ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SEROPREVALENCE STUDY OF TOXOPLASMOSIS IN SMALL RUMINANTS AND HUMANS (HIV/AIDS PATIENTS) IN SELECTED DISTRICTS OF SOUTH WOLLO, ETHIOPIA

BY
YIBELTAL MUHIE MEKONNEN

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A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Veterinary Science in Tropical Veterinary Public Health

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ABBREVIATIONS

AIDS= Acquire Immunodeficiency Syndrome

CFT= Complement Fixation Test

DA= Direct Agglutination test

ELISA= Enzyme-Linked Immunosorbent Assay

HIV= Human immunodeficiency viruse

IgG= Immuno-globulin G

IFAT= Indirect Immunofluorescence Antibody test

IHAT= Indirect Hem-Agglutination Test

MDAT= Modified direct Agglutination Test

PBS= Phosphate Buffer Saline

SFDT= Sabin-Feldman Dye Test

VCT= Volunteer counseling and test center

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ABSTRACT

Toxoplasma gondii has been known to be responsible for abortion, still birth and birth of weak offsprings in susceptible hosts such as sheep and goats and cause subsequent economic losses. The parasite also emerged as a serious opportunistic infection associated with acquired immune deficiency syndrome (HIV/AIDS) in humans. A cross-sectional study on toxoplasmosis in small ruminants and humans (HIV/AIDS patients) were conducted in selected districts of South Wollo, Ethiopia between October 2007 and April 2008. Serological survey of antibodies to *T. gondii* was carried out using modified Direct Agglutination test (MAT) on sheep, goats and humans. Of 426 sheep and 86 goats serum samples analyzed, 45.4% and 37.2% of sheep and goats respectively were found to be positive for anti-*T. gondii* IgG. Higher seroprevalence of toxoplasmosis 49.2% was recorded in Legambo Woreda than in Dessie Municipality Abattoir 28.9%. The sex and age-specific prevalences were found to be higher in females and older animals than in males and younger ones in both species. History of abortion, neonatal mortality and weak births were significantly associated in sheep (P= 0.000) associated with toxoplasmosis and abortion was the most common problems in sheep and goats in the study area. Of the 260 human serum samples analyzed by Modified Direct Agglutination Test (MAT), seropositivity to toxoplasmosis was found in 76.5%. Out of all of HIV-positive sera, 89.6% were positive for anti-*T. gondii* IgG. There was significant association between seroprevalence of toxoplasmosis and HIV infection (P= 0.000). The risk factors associated with seropositivity to *Toxoplasma* infection were consumption of raw/under cooked mutton (P= 0.001) and presence of cat (P= 0.038) in the house holds and more than 68% of the respondents had a history of consumption of raw meat and 25% had close contact with cats. The high seroprevalence of *T. gondii* antibodies found in the present study suggested widespread exposure of sheep, goats and humans to *T. gondii*. Therefore, economic impact of toxoplasmosis in small ruminants should be investigated in detail and early detection of the presence of toxoplasmosis in HIV/ADIS patients is helpful for the initiation of early treatment schemes. Veterinarians should provide advice on possible minimization of contact with cats and avoidance of eating raw or undercooked meat.

Key words: Ethiopia, Goats, Human, MAT, Sheep, South Wollo, *Toxoplasma gondii*

1. INTRODUCTION

Toxoplasmosis is a worldwide zoonosis of increasing concern in both human and veterinary medicine (Tenter *et al.,* 2000; Dubey *et al.,* 2003). The disease is caused by the obligate intracellular organism, *Toxoplasma gondii* (Urquhart *et al.,* 1996; Van Der Puije *et al*., 2000; Naoi and Yano, 2002; Acha and Szyfres, 2003; Sawadogo *et al.,* 2005). Cats and other felids are the only definitive hosts of *Toxoplasma* and warm-blooded animals and humans are intermediate hosts (Dubey, 2004; Sukthana, 2006).

Prenatal problems such as abortion and neonatal mortality in sheep and goats are the major clinical manifestations of infection with *Toxoplasma gondii* and resulted when primary infection occurs during pregnancy. Ovine abortion and neonatal mortality due to toxoplasmosis have been observed in New Zealand, Australia, Canada, United States and in the United Kingdom and in most countries are second in importance to Chlamydial abortion (Radostits *et al*., 1994). A report from Uruguay indicated an annual economic loss of 2 to 5 million US dollars due to abortion and neonatal mortality in sheep caused by toxoplasmosis (Freyre *et al*., 1996). Therefore, the infection has an economic and clinical significance in many sheep and goat producing countries.

Under Ethiopian conditions the most common reproductive problems in small ruminants are abortions, stillbirth, prenatal and neonatal lamb or kid mortality. Over all post natal lamb and kid mortality ranges from about 12% to 68% **(**Kassali *et al*., 1993). The causes of these reproductive wastages have not been elucidated but, it has been established from various countries that toxoplasmosis is one of the major reproductive problems in small ruminants. In Ethiopia few studies carried out previously indicated that prevalence rates ranging from 11% to 56% in sheep and 22.9 to 74.8% in goats (Kassli and Tekelye, 1989; Tilaye and Getachew, 2002; Negash *et al*., 2004; Teshale *et al*., 2007), and in humans 74.4% (Guebre-Xabier *et al*., 1993) and 96.77% (Yimer *et al*., 2005).

In humans, infection with *T. gondii* is chronic and largely asymptomatic; though it may cause stillbirth, blindness, mental retardation and occasional death of congenitally infected infants (Van Der Puije *et al*., 2000). The infection can be acquired both through ingestion of tissue cysts in

under cooked meat and via ingestion of oocysts excreted from the definitive host of *T. gondii* (cat) (Soulsby, 1982; Lucas, 1993; Jones *et al*., 2003). Recently the parasite has emerged as a serious opportunistic infection associated with acquired immune deficiency syndrome (AIDS) (Bisson *et al*., 2000).

In Ethiopia 1.6 million of people living with HIV/AIDS, about 104,000 of them were children age below 15 years. The estimated number of new AIDS cases in the adult population in 2003 was about 105,000 while that in children was about 27,000. About 98,000 adults and 27,000 children have died of AIDS (Berehane *et al*., 2006). Sero-epidemiological study of 6,234 commercial sex workers throughout Ethiopia revealed infection rates between 1.3% and 38.1% in different towns. Rates between 14.7% and 38.1% were found in 14 towns, including Dessie (38.1%), Bahir Dar (35.9%), Mekele (24.1%) and Gondar (14.7%) (Berehane *et al*., 2006).

As in most other African countries, little is known about the natural history of HIV infection in Ethiopia and most of these patients die due to undiagnosed neurological and pneumonic cases each year. There are also a number of mentally retarded children and many others with impaired vision who need special care. The cause of most of these ailments is not investigated. Toxoplasmosis is no doubt to be one of the possible causes of such abnormality. The wide spread nature of toxoplasmosis in animals is a risk factor for human toxoplasmosis as meat from animals is a source of infection for humans. However, detail epidemiological study has not been conducted in Ethiopia in general and no study was carried out in the current study area at all. Therefore the objectives of the present study were to determine the prevalence rate of *T. gondii* in small ruminants and human (HIV/AIDS patients) and to identify risk factors associated with the seroprevalence of *T. gondii.*

2. LITERATURE REVIEW

2.1. *Toxoplasma gondii*

Toxoplasma gondii is an obligate intracellular protozoan pathogen that can cause severe and devastating disease (Michael and John, 2000). The parasite *T. gondii* is classified under kingdom protista, sub-kingdom protozoa, phylum apicomplexa, class conoidasida, order eucoccidiorida, sub-order eimeriorina, family sarcocystidae, sub-family toxoplsmatinae, genus *Toxoplasma* and species *Toxoplasma gondii* (Tenter *et al*., 2002). *Toxoplasma gondii* has nucleus most clearly demonstrated with Giemsa stain, located near one pole of the cell. Its cytoplasm contains mitocondria, microtubules, endoplasmic reticulum, ribosomes, golgi apparatus and rhoptries which are useful in differentiating various Sarcosystidae (Jones *et al.*, 2001). *Toxoplasma gondii* has three infective stages, tachyzoites (endozoites) individual and in-group, bradyzoites (cystozoites) in tissue and sporozoites in sporulated oocysts (Dubey *et al*., 1998).

Tachyzoites are often crescent (banana) shaped and 2μm x 6 μm in size. Tissue cysts vary in size from 5 μm to 7 μm and contain few to several hundred bradyzoites (Dubey *et al*., 1998; Acha and Szyfres, 2003). Bradyzoites differ only slightly from tachyzoites in having a nucleus situated towards the posterior end whereas the nucleus in tachyzoites is more central. Besides, bradyzoites are more slender than tachyzoites and less susceptible to distraction by poroteolytic enzymes. Oocysts in freshly passed feces are unsporulated (none infective) and sub spherical to spherical in shape and 10 μm to 12 μm in diameter (Tenter *et al*., 2000; Acha and Szyfres, 2003). The sporulated oocyst contains two ellipsoidal sporocysts. Each sporocyst contains four sprozoites. The sporozoites are 2 μm x 6-8 μm in size (Dubey *et al.*, 1998; Dubey and Frenkel, 1972).

More recently molecular epidemiological studies have provided evidence that there are three clonal lineages within *T. gondii*, namely Type I, Type II, and Type III. Type I kills mice in less than 7 days (acute virulence in mice) where as those of type II induce chronic pathology in susceptible strains of mice and type III is least virulent (Alexander *et al.,* 2000; Dubey, *et al*., 2003). In humans, the type I is more frequently associated with congenital infection whereas the type II is common in patients in whom disease has reactivated (Alexander *et al.*, 2000).

2.2. Life cycle of *Toxoplasma gondii*

The exact life cycle of *T. gondii* was elucidated in 1970 by Frenkel *et al*. (1995). *Toxoplasma* has two cycle of development, namely, the entroepitelial and extraintestinal, which can occur in the definitive (feline) and intermediate (probably a wide range of animal species) hosts, respectively (Soulsby, 1982).

Enteroepithelial cycle: This is only in the intestinal epithelium of the feline final host. It begins with the ingestion of organs containing viable parasites in the tissue cysts, although direct transmission of oocysts between cats can also occur (Rottman, 1998; Urquhart *et al*., 1996).

Following ingestion, the cyst wall is digested by proteolytic enzymes in the cat's stomach and small intestine and the released bradyzoites then initiate a cycle of schizogonous development in the intestinal epithelium, which result in the formation of schizonts and gamonts, respectively. The gamonts or reproductive cells are responsible for the appearance of sexual stages and contain microgametes and macrogametes (Beverley, 1974; Urquhart *et al*., 1996; Wilkinson, 1984).

The microgamets fertilize the macrogamet resulting in the formation of oocysts. The oocyts are then excreted in the cat's feces. After a sporulation process which is completed within 1-5 days under favorable conditions, two sporocysts each of which containing 4 sporozoites are formed and are infective to the susceptible host (Beverley, 1974; Urquhart *et al*., 1996; Rottman, 1998).

Extraintestinal cycle**:** This commences with the ingestion of either sporulated oocysts or tissue containing cysts by the intermediate hosts and undergoes two phases of asexual development. In the first phase, tachyzoites (or endozoites) multiply rapidly (tachy means fast; zoite means organism) by repeated endodyogeny in many different types of host cells and are responsible for congenital infections (Tenter *et al*., 2000; Jones *et al*., 2003; Urquhart *et al*.,1996; Wilkison, 1984). Tachyzoites of the last generation initiate the second phase of development, which results in the formation of tissue cysts. The encysted stage (tissue cyst) contains relatively slowly multiplying organisms called bradyzoites (or cystozoites). The tissue cyst is a dead end phase

(resting form) of the parasite in the intermediate hosts. Tissue cysts have a high affinity for neural, occular and muscular tissues. They are located predominantly in the central nervous system (CNS), the eye as well as skeletal and cardiac muscles. However, to a lesser extent they may also be found in visceral organs, such as lungs, liver, and kidneys. Tissue cysts are the terminal life-cycle stage in the intermediate host and are immediately infectious. In some intermediate host species, they may persist for the life of the host (Bowmann, 1995; Frenkle, 1990; Hokelek and Safdar, 2004; Rottman, 1998; Singh, 2003; Tenter *et al*., 2000). The life cycle is completed when tissue cysts are ingested by the final host with subsequent development of the oocysts (Fig. 1).

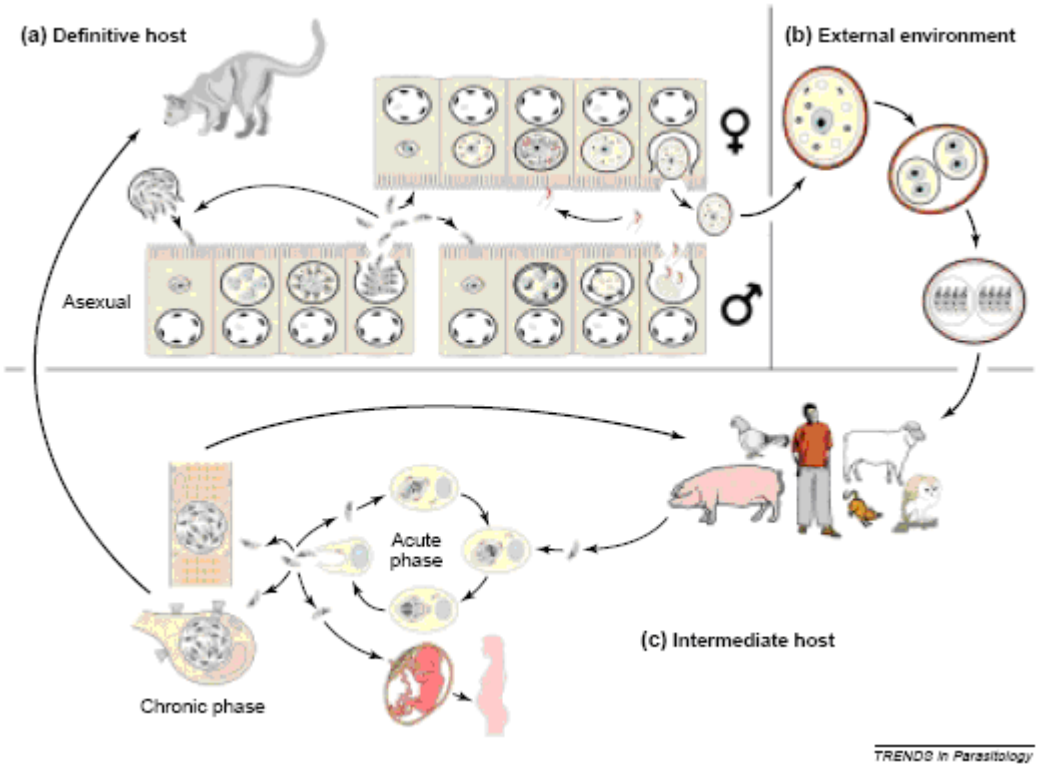

Figure 1: Life cycle of *Toxoplasma gondii*

Source: Ferguson (2002)

2.3. Epidemiology

2.3.1. Geographic distribution

Toxoplasmosis is a true zoonosis occurring naturally in man, domesticated and wild animals and birds throughout the world. However, with higher prevalence found in warm and moist areas than in cool or hot and dry areas (Dubey, 1988). Seroprevalence data on its distribution indicates that a high incidence may occur in particular areas depending on the presence of cats and suitability of environmental conditions such as temperature, humidity and aeration (McCulloch and Remington, 1975; Radostits *et al.*, 1994). Islands with geographical isolation and absence of cats have been found to be free of toxoplasmosis. Otherwise *T. gondii* is known to occur in all continents and countries of the world (Acha and Szyfres, 2003).

2.3.2. Occurrence of toxoplasmosis

The infection has been confirmed in some 200 species of vertebrates, including primates, ruminants, swine, equine, carnivores, rodents marsupials, insectivores, and numerous avian species. In general among domestic animals high reactor rates have been found in cats, sheep, goats and swine; lower levels in horse and dogs; and low level in cattle (Acha and Szyfres, 2003). Several studies indicated that *T. gondii* with higher prevalence has been recorded in defferent countries of the world shown in (Table 1) Tenter *et al* (2000). Domestic cats have a key role in the epidemiology of *T. gondii* infection. Depending on the host species, the geographic area and the season of the year, up to 73% of small rodents and up to 71% of wild birds might be infected with *T. gondii*. In addition to this, the prevalence of *T. gondii* in feral cats is very high, when compared to owned cats, as they are more engaged at predating rodents and birds (Dubey *et al.*, 2002; Sukthana, 2006).

Table 1: Seroprevalence of *Toxoplasma gondii* infection in different species of animals

Species	Country	Year	Prevalence (%)	Test used
Cattle/Buffaloes	Italy	1993	92	DAT
Swine	Germany	1962-64	12-97	SFDT
Domestic/wild fowls				
Chickens	India	1998	40	MAT
Ducks	China	1995	4	-
Pigeons	Iran	1990	33	IHAT
Turkeys	Iran	1990	24	IHAT
Wild turkey	USA	1994	71	MAT
Sheep	France	1997	92	IFAT
Goats	France	1997	0-72	ELISA
Horses	Brazil	1994-96	32	IFAT
Rabbits	Czech Republic	1981-86	53	SFDT
Dogs	Brazil	1999	84	IFAT
Cats	USA	1992	74	ELISA

Source: Tenter *et al* (2000)

In Europe, parasitism rates in excess of 50% have been found in the meat of sheep and swine slaughtered in abattoirs (Acha and Szyfres, 2003). Most infection in animals is clinically unapparent and cases are occurring sporadically, with the following exceptions in sheep and goats in which congenital infection is common and in swine there have been infrequent epizootic outbreaks in several parts of the world. Dogs can develop manifestations that can be mistaken for distemper, but such cases are rare. The greatest damage caused by toxoplasmosis in sheep and goats, and sometimes swine, is abortion and the birth of infected offspring, in which prenatal fatality can be as high as 50% (Acha and Szyfres, 2003).

In Ethiopia the prevalence of *T. gondii* is not well studied. There are only few studies conducted in animals and the highest prevalence was reported in goat 82% (Teshale *et al* 2007) and the lowest in cattle (6.6%) by Kassali and Tekely (1989) and 96.77% of human infection in Addis Ababa abattoir worker (Yimer *et al*., 2005) (Table 2).

Table 2: Prevalence of *Toxoplasma gondii* in Ethiopia.

Study area	Prevalence (%)				Test used	References
	Cattle	Sheep	Goat	Humans		
Central Ethiopia	6.6	11.9	22.9	-	MDAT	Kassali and Tekely (1989)
Debre Birhan	-	35	34	-	MDAT	Tilaye and Getachew (2002)
Nazareth	-	52.6	24	-	MDAT	Negash *et al* (2004)
Nazareth	-	56	25.9	-	ELISA	Negash *et al* (2004)
Various parts of Ethiopia	-	-	-	74.4	ELISA	Guere-Xabier *et al* (1993)
Addis Ababa				96.77	ELISA	Yimer *et al* (2005)
South Omo			82		MAT	Teshale *et al* (2007)
North Omo			79.5		MAT	Teshale *et al* (2007)
East Shewa Zone			62.2		MAT	Teshale *et al* (2007)

2.3.3. Source of infection and mode of transmission

Source of infection

Three important sources of toxoplasmosis are known, this include felines (domestic and wild cats), the environment (soil) and intermediate hosts (Frenkel, 1990). Infected cats shedding oocysts in their feces are the central source of infection. Millions of *Toxoplasma* oocysts may be shed in a single stool and their survival depends on climatic and soil conditions. Oocysts could persist in moist soil for several months (McCulloch and Remington, 1975; Frenkel, 1990).

Fresh meat or carcasses of a wide range of intermediate hosts containing viable parasites in tissue cysts are source of infection for carnivores. Pasture, or hay or stored feeds contaminated with cat feces are the main sources of infection for herbivores (Frenkel, 1990; Radostits *et al*., 1994; Rottman, 1998). Among meat producing animals, pigs, sheep and goats relatively often harbour *Toxoplasma gondii* in edible tissues and, therefore, raw or under cooked meat for these animals constitutes a major risk of infection for humans. On the other hand, as cattle appears to be

naturally resistant and capable of clearing the infection, beef is generally not regarded as an important source of human infection (Figure 2) (Quinn *et al*., 1994).

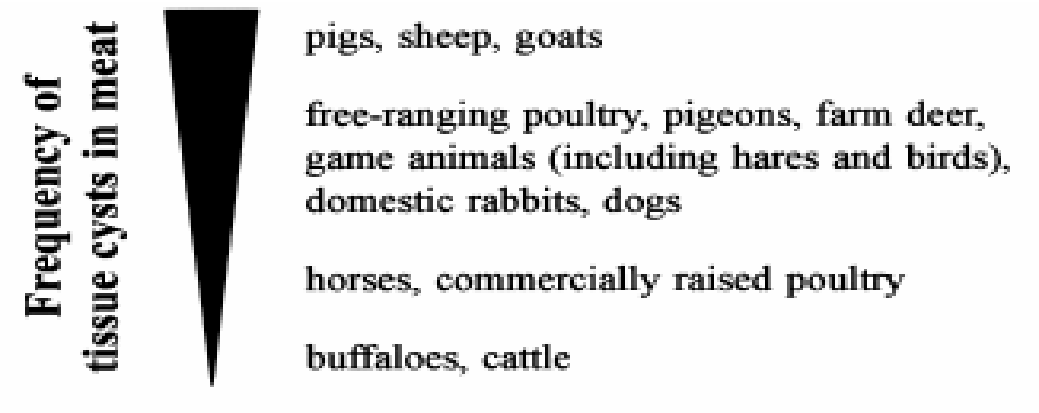

Figure 2: Relative importance of meat-producing and game animals in the transmission of *Toxoplasma gondii* to human

Source: Tenter *et al* (2000)

In Europe and in the USA, pork has generally been considered to be a major source of *T. gondii* infection in humans. This hypothesis is based on the fact that tissue cysts have been found in most commercial cuts of pork (Tenter *et al*., 2000). In areas where goat's milk utilized, unpasteurized milk from acutely diseased goats is also an important source of infection, especially for children. Although tachyzoites in the milk are likely to be destroyed by gastric juices, penetration through sores in the oral mucosa might occur (Frenkel, 1990; Smith and Sherman, 1994).

Mode of transmission

It has been stated that *Toxoplasma* can enter by almost any portal such as conjunctival, nasal, pharyngeal, respiratory and percutaneous route (Beverley, 1974). Humans and animals, including cats could acquire toxoplasmosis by consumption of organs containing tachyzoites or bradyzoites, feed and water contaminated with sporulated oocysts. Besides, oral infection by oocysts and tissue cysts, *Toxoplasma gondii* could also be transmitted transplacentally from mother to the offspring if the infection is contracted during pregnancy (Beverley, 1974; Urquhart *et al*., 1996; Smith and Sherman, 1994; Rottman, 1998).

Ingestion of fresh milk form acutely infected goats has also been implicated as one method of transmission. However, this mode is probably of little significance. The same applies to the risk of infection when assisting infected ewes or does at parturition and handling of raw meat (Beverly, 1974; Wilkison, 1984; Smith and Sherman, 1994). Tachyzoites are sensitive to proteolytic enzymes and usually are destroyed by gastric digestion however; tachyzoites may occasionally survive for a short period of time (up to 2 h) in acid pepsin solutions so it can be transmitted in the milk from the mother to the offspring (Tenter *et al.,* 2000). A number of authors have suggested the possibility of transmission by blood or leucocytes transfusion and with an organ transplant containing viable tissue cyst (McLeod and Ramington, 1980).

Most *T. gondii* infections among humans occur in one of three ways: (1) by eating raw or undercooked meat containing *T. gondii* tissue cysts or eating food that has been cross-contaminated with raw or undercooked meat; (2) by ingesting oocysts from soil (for example, through gardening, handling, eating unwashed vegetables, or changing a cat litter box) or (3) by acquiring congenital infection through the placenta (Fig. 3) (Jones *et al*., 2001; Jones *et al*., 2003; Sukthana, 2006). Of the estimated 750 deaths caused by toxoplasmosis in the United States each year, 375 are thought to occur from eating raw or undercooked meat; this makes toxoplasmosis the third-leading cause of US food borne deaths (Jones *et al*., 2001).

Recent epidemiological studies have identified the following risk factors for *T. gondii* infection: owning a cat, cleaning a cat litter box, eating raw or undercooked pork, mutton, lamb, beef, or minced-meat products, gardening, eating raw or unwashed vegetables or fruits, eating raw vegetables outside the home, having contact with soil, washing kitchen knives infrequently, having poor hand hygiene, and drinking municipal water from a contaminated reservoir (Jones *et al*., 2001; Jones *et al*., 2003).

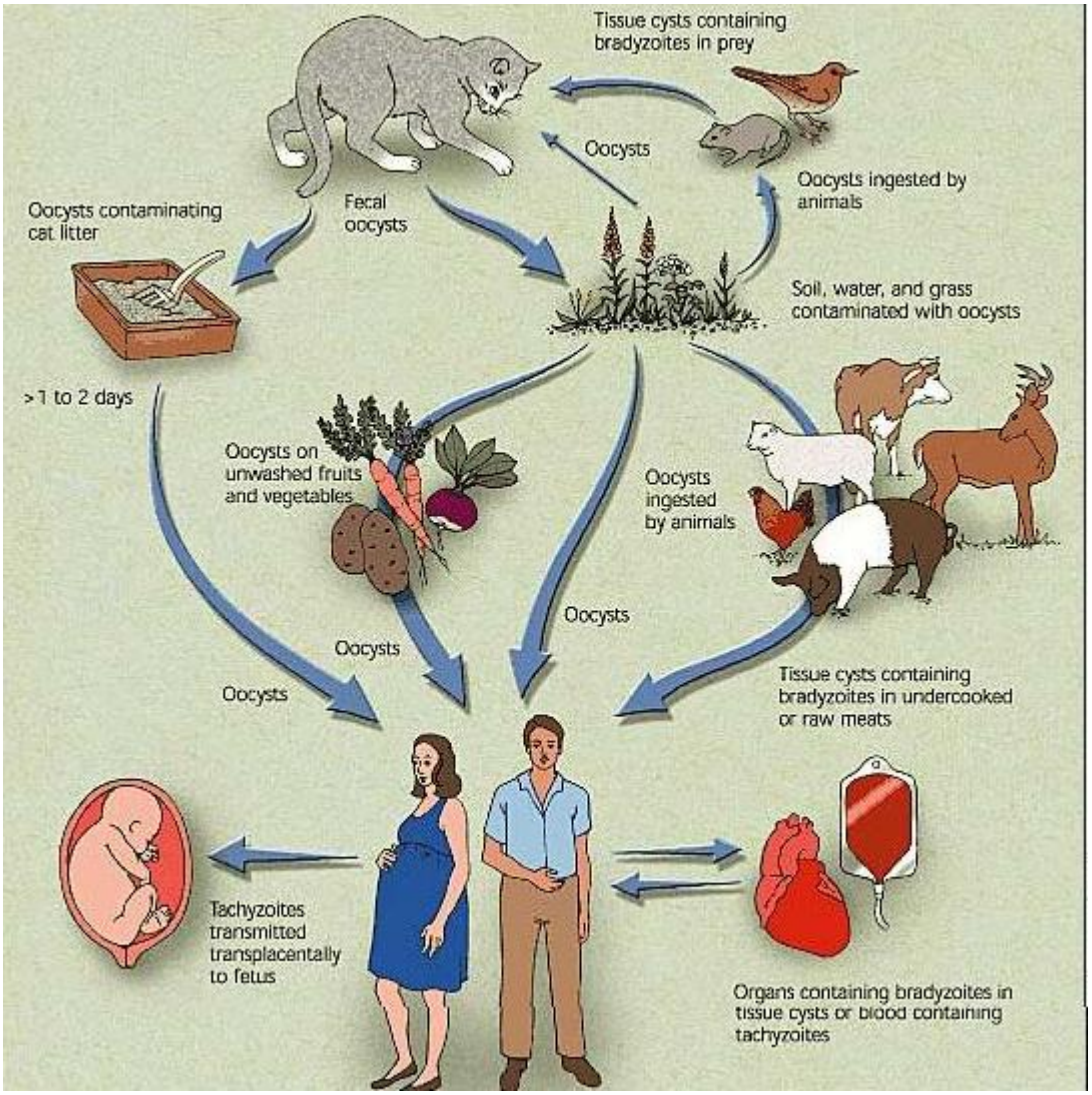

Figure 3: Major routes of transmission of *Toxoplasma gondii* infection

Source: Jones *et al* (2003)

2.5. Pathogenesis

The pathogenicity of *Toxoplasma gondii* is related to the virulency of the strain, species, age and condition of the hosts (Smith and Sherman, 1994). *Toxoplasma gondii* possess the ability to invade and to establish productive infection in almost any nucleated cell (Alexander *et al*., 2000).

Following ingestion, sporozoites from oocysts or bradyzoites from tissue cysts penetrate and multiply in the intestinal epithelium or submucosa and are transferred to tachyzoites. The

tachyzoites then infect tissues throughout the body and replicate intracellularly until the cell bursts, casing tissue necrosis. The librated *Toxoplasma* tachyzoites as a result of rupture of the cell reach other organs through the bloodstream. This stage of parasitemia commences approximately 5 days after initial infection and is associated with fever, increased respiratory rate and loss of appetite and lasts until the development of immunity 2-3 weeks after infection, at which stage the organism localizes in the tissue cysts (Radostits *et al*., 1994; Rottman, 1998).

The clinical character of the disease varies with the organs attacked, which it self varies depending on whether the disease congenital or acquired. However, the vast majority of infections occur without any clinical signs and tissue cysts can be found in many animals and appear to cause no harm (Radostits *et al*., 1994).

When the immunity of the animals fall because of stresses, diseases or immunosuppressive therapy, tissue cysts rupture and the parasites are librated and large number of inflammatory cells invade the surrounding tissue. The characteristic granulomatous lesions observed are thought to be the result of a hypersensitivity reaction (Radostits *et al*., 1994).

In pregnant ewe and doe as the parasites spread throughout body, the placenta and subsequently the fetus may become infected. Parasite multiplication in the placentoms causes focal necrosis and inflammatory reaction. The clinical outcome of infection depends of stage of gestation. Infection acquired early in gestation may result in embryonic death and resorption. Infection during mid pregnancy is also often fatal. Infected fetuses may die in utero, and be aborted or retained and mummified, or they may survive for several weeks, only to be stillborn close to term. However, infection in late pregnancy is rarely fatal, and such lambs or kids are usually born healthy though infected (Radostits *et al*., 1994; Smith and Sherman, 1994).

2.6. Clinical Signs

The tachyzoite is the stage responsible for tissue damage; therefore, clinical sings depend on the number of tachyzoites released, the ability of the host immune system to limit tachyzoites spread, and organs damaged by the tachyzoites. The clinical syndromes also vary between different species and age groups (Radostits *et al*., 1994; Rottman, 1998).

Cat

Cat also high infection rate and replication in the gut is usually asymptomatic, but may rarely cause mild diarrhoea. Tissue cysts of *T. gondii* usually cause no clinical signs in cats (Jones *et al.*, 2003; Soulsby, 1982). Acute and subacute infection occurs mainly in young cats and gives rise to prolonged pyrexia. Most cats may show dyspnoea, cough, icterus, diarrhea, seizures, depression, ascites and death (Jones *et al.*, 2003; Soulsby, 1982; Wilkison, 1984). Chronic infections occur chiefly in older cats. Sings of weight loss, fever, diarrhea, anemia, ataxia, cough, loss of vision and muscle hyperesthesia may be observed (Wilkison, 1984).

Sheep

Although a syndrome of fever, dyspnoea and generalized tremor are seen, the principal manifestations of toxoplasmosis in pregnant ewes are abortions, birth of mummified fetus or stillborn lambs and neonatal death (Urquhart *et al*., 1996; Radostits *et al*., 1994). In Tasmania, Australia, *T. gondii*, was believed to be the etiology in 46% of the outbreaks of abortion and neonatal mortality in sheep between in 1962 and 1968 (Acha and Szyfres, 2003). Congenitally infected lambs lack muscular coordination, they are physically weak, and they are unable to feed themselves (suckling disorder). Congenital toxoplasmosis occurs in lambs only when the ewe is infected during pregnancy. When the fetus is infected between days 45 and 55 of gestation, it usually dies; if the infection is acquired in the third month of pregnancy, the lambs are born but they are sick (Acha and Szyfres, 2003).

Goats

Among domestic food animals, *Toxoplasma gondii* is most pathogenic for goats. Unlike in sheep, *Toxoplasma gondii* can cause encephalitis, nephritis, abomasitis, enteritis and cystitis in adult

goats. Abortion, stillbirth, or birth of weak and non viable kids is the manifestations of clinical toxoplasmosis in pregnant does (Dubey, 1990).

Man

Approximately one-third of world human population has serum antibodies to *T. gondii*. The reported prevalence among general population elsewhere in the world varies from 0% in Eskimos to 94% in Costa Rican and Guatemalan people (Abu-Zeid, 2002; Singh, 2003; Bhopale, 2003). Most cases of *T. gondii* infections in immunocompetent humans are asymptomatic. In immunocompromised humans a previously acquired latent infection can lead to reactivated toxoplasmosis with encephalitis and cause severe disease (Singh, 2003).

Human beings could acquire toxoplasmosis either congenitally, or through the ingestion of food items containing *Toxoplasma* materials, or through organ transplant and blood transfusion. The infection is usually asymptomatic, but a small minority develops one of the following clinical syndromes (McLeod and Remington, 1980).

Congenital toxoplasmosis occurs in approximately one-third of infants born to mothers who acquire infection during pregnancy. In infants born to mother infected during the first trimester, congenital infection is least common (17%), but disease is most sever and results in abortion. However, in infants born to mothers infected during the third trimester, congenital infection is most common (65%), but is usually asymptomatic (McLeod and Remington, 1980; Lucas, 1992). The classic tetrad of congenital toxoplasmosis includes hydrocephalus or microencephaly, chorioretinitis, intracerebral calcification, mental retardation and loss of hearing (Lucas, 1992; Singh, 2003). Loss of vision is the most common sequela in congenitally infected children (Singh, 2003).

Acute acquired toxoplasmosis is asymptomatic; however, in immunologically competent individuals affected with virulent strain lymphadenopathy is the most common recognized clinical manifestation. Cervical lymph nodes are involved most frequently. When mesenteric or retreoperitoneal nodes are involved abdominal pain and significant temperature elevation may occur. Malaise, myalgias, headache, sore throat, maculopapular rash (which spares the palm and

soles), hepathosplenomegaly, pneumonia, convelsion, depression, stiffness of the muscle and pain may be observed (McCulloch and Remington, 1975; McLeod and Remington, 1980; Frenkel, 1990; Lucas, 1992).

Ocular toxoplasmosis has been estimated to be the cause of approximately 35% of cases of chorioretinitis in children and adults. Although chorioretinitis has estimated to occur in approximately 1% of patients with the acute acquired infection, ocular disease is most often a consequence of congenital *Toxoplasma* infection. Blurred vision, scotomas, pain, photophobia, or epiphora, may be due to active chorioretinitis. If the macula is involved, impairment or loss of central vision may lead to blindness (McLeod and Remington, 1980; Lucas, 1992).

Toxoplasmosis of immunocompromised patient is the form of toxoplasmosis occurs in immunocompromised patients receiving immunosuppressive therapy (such as corticosteroids) for lymphoproliferative disorder, for hematologic malignancy, or for prevention of organ graft rejection have the greatest predilection for life-threatening toxoplasmosis. The untreated infection in these patients is frequently culminant and rapidly fatal (McLeod and Remington, 1980).

People who have previously acquired toxoplasmosis, with or without manifestation of the disease, may suffer a devastating relapse if their immune defenses, particularly cell mediated immunity, are impaired as in the case of AIDS (McLeod and Remington, 1980). Reactivation of the infection has greatest impact in late AIDS, where up to 25% of patients will develop toxoplasmic encephalitis (TE), characterized by unrestricted replication of the tachyzoites stage of the parasite this is manly associated with the loss of T-cell function (Alexander *et al*., 2000).

Central nervous system involvement, present in more than 50% of documented cases, is the most characteristic clinical feature of toxoplasmosis in immunocompromised patients. Symptoms are manifestation of diffuse encephalopathy, meningoecephalitis, or cerebral mass lesions and include changes in mental status, headache and seizures (McLeod and Remington, 1980; Lucas, 1992).

2.7. Immunity against *Toxoplasma gondii*

Immunity to *Toxoplasma gondii* is mediated by both humoral and cellular mechanisms; since *T. gondii* is obligate intracellular parasite whose tachyzoites grow within the infected cells; cell-mediated immune response has a crucial role to control the infection (Tizard, 1992). Sensitized T lymphocytes in response to *Toxoplasma* ribonucleoproteins release interferon-γ (IFN- γ), which activates macrophages and enables them to generate reactive nitrogen metabolites by oxidizing the terminal guanidonitrogen atom of L-arginine using NADPH. One of the metabolites is nitric oxide, which can destroy the intracellular form of the parasite (Tizard, 1992). Specific antibodies in conjunction with complement can also destroy the parasite found free in body fluids. They thus reduce the spread of the organism between cells (Tizard, 1992).

2.8. Diagnosis

Definitive diagnosis of toxoplasmosis cannot be achieved through clinicopathological examination because the clinical manifestations are nonspecific to the disease and often the disease run asymptomatic or subclinical course (Sukthana, 2006). The diagnosis of toxoplasmosis is problematic and 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). Generally diagnosis of toxoplasmosis divided into direct and indirect methods.

2.8.1. Direct diagnosis

Isolation and identification

Isolation of *T. gondii* can be made by mouse inoculation, and is considered as the reference method for studies, because of its high sensitivity and specificity. The use of mortality in experimental animals as a measure of virulence and pathogencity is, however, time consuming and expensive and also becoming unacceptable because of increasing concern for animal welfare

(Hove *et al*., 2005). Mouse inoculation removes confusions and misinterpretations of detection of similar parasites like *Sarcosysts* and *Neospor*a (Dubey, 1998).

For successful isolation the samples should be appropriate, fresh and must not be frozen at any stage, as this kills the parasite. The homogenized 2 to 5 gm of tissues from appropriate organs in equal volume of 0.3M sterile phosphate buffered saline (PBS) pH 7.4 with added antibiotic, preferably penicillin and streptomycin is inoculated into *Toxoplasma*-free mice intraperitonially at the dose 0.5ml per mouse. The mice are then monitored for 6 to 8 weeks after inoculation. Smear can be made from one drop (5 μm) of brain suspension of infected mice on slides and stained with Giemsa, to visualize the cyst. The tissue cysts appear as circular structures measuring 5 to 50 μm filled with blue-staining, crescent shaped bradyzoites (Wilson *et al,* 1990; Urquhart *et al.,* 1996).

Direct microscopy

Merozoites or cysts may be demonstrated by direct microscopic examination of tissue fluids such as peritoneal fluids, exudates, blood, vesicular fluids or tissue sections from brain, liver, lung, placenta etc. Impression smears prepared from these samples are usually stained with Giemsa stain to reveal and identify *T. gondii* organisms (Silm *et al.,* 1963; Pelloux *et al*., 2000). Microscopic examination of feacal smears obtained by sugar or salt floatation is useful in detecting the oocysts of *T. gondii*. But it is very difficult to differentiate *T. gondii* and other protozoan parasites that use felines as definitive hosts (Wallece, 1973).

Molecular diagnosis

Recently polymerase chain reaction with high specificity and sensitivity has appeared in the field of toxoplasmosis (Ajzenbergh *et al*., 2004). Various PCR techniques such as RE-FLP microsatellite analysis and multiplex PCR have been approved and employed for direct diagnosis and genotyping of *T. gondii*. Most of the molecular diagnostic techniques are applied on body fluid such as ocular fluids, amniotic fluids and cerebrospinal fluids in human medicine. (Pelloux *et al*., 2000).

2.8.2. Indirect diagnosis

The detection of *Toxoplasma*-specific antibody is the primary diagnostic method to determine infection with *Toxoplasma* (Singh, 2003). Antibodies have been detected by numerous serologic tests and most of the test kits are commercially available to detect *T. gondii* specific IgG, IgM, or IgE antibodies. Animal studies indicated that prasitemia is present for only a very limited time following acute infection (Derovin and Garin, 1991; Singh, 2003). Some of these tests which have been extensively used for the detection of *T. gondii* antibodies are Sabin Feldman Dye Test (SFDT), Indirect Fluorescent Antibody Test (IFT), Indirect Haemaglutinition test (IHAT), Latex Agglutination Test (LAT), Direct Agglutination Test (DAT), Modified Agglutination Test (MAT), Enzyme Linked Immunosorbent Assay (ELISA). Most of these tests employ intact tachyzoites as antigen; hence mainly antibodies to surface antigens are detected (Acha, and Szyfres, 2003; Singh, 2003; Jones *et al.*, 2003; Dubey, 2004; Urquhart *et al.*, 1996; Wilson *et al*., 1990).

Sabin-Feldman Dye Test (DT)

The Sabin-Felman test is the oldest, usually considered as "the gold standard" quantitave test characterized by high sensitivity and specificity (Silm *et al*., 1963). It is based on the principle that extracellular *Toxoplasma* organisms are killed and lose their normal ability to stain with alkaline methylene blue when exposed to serum containing anti-*Toxoplasma* antibodies. Live *Toxoplasma* tachyzoites are incubated with a complement like accessory factors and the serum at 37^{o}c for one hour before methylene blue added. Specific antibody induces membrane permeability in the parasite so that cytoplasm is able to leak out and the tachyzoites does not incorporate the dye and so appears colorless. Tachyzoites not exposed to specific antibody (i.e. a negative serum sample) take up the dye and appear blue (Sabin and Feldman, 1948).

Indirect Immunofloresent Antibody Test

Like the dye test, indirect immunoflorescsent test detects antibodies direct against intact tachzoites of *T. gondii* but it overcomes some of the drawbacks associated with the dye test. Indirect immunoflorescent test utilizes killed and formalin-fixed tachyzoites as an antigen. It has similar performance characterstics as the dye test. It allows the expression of the results of the tests in International Unit (IU) since calibrated gold standard can be used during the tests. However, it requires the availability of fluorescent anti-species antibody for each species of host to be tested and microscope with Ultra Violet light. As a result its application has been handicapped by the aforementioned factors (Masali *et al*., 2003).

Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme linked immunosorbent assay is a serologic test used to detect specific antibody against Toxoplasma cytoplasmic or membrane antigens obtained after lysis of the parasites. It is one of the most widely used serologic techniques that made toxoplasmic serology easier to perform. Enzyme linked immunosorbent assay is highly sensitive and specific serology test that allows the expression of the results in quantitave terms using international unit. Different types of enzyme linked immunosorbent assay are available currently but no uniformity or standardization is in place to compare the cut-off or antibody kinetics. As the results of this test are read using automatic reader it is free from subjective bias. Currently ELISA was found to be the most sensitive and specific serologic test used to investigate toxoplasmosis (Gamble *et al*., 2005).

Modified Agglutination Test (MAT)

Modified agglutination test is becoming the most commonly used serologic test in the epidemiological studies of toxoplasmosis. It was described and recommended for field studies by Desmonts and Remington (1980). Modified agglutination test employs intact tachyzoites as antigen and 2-Mercaptoethanol to eliminate IgM (non specific antibody) to ease detection of specific anti-*T. gondii* IgG antibodies. The results of DA test have been shown to correlate well with that of the dye test and IFAT and it does not require the use of species specific conjugates,

which made it possible to use in any species. The test is performed in micro plate unlike the dye test and IFAT, which are performed on microscope slide and permits the manipulation of sera at a time. The reaction occurs at room temperature and does not require the use of incubator. Reading of the test results can also be made with naked eye. It is also possible to transform the results in quantitative terms (Desmonts and Remington, 1980).

These serological tests have been known by their failure to discriminate between resent and past infections, inability to differentiate between maternal antibodies and antibodies due to congenital infections and between reinfection and reactivation.

2.9. Treatment

Mainly ingesting bradyzoites or oocysts infects humans and animals. After ingestion, both bradyzoites and sporozoites convert to tachyzoites inside tissues. The conversion of tachyzoites to bradyzoites and bradyzoites to tachyzoites is of biological and clinical significance because bradyzoites are less susceptible to chemotherapy and reactivation of bradyzoites to tachyzoites is considered the cause of fatal toxoplasmosis in AIDS patients (Dubey *et al.*, 2003). In general there is no satisfactory treatment. A combination of chemotherapeutic drug pyrimethamine and sulphadiazen has been reported to be effective against tachyzoites, but not bradyzoites, in humans, but rather toxic to cats (Urquhart *et al.*, 1996). If the presence of acute *T. gondii* infection in a pregnant woman is confirmed, treatment with spiramycin (rovamycine) can be initiated in an effort to prevent transmission to the fetus. If fetal infection is confirmed through amniocentesis, the woman may be switched to pyrimethamine (daraprim) and sulfadiazine after the first trimester or, according to some experts after the 18th week of gestation. Folinic acid (leucovorin) is given with pyrimethamine and sulfadiazine to protect bone marrow from the suppressive effects of pyrimethamine (Jones *et al*., 2003).

2.10. Prevention and control

Because of the economic and zoonotic significance, great effort should be applied towards the prevention and control of toxoplasmosis.

Cats

As domesticated cats play a pivotal role in the epidemiology of toxoplasmosis through shedding oocysts, control and prevention measures should target them. Domesticated cats should be provided with adequately cooked meat and should also be prevented from hunting birds and rodents; however, it is not practical especially in developing countries (Urquhart *et al*., 1996).

Chemoprophylaxis with sulfadiazine-pyrimethamine or with monensin is also useful as they prevent the shedding oocysts (Frenkel and Smith, 1982).

Sheep and goats

The control of toxoplasmosis in sheep and goats can be approached in several ways. The first is to prevent exposure of susceptible goats and sheep to the oocyst in cat feces during pregnancy. This could be achieved by keeping feeds in a closed container and by proper disposal of cat feces. The second method, which could be useful to prevent reproductive losses due to toxoplasmosis, is through encouraging exposure of ewe and does to infection before breeding to develop protective immunity. Chemoprophylaxis by adding the anticoccidial drug such as monensin to the feed is effective in reducing lamb losses (McCulloch and Remington, 1975; Smith and Sherman, 1994).

Good immunity obtained because of prior infection indicates that vaccination is realistic strategy to prevent clinical toxoplasmosis. Vaccination trial with the incomplete strain subgroug 48 showed a significant reduction in reproduction wastage. However, fears that a live vaccine could result in the presence of tissue cysts in the meat used for human consumption were shown to be unwarranted (Wilkins and O'connel, 1992). Repeated administration of killed vaccine is found to be infective in preventing lambing losses (Wilkins *et al*., 1987).

Humans

As toxoplasmosis is clinically important in pregnant women and in immunodeficient patients, measures for prevention involve in general increasing both the public awareness about the disease

and several precautions. Avoidance of eating raw or under cooked meat, all red meat need to be cooked until its internal temperature reaches 66 to 71.1°C throughout. Cats should be fed only canned or dried commercial cat food or well-cooked table food; they should not be given raw or undercooked meat, cats should be kept indoors and litter boxes changed daily so that excreted oocysts do not have access to food and environment (Dubey, 1994; Pipano *et al.,* 1994).

Disinfections of the litter box with scalding water, chemical disinfectants that are effective against *T. gondii* is useful preventive measure. Wearing of gloves when working in garden and handling soil and washing hands thoroughly with soap and after handling suspected materials (Jone *et al*., 2003). Cat faces should be flushed down in to the toilet or burned. Fruits and vegetables should be peeled or thoroughly washed before they are eaten. Cutting boards, dishes, counters, utensils, and hands should be washed with hot soapy water after they have been in contact with raw meat, poultry, or seafood, or with unwashed fruits or vegetables (Jone *et al*., 2003; Sukthna, 2006).

Pregnant women should be encouraged to keep their cats inside and not to adopt or handle stray cats. Health education for women of childbearing age should include information about preventing *T. gondii* transmission from food and soil. At the first prenatal visit, health care providers should educate pregnant women about food hygiene and avoiding exposure to cat feces. Avoidance of consumption of unpasteurized goat's milk and milk product that harbors *Toxoplasma* parasite (Dubey, 1994; Pipano *et al.,* 1994; Jone *et al*., 2003; Sukthna, 2006).

3. MATERIALS AND METHODS

3.1. Description of the study areas and study population

3.1.1. Study areas

The study area, South Wollo Zone, is located 401 km North East of Addis Ababa. South Wollo Administrative Zone is geographically categorized as highland (47%), mid-altitude (45%) and mountainous (8%). The altitude ranges from 1700 3800 meters above sea level (Dessei Zuria Woreda Agricultural and Development Office, 2007). South Wollo Zone experiences a bimodal pattern rainfall: the short between March to April and long (July to September), with annual rainfall of 39.63 mm and 1000 mm, respectively. The average maximum and minimum temperatures during short and long rains are 23.9 ^{o}C and 11.7 ^{o}C, respectively. The relative humidity of the region varies from 23.9 % to 79 %. From the district found in South Wollo Zone three districts were selected for study (Figure 4) namely, Dessie Zuria Woreda. Legambo Woreda and Debresina Woreda. Dessie Zuria Woreda has a total land mass of the area 123,162 hectares. Proportionally 41.5 % and 9.4% of this area are used for cropping and grazing respectively. The livestock population comprises of 121,186, 152,234, 59,101, 35,394, 24,000 and 138,411 heads of cattle, sheep, goats, equine, camel, and poultry respectively (Dessei Zuria Agricultural and Rural Development Office, 2007).

Legambo district is characterized by smallholder mixed crop-livestock farming. The topography of the area is characterized by flat to undulating and hill types. The district is densely populated and most of the land intensively cultivated during spring (belg) from (March to April). The rainfall pattern is binomial and the annual rainfall ranges from 500 to 9000 mm. The mean annual minimum and maximum temperature are 10 ^{o}C and 20 ^{o}C, respectively. The mean relative humidity is 68%. In the district there are 93,193 bovines, 22,531 caprines, 255,242 ovines, 28,668 equines, and 92,439 poultry (Legambo Woreda Agricultural and Rural Development Office, 2007).

Figure 4: Map of Ethiopia showing the study area

3.1.2. Study population

The study was conducted in small ruminants (sheep and goats) and in human (HIV/AIDS patients). In small ruminants age, sex, presence of cat, abortion or neonatal mortality or weak births were considered as risk factors. In human beings consumption of raw/or under cooked mutton, contact with cats and HIV/AIDS infection were taken into account. These study subjects were classified into different age groups based on dentition and questionnaire as described in Shively (1987). Sheep and goats of more than 6 months of age were included in the study to avoid measuring antibodies passively transferred in colostrum.

3.2. Study design

A cross-sectional study was conducted to determine the presence of anti-*Toxoplasma gondii* IgG antibodies in sheep, goats and humans. Blood samples from sheep and goats were collected through a systematic random sampling procedure (Thrusfield, 2005). Similarly, blood samples were collected from humans who were visiting volunteer, counseling and testing center (VCT) in collaboration with medical personnel in the study area.

A systematic questionnaire was designed and instituted to obtain relevant information on the potential risk factors. Animal owners (farmers) were interviewed during collection of blood about history of study variables including abortion, neonatal mortality, herd size and management. Humans visiting VCT center were also interviewed at the same time during taking samples about their feeding habits and presence or absence of cats in their households.

3.3. Sampling

Systematic random sampling sheep and goats was used. Determination of sample size was done using the formula described by Thrusfield (2005).

$n = (1.96)^{2} * Pexp (1- Pexp)/d^{2}$

Where:

n= required sample size

Pexp = expected prevalence

d= desired absolute precision

An expected prevalence of 50 % and a 5% desired absolute precision was considered. A prevalence of 50% of toxoplasmosis was used because no published data in the study area was available. Accordingly, the estimated sample size was 384. However, the low goat population density in the study area forced us to sample only available ones. A total of 512 animals were then sampled from Legambo Woreda (384 serum samples) and Dessie Municipality Abattoir (128 serum samples). Similarly, a total of 260 sera from volunteer humans was purposively collected from Mekaneselam Health Center (64 serum samples) and two referral hospitals namely, Dessie (127 serum samples), Hidar 11 (69 serum samples).

Approximately 5 ml of blood from jugular vein of each sheep and goat, and 4 ml of blood from brachial vein of each human visiting VCT centers were collected aseptically using sterile plain vaccutainer tubes and needles and properly labeled with the necessary information. All the tubes were left for 8 hours at room temperature to allow clotting and the sera separated within 12 hours and transferred to other sterile vials and kept at -20 ^{o}C until tested for anti-*T. gondii* antibodies.

3.4. Serologic analysis

The antibodies to *T. gondii* were determined by using modified (2-mercaptoethanol) direct agglutination test using a commercial kit (Toxo-Screen DA Biomerieux® SA, RCS LYON, Marcy-I'Toile / France). The test was performed as previously described by Patton *et al*. (1990). The sensitivity and specificity of the test were 96.22% and 98.8%, respectively. Each serum sample was tested in parallel with positive and negative control sera. The test sera were screened for the presence of anti-*T. gondii* IgG antibodies at 1/40 and 1/4000 dilutions as recommended by the manufacturer. Positive serum samples were identified by the presence of an agglutination mat. Any serum sample with detectable agglutination at 1/40 dilution or greater was considered positive for anti-*T. gondii* IgG antibodies. Negative sera were demonstrated by the presence of a button at the bottom of the well.

3.5. Data management and analysis

The establishment of the database files and necessary manipulations, for example validation, correction, recording, etc, were performed in Micro soft excel spread sheets before transferring the files to SPSS 15.0.0. for Windows (SPSS, 2006) for analysis. The database included serological tests and questionnaire responses. The prevalence of toxoplasmosis was calculated as the number in study population testing positive to the serological test divided by the total study units tested. The differences in prevalences were done using chi-square (χ^2) test. The associations between each risk factor were further analyzed and test outcome was assessed using univariate logistic regression. All significantly associated risk factors were further analyzed using multivariable stepwise logistic regression. For all analysis, a P-value of less than 0.05 was taken as significant.

4. RESULTS

4.1. Overall prevalence of toxoplasmosis in small ruminants

In the current study, a total of 426 sheep and 86 goat serum samples were analyzed using Toxo-Screen DA kit from Legambo Woreda and Dessie Municipality Abattoir. An overall positivity of 44.1% was recorded (Table 3). Of this 45.4% and 37.2% of sheep and goats, respectively were found to be positive. The prevalence of toxoplasmosis from the two study sites were 49.2% in Legambo Woreda and 28.9% in Dessie Municipality Abattoir and the prevalence is significantly different (P=0.000) between the two sites.

Table 3: Results of toxoplasmosis seroprevalence in small ruminants in the study sites

Study site	Number tested	Prevalence (%)
Legambo Wereda	384	49.2
Dessie Municipality Abattoir	128	28.9
Overall	512	44.1

4.2. Seroprevalence by sex and age

Seroprevalence of toxoplasmosis was analyzed based on sex and age of the study animals. The prevalence was 45.2% and 42.6% in female and male animals respectively. No statistically significant difference was found between the two sex-specific prevalences in both species. However, statistically significant difference was observed in prevalence of toxoplasmosis among different age-groups. Of 426 sheep and 86 goat sera tested the age-group-specific prevalence were 15.5% and 25% in animals aged less than 1 year, 48.9% and 33.3% in animals aged 1-3 years and 51.6% and 45% in animals aged more than 3 years, respectively (Table 4). Generally the prevalence of *T. gondii* antibodies increases as the age of animal increase in both species (Figure 5). But no significant (P> 0.05) difference in seroprevalence was observed among animals aged 1-3 years and those greater than 3 years old (Table 4). The age-group-specific prevalence was found to be significantly (P= 0.000) higher in animals those of less than 1 year

(lambs and kids) compaired to older ones in sheep. However, there was no statistically significant difference in prevalence in similar age group in goat in all age categories (Table 4).

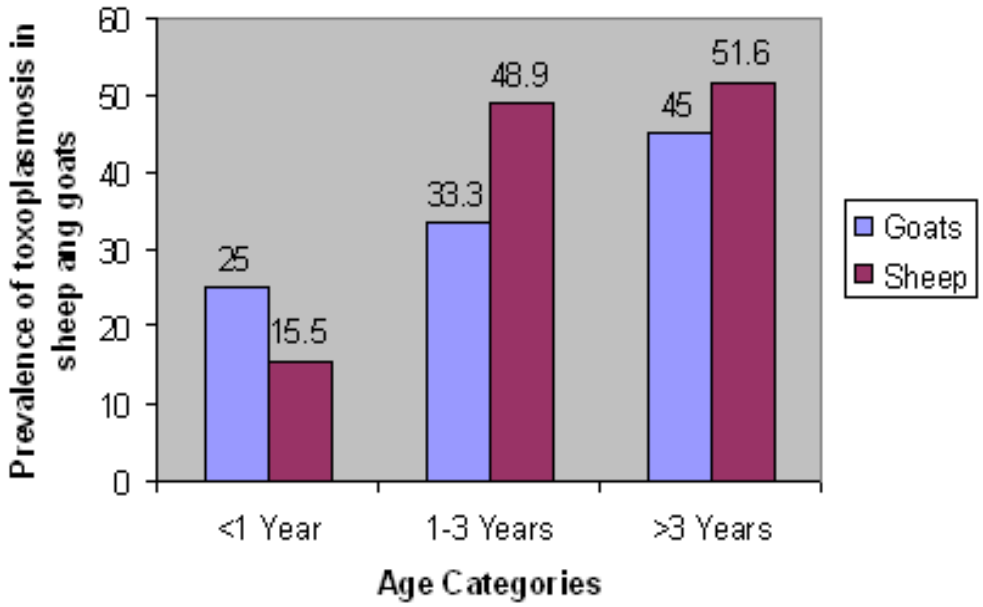

Figure 5: Distribution of age-group-specific seroprevalence for toxoplasmosis and test samples in sheep and goats

4.3. Factors associated with seropositivity of *T. gondii* in sheep and goats

Information was collected for some risk factors potentially associated with toxoplasmosis using questionnaire. The selection of these factors was based on reviewing available published literature. The analysis of the data was undertaken to determine if significant associations between these factors and seropositivity existed. Factors that are closely related to the natural history of toxoplasmosis, for example age of animals, history of abortions, neonatal mortality, weak births and presence of cats, were analyzed. By univariate analysis all the variables were found to be significantly associated with seroprevalence of *T. gondii* in sheep and none in goats (Table 4). Thus in goats, all risk factors occurred independently of the *T. gondii* infection. In sheep, the presence of cat was significantly associated with seroprevalence of toxoplasmosis **(**P= 0.008). Higher prevalence (67.3%) was observed in sheep flock raised in the presence of cats than those that was reared in the absence of them (46.5%). Similarly, the seroprevalence was higher (74.1%) in sheep flock with history of abortion, neonatal mortality and weak lambs than in

sheep flock without such histories (45.1%). These association was statistically significant (P= 0.000).

Table 4: Results of univariate logistic regression analysis to determine whether *T. gondii* infection in sheep and goats was independent or associated with potential risk factors studied from October, 2007 to April, 2008.

Risk factors		Number tested	Prevalence (%)	P-value	OR	95% C.I. (OR) Lower	Upper
Species	Ovine	426	45.5				
	Caprine	86	37.2	0.157	0.709	0.440	1.142
Area	Legambo	384	49.2				
	Dessie abattoir	128	28.9	0.000	0.420	0.273	0.646
Sheep	Female	265	46.4	0.642	1.098	0.741	1.628
	Male	161	44.1				
Age	<1 year	58	15.5	0.000			
	1-3year	141	48.9	0.000	0.173	0.080	0.371
	>3year	227	51.6	0.605	0.898	0.596	1.352
Cat	Absence	286	46.5	0.008	0.422	0.222	0.800
	Present	49	67.3				
ANW	Absent	142	45.1				
	Present	58	74.1	0.000	3.494	1.780	6.857
Goat	Female	45	37.8				
	Male	41	36.6	0.909	0.950	0.396	2.281
Age	<1 year	16	25	0.331			
	1-3year	30	33.3	0.173	0.407	0.112	1.483
	>3year	40	45	0.326	0.611	0.229	1.631
Cat	Absent	37	48.6	0.674	1.326	0.356	4.947
	Present	12	41.7				
ANW	Absent	16	50	0.258	0.250	0.023	2.757
	Present	5	80				

ANW= abortion, neonatal mortality, weak birth, SE= standard error, OR= odd ratio

All the significantly associated risk factors with sheep toxoplasmosis were all further analyzed using multivariable stepwise logistic regression. The results showed that abortion, neonatal mortality and weak birth of lambs were again significantly associated with seroprevalence of *T. gondii* in sheep (P= 0.000; Table 5).

Table 5: Results of multivariable logistic regression analysis to determine whether *T. gondii* infection in sheep was independent or associated with potentially risk factors studied from October, 2007 to April, 2008.

Risk factors	Coefficient estimate	SE	P-value	OR	95% C.I. of (OR)	
					Lower	Upper
Age	0.213	0.239	0.371	1.238	0.776	1.976
Cat	0.423	0.409	0.301	1.526	0.685	3.399
ANW	1.204	0.347	0.001	3.332	1.689	6.576

ANW= abortion, neonatal mortality, weak birth, SE= standard error, OR= odd ratio

4.4. Prevalence of toxoplasmosis in humans

Human serum samples collected from Dessie hospital, Hidar 11 hospital and Mekaneselam Health Center submitted to VCT center for HIV testing using HIV Rapid Test Algorism (Annex 1) were tested. Out of all the sera tested 48.1% were HIV-positive of which 55 (55.3%) were females and 70 (44.6%) were males. From analysis, higher seroprevalence was recorded in females than males but they were not statistically significant (P= 0.165; Table 6).

Table 6: Results of HIV infection in humans from October, 2007 to April, 2008**.**

Sex	Number tested	Prevalence (%)	P-value	OR	95% C.I. (OR)	
Female	103	53.4	0.165	0.702	0.426	1.156
Males	157	44.6				
Total	260	48.1				

SE= standard error, OR= odd ratio

Out of the 260 human sera examined for anti-*Toxoplasma gondii* IgG antibodies by Toxo-Screen DA kit, serologic evidence of toxoplasmosis was found in 76.5%. An overall seroprevalence in the current study was 54.7% in Debresina, 59.5% in Legambo and 82.7% in Dessie, respectively. Although proportionally large number of seropositive people were females (79.6%) than males (74.5%), nevertheless the difference was not statistically significant (P= 0.344). In addition, the positivity rate was not significant in different age groups but higher prevalence rate was observed in middle and older age-groups. Out of the total of HIV positive sera, 89.6% were positive for anti-*T. gondii* IgG. There was statistically significant association between seroprevalence of toxoplasmosis and HIV infection (P= 0.000). Notably, significantly, more of people who consumed raw/or partially undercooked mutton were seropositive for *T. gondii* than those known to consume well served mutton (P= 0.001; Table 7).

Table 7: Comparative results of risk factors to *Toxoplasma* infection in humans using univariable logistic regression analysis (studied from October, 2007 to April, 2008)

Risk factors/categories		Number tested	Prevalence (%)	P-value	OR	95% C.I.(OR)	
						Lower	Upper
Sex	Female	103	79.6	0.344	1.335	0.733	2.430
	Male	157	74.5				
Year	<25 years	94	72.3	0.315			
	25-35years	97	76.3	0.128	0.551	0.255	1.188
	>36years	69	82.6	0.327	0.677	0.311	1.476
Area	Debresina	64	54.7	0.000			
	Dessie	127	82.7	0.000	0.205	0.089	0.470
	Legambo	69	85.5	0.610	0.809	0.359	1.824
Meat	Well served mutton	81	63	0.001	0.356	0.197	0.645
	Raw/under cooked	179	82.7				
Cat	Absence	195	73.3				
	Presence	65	86.2	0.038	2.261	1.045	4.893
HIV	Negative	135	64.4	0.000	0.210	0.107	0.413
	Positive	125	89.6				

The risk factors associated with seropositivity to toxoplasmosis, for example consumption of raw/ or undercooked meat, HIV infection and the presence of cats (Table 7) were multivariably analyzed using logistic regression. The results of the multivariable stepwise logistic regression further show that consumption of raw or under cooked mutton (P= 0.008), presence of cat at home (P= 0.041) and people living with HIV/AIDS (P= 0.002) still remained significantly associated with seroprevalence to *T. gondii* in humans (Table 8).

Table 8: Risk factor associated with seropositivity to *T. gondii* in humans in stepwise multivariable logistic regression

Factors	Coefficient	SE	P-value	OR	95% C.I. (OR)	
HIV	1.328	0.427	0.002	3.773	1.634	8.714
Co. Raw.M	0.870	0.326	0.008	2.388	1.261	4.524
Cat	0.854	0.418	0.041	2.349	1.036	5.337
Area	0.300	0.294	0.309	1.349	0.758	2.403

Co. Raw.M. = consumption of raw meat, SE= standard error, OR= odd ratio

Humans living with HIV were 3.773 times (OR= 3.773, 95% C.I.= 1.634, 8.714) at risk of being seropositive to *T. gondii* than non-HIV ones. Further, those who consumed raw meat or partially cooked as well as those who had cats were 2.388 times (P= 0.008, OR= 2.388, 95% C.I.= 1.261, 4.524) and 2.349 times (P=0.041, OR= 2.349, 95% C.I.=1.036, 5.337) at risk of being seropositive to *T. gondii* than who were not exposed to these factors, respectively.

5. DISCUSION

5.1. Toxoplasmosis in small ruminants

Overall prevalence

This study was conducted with the aim of determining the prevalence of antibodies against *T. gondii* in small ruminants and humans in South Wollo at the Dessie Municipality Abattoir and Legambo Woreda. The study has shown that higher prevalence of toxoplasmosis in sera of animals from Legambo Woreda than sera obtained from abattoir samples. The overall seroprevalence recorded in sheep was found to be higher (45.5%) than in goats (37.2%). This finding agrees with previous reports with higher prevalences in sheep and goats Ethiopia (Negash *et al*., 2004). Similarly, higher seropevalence of *T. gondii* was recorded in sheep than in goats in Ghana (Van Der Puije *et al*., 2000). These observations might be due to the differences in feeding habits since sheep are more likely to get infection from the pasture as they graze close to the ground than goats which prefer browsing.

Relatively lower prevalence was observed in abattoir samples than in field samples. However, the findings from the abattoir samples support the health hazards linked to consumption of raw or under cooked meat as principal modes of transmission of toxoplasmosis to humans. Elsewhere it has been shown that parasitism in excess of 50% has been reported in mutton and pork from abattoirs (Acha, and Szyfres, 2003). The difference in prevalence of toxoplasmosis among different animal species may be explained by differences in their susceptibility to *T. gondii* infection.

The overall prevalence recorded in sheep in the present investigation is relatively higher (45.5%) compaired to the previous studies in Ethiopia (Kassali and Tekely, 1989; Tilaye and Getachew, 2002). Similarly, lower seroprevalence were reported in Ghana and Morocco (Van Der Puije *et al*., 2000; Sawadogo *et al*., 2005). However, Negash *et al*. (2004) found *T. gondii* infection in 56% of sheep in Nazareth which was slightly higher than that recorded in this study. The seroprevalence obtained in this study is slightly greater than continental African seroprevalence

which ranges between 11.5% and 34% according to data obtained from various African countries (Martins *et al*., 1997) and the seroreaction rate of toxoplasmosis in sheep sera in Brazil (Tenter *et al*., 2000). The current seroprevalence is however; lower than the prevalence recorded in certain European countries like France of 92% (Tenter *et al*., 2000).

An overall *T. gondii* seroprevalence of 37.2% was recorded in goats. This is consistent with the results of Tilaye and Getachew (2002) who reported prevalence of 34% in Debreberhan. But it is greater than that reported by Kassali and Tekelye (1989) and Nagash *et al*. (2004) of 26.7% and 22.9%, respectively. Similarly, lower prevalence than this finding has been recorded elsewhere in the world such as Ghana and Thailand in goats (Van Der Puije *et al*., 2000; Jittapalapong *et al*., 2005) of 26.8% and 27.9%, respectively. However, Teshale *et al*. (2007) found infection in 74.8% in Southern and Central Ethiopia which was significantly higher than obtained by this study.

The difference in seroprevalence of *Toxoplasma* infection in sheep and goats between the present work and the previous reports from Ethiopia or elsewhere may be attributed to differences in climatic conditions. *Toxoplasma* seroprevalence is highly associated with climatic conditions of the study areas which are affected by agro-ecological factors. Higher prevalences of toxoplasmosis have been known to occur in warm and moist areas than in cool and dry ones. This is due to the fact that the oocysts of *T. gondii* survive and sporulate better under moist and humid environments (Van Der Puije *et al*., 2000). Apart from differences in weather conditions, variations may also by chance, the sampling method, difference in sensitivity and specificity of serological tests used and the relative cat densities in the areas and the access of cats to contaminate feed and water with oocysts. In the present study, Modified Direct Agglutination Test was used to screen the sera for anti *T. gondii* antibodies. The cut off value we used was 1/40 as indicated by the manufacturer while the previous workers used 1/20 and sometimes 1/32. Studies performed in other parts of the world have been based on other serologic methods such as IFAT, DAT, ELISA and others. It has been well known that the sensitivity and specificity of various tests vary greatly.

In this study, even though there was no statistically significant difference in prevalence of toxoplasmosis between sexes in sheep and goats it was nevertheless higher in females than males in both animal species. This is consistent with previous reports in Ethiopia in which significantly higher seroprevalence was recorded in females than males for both species (Negash *et al*., 2004; Teshale *et al*., 2007). Similarly, reports from Satun Province, Thailand indicate that female goats were more prone to toxoplasmosis than males (Jittapalapong *et al*., 2005). The higher prevalence of the infection in females can partly be explained by the fact that female animals are kept for longer period in farms for breeding purposes. Ewes and does were kept for longer periods than bucks and rams and the high proportions of the flocks and herds were made of females while only few males were found.

The age-specific prevalence showed that the infection pressure increase with age. Highest prevalence was observed in sheep and goats of more than 1 year of age. This is in agreement with the report of Teshale *et al*. (2007) who reported lower prevalence in the younger goats (<1 year) of age than in the yearlings (>1 year). Similarly, Singh and Msolla (1984) reported prevalence of 25.2% in the one and half years of goats and 38.3% in the older age groups of at least three years old. Dubey and Welcome (1988), Opel *et al*. (1991) and Dorny *et al*. (1993) also found higher prevalence of *Toxoplasma* infection in older sheep and goats than in kids and lambs. This difference in prevalence among age groups is partly due to the cumulative effects of age. Older sheep and goats as they live longer are more likely to encounter oocysts of *T. gondii* from the environment. Risk factors associated with the seropositivity to *T. gondii* in sheep and goats were analyzed from information obtained by the questionnaire from the animal owners (farmers). The seropositivity of *T. gondii* infection in sheep was significantly (P= 0.008) associated with the presence of cats in the households or in the grazing areas. This supports the previous findings and established scientific ideas. For instance, Acha, and Szyfres (2003) reported that cats are significant risk factors in contaminating the pasture, where *T. gondii* antibodies were reported higher in sheep (32%) raised in the presence of cats than sheep raised (2%) in the absence of cats.

Results of questionnaire survey revealed that higher reproductive problems such as abortion, neonatal death and birth of weak lambs occur in ewes in the study area. There was statistically significant association between seroprevalence of toxoplasmosis and these reproductive

abnormalities in sheep. There has been no systematic screening of small ruminants in the area for the diseases known to cause these problems. This goes in line with the previous report of Negash *et al*. (2004) who observed abortion, neonatal mortality and weak birth being associated with *T. gondii* infection in sheep. Ovine abortion and neonatal mortality due to toxoplasmosis have been published from New Zealand, Australia, Canada, United States and United Kingdom and in most of these countries it is second in importance to chlamydial abortions (Radostits *et al*., 1994). *T. gondii* has been known to be responsible for abortion, still birth and birth of congenitally infected weak offsprings in more susceptible hosts such as sheep, goats and pigs (Masala *et al*., 2003).

5.2. Human toxoplasmosis

The prevalence of antibodies to *T. gondii* in humans in this study was high. Out of 260 sera tested 199 (76.5%) people had anti-*Toxoplasma gondii* IgG antibodies. The present findings agree with previous reports from different parts of Ethiopia that include the work of Guebre-Xabier *et al*. (1993). Our result showed that the current seroprevalence in human subjects is lower than the prevalence recorded in Addis Ababa Abattoir workers which was 96.77% (Yimer *et al*., 2005). However, lower prevalence was reported in Burundi (44.1%), Tanzania (40%), Somalia (53%), and Kashmir women with history of repeated abortions (49.47%) (Excler *et al*., 1988; Zargar *et al*., 2007).

One of the reasons for high prevalence in this study may be due to the habit of consumption of raw or under cooked meat. Based on questionnaire survey analysis, most of the people who consumed raw or partially cooked mutton were seropositive for *T. gondii* than those who consumed well cooked mutton. The prevalence was higher in people who consume raw meat than in those who were known to consume well served mutton (P= 0.008). Higher seropositivity to *T. gondii* antibody is commonly associated with the consumption of raw or under cooked meat that facilitates higher transmission rates as reported by different authors (Guebre-Xabier *et al*., 1993; Tenter *et al.,* 2000; Dubey, 2002; Acha and Szyfres, 2003) whose results agree with the present study. Similarly, higher prevalence was recorded in Addis Ababa Abattoir workers who ate raw meat (Yimer *et al*., 2005). Viable *T. gondii* cysts may remain in the edible tissues of sheep and goats for the life of the animals where as (Smith, 1991; Dubey 1998) whereas, cattle appears to

be naturally resistant and capable of clearing the infection, beef is generally not regarded as an important source of human infection (Quinn *et al*., 1994). The higher prevalence of toxoplasmosis in sheep and goats in the study areas present another factor risk for human infection.

Another factor that probably contributed to the higher prevalence of toxoplasmosis in humans is the presence of close contact with domestic cats. In the present study, more than 25% respondents indicated history of close contacts with cats. Almost all the cat owners stated that they keep them as biosecurity for rodents and mice and none of the respondents practice hygienic procedures such as proper disposal of cat feces. The analysis has shown that people with a known history of presence of cats in their home were 2.350 times more likely to be seropositive than those who do not have cats in their home (P= 0.041; Table 6). Acha and Szyfres (2003) reported that the prevalence of toxoplasmosis in women (31.1%) that had cats in their home was greater than in women (19.3%) who do not have cats in Thailand. Generally, domestic cats are the only animals that are definitive hosts of *T. gondii*, and play a key role in the epidemiology of *T. gondii* infection. After primary infection with *T. gondii*, cats that are kept inside the houses shed large number of oocysts thereby putting their owners at risk of infection (Tenter *et al*., 2000).

In the present study it was shown that seropositivity against *T. gondii* antibody was associated with positivity to HIV. Out of 125 HIV positive sera 89.6% were reactive to anti-*Toxolasma gondii* IgG antibodies which was higher than the proportion found in HIV negative individuals (64.4%) (P= 0.002). The current result showed that high *Toxoplasma* infection in HIV-positive individuals fairly agrees with toxoplasmosis found in HIV/AIDS patients (67.4%) in Bombay, India (Meisheri *et al*., 1997). Similarly, out of 54% *Toxoplasma* IgG positive, high prevalence of 22% was recorded in HIV-positive group than HIV negative groups (1%) in Nairobi (Brindle *et al*., 1991). Also the infection rate in Thailand revealed that 13.1% in pregnant women who were HIV-negative and 21.1% in women who were HIV-positive (Chintana *et al*., 1998). Toxoplasmosis is more sever in immunodeficient individuals, 24.5% had encephalitis caused by *Toxoplasma* in Nairobi (Brindle *et al*., 1991).

7. CONCLUSION AND RECOMMENDATIONS

The questionnaire survey revealed that close contacts between cats and the household members and the habit of consuming raw meat or undercooked mutton were significantly associated with *Toxoplasma* infection in humans. Similarly, reproductive problems and the presence of cats in the house or in the nearby proximity were closely correlated to the seroprevalence of *Toxoplasma gondii* infection in sheep. Cross-sectional investigation of ovine and caprine toxoplasmosis in the study areas showed a relatively higher prevalence from previous reports. Females and older animals were comparatively more affected. Since sheep and goats become infected chiefly by ingestion of oocysts, the high prevalence of *T. gondii* in sheep and goats suggests that oocysts are widely dispersed in the environment and thus pose risks to humans.

Results of toxoplasmosis in humans in this highlight the zoonotic importance of this infection. With the ever increasing rate of HIV/ADIS infection in Ethiopia, raw eating meat predisposes many HIV/AIDS infected persons to serious complications from opportunistic pathogens. In line with the above observations the following recommendations are put forward.

- The economic impact of toxoplasmosis infection under small scaled farms should be further investigated in details
- Investigation should be undertaken to identify other possible causes of abortions in small ruminants.
- Continuous community–based heath education programmes should be set and all communication media utilized for creating awareness about toxoplasmosis and its associated risk factors and transmission pathways.
- To increase the public awareness of the infection in rural and urban communities, veterinarians should provide advice on possible minimization of contacts with cats or consumption of raw or undercooked meat.
- There should be isolation and identification of the genotype of *T. gondii* circulating in Ethiopia.

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9. ANNEXES

Annex 1: Modified Direct Agglutination Test (Toxo-Screen DA kit)

1. In tubes, dilute the serum samples and control sera R4 (positive control) and R5 (negative control):
    - 1/20 : 100 µl of serum + 1.9 ml of PBS (reconstituted R6)
    - 1/2000: 25 µl of the 1/20 dilution + 2.5 ml of PBS (reconstituted R6)

2. Locate the patient sera on the report sheet then dispense 25 µl of each serum dilution into the wells provided.
3. Add 25 µl of 0.2 mol/l 2-mercaptoethanol to each well, thus diluting the sera 1/40 and 1/4000
4. Add 50 µl for R1 antigen suspension diluted 1/5 to each well.

    Allow 1 well for the antigen control:

    - 2-mercaptoethanol (0.2 mol/l) 25 µl
    - PBS 25 µl
    - Antigen (R1) diluted 1/5 50 µl

5. Homogenize using the vibrator or Kline rotator
6. Cover with a self-adhesive sheet. Leave for 5-18 hours at room temperature away from vibrations and sources of drying.

7. Perform reading

Antigen control: sedimentation of the *Toxoplasma* in the button or ring.

Positive reaction**:** e.g. (positive control serum R4 at 1/40). 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 reaction: (e.g. negative control serum R5). Sedimentation of the *Toxoplasma* in a bottom or ring.

RESULT-INTERPRETATION

According to Desmonts G. and Remington, results are almost identical to those obtained with the dye test.

- If the test is negative at both 1/40 and 1/4000 dilution b(or borderline at 1/40 and negative at 1/4000): the serum is considered free of IgG antibodies as with the dye test
- If the test is positive at the 1/40 dilution and negative, border line or positive at the 1/4000 dilution: the serum contains IgG antibodies. Perform the quantitative test to determine the titer. In a normal population, a marked titer is more frequently observed with Toxo-Screen DA than with other serological techniques as antibody kinetics differ. This high titer is not indicative of recent infection as it can persist for long period.

Some sera cause prozon phenomena, indicated by a positive reaction at the 1/4000 dilution and a negative or borderline reaction at a 1/40 dilution.

At the onset of toxoplasmosis, sera may be found to be negative by this technique, but detection of IgM antibodies will be positive in such cases.

Annex 2**:** HIV Rapid Test Algorism

Step 1: Screening test by Determine HIV1/2

Test result is negative client receive negative result, if Positive use the second test to confirm the result of the first test.

Step 2: Confirmatory test by Capillus

The test result was positive client receives positive result, if negative use the third test to break the tie.

Step 3: Tie breaker by Uni-Gold test

The test result is negative the result receives negative result, if positive the client receives positive result.

Annex 3: Age determination

Age will be determined by looking the erupted permanent incisor teeth.

Incisor	Age (months)
0/1	12-21
0/2	21-24
0/3	24-36
0/4	36-48

Annex 4: Questionnaire format

Respondents: Animal owners during sample collection and humans visiting VCT center

1. Name or code of respondents_____ _____
2. Age (<15, 15-45, >45).
3. Sex (Male, Female)
4. Address: a. Urban (city _____; _____; Kebele _____;
   House no _____)
   b. Rural (_____; PA _____; Village _____)
5. Occupation (Farmer, Civil servants, others)

Management information

1. What types of livestock species are keep in the area? (cattle, sheep, goats, equine, camels, others)
2. Flock/Herd size
3. Purpose of raising small ruminants and others?
4. What is your grazing system? (communal, individual, both)
5. What is the water source of your animals? (river, spring, pond, well, borehole, other)

6. What type of house do you use for your animals? (only fenced, fenced with shed, other)
7. Is there the history of abortion/still birth in the flock?
8. Is there the history of neonatal mortality?
9. Is there the history of reproductive?
10. Do you have cat (s) in your house? Yes ; No
11. How do you manage owned cats?
12. Do you properly dispose cat faces? Yes; No
13. Presence or absence of stray cats?

Questionnaire format for humans available in VCT center

1. Do you have cat (s) in your house? Yes ; No
2. How do you manage owned cats?
3. Do you properly dispose cat faces? Yes; No
4. Presence or absence of stray cats
5. How do you consume meat? Cooked , Raw, Other treatment
6. How do you consume milk? Raw, boiled, other treatment
7. Is there history of consumption of raw fruits

10. CURRICULUM VITAE

I. Personal data

Name: Yibeltal Muhie Mekonnen

Sex: Male, Age: 28

Marital Status: Not married

Place of birth: South Wello, Mekaneselam, Ethiopia

Language (speaking and writing): Amharic and English

II. Educational Background

1987-1995: Primary and Junior secondary school

- Borena primary and junior school

1996-1999: High school

- Borena high school

Sep, 2000-July, 2005 Higher Education

- Addis Ababa University, Faculty of Veterinary Medicine
  Degree in Doctor of Veterinary Medicine (DVM)

Sep, 2006- July, 2008: Post Graduate

- Addis Ababa University, Faculty of Veterinary Medicine
  Degree of Master of Science in Tropical Veterinary Public Health

Certificate: Computer MS DOSE

III. Work Experience

August, 2005-January, 2006: Benshangul Gumuze, Bambasi rural and agricultural office

February, 2006- September, 2007: Alage ATVET College as Instructor II

IV: Research activities

- Published one research paper: Seroprevalence of small ruminant Brucellosis in selected districts of Afar and Somali pastoral areas of Eastern Ethiopia: the impact of husbandry practice (*Rev. Med.* 2006, **157**, 11, 557-563)

V: Research interest

Zoonotic and communicable diseases

VI: Address E-mail: yibe4@yahoo.com.

11. SIGNED DECLARATION SHEET

"I, the signed, declare that the thesis is my original work and has not been presented for a degree in any University and that all sources of materials used for the thesis have been duly acknowledged"

Name Yibeltal Muhie Mekonnen

Signature **.....**

Date of submission **.....**

This thesis has been submitted for examination with our approval as University advisors.

Dr. Mosses Kyule (BVM, MSc, MVPM, PhD) **.....**
Dr. Teshale Sori (DVM) **.....**
Dr. Arega Firew (MD) **.....**