ trimester | 19 (44.2) | 12 (63.2) | 0.117 | 19 (44.2) | 3 (15.8) | 0.677 |
| | $3^{rd}$ trimester | 14 (32.6) | 12 (85.7) | | 14 (32.6) | 1 (7.1) | |
| | $1^{st}$ trimester | 10 (23.2) | 9 (90.0) | | 10 (23.3) | 0 (0.00) | |

‡Shoats meat (sheep and goat), †Mixed (shoats meat and beef), §Both (boiled and raw), ‖Mixed (stream and well water), *Significant

ref.: reference

Table 14: Seroprevalence of anti-*T. gondii* antibodies in pregnant women

| Risk factors | Category | Number Tested | DAT IgG Positive (%) | ISAGA IgM Positive (%) |
|---|---|---|---|---|
| District | Haramaya | 13 | 11 (84.6) | 1 (7.7) |
| | Gursum | 7 | 6 (85.7) | 2 (28.6) |
| | Babile | 23 | 16 (69.6) | 1 (4.35) |
| Age | Teenagers | 2 | 1 (50.0) | 0 (0.0) |
| | Youth | 37 | 28 (75.7) | 6 (8.1) |
| | Adult | 4 | 4(100.0) | 1 (25.0) |
| Stage of pregnancy | 2nd trimester | 19 | 12 (63.2) | 3 (15.8) |
| | 3rd trimester | 14 | 12 (85.7) | 1 (7.1) |
| | 1st trimester | 10 | 9 (90.0) | 0 (0.0) |

*4.3.2. Risk factor analysis*

Univariable analysis was used to measure the strength of the association with *T. gondii* sero-positivity among different group of risk factors to compare the importance to acquire the infection. To ease the analysis the first category/level was taken as a reference. The result of univariable analysis showed that variables including age, residence, district, source of meat, cats holding, presence of feral cats and pipe water source had strong association (Table 15) with seropositivity to *T. gondii* IgG antibodies ($P < 0.05$). On the other hand none of the risk factors showed association with IgM seropositivity in chi square analysis. However, to determine the predictive value of the risk factors with *T. gondii* IgG seropositivity multivariable logistic analysis was employed. Accordingly, non-collinear variables that presented $P \leq 0.20$ in univariable analysis were offered to the multivariable regression model (Table 16).

Table 15: Univariable analysis for predictors of anti-*T. gondii* IgG seropositivity in humans

| Variables | Category | Number Tested | Positive (%) | Crude OR (95% CI) | *P*-Value |
|---|---|---|---|---|---|
| Districts | Haremaya | 120 | 70 (58.3) | 1.00 (ref.) | - |
| | Gursum | 105 | 70 (66.7) | 1.43 (0.83–2.46) | 0.119 |
| | Babile | 129 | 93 (65.8) | 1.85 (1.09–3.13) | 0.023* |
| Residence | Rural | 284 | 174 (61.3) | 1.00 (ref.) | |
| | Urban | 70 | 59 (84.3) | 3.39 (1.71–6.74) | <0.001* |
| Sex | Male | 164 | 107 (65.2) | 1.00 (ref.) | - |
| | Female | 190 | 126 (66.3) | 1.05 (0.68–1.63) | 0.832 |
| Age | Kid and Teenager | 36 | 14 (38.9) | 1.00 | - |
| | Young | 206 | 141 (68.5) | 3.41 (1.64–7.09) | 0.001* |
| | Adult | 112 | 78 (69.5) | 3.61 (1.65–7.88) | 0.001* |
| Meat consumption | Cooked | 259 | 167 (64.5) | 1.00 (ref.) | - |
| | Raw | 95 | 66 (69.5) | 1.25 (0.76–2.08) | 0.380 |
| Source of meat | Shoats‡ meat | 29 | 16 (55.2) | 1.00 (ref.) | - |
| | Mixed† | 300 | 194 (64.7) | 1.49 (0.69–3.21) | 0.312 |
| | Beef | 25 | 23 (92.0) | 9.34 (1.85–7.20) | 0.007* |
| Milk consumption | Boiled | 105 | 66 (62.9) | 1.00 (ref.) | - |
| | Both§ | 178 | 116 (65.2) | 1.11 (0.67–1.83) | 0.695 |
| | Raw | 71 | 51 (71.8) | 1.51 (0.79–2.89) | 0.217 |
| Vegetable consumption | Cooked | 146 | 94 (64.4) | 1.00 (ref.) | - |
| | Raw | 208 | 139 (66.8) | 1.11 (0.71–1.74) | 0.633 |
| Cat holding at home | No | 258 | 160 (62.0) | 1.00 (ref.) | - |
| | Yes | 96 | 73 (76.0) | 1.94 (1.14–3.31) | 0.014* |
| Presence of feral cats | No | 160 | 95 (59.4) | 1.00 (ref.) | - |
| | Yes | 194 | 138 (71.1) | 1.69 (1.08–2.63) | 0.021* |
| Water source | Stream | 63 | 33 (52.4) | 1.00 (ref.) | - |
| | Mixed‖ | 221 | 141 (63.8) | 1.60 (0.91–2.82) | 0.102 |
| | Pipe water | 70 | 59 (84.3) | 4.88 (2.17–0.98) | <0.001* |
| Status of Pregnancy | No | 124 | 77 (62.1) | 1.00 (ref.) | - |
| | Yes | 43 | 33 (76.4) | 1.87 (0.85–4.09) | 0.120 |
| Stage of pregnancy | 2nd trimester | 19 | 12 (63.2) | 1.00 (ref.) | - |
| | 3rd trimester | 14 | 12 (85.7) | 3.50 (0.60–20.4) | 0.164 |
| | 1st trimester | 10 | 9 (90.0) | 5.25 (0.54–0.64) | 0.152 |

‡Shoats meat (sheep and goat), †Mixed (shoats meat and beef), §Both (boiled and raw), ‖Mixed (stream and well water), *Significant, ref.: reference

Accordingly, by multivariable analysis, age, sex and cats kept at home were identified as predictors of IgG seropositivity (Table 16) and variables such as residence and pregnancy in the model were dropped relative to water source and sex due to collinearity. Living in Babile district (OR 2.24, 95% CI: 1.25–4.01, P = 0.007) increased the chance of *T. gondii* infection by twofold compared to other districts and individuals aged from 19 to 35 years (OR 4.32, 95% CI: 1.91–9.75, P < 0.001) were more likely to be infected with *T. gondii* compared to other age groups. On the other hand, consumption of beef meat (OR 5.67, 95% CI: 1.00– 32.21, P = 0.050) resulted in a sixfold increase in the chance of an individual being infected than consumption of any other meat source. Furthermore, keeping cats at home (OR 2.01, 95% CI: 1.11–3.65, P = 0.021) increased by twofold the likelihood of acquiring *T. gondii* infection in the household, whereas drinking pipe water was almost six times more likely to result in infection (OR 6.70, 95% CI 2.70–16.64, P < 0.001) compared to drinking water from other sources (Table 16). Analysis of the model fitness showed a difference between the observed and predictive value. The Hosmer–Lemeshow $\chi 2 = 2.84$, area under curve (AUC) = 0.7272 and P = 0.9439 indicated that the model fitted the data.

Table 16. Multivariable analysis of predictors of anti-*T. gondii* IgG seropositivity in humans

| Variables | Category | Adjusted OR (95%CI) | *P*-Value |
|---|---|---|---|
| Districts | Haremaya | 1.00 (ref) | |
| | Gursum | 1.20 (0.64–2.27) | 0.564 |
| | Babile | 2.24 (1.25–4.01) | 0.007* |
| Age | Kids and Teenagers | 1.00 (ref) | |
| | Youth | 4.32 (1.91–9.75) | <0.001* |
| | Adults | 4.21 (1.74–9.87) | <0.001* |
| Source of meat | Shoats‡ meat | 1.00 (ref) | |
| | Mixed† | 1.77 (0.76–4.14) | 0.187 |
| | Beef | 5.67 (1.00–32.21) | 0.050 |
| Cat holding at home | No | 1.00 (ref) | |
| | Yes | 2.01 (1.11–3.65) | 0.021* |
| Presence of feral cats | No | 1.00 (ref) | |
| | Yes | 1.63 (0.99–2.70) | 0.055 |
| Water source | Stream | 1.00 (ref) | |
| | Mixed‖ | 2.18 (1.17–4.07) | 0.014* |
| | Pipe water | 6.70 (2.70–16.64) | <0.001* |

‡Shoats meat (sheep and goat), †Mixed (shoats meat and beef), ‖Mixed (stream and well water), *Significant, ref.: reference

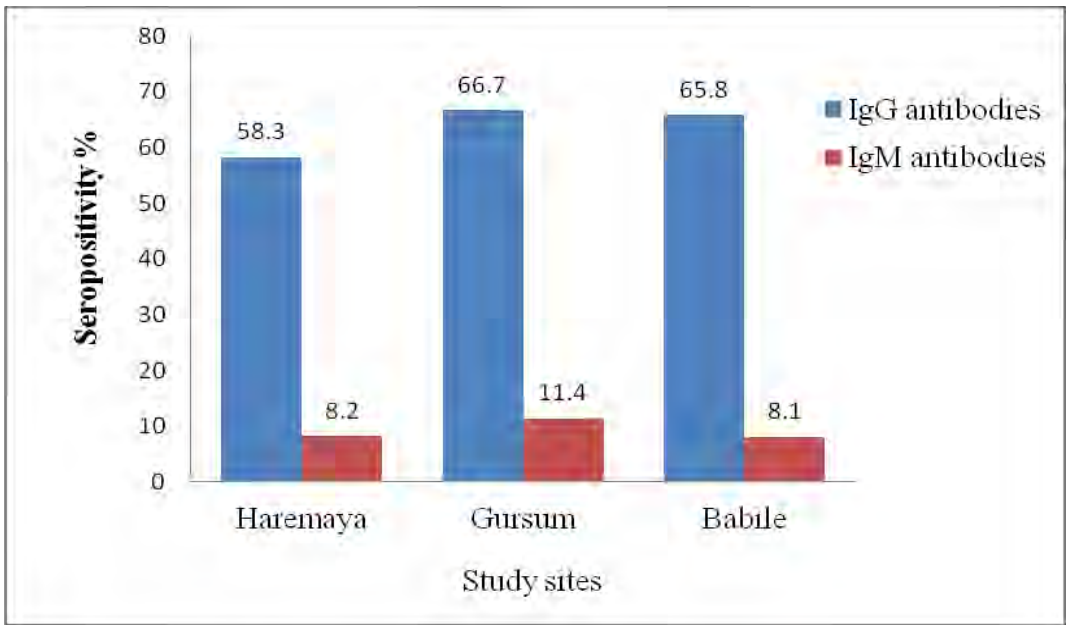


Figure 5: Distribution of anti-*T. gondii* IgG and IgM antibodies among the examined women (n = 167) in three selected study districts

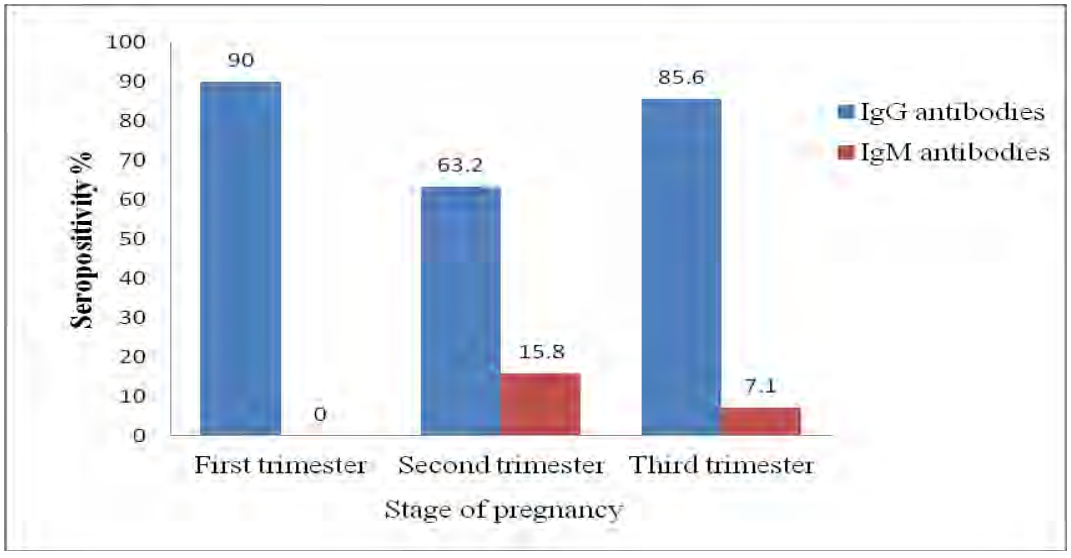


Figure 6: Distribution of anti-*T. gondii* IgG and IgM antibodies of pregnant women (n = 43) in the study districts

## 5. DISCUSSION

The present study determined a widespread occurrence of *T. gondii* infection among the domestic ruminants of East Hararghe Zone. At animal level, the seroprevalence of infection in sheep, goats, cattle and camels was 33.7%, 27.6%, 10.7% and 14.4% respectively. The observed seroprevalence in sheep is in agreement with those previously reported in Ethiopia that of 34% in Debrebirhan (Demessie and Tilahun, 2002) and 31.59% in East and West Shewa Zones of Oromia Region (Gebremedhin *et al*., 2013a) and elsewhere in the world, Morocco 27.6% (Sawadogo *et al.,* 2005), Brazil 29.41% (Clementino *et al.,* 2007), Italy 28.5% (Fusco *et al.,* 2007) and Brazil 32.9% (Pinheiro *et al.,* 2009), but slightly higher than the prevalence of Central Ethiopia 22.9% (Bekele and Kasali, 1989), Pakistan 11.1% (Ramzan *et al.,* 2009) Nigeria 6.7% (Kamani *et al.,* 2010), and northeastern China 3.0% (Wang *et al.,* 2011), while lower than that of 2.6% using MDAT and 56% with ELISA in Nazareth, Ethiopia (Negash *et al.,* 2004), 48.2% in Bulgaria (Prelezov *et al.,* 2008) and 57% in Spain (Panadero *et al.,* 2010).

In goats, the percentage of *T. gondii* infection is consistent with the reported prevalence of 24.1% using MDAT and 25.9% by ELISA in Nazareth, Ethiopia (Negash *et al.,* 2004) and 27.9% in Thailand (Jittapalapong *et al.,* 2005) and higher than 11.6% (Bekele and Kasali, 1989) and 15.48% (Gebremedhin *et al.,* 2014a) in Central Ethiopia and 17% in Norway (Stormoen *et al.,* 2012). However, on the contrary, it was lower than the reported prevalence of 35% in Debrebirhan (Demessie and Tilahun, 2002) and 74.8% in south omo (Teshale *et al.,* 2007) in Ethiopa; 59.8% in Bulgaria (Prelezov *et al.,* 2008) and 44.3% in Egypt (Shaapan *et al.,* 2010).

The seroprevalence of *T. gondii* infection in cattle (10.7%) observed in the current study is similar to the reported 6.6% in Central Ethiopia (Bekele and Kasali, 1989), 10.75% in Egypt determined using ELISA for detecting the antibodies against the recombinant *Tg*SAG2t (Ibrahim *et al*., 2009), 15.77% in Iran (Hamidinejat *et al.,* 2010), 7.3% in Spain (Panadero *et al.,* 2010) and 13% in Tanzania (Schoonman *et al*., 2010) and in contrary, it was higher than 2.68% in Brazil (Fajardo *et al.,* 2013) and 3.92% in Algeria (Dechicha *et al*., 2015) but lower than that of 54.16% in Turkey (Acici *et al*., 2008) 22.3% in Thailand (Jittapalapong *et al.,* 2008), 32% in Sudan (Khalil and Elrayah, 2011) and 43.5% in Pakistan (Tasawar *et al*., 2013). In the present study the proportion of camels seropositive for anti-*T. gondii* antibodies (14.4%) is in consistent with the

proportion, in Sudan 20% (Khalil and Elrayah, 2011) while higher than that of 3% in China (Wang *et al.,* 2013).

The analysis for risk factors indicated that female sheep were infected 2.63 times more than the male sheep; and those that drunk pond water and pipe water only were 4.25 times and 9.57 times at risk of infection compared with those using other water sources respectively (Table 4). In this study, a significant difference in *T. gondii* seropositivity was observed between the two sexes, female sheep being highly seropositive compared to males. This finding is in accordance with that reported by Ramzan *et al*. (2009). The increased susceptibility of females might be associated with their lower immunologic resistance in certain periods of their lives (Guimarães *et al.,* 2013). In contrast, Silva *et al.* (2003) and Lashari and Tasawar (2010) observed higher seroprevalences in male sheep than in females that is attributed to androgen production lowering their immunity. In goats, a high seroprevalence was found in females (30.6%) than in males (17.2%), but this difference was statistically insignificant. In addition, $>1$ year goats were 3.45 times more infected than $\leq 1$ year ones. Besides, goats that drunk pond water and pipe water had 6.03 times and 11.61 times more chance to acquire the infection respectively. Alike, cattle that obtained drinking water from ponds and pipe were 5.60 times and 10.68 times more chance to be infected by *T. gondii* compared with those that used other water sources. Adult camels were 2.49 times more likely to be infected compared with the young group.

The observed higher risk of infection in sheep, goats and cattle that were given pipe water might be explained by the increased number of cats roaming in the area contaminating the source of pipe water with infective oocysts. Silva *et al*. (2003), Dubey (2004) and Tenter (2009) suggested that few cats are sufficient to contaminate a wide field area in short time since one infected cat sheds millions of oocysts. In addition, poor sanitation of watering troughs and the storage of animal feeds outside in the open air accessible to cats could also contribute the increased chance of *T. gondii* infection to domestic ruminants. In consistent with our finding, Gebremedhin *et al.,* (2013a) reported high risk of infection in sheep given pipe water. However, contrary to our finding, Pinheiro *et al.* (2009) observed increased chances of infection in animals living on properties with running water systems than those living with stagnant water sources. The similarities and differences might be attributed to the resemblance and variations in agroecological situation and rate of contamination of the water properties by infective oocysts.

The progressive increase in seroprevalence with age seen in sheep, goat, cattle and camel indicates sustained exposure to the *T. gondii* infection in the environment. Moreover, in the present study a significantly higher *T. gondii* infection was found in adult goats and camels compared with young. Older animals as they lived longer might be more likely exposed to the infectious agent from different sources (Jittapalapong *et al*., 2005; Teshale *et al*., 2007).

The flock/herd level seroprevalences in sheep (60.8%), goats (55.8%), cattle (23.2%) and camels (53.2%) observed in the current study are high. In sheep the seroprevalence is similar to that of previously reported 70.48% in East and West Shewa Zones of Oromia Region, Ethiopia (Gebremedhin *et al.,* 2013a). In goats, the flock seroprevalence was higher than that of 45.17% reported by Swai and Kaaya (2013) in Tanzania, but lower than 75% recorded in Norway by Stormoen *et al*. (2012). The variation in seroprevalence of *T. gondii* infection among domestic ruminants in the current study, and those previously reported in Ethiopia and elsewhere, might be attributed to differences in geographical location, animal management practices and the sensitivity and specificity of serological diagnostic tests used for detecting the infection.

Sheep and goat flock that drunk pipe water were found at sixfold and fourfold risk of acquiring *T. gondii* infection respectively compared with those used other water sources (Table 8 and 9). Unlike, cattle herd that used pond water were eightfold more infected than those herd which used other water sources (Table 10). while no significant relationship was discerned regarding camel herd seropositivity with the tested possible risk factors (Table 11).

In the current study two out of the 12 *T. gondii* isolates of sheep were observed as virulent to mice. This result in agreement to the reported by Gebremedhin *et al.* (2014b) about the virulence of the *T. gondii* isolates from sheep in central Ethiopia. Elsewhere in the world the observed virulence of the isolates from sheep is in agreement with *T. gondii* isolates from France (Dumetre *et al*., 2006), but lowerd compared to the reported from Iran (Zia-Ali *et al*., 2007) and Brazil (Ragozo *et al*., 2008). In our finding none of the *T. gondii* isolates from goats were showed virulence to mice. Incontrary, Gebremedhin *et al.* (2014b) reported the virulence of the isolates from goats to mice.

The serprevalence of toxoplasmosis (anti-*T. gondii* IgG antibodies) in human population in the study areas was 65.82% and anti-*T. gondii* IgM antibodies were detected in 8.98% of women. This rate is consistent with the 60% seroprevalence reported from the previous study in the Adama hospital (Negash *et al*., 2008) but it is lower than the rate range (81.4%–96.7%) found most vulnerable groups from different parts of the country (Gebre-Exabier *et al*., 1993; Woldemichael *et al.*, 1998; Yimer *et al*., 2005; Shimelis *et al*., 2009; Gebremedihin *et al*., 2013b; Walle *et al*., 2013). Nevertheless, this and other studies justified the fact that the seroprevalence of toxoplasmosis is substantially higher in the country than other parts of the world, where reported rates range from 9·3% to 43·8% (Berger *et al*., 2009; Xiao *et al*., 2010; Chiang *et al*., 2012; Mwambe *et al*., 2013). The difference might be due to the variation of the geographic area, the sensitivity and specificity of the test employed, demographic characteristics and economic status of the studied population. The present study showed increased seropositivity with an increase in age, which might reflect the longer exposure of older individuals to infection from various sources leading to life-long elevated IgG antibody levels as similarly recorded by others (Gebre–Exabier *et al*., 1993; Alvarado Esquivel *et al*., 2011; Zemene *et al*., 2012; Gebremedihin *et al*., 2013b)

It was observed that people living in Babile district were twice likely to be infected with *T. gondii* than in other districts. The rural population in Babile district is semi-pastoralists, moving frequently with animals seeking pasture and water thus possibly increasing exposure to sources of infection. The district has a relatively hot climate and is at a lower altitude compared to the other districts. These conditions favour the sporulation and long-term survival of oocysts better than colder climates at high altitudes (Dubey and Beattie, 1988). The observed high seroprevalence in people residing in urban (84.3%) compared to rural (61.3%) areas, might be connected with increased consumption of raw meat in the former (52.9%) than the latter (20.4%), which is facilitated by the availability of retail meat. By contrast, in China no significant association was evident between *T. gondii* infections and urban living; consumption of raw meat and keeping pet animals are not popular practices in China (Xiao *et al*., 2010). Viable *T. gondii* cysts are rarely found in beef and isolation of the parasite from the tissue of cattle has not to our knowledge been documented (Dubey, 1996a). Nevertheless, in this study, the likelihood of acquiring infection through consumption of raw beef was 5.67 times (95% CI: 1.00–34.21) higher than for other raw meat sources (P = 0.05), and is probably explained by cultural differences in different regions of

the country. Similarly, several authors have noted an association between seropositivity and raw meat consumption (Yimer *et al*., 2005; Negash *et al*., 2008; Berger *et al*., 2009; Dubey, 2010; Chiang *et al*., 2012).

The observed difference in seroprevalence of *T. gondii* between people having or not having domestic cats in the household (76.0% vs. 62.0%, respectively) indicated statistical significance ($P < 0.05$) and suggests high environmental contamination with shedding of infective oocysts by cats. This finding is consistent with some previous studies (Zemene *et al.,* 2012; Gebremedhin *et al*., 2013b). However, other studies have reported the absence of such an association (Gebre–Exabier *et al*., 1993; Nijem and Al-Amleh, 2009). However, the contribution of feral cats to environmental contamination and subsequent exposure of people to the infection should not be discounted (Gebremedhin *et al*., 2013b). We found a slightly higher IgM response in women (8.98%) compared to previous local reports (2.5–4.2%) (Zemene *et al*., 2012; Gebremedhin *et al*., 2013b), which could be due to the demographic variables and degree of exposure to the source of infection. The detection of anti-*T. gondii* IgM antibodies on serology in 9.3% of pregnant womens signifies the increasing risk of congenital transmission during pregnancy, particularly if the woman acquires the infection for the first time. This finding implies an emphasis has to be made the requirement of antenatal healthcare practices against toxoplasmosis throughout East Haraghe Zone. The reduction in anti-*T. gondii* IgG antibodies from the first to the second trimester and that rise of anti-*T. gondii* IgM in that order may be attributed either to a recent infection or reactivation of an existing chronic infection and merits future investigation.

# 6. CONCLUSION AND RECOMMENDATIONS

East Haraghe Zone is characterized by different agroecological zones and exhibit diversified climatic conditions. The study area is inhabited by different tribes that are accustomed to varied traditional feeding habits. Many if not all of the inhabitants rear livestock for various purposes and the area consists of various wild animals. This geographical and climatic differences, the interface between wild animals (wild cats) with domestic ruminants and the feeding habit of the people might have influenced the epidemiological picture of *T. gondii* infection and the circulating genotype in the area. The current serological study determined that *T. gondii* infection is moderately prevalent in domestic ruminants of East Haraghe Zone. The free acess of feral and free roaming owned cats to grazing areas, animal feed and watering points might have resulted in contamination with cat faeces containing *T. gondii* oocyst which increases the chance of infection in domestic ruminants and humans. The encountered higher seroprevalence in all studied food animals, and the isolation of the viable *T. gondii* cyst from sheep and goat heart tissue indicates the increased public health concern of the disease.Additionally,the consumption of raw/undercooked/meat and milk from such infected animals is among potential risks to transmit the infection to humans. The result showed Sex, age, pond and pipe water sources were found a potential risk factors for *T. gondii* infection in domestic ruminamts. In humans, the seroprevalence of anti-*T. gondii* IgG antibodies was found considerably high,while water source, age, district and presence of cats at home were found to be risk factors for acquiring *T. gondii* infection in humans. The high level of anti *T. gondii* IgM seropositivity determined among women of child-bearing age indicates the presence of either current or latent infection and possible occurrence of congenital transmission during pregnancy.

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

- Feeding and watering troughs for domestic animals should be treated regularly and be protected from being accessed by cats to avoid contamination by *T. gondii* oocysts

- The public health sector of the study areas should initiate a study on the health impact of toxoplasmosis so as to formulate guidelines for the prevention and control of the disease.

- People in the study areas should be advised whenever possible either to boil or filter the pipe water before using for drinking.

- The finding of high level of IgM sero-positivity in women of child bearing age and associated impacts on the congenital transmission of the *T. gondii* infection should be investigated in future.

- Health education on the zoonotic significance of toxoplasmosis, mode of transmission and maintenance of a high standard of personal hygiene should be introduced

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# 8. APPENDICIES

## Appendix I: Tables and Figures

a) Best fitting model for predictors of *T. gondii* infection in sheep

| Variables | Category | Adjusted OR (95% CI) | *P*-Value |
|---|---|---|---|
| Sex | Male | 1.00 (ref.) | |
| | Female | 2.71 (1.28–5.74) | 0.009* |
| Cats contact | No | 1.00(ref.) | |
| | Yes | 1.27 (0.74–2.19) | 0.383 |
| Water source | Mixed‡ | 1.00(ref.) | |
| | Pond† | 4.07 (2.09–7.95) | <0.001* |
| | Tap water | 9.22 (5.00–17.01) | <0.001* |

‡Mixed, †Pond, *Significant, ref.: reference

Hosmer Lemeshaw chi-square (HCL $\chi^2$) = 3.89; ROC=0.7682; P=0.566

b) Best fitting model of predictors for *T. gondii* seropositivity in goat

| Variables | Catagory | Adjusted OR (95% CI) | *P*-Value |
|---|---|---|---|
| District | Haremaya | 1.00 (ref.) | |
| | Gursum | 0.54 (0.21–1.35) | 0.185 |
| | Babile | 0.19 (0.06–0.57) | 0.003* |
| Sex | Male | 1.00 (ref.) | |
| | Female | 1.73 (0.90–3.31) | 0.099 |
| Age in years | Kids and yearlings | 1.00 (ref.) | |
| | Adults | 3.19 (1.25–8.12) | 0.015* |
| Flock size | Small | 1.00 (ref.) | |
| | Large | 0.78 (0.42–1.47) | 0.443 |
| Water source | Mixed‡ | 1.00 (ref.) | |
| | Pond‡ | 6.51 (262–16.17) | <0.001* |
| | Tap water | 10.78 (4.07–28.55) | <0.001* |

‡Mixed, ‡Pond, *Significant, ref.: reference

Hosmer Lemeshaw chi-square (HCL $\chi^2$) = 1.42; ROC=0.7317; P=0.994

c) Best fitting model of predictors for *T. gondii* seropositivity in cattle

| Variables | Category | Adjusted OR (95%CI) | *P*-Value |
|---|---|---|---|
| Herd size | Small | 1.00 (ref.) | |
| | Large | 0.32 (0.12–0.85) | 0.022* |
| Cats contact | No | 1.00 (ref.) | |
| | Yes | 2.38 (1.05–5.38) | 0.038* |
| Source of water | Mixed‡ | 1.00 (ref.) | |
| | Pond‡ | 4.77 (2.15–10.57) | <0.001* |
| | Tap water | 4.68 (1.43–14.67) | 0.010* |

‡Mixed, ‡Pond, *Significant, ref.: reference

Hosmer Lemeshaw chi-square (HCL $\chi^2$) =7.95; ROC=0.7439; P=0.159

d) Best fitting model for predictors of IgG seropositivity in humans

| Variables | Category | Adjusted OR (95%CI) | *P*-Value |
|---|---|---|---|
| Residence | Rural | 1.00 (ref.) | |
| | Urban | 3.76 (1.82–7.73) | <0.001* |
| Age in years | Kids | 1.00 (ref.) | |
| | Youngs | 4.38 (1.98–9.71) | <0.001* |
| | Adults | 4.23 (1.82–9.84) | <0.001* |
| Cat holding at home | No | 1.00 (ref.) | |
| | Yes | 1.78 (1.01–3.13) | 0.043* |
| Presence of feral cats | No | 1.00 (ref.) | |
| | Yes | 1.47 (0.92–2.35) | 0.107 |

Hosmer Lemeshaw chi-square (HCL $\chi^2$) = 0.90; ROC = 0.6812; P = 0.9890;

e) Mice sacrificed and examined for presence of *T. gondii* cysts in the brain tissue after IP inoculation

| No. | Date inoculated | Host type and ID | Mice ID | Days after inoculation | Wt of mice brain | Solution PBS 10% | Cyst count | Cyst size | Remark |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3/02/07 | Gt2 | Gt2A | 60 days | 0.25 | PBS | 17 | | |
| | | | Gt2B | | 0.27 | | - | | |
| | | | Gt2C | | 0.40 | | 9 | | |
| | | | Gt2D | | 0.56 | | - | | |
| | | | Gt2E | | 0.34 | | 7 | | |
| | | | Gt2F | | 0.40 | | 5 | | |
| 2 | 3/02/07 | Gt7 | Gt7A | 60 days | 0.44 | PBS | 41 | | |
| | | | Gt7B | | 0.47 | | 91 | | |
| | | | Gt7C | | 0.52 | | 49 | | |
| | | | Gt7D | | 0.44 | | 32 | | |
| | | | Gt7E | | 0.47 | | 26 | | |
| 3 | 9/02/07 | Gt1 | Gt1A | 66 days | 0.57 | PBS | - | | |
| | | | Gt1B | | 0.50 | | 14 | | |
| | | | Gt1C | | 0.59 | | 27 | | |
| | | | Gt1D | | 0.42 | | 5 | | |
| | | | Gt1E | | 0.58 | | 53 | | |
| 4 | 9/02/07 | Gt4 | Gt4A | 66 days | 0.44 | PBS | - | | |
| | | | Gt4B | | 0.53 | | 5 | | |
| | | | Gt4C | | 0.43 | | 11 | | |
| | | | Gt4D | | 0.49 | | 4 | | |
| | | | Gt4E | | 0.54 | | - | | |
| 5 | 26/12/07 | Gt16 | Gt16A | 50 days | 0.51 | PBS | - | | |
| | | | Gt16B | | 0.48 | | - | | |
| | | | Gt16C | | 0.52 | | 34 | | |

*Continued….*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Gt16D | | 0.47 | | 64 | | |
| | | | Gt16E | | 0.57 | | 13 | | |
| 6 | 26/12/07 | Gt26 | Gt26A | 50 days | 0.42 | PBS | 7 | | |
| | | | Gt26B | | 0.45 | | - | | |
| | | | Gt26C | | 0.44 | | - | | |
| | | | Gt26D | | 0.43 | | - | | |
| | | | Gt26E | | 0.43 | | - | | |
| 7 | 26/12/07 | Gt21 | Gt21A | 50 days | 0.51 | PBS | - | | |
| | | | Gt21B | | 0.36 | | 65 | | |
| | | | Gt21C | | 0.44 | | 21 | | |
| | | | Gt21D | | 0.41 | | 27 | | |
| | | | Gt21E | | 0.43 | | - | | |
| | | | Gt21F | | 0.46 | | 1 | | |
| 8 | 26/12/07 | Gt29 | Gt29A | 50 days | 0.41 | PBS | - | | |
| | | | Gt29B | | 0.49 | | - | | |
| | | | Gt29C | | 0.42 | | - | | |
| | | | Gt29D | | 0.49 | | - | | |
| | | | Gt29E | | 0.42 | | - | | |
| 9 | 30/1/07 | Gt36 | Gt36A | 48 days | 0.46 | PBS | - | | |
| | | | Gt36B | | 0.53 | | 3 | | |
| | | | Gt36C | | 0.55 | | - | | |
| | | | Gt36-D | | 0.54 | | 60 | | |
| | | | Gt36E | | 0.50 | | - | | |
| 10 | 30/1/07 | Gt41 | Gt41A | 49 days | 0.48 | PBS | 4 | | |
| | | | Gt41B | | 0.57 | | - | | |
| | | | Gt41C | | 0.49 | | - | | |
| | | | Gt41D | | 0.54 | | - | | |
| | | | Gt41E | | 0.54 | | - | | |
| 11 | 30/1/07 | Gt34 | Gt34A | 49days | 0.57 | PBS | - | | |

*Continued….*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Gt34B | | 0.43 | | - | | |
| | | | Gt34C | | 0.49 | | - | | |
| | | | Gt34D | | 0.47 | | - | | |
| | | | Gt34E | | 0.45 | | - | | |
| 12 | 30/1/07 | Gt42 | Gt42A | 49days | 0.50 | PBS | - | | |
| | | | Gt42B | | 0.56 | | - | | |
| | | | Gt42C | | 0.56 | | - | | |
| | | | Gt42D | | 0.45 | | - | | |
| | | | Gt42E | | 0.43 | | - | | |
| 13 | 7/02/07 | Sp48 | Sp48A | 46days | 0.44 | PBS | - | | |
| | | | Sp48B | | 0.58 | | - | | |
| | | | Sp48C | | 0.63 | | - | | |
| | | | Sp48D | | 0.56 | | - | | |
| | | | Sp48E | | 0.50 | | - | | |
| 14 | 7/02/07 | Sp53 | Sp53A | 47days | 0.59 | PBS | 10? | | 2died |
| | | | Sp53B | | 0.48 | | - | | |
| | | | Sp53C | | 0.54 | | - | | |
| 15 | 7/02/07 | Sp55 | Sp55A | | 0.62 | | 1 | | |
| | | | Sp55B | 47days | 0.60 | PBS | - | | |
| | | | Sp55C | | 0.46 | | 30 | | |
| | | | Sp55D | | 0.53 | | 12 | | |
| | | | Sp55E | | 0.59 | | 10 | | |
| 16 | 7/02/07 | Sp61 | Sp61A | 47days | 0.47 | PBS | - | | |
| | | | Sp61B | | 0.40 | | - | | |
| | | | Sp61C | | 0.61 | | - | | |
| | | | Sp61D | | 0.63 | | 8 | | |
| | | | Sp61E | | 0.55 | | 25 | | |
| 17 | 13/02/07 | Sp64 | Sp64A | 47days | 0.43 | PBS | - | | |

*Continued….*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sp64B | | 0.47 | | 10 | | |
| | | | Sp64C | | 0.43 | | - | | |
| | | | Sp64D | | 0.52 | | 2 | | |
| 18 | 13/02/07 | Sp73 | Sp73A | 47days | 0.51 | PBS | 32 | | |
| | | | Sp73B | | 0.52 | | 1 | | |
| | | | Sp73C | | 0.42 | | 5 | | |
| | | | Sp73D | | 0.52 | | 15 | | |
| | | | Sp73E | | 0.51 | | - | | |
| | | | Sp73f | | 0.51 | | 13 | | |
| 19 | 13/02/07 | Sp65 | Sp65A | 48days | 0.57 | PBS | 5 | | |
| | | | Sp65B | | 0.45 | | 15 | | |
| | | | Sp65-C | | 0.41 | | 6 | | |
| | | | Sp65D | | 0.52 | | 11 | | |
| | | | Sp65E | | 0.42 | | 10 | | |
| 20 | 13/02/07 | Sp63 | Sp63A | 48days | 0.55 | PBS | - | | 1died |
| | | | Sp63B | | 0.46 | | - | | |
| | | | Sp63C | | 0.47 | | 46 | | |
| | | | Sp63D | | 0.54 | | 98 | | |
| 21 | 17/02/07 | Sp72 | Sp72A | 49 days | 0.40 | PBS | 19 | | |
| | | | Sp72B | | 0.49 | | - | | |
| | | | Sp72C | | 0.46 | | - | | |
| | | | Sp72D | | 0.49 | | 25 | | |
| | | | Sp72E | | 0.40 | | - | | |
| 22 | 17/02/07 | Sp66 | Sp66A | 49 days | 0.40 | PBS | - | | |
| | | | Sp66B | | 0.47 | | - | | |
| | | | Sp66C | | 0.56 | | - | | |
| | | | Sp66D | | 0.55 | | - | | |
| | | | Sp66E | | 0.58 | | - | | |
| 23 | 17/02/07 | Sp62 | Sp62A | 49days | 0.53 | PBS | - | | |

*Continued……..*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Sp62B | | 0.58 | | - | | |
| | | | Sp62C | | 0.52 | | - | | |
| | | | Sp62D | | 0.50 | | - | | |
| | | | Sp62E | | 0.40 | | - | | |
| 24 | 17/02/07 | Sp70 | Sp70A | 49 days | 0.43 | PBS | - | | |
| | | | Sp70B | | 0.44 | | - | | |
| | | | Sp70C | | 0.44 | | - | | |
| | | | Sp70D | | 0.43 | | - | | |
| | | | Sp70E | | 0.49 | | - | | |
| 25 | 03/04/07 | Sp86 | Sp86A | 67 days | 0.47 | PBS | - | | |
| | | | Sp86B | | 0.55 | | - | | |
| | | | Sp86C | | 0.44 | | - | | |
| | | | Sp86D | | 0.45 | | - | | |
| | | | Sp86E | | 0.40 | | 16 | | |
| 26 | 03/04/07 | Sp76 | Sp76A | 67days | 0.55 | PBS | 21 | | |
| | | | Sp76B | | 0.51 | | 5 | | |
| | | | Sp76C | | 0.53 | | - | | |
| | | | Sp76D | | 0.55 | | - | | |
| | | | Sp76E | | 0.43 | | - | | |
| 27 | 03/04/07 | Sp83 | Sp83A | 68days | 0.51 | PBS | 18 | | |
| | | | Sp83B | | 0.42 | | 13 | | |
| | | | Sp83C | | 0.47 | | 35 | | |
| | | | Sp83D | | 0.54 | | - | | |
| | | | Sp83E | | 0.50 | | 17 | | |
| 28 | 03/04/07 | Sp90 | Sp90A | 68days | 0.55 | PBS | - | | |
| | | | Sp90B | | 0.58 | | 15 | | |
| | | | Sp90C | | 0.54 | | 43 | | |
| | | | Sp90D | | 0.60 | | - | | |
| | | | Sp90E | | 0.59 | | - | | |

*Continued…….*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 29 | 07/04/07 | Sp75 | Sp75A | 64days | 0.40 | PBS | 78 | | |
| | | | Sp75B | | 0.48 | | 10 | | |
| | | | Sp75C | | 0.50 | | 98 | | |
| | | | Sp75D | | 0.58 | | 76 | | |
| | | | Sp75-E | | 0.42 | | - | | |
| 30 | 07/04/07 | Sp78 | Sm78A | 64days | 0.52 | PBS | 35 | | |
| | | | Sp78B | | 0.55 | | 75 | | |
| | | | Sp78C | | 0.48 | | - | | |
| 31 | 22/08/07 | Bn94 | Bn 94A | 66days | 0.37 | PBS | - | | |
| | | | Bn94B | | 0.35 | | - | | |
| | | | Bn 94C | | 0.40 | | - | | |
| | | | Bn94D | | 0.40 | | - | | |
| | | | Bn94E | | 0.40 | | - | | |
| 32 | 22/08/07 | Bn92 | Bn92A | 66days | 0.42 | PBS | - | | |
| | | | Bn92B | | 0.40 | | - | | |
| | | | Bn92C | | 0.40 | | - | | |
| | | | Bn92D | | 0.37 | | - | | |
| 33 | 22/08/07 | Bn102 | Bn102A | 67days | 0.50 | PBS | - | | |
| | | | Bn102C | | 0.40 | | - | | |
| | | | Bn102D | | 0.45 | | - | | |
| | | | Bn102E | | 0.40 | | - | | |
| 34 | 22/08/07 | Cm103 | Cm103A | 67days | 0.40 | PBS | - | | |
| | | | Cm103B | | 0.38 | | - | | |
| | | | Cm103C | | 0.45 | | - | | |
| | | | Cm103D | | 0.37 | | - | | |
| | | | Cm103E | | 0.40 | | - | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

Appendix II: Toxo-diagnostic kits procedures and other protocols

**a) Instruction for use TOXO ISAGA**

Label the stripe to be used appropriately on the frosted part provided (include 2 wells for each serum sample).

**1. Incubation of sera**

1. At time of the test dilute the sera in PBS (R5)
   Serum samples 1/100
   Positive serum (R4) 1/10
   Serum sample from neonate 1/20
2. Dispence 100 µl of diluted R4 in 2 adjacent wells.
3. Dispence each diluted serum sample in the same way.
4. Replace sera by PBS in 2wells for each bach of tests (antigen control).
5. Cover the strips with the self-adhesive sheet and incubate at 35–39$^{o}$C for 2 hours.

**2. Washing (wash bottle only)**

1. Empty the wells by inverting the plate over the sink with a collection tray (containing bleach solution).
2. Take care to squeeze the central part of the stand to hold the strips in place.
3. Wash wells once in PBS–Tween avoiding over spilling.
4. Empty immediately.
5. Wash twice for 5 minutes in PBS–Tween and then wash twice for 5 minutes in PBS only.
6. After each washing step drain the strip thoroughly on each filter paperwithout allowing to dry. An automatic washer must not bu used.

**3. Demonstration**

1. Dispence antigen (R2) diluted 1/20 in BABS buffer (R3) as follows for each serum sample and antigen control.
   100 µl in the first well.
   150 µl in the second well.

2. Cover the stripe with a self – adhesive sheet and incubate overnight at 35–39$^{o}$C in a moist chamber.

**4. Reading**

Reading is done by either with a reading system or with the naked eye.

With the naked eye: eye read wells by placing the strip approximately 50cm above a suitably it white background. Each serum is tested with increasing concentration of toxoplasma antigens. If the reaction is negative sedimentation of the toxoplasma occurs. If the reaction is positive, the toxoplasma are agglutinated in a mat and any antigen in excess of the bound specific IgM forms an equivalent amount of sedimentation.

Total sedimentation in a button similar to the antigen control.

1+ large sedimentation button

2+ medium sized sedimentation button

3+ very small sedimentation buttons.

4+ mat covering the base of the well similar to the positive serum (R4).

Antigen control total sedimentation of the toxoplasma (button).

Positive serum (R4) agglutination of the toxoplasma in a mat covering the base of the well.

Serum sample compare the size of the button with that of the corresponding antigen control and not the result obtained by referring the to the scale given above.

**5. Results**

The ISAGA index of a serum corresponds to the sum of the values obtained for the 2 antigen volumes used.

Examples of ISAGA index calculation

| | 100 µl of antigen | 150 µl of antigen | ISAGA index |
|---|---|---|---|
| Serum no 1 | 3+ | 2+ | 5 |
| Serum no 2 | 4+ | 4+ | 8 |

Interpretation

0-5+ negative reaction

$\geq 6^{+}$ perform confirmatory test

| | 100 µl of antigen | 150 µl of antigen | 200µl of antigen | ISAGA INDEX |
|---|---|---|---|---|
| Serum No. 1 | 3+ | 2+ | 2+ | 7 |
| Serum No. 2 | 4+ | 4+ | 3+ | 9 |

**Confirmatory test**

Label the strips appropriately on the frosted part provided (include 2 wells for each serum sample).

i) Incubation of sera –see point 1 of the screening section.

ii) Washing – see point 2 of the screening section.

iii) Demonstration

Dispence R2 antigen diluted 1/20 in BABS buffer (R3) as follows for each serum sample and antigencontrol.

- 150 µl in the first well.
- 200 µl in the second well.

Cover the strips with the self adhesive sheet and incubateover night at 35 - $39^0$C , in a moist chamber.

iv) Reading – see point 4 of the screening section.

v) Results

The ISAGA index of a serum corresponds to the sum of the values obtained for the 3 antigens used (100µl and 150µl from the screening and 200µl from the concentration test)

Examples of ISAGA index calculation.

Results and Interpretation

Acquired toxoplasmosis (in adults and children)

ISAGA index

0 – 5 negativereaction

6 - 8 borderline reaction

9 –12 positive reaction

b) Instruction for use of Toxo DA (DAT) diagnostic kit:

Screening:

1. In tubes, dilute the serum samples and control sera (R4 and R5):

1/20: 100µl of serum + 1.9 ml of PBS (R6)

1/2000: 25µl of the 1/20 dilution +2.5ml of PBS

2. Locate the patient sera on the report sheet then dispense 25µl of each serum dilution into the 2 wells provided.

3. Add 25µl of 0.2 mol/l 2-mercaptoethanol to each well, thus diluting the sera 1/40 and 1/4000.

4. Add 50µl R1 antigen suspension diluted 1/5 to each well.

Allow 1 well for antigen control:

| | |
|---|---|
| 2-Mercaptoethanol(0.2mol/l) | 25µl |
| PBS | 25µl |
| Antigen | 50µl |

5. Homogenize using the vibrator or Kline rotar.

6. Cover with a self- adhesive sheet. Leave for 5-18 hours at room temperature away from vibration and sources of drying.

6. Perform reading

Reading:

Antigen control: sedimentation of the toxoplasma in button or ring.

Positive control: agglutination of the toxoplasma in a mat covering about half of the well base. The mat may show slight shrinking around the edges (irregular shape).

Negative control: sedimentation of the toxoplasma in a button or ring.

Border line reaction: agglutination of the toxoplasma in a mat covering less than half of the well base.

Quantitative test (to determine the titer)

1. Depending on the result of screening test (serum positive at 1/40 or 1/4000) use either 1/20 or 1/2000 dilution in PBS to make serial threefold dilution

2. Dispense:

50µl of 0.2 mol/l 2-mercaptopethanol in successive wells

25µl of the chosen serum dilution (1/20 or 1/2000) in the 1$^{st}$ well.

3. Transfer 25µl from 1$^{st}$ well to the 2$^{nd}$ well. Repeat up to the 4$^{th}$ well

A dilution series is obtained:

Either: 1/60,1/180,1/540, 1/1620

Or: 1/6000,1/18000.1/54000.1/162000.

4. Procedure and reading are performed as for the screening test.

| Result | Status |
|---|---|
| S/P $\leq$ 40% | NEGATIVE |
| 40% < S/P < 50% | DOUBTFUL |
| S/P $\geq$ 50% | POSITIVE |

c) Toxo ELISA diagnostic kit testing procedures

For sera and plasma - dilution at 1/10

1. Add:

90 µl of Dilution Buffer 2 to each microwell.

10 µl of the Negative Control to wells A1 and B2.

10µl 0f the Positive Control to wells C1 and D1.

10µl of the sample to be tested to the remaining wells.

2. Incubate 45 min $\pm$ 4min at $21^0$C ($\pm$ $5^0$C)

3. Empty the wells wash each well 3 times with approximately 300µl of the wash solution. Avoid drying of the wells between washings.

4. Prepare the conjugate 1x by diluting the concentrated conjugate 10x to 1/10 in dilution buffer three

5. Add 100µl of the conjugate 1x to each well.

6. Incubate 30 min $\pm$ 3 min at $21^0$C ( $\pm$ $5^0$C).

7. Empty the wells. Wash each well 3 times with approximately 300µl of the wash solution. Avoid drying of the wells between washings.

8. Add 100µl of the substrate solution to each well.

9. Incubate 15 min $\pm$ 2min at $21^0$C ( $\pm$ $5^0$C ) in the dark.

10. Add 100µl of the stop solution to each well in order to stop the reaction.

11. Read and rescored the O.D.at 450nm.

Validation: The test is validated if:

The mean value of the positive control O.D. (ODPC) is greater than 0.350. OD > 0.350.

The ratio of the mean O.D. values of the positive and negative controls ( ODPC and ODNC) is greater than 3.5 ODPC/ODNC >

**Interpretation:**

For each sample calculate the S/P percentage

$$S/P = \frac{ODsample - ODNC}{ODPC - ODNC} \times 100$$

Samples presenting a S/P:

Less than or equal to 40% are considered negative.

Between 40% and 50% are considered doubtful.

Greater than or equal to 50% are considered positive.

d) Instruction to use Toxo-latex agglutination test

Qualitative method:

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperature.
2. Place 50µl of the sample and one drop of each positive and negative control into separate circles on the slide test.
3. Swirl the Toxo-latex reagent gently before using and add 25µl of this reagent next to the samples to be treated.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place slide on a mechanical roteter at 80-100 rpm for 4 minutes. False positive results could appear if the test is read later than four minutes.

**Semi-quantitative method**

1. For each specimen to be tested place 50µL of 0.9% saline solution into each of the circleof the card. Do not spread diluents.

2. To circle one add 50µL of specimen to the saline solution and using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 50µL of the mixture to the saline solution in the second circle.
3. Control with 2- fold serial dilution in a similar manner up to the sixth circle and discard 50µL from this circle. Final sample dilution will be 1:2, 1:4, 1:16, 1:32, 1:64

**Reading and interpretation**

Examine macroscopically the presence or absence of viable agglutination immediately after removing the slide from the rotator.
The presence of agglutination indicates an antibody concentration equal or greater than 4IU/mL. The titer, in the semi-quantitative method,is defined as the highest dilution showing a positive result.

e) Bioassay procedure in mice.

For the isolation of *T. gondii* from Animal tissues, often muscular in nature such as tongue, heart, and diaphragm, can be used to isolate *T. gondii* in mice.
1. Trim connective tissue, fat, epithelium (e.g., from tongue) from muscle tissues using nonporous, hard plastic cutting boards, scissors or disposable razors. Cut muscle into small (1 – 2 cm) pieces and store in plastic bags or cups.
2. Grind muscle (50 g) in a blender for 15 s at low speed without saline. Then add 125 ml of saline and blend at top speed for about 30 s. Rinse blender with 125 ml of saline and add the washings to the muscle homogenate. To save expense and time in cleaning the lid of the blender every time to avoid cross contamination, line the lid of the blender with a disposable plastic sheet (such as commercially available 16.5 × 14 cm sandwich bags are convenient to use).
3. Pour the tissue homogenate into a 1000 ml wide – mouth plastic jar with a disposable plastic liner. Make two labels for each jar with the type of tissue, and the animal no. using a good adhesive tape and water-resistant marker; transfer of one of these labels throughout the procedure helps to reduce mislabeling. Homogenates can be left at room temperature for 1 – 3 hrs until all specimens have been processed.

4. To the prewarmed (37°C) homogenate, add 250-ml of freshly prepared, prewarmed (37°C) acid pepsin solution (pepsin, 2.6 g; NaC1, 5.0 g; HCI, 7.0 ml and distilled water to make 500 ml, pH ~ 1.10 – 1.20). Incubate at 37°C in a shaking water bath for 60 min. The source and purity of pepsin used is probably not critical but porcine stomach pepsin (1:10000 biological activity, Sigma Chemical, St. Louis, MO, USA) has been used routinely and successfully.

5. Filter the homogenate through two layers of gauze and centrifuge 250 ml of filtered homogenate in a 250-ml wide-mouth polycarbonate centrifuge bottle (Specor, 9153 Industrial Court, Gaithersburg, MD 20877, USA) at 1200 × g for 10 min.

6. Pour off the supernatant. Depending on the tissue, fatty scum may stick to the rim of the centrifuge bottle. To prevent this, suspend the sediment in ~ 20 ml of phosphate buffered saline (PBS, pH 7.2) using disposable plastic pipettes. Transfer the homogenate in a 50-ml centrifuge tube with a conical bottom. Neutralize the homogenate with 12 to 15 ml of freshly prepared 1.2% sodium bicarbonate (pH ~ 8.3) with neutral red as a Ph indicator until the color changes to orange. After mixing, centrifuge at 1200 × g for 10 min.

7. Pour off the supernatant and add ~5-10 ml of saline that contains 1000 units penicillin and 100mg of streptomycin per ml.

*8. T. gondii* stages (tachyzoites, bradyzoites) in tissues are killed by water and by heating to 60°C (Jacobs *et al*., 1960b), and so blenders, cutting boards, and other materials can be cleaned with soap and hot water, then rinsed with cold water and finally with saline before using for the next specimen.

9. Inoculate 1 ml of tissue homogenate subcutaneously into each of 5 to 10 mice (Swiss-Webster weighing 20 – 25 g) over the back using a 4-cm long 21 – 23 gauge needle. It is preferable that each mouse is identified with a rodent ear tag (Ear tag size No. l, National Band and Tag, P.O. Box 430, Newport, KY 41092, USA). For additional identification, each mouse can also be marked with a streak of alcoholic picric acid in case the ear tag was lost accidentally.

10. Examine all inoculated mice for *T. gondii* infection as described (Dubey *et al.*, 1995; Dubey and Beattie, 1988).

**Appendix III: Forms and Questionnaires**

a) Blood sampling format for individual animals

Region …… Zone…… District ……PA……Date……..

| Owners name | Code No. | Sex | Age | Breed | Herd/Flock size | | | | Cont with cats | Water source | House. type | History of female Reprodu. Problem in the herd | | | | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cat. | She. | Goa. | Cam. | | | | Abortion | Stillbirt. | Neona. mortal. | Other | |
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b) Sera collection from domestic ruminant to test *T. gondii* antibodies using Latex for isolation of the parasite

| No. | Date | Place | ID No. | Species | Age (mons). | Sex | Breed | Latex status | | | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | +ve | -ve | Titer | |
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c) Data collection format of *T. gondii* cyst from mice brain after inoculation

| No. | Date | Mice ID | Date after IP inf. | Host type & ID | Wt. of brain | Solu.PBS | Cyst weight | Cyst size | Remark |
|---|---|---|---|---|---|---|---|---|---|
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d) Data collection format to monitor IP infected mice

| Date | Mice ID | Initl. Wt. | Final Wt. | Rough coat | Ascites | Wt .lose | dullness | Inappetance | Arched back | Tachycardia | Circling | Paralysis | Lethargy | Other signs | Remark |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | |
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**e) Questionnaire administered to animal owners**

Date _____

Code of respondents _____

1. Name_____ 2. Age _____ 3. Sex_____

4. Address a) Urban: Town _____ Kebele _____ House No. _____

b) Rural _____ PA _____ Village _____

5. Occupation a) Farmer _____ b) Civil servant _____ c) Others_____

6. What types of livestock species are kept in the area?

a) Sheep b) Goat c) Cattle d) Camel

e) Sheep and goat f) Sheep, goat and cattle g) Sheep, goat and camel

h) Sheep, goat, cattle and camel

7. Types of Animals and flock/herd size owned by the respondant.

a) Sheep b) Goat c) Cattle d) Camel

8. Purpose of raising ruminants:

8.1. Sheep

a) Milk b) Meat c) Draught power d) Draught mitigation

e) Milk and Meat f) Milk, Meat, draught power g) Milk, meat, and draught mitigation

8.2. Goat

a) Milk b) Meat c) Draught power d) Draught mitigation

e) Milk and Meat f) Milk, Meat, draught power g) Milk, meat, and draught mitigation

8.3. Cattle

a) Milk b) Meat c) Draught power d) Draught mitigation

e) Milk and Meat f) Milk, Meat, draught power g) Milk, meat, and draught mitigation

8.4. Camel

a) Milk b) Meat c) Draught power d) Draught mitigation

e) Milk and Meat f) Milk, Meat, draught power g) Milk, meat, and draught mitigation

9. Type of grazing system used a) Communal b) Individual c) both

10. Source of water for the animals

a) River b) Pond c) Well d) Spring e) Borehole

f) River and pond g) Pond and well h) River, pond and well

i) River, pond and borehole j) River, pond, well and borehole

11) How is night resting for animals?

a) Barn b) Fence c) Open field

12) What method of animal feed storage used?

a) Open sided building b) outside in the open air c) I n closed building

13) Are there rodents in the field? a) Yes _____ b) No _____

14) Which reproductive problems did you see in the past twelve months?

| | | | |
|---|---|---|---|
| In Sheep | a) Abortion | b) Stillbirth | c) neonatal mortality |
| In goats | a) Abortion | b) Stillbirth | c) Neonatal mortality |
| In Cattle | a) Abortion | b) Stillbirth | c) Neonatal mortality |
| In Camels | a) Abortion | b) Stillbirth | c) Neonatal mortality |

15) Do you hold cats in your house? a) Yes b) No

16) How do you manage owned cats in relation to other animals?

a) Separately and tied in pens b) Together with the animals c) Free and untied

17) Do you properly dispose cat feaces?

a) Yes b) No c) Not given attention d) Free and untied

18) Is there stray cats in the vicinity?

a) Yes b) No

19) Is there wild cats in the area?

a)Yes b) No

20) What disposal method of dead animals' body did you use?

a) Buried immediately b) burned immediately

c) Give to flesh eaters immediately

21) Do special care given to pregnant animals, like feeding and watering separately?

a) Yes b) No

**f) Questionnaire to study attributes of *T. gondii* infection in human**

1. Name/code of participant _____

2. Age _____

3. Sex _____

4. Address a) Urban city_____ Kebele _____ House No. _____

b) Rural _____ PA _____ Village _____

5. How do you consume meat?

a) Raw b) Cooked c) Both d) Raw dry

6. If the participant consumed raw meat, which animal meat?

a) Cattle b) Sheep c) Goat d) Camel

7. How do you consume milk?

a) Raw b) Boiled c) Both d) Femented milk/Yoghurt

8. How do you consume vegetables?

a) Raw b) Cooked c) Both

9. Do you hold cat in your home? a) Yes b) No

10. Purpose of holding cats in the house.

a) For clearing rodents b) As pet animals c) For both

11. How did you manage owned cats?

a) Separately and tied in pens b) Together with the house members

12. Do you properly dispose cats faeces?

a) Yes b) No c) Not given attention

13. Are there stray cats in the vicinity?

a) Yes b) No

14) What source of water used the family for drinking?

a) Tap water b) Hand pump c) stream water d) Well

e) Bore hole f) Other sources

15. If the respondent is female what is the current physiological status?

a) Pregnant b) Not Pregnant

16. If pregnant, what stage of pregnancy?

a) 1st trimester b) 2nd trimester ---------- c) 3rd trimester

17. Complications during delivery in the past 12 months

a) Yes b) No

**g) Subject information sheet and consent form on human *T. gondii* infection**

**Title of the project:** Serepidemiology of Toxoplasmosis in Domestic Ruminants, Public Health Significance and Isoolation of *Toxoplasma gondii* from Animal Tissues In Selected Districts of East Hararghe Zone of Oromia Region, Ethiopia.

**Principal investigator:** Berhanu Tilahun a student in Addis Ababa University, College of Veterinary Medicine and Agriculture.

**Introduction:** Toxoplasmosis is one of the important zoonotic disease affected all warm blooded animals and humans. The disease is very severe in pregnant women and immunocompromised people with a disease like HIV AIDS. This disease caused by protozoal parasite and transmitted to humans through consumption of raw meat, raw vegetables and contaminated water. It is also transmitted from mother to foetus. So you have been invited to participate in a study about the "public health significance of Toxoplasmosis in Eastern Hararghe" This study is being conducted by a PhD student from the Addis Ababa University, College of Veterinary Medicine and Agriculture in collaboration with the health beuro of Eastern Hararghe Zone and local partners.
This form provides you with information about the study. So reading the information below is very useful. More explanation can be given to you by the research participant professionals and can answer your questions before you decide whether or not to take part.

**Objective of the study:** The purpose of the study is to know the situation of toxoplasmosis in the communities of the study areas. Therefore, your participation will help us to achieve concrete information that can be used to improve the toxoplasmosis prevention and control program and to provide better health care in the area.

**Duration of the study:** The duration of the study is about three months and as part of this study you will be asked to give 3ml of blood drawn from your arm vein by trained health care professional using syringe and needle to conduct serological examination for *Toxoplasmosis,* also you will be requested to answer the questions focusing contacts with cats and about your feeding

habits of meat and milk. Total estimated time to participate in this study is approximately half an hour.

**Benefits of being in the study:** By participating in this study you will not receive any direct benefit, except the clinical service related to serological study.

**Potential risks of being in the study:** There are professionals well trained to take the minimum amount of blood to obtain the required test result without any risk on your health. However, there will be minor irritation and bleeding at the site of injection, but this conducted as it is done in any health center for similar activities. Also such condition is properly handled by the health professional.

**Right of the participant:** Your participation is entirely voluntary. You can refuse to participate without penalty or loss of benefits to which you are otherwise entitled. You can stop your participation at any time and your refusal will not have impact current or future relationships with the people or institutions carrying out this study. If you do not wish to participate, simply tell the researcher you wish to not participate or you can stop participation at any time during the process.

**Confidentiality and Privacy Protections:** The records of this study will be stored securely and kept confidential. All publications will exclude any information that will make it possible to identify you. But if you are found to have active toxoplasmosis health workers who are going to treat or diagnose will be informed about the findings of the examination for the purpose of your benefit to get treated and/or get appropriate advice.

**Contact address:** If you have any questions about the study, you will have an opportunity to ask the local health care worker involved in the study area. Also, in case if you have any unclear idea regarding to the issue you can use the following address.

Dr. Berhanu Tilahun: Addis Ababa University, College of Veterinary Medicine and Agriculture
Mobile No. 0911 01 18 81
Oromiya Health Bureau Tel. No. 0113 69 01 49
Consent Form

I, _____the undersigned, the above document describing the purpose, benefits, risks, and confidentiality for the research study on ‘’ the public health significance of toxoplasmosis” that is being conducted at/in _____ district, has been read and explained to me. Hence, I understood that the risks are minimal and may include only minor pain at the site of vein puncture during blood sample collections. I have also been informed that other people will not know my results as it is coded with number rather than writing my name. I understand that there may be no benefit to me personally apart from the clinical service related to serological studies. Also, I have been encouraged to ask questions about the above subject information and answered to me properly and understand that I have the right to withdraw from the study at any time with out in any way affecting my further life. Therefore, with full understanding of the importance of the study, I voluntarily agreed to participate in the study/research and confirm with my signature below.

Signature of the participant _____ Date _____

Kebele _____ Urban_____ Rural _____Village/ketena _____

## Appendix IV. Scope Of Future Studies

- The presence of *T. gondii* infection in domestic ruminants of EHZ necessitates future study on its impact on animal production and reproductive performance as well as its significance for the health of the inhabitants.

- The high risk for acquiring *T. gondii* infection identified among domestic ruminants and humans using pipe water in EHZ requires future study on the point and level of contamination with *T. gondii* oocysts.

- The increased level of anti-*T. gondii* IgM seropositivity found among women of child bearing age in EHZ either from current or reactivation of latent infection calls for future study on the status of congenital toxoplasmosis during pregnancy.

- Isolation and molecular characterization of *T. gondii* isolates should be performed to better understand the diversity and structure of *T. gondii* population inEHZ.

## Appendix V: Curriculum Vitae

### 1. Bio data


Name: Berhanu Tilahun Chefek
Nationality: Ethiopian
Sex: Male
Date of birth: Nov. 27, 1959
Place of birth: Harar
Marital status: Married
Adress: Mobile: + 251911011881,
Email address: btilahun11@gmail.com
berhanutilahun@ymail.com

## 2. Educational background

### 2.1. Elementary and High School Education

1967-1971 Elenentary education completed at Harar 2nd Model school

1972-1973 Junior secondary schools completed at Harar Hailesilasie 1st Elementary School

1974-1978 High school completed at Harar Secondary School

### 2.2. Higher Education

1979-1980 Awarded Diploma in Animal health from Debrezeite Assistant Veterinarian Training College.

1983-1989 Awarded Degree of Doctor of Veterinary medicine/DVM/from Kishinov Agricultural institute of the former USSR /MSSR/.

2005-2006 Awarded MSc degree in Tropical Epidemiology from Addis Ababa University Faculty of Veterinary Medicine.

## 3. Thesis

Berhanu Tilahun (1989). Cattle Eimeriosis and its control in Ketros. DVM thesis, Kishinov Agricultural Institute of the former USSR/MSSR/.

Berhanu Tilahun (2006). Camel brucellosis and management at Babile and Jijiga districts, Eastern Ethiopia, MSc thesis, FVM, AAU.

Berhanu Tilahun (2015). Seroepidemiology of toxoplasmosis in domestic ruminants, public health significance, isolation and characterization of *Toxoplasma gondii* isolate from selected districts of Eastern Hararghe zone in Oromia region, Ethiopia, PhD dissertation, CVMA, AAU.

**4. Research Publications in Peer Reviwed Scientific Journals**

Tilahun B., Bekana M., Belihu K and E.2013.Camel brucellosis and management practices in Jijiga and Babile districts, Eastern Ethiopia. Journal of Veterinary Medicine and Animal Health Vol. 5(3), pp.81-86 DOI.10.5897/JVMAH 2013. 0206

Tilahun B., Yacob H., Tilahun G., Hagos. A., Vitale M., DI Marco V and Gebremedhin E.Z.2015. Seroprevalence and risk factors of Toxoplasma gondii infection in humans in East Hararghe Zone, Ethiopia.Cambrige University press. 2015. doi:10.1017/S0950268815001284

**Short Communication**

Shimelis S., Gebremeskel M., Abdella A. and Tilahun B. (2013): A bilobed Gallbladder (Vesica Fellea Divisa) in Cattle Slaughtered at Jimma Municipal Abattoir, West Oromiya, Ethiopia, Ethiop.Vet.J. 106*, 17(1), 105-108*

**5. Work Experience**

**From 1980 to 1983**

- Worked as Assistant Veterinarian in various provinces under the Ministry of Agriculture taking the following responsibilities.
- Organizing Veterinary activities at field, assessing outbreaks, reporting and participating in disease controlling program.
-

**From 1990 to 1994**

- Served as zonal head of Animal health section in South East Range land project /SERP/ at Gursum district. Organizing and supervising field veterinary activities, controlling and supervising the proper utilization of medical instruments and drugs and participating in treating sick animals in the clinic as well as at field.

**From 1994 to 1996**

- Worked at the position of zonal project coordinator in addition to the responsibility of head of Animal health
- 2001-2003

- Served as district head of veterinary team under Gursum MoARD office from September 2003–September 2005
- Served as the coordinator in the Pan African Control de Epizootics Harar branch office.
- Since 2006
- I have been working at Haramaya University College of Veterinary Medicine as an instructor for various disciplines.

**4. Others**

Attended the advanced level training course in goat medicine at Diredawa veterinary laboratory organized by FARM Africa dairy goat project unit from Nov.29 – Dec.14, 1995.
Attended a participatory rural appraisal (PRA) training in Jijiga organized by SCF,UK and SERP from august 23 – sep. 1, 1995.
Attended a training program on participatory disease search (PDS) organized by PACE in Dolo-odo from March 19 – 25, 2004

**5. Language ability**

Amharic, Oromifa, English and Russian languages – Reading, writing and speaking

**6. References**

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