SERO-PREVALENCE AND ASSOCIATED RISK FACTORS OF *T.GONDII* INFECTION IN SHEEP IN SELECTED DISTRICTS OF KAFA ZONE AND PREGNANT WOMEN ATTENDING ANTE NATAL CARE AT BONGA HOSPITAL, SOUTH WESTERN ETHIOPIA

MSc. THESIS

BY

JALEL NEGERO

SEPTEMBER, 2016

JIMMA, ETHIOPIA

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A Thesis Submitted to School of Graduate Studies, Jimma University, College of Agriculture and Veterinary Medicine.

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JIMMA, ETHIOPIA

DEDICATION

This thesis is dedicated to my beloved wife Sister Jalale Hika for her endless love, support and encouragement.

STATEMENT OF THE AUTHOR

I hereby declare that this Thesis is my original work and that all sources of materials used for this thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirements for MSc degree in Veterinary Epidemiology at Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the Library. I critically declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Quotations from this Thesis are allowable with accurate acknowledgement of source.

Name: Jalel Negero Signature: _____ Date_____

Place: Jimma University, Jimma, Ethiopia

Date of Submission: September, 2016

BIOGRAPHICAL SKETCH

The author was born in Aira district, West Wollega Zone of Oromia National Regional State, in March 1986 G.C. He attended his elementary school at Chalte Aira elementary school. Then, he attended his high school and preparatory school at Lalo Aira Secondary School (LASS) from 2002/3-2006/7 G.C. The author successfully passed the 'Ethiopian Higher Education Entrance Examination' and joined Gondar University in 2008 G.C. He was graduated from Gondar University by DVM in 2012 G.C. He worked at Genji district Livestock development and fisheries office of West Wollega Zone as a field Veterinarian for three years. In 2015, he joined Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) to pursue a study toward his MSc in Veterinary Epidemiology.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ANC	Ante Natal Care
AOR	Adjusted Odds Ratio
ART	Anti-Retroviral Therapy
BPED	Bureau of Planning and Economic Development
CDC	Center of Disease Control and Prevention
CFT	Complement Fixation Test
CI	Confidence Interval
CMV	Cytomegalovirus
COR	Crude Odds Ratio
c-PCR	Conventional Polymerase Chain Reaction
CSA	Central Statistical Agency
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immune sorbent assay
G.C	Gregorian calendar
HIV	Human Immune Virus
IFAT	Indirect Fluorescent Antibody Test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect Hemagglutination Test
JUCAVM	Jimma University College of Agriculture and Veterinary Medicine
LASS	Lalo Aira Secondary School
LAT	Latex Agglutination Test
M.a.s.l	Meter above Sea Level
MAT	Modified Agglutination Test
MLST	Multi Locus Sequence Typing
n-PCR	Nested Polymerase Chain Reaction
OHCEA	One Health in Central and East Africa

OR	Odds Ratio
PA’s	Peasant Associations
Pexp	Expected Prevalence
q-PCR	Real Time Polymerase Chain Reaction
RPM	Revolution per Minutes
SD	Standard Deviation
SFDT	Sabin-Feldman Dye Test
SNNPRS	Southern Nations Nationalities and Peoples Regional States
SPSS	Statistical Package for Social Sciences
T. gondii	*Toxoplasma gondii*
VIF	Variance Inflation Factor
X^2	Chi- Square

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ABSTRACT

Toxoplasmosis caused by Toxoplasma gondii is a primary cause of abortions, disabilities and neonatal deaths in animals and humans. A cross-sectional study design was carried out from February to May 2016 to estimate seroprevalence and associated risk factors of T. gondii infection in sheep in selected districts of Kafa Zone and pregnant women attending ante natal care at Bonga Hospital, South Western, Ethiopia. Multistage and systematic sampling techniques were used to collect sera from 400 sheep and 210 pregnant women respectively. Serum collected was tested for anti T. gondii antibodies by latex agglutination slide test. Risk factors associated with T. gondii infection in both sheep and pregnant women was assessed through structured questionnaire. Univariate and multivariate logistic regression analysis was used to check association of T. gondii infection with different risk factors using SPSS version 20. The overall flock and animal level seroprevalence of T. gondii infection in sheep was 86.25% (95% CI: 78.8-93.8) and 67.25% (95% CI: 62.65-71.85) respectively. Multivariate analysis revealed that the odds of being seropositive was significantly high in adult sheep (AOR=1.69; 95% CI: 1.01-2.85; P= 0.050), in female (AOR=1.77; 95% CI: 1.04-2.99; P= 0.035) and in the presence of cats (AOR=1.74; 95% CI: 1.02-2.95; P= 0.041). The overall seroprevalence of T. gondii infection in pregnant women was 75.7% (95% CI: 69.9-81.5). Multivariate analysis indicated that the odds of acquiring T. gondii infection was significantly high in pregnant women between age range of 36-44 (AOR=2.82; 95% CI:1.2-7.82; P=0.031), in multigravidae (AOR=3.3; 95% CI:1.36-8.04; P=0.009), in those eating raw meat (AOR=5.1; 95% CI:2.22-11.68; P≤ 0.001), in those eating raw vegetables (AOR=5.5; 95% CI:1.03-29.5; P=0.046), in those who have history of abortion (AOR=4.4; 95% CI: 1.10-17.49; P=0.036), in women who drink river/streams water (AOR=5; 95% CI: 1.67-15.44; P=0.004) and in those who didn't wash their hands after handling of raw meat (AOR=2.4; 95% CI: 1-5.56; P=0.049). The present result shows high seroprevalence of Toxoplasma gondii infection in sheep and pregnant women. High seroprevalence in sheep is a good indicator of the potential risk for human infections and therefore, appropriate preventive measures, mainly public education on identified risk factors and screening of pregnant women during their antenatal care and further molecular level epidemiological studies are recommended to reduce associated morbidities and mortalities.

Keywords: Kafa zone, LAT, Pregnant women, Risk factors, Seroprevalence, Sheep, T. gondii

1. INTRODUCTION

Toxoplasmosis is one of the most important food borne zoonotic parasitic diseases (CDC, 2011) with worldwide distribution and caused by the cyst forming intracellular protozoan parasite, *Toxoplasma gondii* (Sudan *et al.,* 2013). It has the capacity of infecting all warm blooded animals including humans and approximately, one third of the world's human populations are reported to be seropositive for anti *T. gondii* antibodies (Tenter *et al*., 2000). *Toxoplasma gondii* has a complex reproductive cycle involving two hosts in which Felidae family are final hosts, whereas a wide range of warm blooded animals, including humans and cats serve as intermediate hosts of the parasite (Dubey, 2010a). Asexual phase of reproduction takes place in various tissues of intermediate hosts and sexual phase takes place in digestive epithelium of cats (Elmore *et al*., 2010).

Toxoplasma gondii is identified as the known causes of abortions or neurological symptoms in intermediate hosts. Among food producing animals, sheep is the most sensitive to *T. gondii* infection (Cenci-Goga *et al.*, 2011) and large amount of tissue cysts can be found in meat of seropositive sheep (Buxton *et al*, 2007; Dubey, 2010b). Sheep became infected by accidental ingestion of pastures or water containing *T. gondii* oocysts and transplacentally from ewes to lamb if infection occurs during pregnancy (Innes *et al.,* 2009). Once infection occur, it results in neonatal death, still births and abortions, economic losses and threats to human health via contaminated meat and milk and limit the productivity due to the associated morbidity and mortality (Panadero *et al.*, 2010; Edwards and Dubey, 2013).

Humans acquire the infection through accidental ingestion or handling of raw meat containing bradyzoites stage and vegetables and water contaminated by *T. gondii* oocysts (Montoya and Liesenfeld, 2004) and unpasteurized milk of small ruminants (Higa *et al.,* 2010; Qui *et al.,* 2012), blood transfusion, organ transplantation, laboratory accidents with biological handling of contaminated feces and soils (Pereira *et al.,* 2010) and placental transmission from mother to fetus if infection is contracted during pregnancy (Dubey, 2008; CDC, 2015). After infection by *T. gondii*, immune competent individuals are usually asymptomatic but, it is grave in immune-compromised individuals like AIDS patients and pregnant women (Dubey,

2009; Innes *et al.*, 2009). Primary infection during pregnancy results in severe damage to the fetus manifested by mental retardation, seizures, blindness, hydrocephalus, intracranial calcification and retinochoroiditis whereas, in late infection it causes premature birth, adverse complications and even death of fetus (Pereira *et al.*, 2010; Andiappan *et al.*, 2014). Congenital *toxoplasmosis* occurs when a woman is newly infected with *T. gondii* during pregnancy. The rate of congenital transmission and the degree of severity of *toxoplasmosis* in fetuses vary depending on the stage of gestation; the risk of transmission is lower in the first trimester and higher in the last trimester of pregnancy (Lopes *et al.*, 2012).

Toxoplasmosis in animals or humans is mostly diagnosed by detecting anti *T. gondii* specific IgM and IgG antibodies serologically and avidity of *T. gondii*-specific IgG antibodies. Serological tests, such as the latex agglutination test (LAT), Enzyme-linked Immunosorbent Assay (ELISA) and Immune fluorescence antibody test (IFAT) are used in many clinical laboratories (Thiebau *et al.*, 2007). Compared to the gold standard (the Sabin-Feldman dye test), Latex agglutination test has been reported to show high specificity (95%) and sensitivity (92%). Hence; it has been widely used in remote areas in developing countries (Montoya and Liesenfeld, 2004).

Anti *T. gondii* antibodies in sheep has been found worldwide with the range of 0 to 100% (Tenter *et al.*, 2000) but there are only a few studies on seroprevalence and risk factors associated with *T. gondii* infection in sheep in different environmental conditions (Dubey 2010a). In Ethiopia, limited studies conducted in northern and central parts of the country have confirmed the seroprevalence of *T. gondii* in sheep that varies from 20% to 56% (Tamiru *et al.*, 2004; Endrias and Daniel, 2014). However, there is no any reported data from Southern part of the country including in Kafa Zone; current study area. Similarly, the causes of most abortions, stillbirths and neonatal mortalities in sheep remain unexplored and the relationship with seroprevalence of *T. gondii* infection has not been well studied.

Epidemiological studies of *T. gondii* infection in pregnant women conducted around the world indicate considerable variation of seroprevalence between continents and countries. In Europe seroprevalence of anti *T. gondii* antibodies in pregnant women vary from 9% to 67% (Cong *et al.*, 2015) and in Asia 0.8% to 63.9% seroprevalence were reported (Song *et al.*, 2005).

In Africa different scholars were reported 18.5% to 92.5% seroprevalence (Ayi *et al.,* 2009; Kefale *et al.,* 2015). In Ethiopia, seroprevalence of *T. gondii* infection in pregnant women varies from 18.5 to 88.6% (Mengistu *et al.,* 2014; Kefale *et al.,* 2015). Nonetheless; the information on seroprevalence and associated risk factors of *T. gondii* infection among pregnant women is still limited in many parts of Ethiopia and unavailable in the current study area. Additionally, serological screening of *T. gondii* infection as part of routine antenatal care of pregnant women is not a common practice in Ethiopian health institutions (Anteneh *et al.,* 2014).

In general, there is lack of adequate epidemiological data on seroprevalence and associated risk factors with *T. gondii* infection in food producing animals in general and sheep in particular in the study area. Similarly, there is also absence of epidemiological data on *T. gondii* infection in pregnant women, who are at high risk groups. Therefore, this study was initiated with the following objectives.

General objective:

The main objective of this study was to estimate seroprevalence of *T. gondii* infection in sheep and pregnant women using latex agglutination test and assess the potential risk factors associated with seropositivity in these study populations in selected districts of Kafa zone, South Western Ethiopia.

Specific objectives:

- To estimate seroprevalence of *T. gondii* infection in sheep in Adiyo, Bonga and Decha districts of Kafa Zone and in pregnant women attending antenatal care at Bonga hospital.

- To assess the potential risk factors associated with the occurrence of *T. gondii* infection in sheep in Adiyo, Bonga and Decha districts of Kafa Zone and in pregnant women attending antenatal care at Bonga Hospital.

2. LITERATURE REVIEW

This section includes the general overview of *T. gondii*, life cycle, epidemiology, clinical manifestations, diagnosis, treatment, and control and prevention methods of *toxoplasmosis* in animals and humans.

2.1. *Toxoplasmosis*

Toxoplasmosis is a disease caused by *T. gondii* which is a protozoan parasite that infects all warm-blooded animals, including humans and it is considered as one of the most successful eukaryotic pathogens (Liu *et al.,* 2012) worldwide. Approximately one third of world human populations are chronically infected with *T. gondii* (Moncada and Montoya, 2012). The word *toxoplasma* originated from the Greek word toxon, means "bow" and plasmid means "form" and it was first discovered in 1908 in Tunis by Nicolle and Manceaux within the tissues of the *gondii* (Ctenodoactylus *gondii*). In the same year it was also described in Brazil by splendor within the tissues of a rabbit. Ultrastructure of *Toxoplasma gondii* morphology seen under the electron microscope is shown in Figure 1.

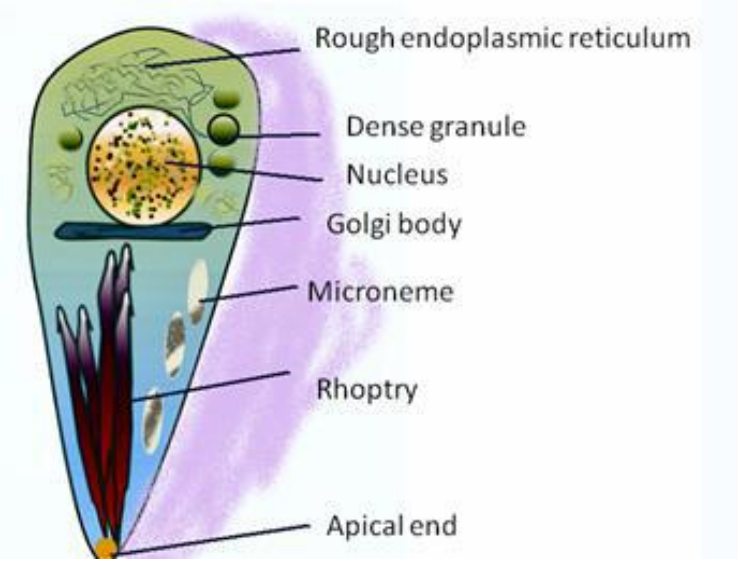

Figure 1: Schematic representation *T. gondii* morphology

Source; Dubey *et al.,* 1998

2.2. Life cycle

In the life cycle of *T. gondii* both definitive and intermediate hosts are involved. The sexual phase of the parasite life cycle occurs only in cats and other felids and the asexual phase occurs in other warm-blooded hosts and cats (Sudan *et al.*, 2013). In cats and other felids sexual cycle continues following ingestion of tissue cysts of infected mouse and then cysts survive and passage through the stomach of the cat, cyst wall is digested by gastric acid, bile and lytic enzymes of the upper digestive tract, which results in releasing bradyzoites and the released bradyzoites, invade the intestinal epithelium where they undergo sexual reproduction and oocyst formation (Bayarri *et al.*, 2012).

Besides systemic dissemination after conversion to the invasive tachyzoites stage, some organisms inside the epithelium of definitive host undergo five different developmental stages that reproduce asexually by endodyogeny, where two daughter cells are created inside one and by schizogeny to differentiate into micro and macro gametocytes within 2 days of infection and involving the formation of multiple merozoite cells around a previously divided nucleus (Tenter *et al*., 2000). The gametes fuse to form a zygote, which subsequently secretes a cyst wall to develop into oocysts and oocysts rupture the intestinal epithelial cells to disseminate into the lumen and hundreds of millions are excreted in feces of cats for days or weeks. Oocysts undergo sporulation outside of the body and become infective to other hosts (Dabritz and Conrad, 2010). Prepatent periods and frequency of oocyst shedding vary according to the stage of *T. gondii* ingested. Prepatent periods are 3 to 10 days after ingesting tissue cysts, and more than 18 days after ingesting oocysts (Dubey, 2008). Fewer than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts (Dubey, 2009).

The asexual phase occurs in any warm blooded hosts after infection by any infectious stage of the parasite (Dubey, 2010b). After ingestion, sporozoites are released and invade the macrophages of the intestine. The sporozoites are differentiated into motile tachyzoites and multiply rapidly by asexual process (endodyogeny) in a variety of cells and eventually encyst in several tissues, particularly in the brain and muscle tissues. They are also responsible for congenital infections during pregnancy. When host immunity develops, the process slow

down leading to chronic infection and the tachyzoites changed to bradyzoites (or cystozoites) stage, results tissue cysts and persist for a long time maybe for the life of the host (Tenter *et al.*, 2000; Jones *et al.*, 2003). In immune competent hosts, tissue cysts rupture occasionally and the released bradyzoites are killed but in immune suppressed hosts, bradyzoites released from tissue cysts may multiply locally and spread to other organs. Encephalitis is the predominant clinical manifestation of *toxoplasmosis* in AIDS patients and is believed to be due to reactivation of latent infections (Ira *et al.*, 2009). The life cycle of *T. gondii*, its final and intermediate hosts were illustrated in Figure 2 below.

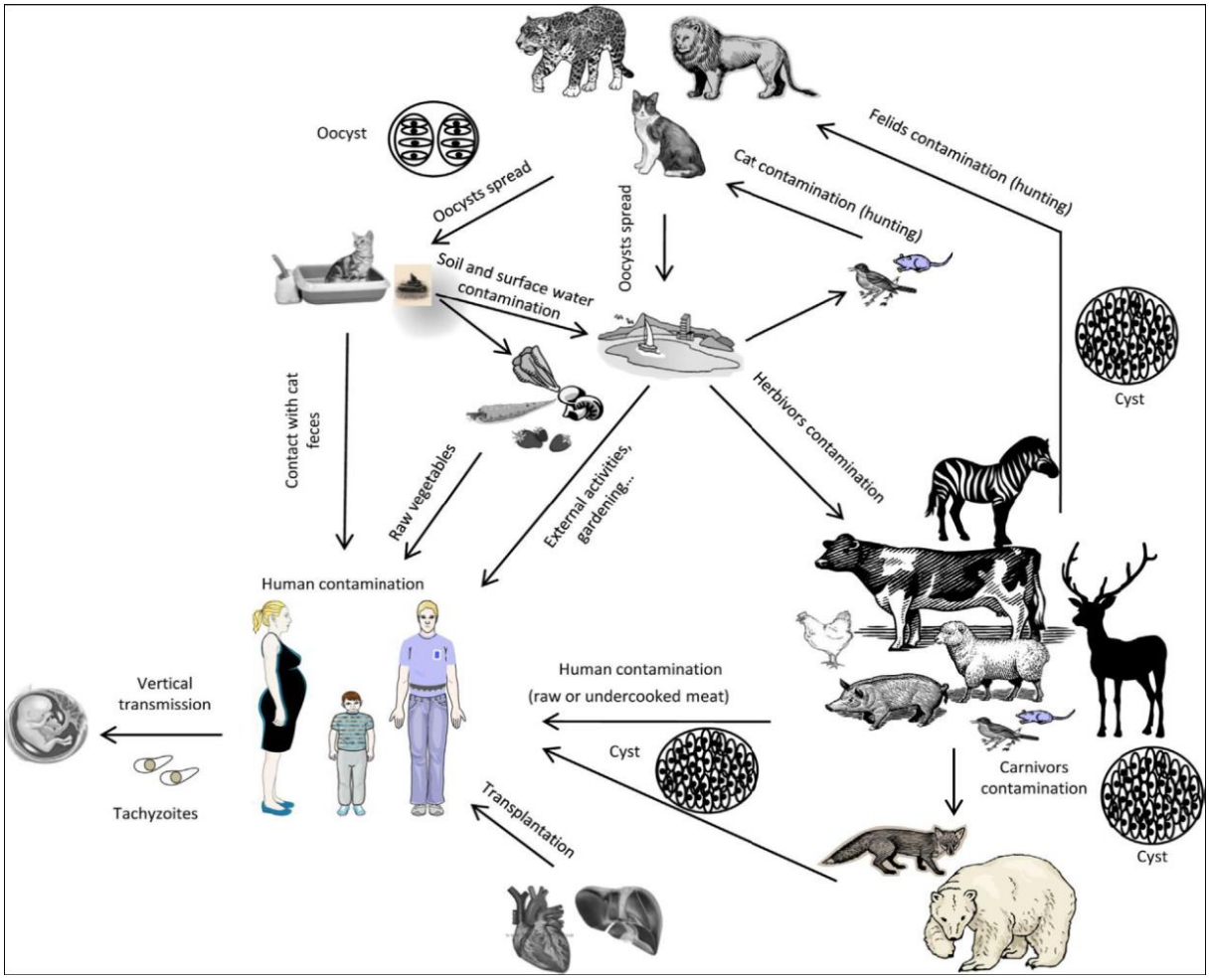

Figure 2: Life cycle of *Toxoplasma gondii*

Source; Robert-Gangneux, 2014.

2.3. Epidemiology

2.3.1. Host range and distribution

T. gondii has been confirmed from 200 species of vertebrates (Pal, 2007; Dubey, 2010a) and distributed worldwide. 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 (Sukthana, 2006). In the epidemiology of *T. gondii* infection, chickens are considered as one of the most important hosts since they are an efficient source of infection for cats that excrete the environmentally resistant oocysts due to their habit of feeding close to the ground (Lehmann *et al.,* 2006). All intermediate hosts may carry an infective stage of encysted *T. gondii* in their tissues (Dubey, 2009).

2.3.2. Source of infection and transmission

Ingestion of environmentally tough stages (sporozoites in oocysts) or eating raw/ undercooked meat containing tissue stages of tachyzoites or bradyzoites are the main source of *T. gondii* infection in humans (EFSA, 2007; Cenci-Goga *et al.,* 2013). Although the prevalence is low in cattle, consumption of beef also identified as an important source of *T. gondii* infection (Opsteegh *et al.,* 2011; Zhou *et al.,* 2011). The storage of animal feed may increase the presence of rodents that make the cats to spending length time looking for rodents in the feed storage locations and then cat's litters containing oocyst contaminate animal feeds which is potential source infection to the animals (Gharekhani, 2013). A cat's natural instinct to bury or hide its feces provides millions of infective oocysts into the environment which is potential source of infection. Oocyst contaminated pastures; fodder and drinking water are regarded as potential sources of postnatal infection in animals. The meat of infected sheep is a major source of *T. gondii* infection for humans and carnivore animals (Dubey, 2009).

Transmission in sheep occurs through ingestion of contaminated feed stuffs or grazing land with sporulated oocysts (Innes *et al.,* 2009) and transplacental transmission from ewes to lamb through infected placenta (Dubey and Jones, 2008). Although exact data are not available, it is thought that < 2% of sheep become congenitally-infected with *T. gondii* and less than 4% of

persistently infected sheep transmit it to the next generation which occurs between days 45 and 55 of gestation (Dubey, 2009; Higa *et al.*, 2010). But if infection is acquired in the third month of pregnancy, the lambs are born but they are sick; if it occurs after four months, the lambs may be born with the infection but they are asymptomatic (Lopes *et al.*, 2010).

In humans, horizontal transmission usually occurs through accidental ingestion of fruits, vegetables and water contaminated with *T. gondii* oocyst, blood transfusion, organ transplant, handling of contaminated feces by cleaning cat's litter, gardening and contaminated soils (Pereira *et al.*, 2010), ingestion of tissue cysts in undercooked/raw meat infected with *T. gondii*, drinking of unpasteurized sheep and goat's milk (Tenter *et al.*, 2000; Dubey, 2008). Vertical transmission from mother to fetus through infected placenta if infection is contracted during pregnancy (Dubey, 2010a). The possible mode or routes of transmission and source of infections of *T. gondii* are summarized in Figure 3.

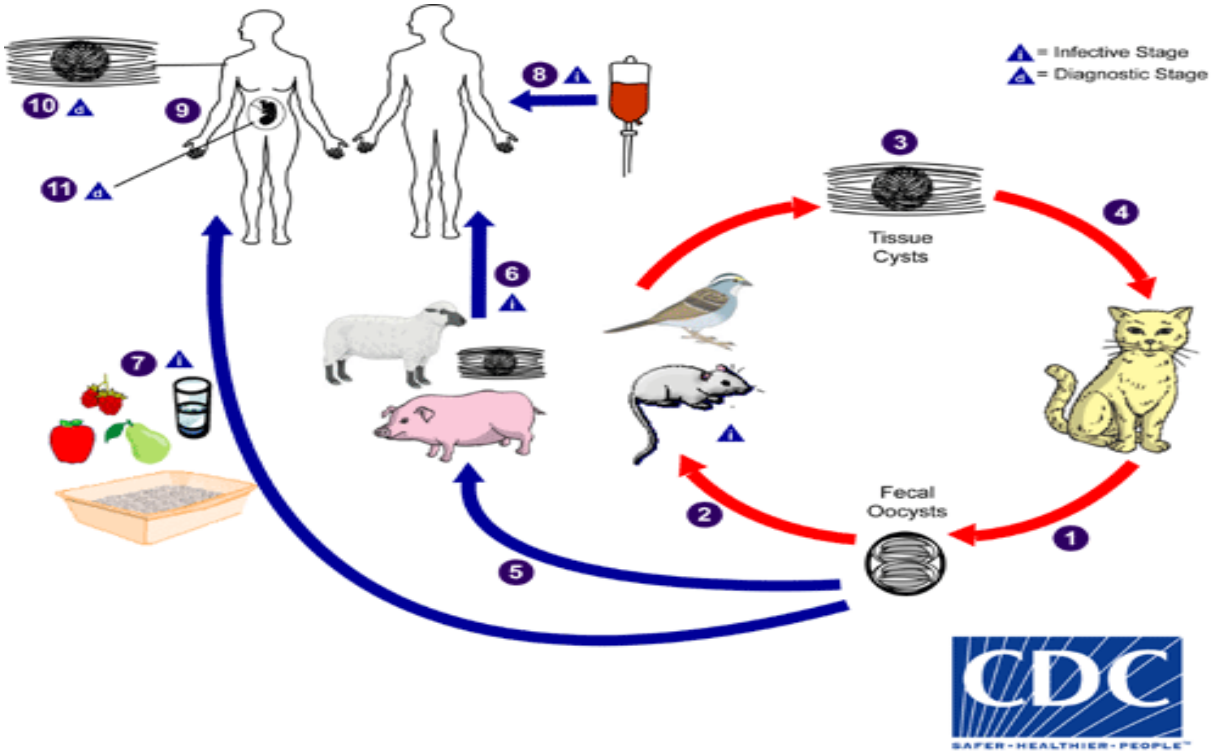

Figure 3. The possible source and transmission routes of *T. gondii* infection

Source: CDC, 2015.

2.3.3. Risk factors of *T. gondii* infection in sheep

Age

It has been reported that age is associated with the seroprevalence of *T. gondii* infection as adult sheep had a higher prevalence of *T. gondii* infection compared to young sheep (Endrias *et al*., 2013a; Ahmad *et al.,* 2015). This might be due to increased opportunities to exposure to several predisposing factors from environment (Dubey, 2010a). It may be also due to the fact that older animals are usually less immune to parasitic infections than younger animals (Roberts *et al*., 2001). But, different studies did not show positive association between age of animal and *T. gondii* infection (Lopes *et al.,* 2013; Yin *et al*., 2015; Arye *et al.,* 2015).

Sex

Earlier studies from Ghana and Ethiopia indicated that *T. gondii* infection is more common in female sheep compared to male (Teshale *et al*., 2007; Lashari and Tasawar, 2011; Endrias *et al*., 2013a). Nonetheless, in other studies higher seroprevalence of *T. gondii* infection were reported in male sheep than females (Ueno *et al*., 2009; Cosendey Kezen Leite *et al.,* 2014). The difference in susceptibility of infection between different sexes may be attributed to hormonal differences which affect the immune system (Roberts *et al*., 2001).

Herd/ flock size

According to Gazzonis *et al.* (2015), seroprevalence of *T. gondii* infection was positively correlated with flock size; large flocks possibly contribute to the maintenance of the infection within the animals. In the same way, the different studies reported that higher seroprevalence in large herds (Klun *et al.,* 2006; Vesco *et al.,* 2007; Anderlini *et al*., 2011; Ahmad *et al.,* 2015). In contrast, result reported by Cenci-Goga *et al.* (2013) from sheep reared in Tuscany, Italy and Endrias *et al.* (2013a) from central Ethiopia showed that higher seroprevalence in small flocks which may be due to the fact that small flocks are often repeatedly tethered or allowed to graze on a small area close to the farm and households where domestic cats have an easy access and may contaminate the pasture and feed reserves.

Presence of cats

The domestic cat is the only domestic animal that is used as a definitive host for the completion of *T. gondii* life cycle; thus, play a key role in the epidemiology of *T. gondii* infection. After primary infection with *T. gondii,* cats shed large numbers of oocysts into the household, thereby putting their owners at risk of infection. Stray cats may contaminate the environment with oocysts which may infect livestock (Tenter *et al.,* 2000). Several previous risk assessment studies of *T. gondii* infection in sheep revealed the significant association between *T. gondii* seropositivity and presence of domestic cats at home (Vesco *et al.,* 2007; Tamiru *et al.,* 2008; Sechi *et al.,* 2013; Ahmad and Qayyum, 2014). In contrast, absence of significant association between presence of cats in the household and *T. gondii* seropositivity were reported in other studies (Endrias *et al*., 2013a; Cosendey KezenLeite *et al.,* 2014).

Season

The difference in weather condition has a clear influence on the habitat of *T. gondii*; an increase in ambient temperature and precipitation can change the soil humidity, so that the sporulated oocysts persist for a long time viable in the moist environment (Meerburg and Kijlstra, 2009). Higher prevalence of *T. gondii* infection in warm climates, moist and humid areas is associated with the longer viability and survival of *T. gondii* oocysts in moist or humid environments (Dubey, 2010b). Study conducted in sheep in tropical areas of Mexico show that increased seroprevalence of *T. gondii* infection in humid areas as compared to the cold areas (Caballero-Ortega *et al.,* 2008). This explained the high frequency of *T. gondii* in this region, which apparently had favorable climatic conditions for the transmission of this protozoan, beside the presence of both domestic and wild cats. It is well known that a dry climate has an adverse effect on the persistence and dissemination of oocysts of *T .gondii* (Jones *et al.,* 2001; Dubey, 2010a).

Altitude

Endrias *et al.* (2013a) states that sheep sampled from the highland and midland were significantly at high risk of *T. gondii* infection compared to sheep sampled from lowland

areas because, highland and midland areas receive more rainfall; evaporation is relatively less and abundance of forest canopies or vegetation in the area which results high chance of survival of *T. gondii* oocysts in the environment. Similarly, in Nigeria (Kamani *et al.,* 2010) reported that a milder climate with higher rainfall and relative humidity favors a higher seroprevalence as compared northern zones. However, a study conducted in Mexico (Caballero-Ortega *et al.,* 2008) revealed that, infection rate was higher at low altitudes.

Management system

Gazzonis *et al.* (2015) revealed that management system is a common risk factor associated with the occurrence of *T. gondii* infection in animals: extensive or semi-intensive management are at higher risk of *T. gondii* infection due to inadequate hygienic standard and consequent spread of *T. gondii* oocysts among animals. The report of Clementino *et al.* (2007) show that significantly higher seroprevalence of *T. gondii* infection was observed in sheep flocks reared under extensive management system. This suggests that animals reared under extensive management may be more exposed to cats in the environment or to contaminated stagnant pools, which makes oocysts more dispersed in the environment. Furthermore, animals kept on pastures are more exposed to infection due to contamination of the environment with oocysts of *T. gondii* (EFSA, 2007).

However, different scholars reported higher prevalence of *T. gondii* infection in intensive management system. They explained that in intensive management, the animals could possibly be more exposed to oocyst shed by cats than extensive farms (Dubey, 2009; Al mabruk *et al.,* 2013). In contrast of both observation, Teshale *et al.* (2007) reported as management has no significant effect on seroprevalence of the infection rather the feces and urine of animals, waste feed and water supplied to animals might contribute to the maintenance of viability of oocysts (Cenci-Goga *et al*., 2013; Bawn *et al.,* 2016).

2.3.4. Risk factors of *T. gondii* infection in humans

Consumption of raw meat

Although the relative importance of the risk factor and the type of meat associated with it varied among different countries (Cook *et al.,* 2000), many scholars report that about 50% of all human *toxoplasmosis* cases are related with food borne infection (Slifko *et al.,* 2000). Consumption of undercooked meat is most likely an important source of *T. gondii* infection in many countries and reported as the main risk for *T. gondii* infection in humans (Dubey, 2010b). A study conducted in France, revealed that the seroprevalence of *T. gondii* infection in human is as high as 75-80% due to a desire of eating raw or undercooked meat (Miller *et al.*, 2009). In Sudan, (Mohammad *et al.,* 2013), in Ethiopia, (Kasim and Zinabu, 2015; Kefale *et al.,* 2015; Birihanu *et al.,* 2016; Dechassa *et al.,* 2016), in Rwanda, (Esperance, 2014) were reported statistically, significant association between consumption of raw meat and being seropositive for anti *T. gondii* antibodies. In contrast, other studies (Endalew *et al.,* 2012; Endrias *et al.,* 2013b; Woyneshet *et al.,* 2015) revealed the absence of statistically significant association between seropositivity of *T. gondii* and ingestion of raw meat.

Consumption of raw vegetables

Eating of unwashed raw vegetables or fruits was associated with an increased risk of primary infection of *T. gondii* (Berger *et al.,* 2009). Some previous reports (Mohammad *et al*., 2013; Endrias *et al*., 2013b; Kefale *et al*., 2015) revealed the presence of significant relationship between anti *T. gondii* seropositivity and consumption of raw vegetables or fruits. In contrary, the reports of Endalew *et al.* (2012) and Woyneshet *et al.* (2015) revealed that absence of statistically positive association between *T. gondii* infection and eating of raw vegetables.

Contact with soil

Multicenter case-control study from Europe revealed that contact with soil was identified as a strong risk factor of *T. gondii* infection and 6 to 17% of primary infections in humans were attributed to this risk factor (Cook *et al*., 2000). The study of Jafari *et al.* (2012) and

Kudakwashe and Yesuf, (2014) states the presence of statistically, significant association between contact with soil and being seropositive for anti *T. gondii* antibodies. But, the finding of Doudou *et al.* (2014) and Puccio *et al.* (2014) did not show significant association between *T. gondii* seropositivity and contact with soil.

Presence and contact with cats

Reports from Ghana (Ayi *et al.,* 2009), Brazil (Andrade *et al.,* 2013), South western Ethiopia (Endalew *et al.,* 2012; Dechassa *et al.,* 2016); North western Ethiopia (Birihan *et al.,* 2015; Kefale *et al.,* 2015) showed statistically, significant association between *T. gondii* infection and presence of domestic cats at home. In contrast, studies reported from Sudan (Raouff and Elbasheir, 2014), Tanzania (Mwambe *et al.,* 2013; Shao *et al.,* 2015) and Ruwanda (Esperance, 2014) showed that absence of statistically significant association between *T. gondii* infection and presence of cats in the household. This variation among different studies indicates the risk of contracting *T. gondii* infection might not be due to the presence of cats in the households but it could be due to contact with cats' fecal material while gardening.

Hygienic condition

Better hygienic conditions reduce the risk of water and food being contaminated with cat feces as a result, the risk of transmission of the infection will be reduced. Endrias *et al.* (2013b) reported that vegetables transported and sold under poor hygienic practice and poor quality water used to wash vegetables might have provided the opportunities for contamination by *T. gondii* oocysts. The role of surface water in outbreaks of *toxoplasmosis* has been reported previously (Romanelli *et al*., 2007). The same authors concluded that surface water sources can be simply contaminated with oocysts of *T. gondii*.

Age

The other important risk factor identified for occurrence of *T. gondii* infection in human is age. Statistically, significant relationship between age and *T. gondii* seropositivity was reported in some investigations (Endalew *et al.,* 2012; Endrias *et al.,* 2013b; Birihan *et al.,*

2015) and suggest that as age increases the possible change of exposure to the oocyst also increases. In contrast, comparative cross sectional study done in Addis Ababa, Ethiopia (Woyneshet *et al.,* 2015) in Southern Thailand (Nissapatorn *et al.,* 2011) and in Taiwan (Chiang *et al.,* 2014) revealed that being seropositive for anti *T. gondii* antibodies and increased age of individuals were not found to be significantly associated.

Residential places

Study conducted in pregnant women in northern Iran (Panah *et al.,* 2013) indicates that sero-prevalence of *T. gondii* infection is found to be high in rural areas than urban areas. Correspondingly, the seroprevalence of *T. gondii* infection among pregnant women in northeast Iran living in urban and rural areas was found to be 29.1% and 47.5%, respectively (Babaie *et al.,* 2013). High seroprevalence observed in rural dwellers might be associated with farms buildings and their surroundings which provide shelter for cats, agricultural activity which is the major source of infection for animals and humans. On top of this, presence of large mechanical vectors like sow bugs, earthworms, houseflies, cockroaches and snails are known to harbour and disseminate oocysts; thus, increase prevalence of *T. gondii* infection in rural area (Dubey, 2009). In contrast, the reports of Sucilathangam *et al.* (2013) and Kudakwashe and Yesuf, (2014) revealed high seroprevalence of *T. gondii* infection in pregnant women who live in urban than rural areas.

2.4. Worldwide Prevalence of *T. gondii* infection in Sheep

The seroprevalence of anti *T. gondii* antibodies in sheep found worldwide (Dubey, 2009) and reported with different seroprevalence which ranges from 0 to 100% (Tenter *et al.,* 2000). *Toxoplasmosis*, besides causing reproductive problems in sheep, it is reported as an important zoonotic (Moreno *et al.,* 2012; Silva *et al.,* 2013). Seroprevalence of 44.1% has been reported in sheep from West Indias (Chikweto *et al*., 2011) and 18.6% in Sao Paulo, Brazil (Langoni *et al*., 2011). 67.7% of seroprevalence of anti *T. gondii* antibodies in sheep has been also reported from Zimbabwe (Hove *et al.,* 2005), 71% of seroprevalence from Grenada, West Indies (Sharma *et al.,* 2015), 71% from Libya (Al- mabruk *et al*., 2013), 20.3% from North western China (Yin *et al.,* 2015), 17.9% from North Eastern China (Xu *et al.,* 2015).

Correspondingly, from Italy, (Vitale *et al*., 2008; Sechi *et al.,* 2013; Cenci Goga *et al.,* 2013; Gazzonis *et al.,* 2015) reported 95.7%, 33.97%, 33.3% and 59.3% the overall seroprevalence of anti *T. gondii* antibodies in sheep respectively. From Iran (Moazeni Jula *et al*. (2013) and Havakhah *et al.* (2014) reported 36.8% and 27.6% seroprevalence of *T. gondii* infection in sheep respectively. On the other hand, Arye *et al*. (2015) from South Africa, Correia *et al.* (2015) from Brazil, Klun *et al.* (2006) from Serbia, Mor and Arslan, (2007) from Turkey reported 8%, 11.1%, 84.5% and 95.7% of the overall seroprevalence of anti *T. gondii* antibodies in sheep respectively. In Egypt, 43.3%, 43.7%, 53.3% seroprevalence of sheep *T. gondii* infection were reported respectively by Khalifa *et al.* (2005); Shaapan *et al.* (2008) and Khater *et al.* (2013).

In Bulgaria, (Prelezov *et al.,* 2008) reported 48.2%, in Scotland, (Katzer *et al.,* 2011) reported 56.6%, in Sudan, (Khalil and Elrayah, 2011) reported 57.5%, in Nigeria (Kamani *et al*., 2010), reported 22.1% in Brazil (Andrade *et al.,* 2013) describe 24.8% and in USA (Edward and Dubey, 2013) reported 94.8% seroprevalence of *T. gondii* infection in sheep. Different studies undertaken in Ethiopia to investigate the importance of *T. gondii* infection in sheep indicated high seroprevalence ranging from 20 % to 56% (Tamiru *et al.,* 2004; Muhie, 2008; Endrias *et al.,* 2013a; Endrias and Daniel, 2014).

2.5. Worldwide Prevalence of *T. gondii* infection in Humans

Human *toxoplasmosis* can result from a congenital or an acquired infection (Dubey, 2010b) and varies from 7.5 to 95% in different parts of the world, and in fact between different populations groups within the same country (Asthana *et al.,* 2006). This variation may be due to geographical, socio-economic and environmental factors, age, genetic and immune status of host and parasite (Furtado *et al.*, 2013; Ferreira *et al.,* 2014; Abbas, *et al.*, 2014), parasite genotype, parasite load and stage of parasite development, exposure to cat feces and soil, level of education, cultural habits of the people, occupation, serological techniques used, sample size, increased globalization of the society, population increase and residential places (Akhlaghi *et al.*, 2014).

Several surveys revealed that human *toxoplasmosis* is higher in Africa, the Middle East, Southeastern Asian countries, Latin America and parts of Eastern and Central Europe than in the United States and most Western European countries (Pappas *et al.,* 2009). The incidence of ocular *toxoplasmosis* is also higher in Africa and South America compared to that of Europe (Peterson *et al.,* 2012). In pregnant women, the risk of congenital infection from acute infection by *T. gondii* ranges between 20% and 50% when strict treatment was not undertaken (Jones *et al*., 2003). Nevertheless, it will be reduced when acute maternal infection (IgM) is detected during the first trimester of pregnancy which ranges between 10% to 25% (Remington *et al.,* 2001) and high (65-90%) when acute maternal infection is detected during the third trimester of pregnancy. Dubey, (2009) reported that, transmission of *T. gondii* from mothers to fetus occurs between one and four months after placenta has been infected by tachyzoites. According to the report of Furtado *et al*. (2013) the risk of mother-to-child transmission increases as pregnancy progresses.

Hospital based survey of seroprevalence of anti *T. gondii* antibodies in pregnant women attending Ante Natal Care (ANC) were reported from different parts of the world. For instance: Raouff and Elbasheir (2014) from Sudan, Esperance, (2014) from Rwanda, Bessong *et al.* (2010) from South Africa reported 20.2%, 12.2% and 18.1% seroprevalence of anti *T. gondii* antibodies among pregnant women respectively. Likewise, Mwambe *et al.* (2013) and Shao *et al.* (2015) reported 30.9% and 41.67% seroprevalence of anti *T. gondii* antibodies in pregnant women from Tanzania respectively. Nasir *et al.* (2015) from Nigeria and Koffi *et al.* (2015) from Ivory Coast were reported 48.9% and 58.7% seroprevalence of *T. gondii* infection in pregnant women respectively. From India, Sarkar *et al.* (2012) and from Yemen, Al- Eryani *et al.* (2016) reported 49.5 and 45.4% of the overall seroprevalence of pregnant women *toxoplasmosis.*

Studies conducted in Ethiopia by different scholars also revealed different report of seroprevalence of anti *T. gondii* antibodies in pregnant women. Woyneshet *et al.* (2015) reported 85.4% seroprevalence of anti *T. gondii* antibodies in pregnant women attending ANC at two hospitals of Addis Ababa, Ethiopia. Endalew *et al.* (2012) and Endrias *et al.* (2013b) reported 83.6% among pregnant women in Jimma town and 81.4% among women of child

bearing age in Central Ethiopia respectively. Similarly, Kefale *et al.* (2015) and Birihan *et al.* (2015) described 18.5% and 68.4% of seroprevalence of anti *T. gondii* antibodies among pregnant women attending antenatal care at Felege Hiwot Referral Hospital and Debre Tabor town northwestern Ethiopia respectively.

2.6. Clinical Manifestation of *Toxoplasmosis* in Sheep

In sheep clinical symptoms include early embryonic death, mummification, stillbirths, neonatal death or birth of alive but weak lambs (Buxton and Rodger, 2008). Up to 50% of sheep may develop fever, tremor, dyspnea and abortion in the last 4^{th} weeks of pregnancy. Severity of infection is associated with the stage of pregnancy at which the ewe becomes infected. Infection during the early stage of gestation can result in fetal death, resorption and abortion, while infection in the latter stage of gestation (fetal immunity is relatively well developed), may have no clinical effect and lambs are usually born normal but infected and immune (Buxton *et al.,* 2007).

2.7. Clinical Manifestation of *Toxoplasmosis* in Pregnant Women

The clinical spectrum of *T. gondii* infection varies from an asymptomatic state to severe illness. The parasite can affect the host's lymph nodes, eyes, central nervous system, liver, and heart (Alvarado-Esquivel *et al.,* 2009). Primary infections with *T. gondii* acquired during pregnancy are usually asymptomatic for the pregnant women but can lead to serious neonatal complications (Mwambe *et al.,* 2013). Congenital *toxoplasmosis* is considered as a serious health problem in pregnant women, who can pass the infection to the fetus or newborn and cause severe consequences in the infant (e.g. mental retardation, blindness, and epilepsy). If infection is acquired by mother during pregnancy, severe neurological abnormalities like intracranial calcification, microcephalus, hydrocephalus, convulsion and severe restriction of intrauterine growth (Jones *et al.,* 2003; Dubey and Jones, 2008).

In contrary, if infection occurred before pregnancy the fetus is protected from such clinical manifestations. Therefore, sero-negative women should routinely check up their status of infection during pregnancy. The risk of congenital infection is lowest when maternal infection

is in the first trimester (10–15%) and highest when infection occurs during the third trimester (60–90%). The highest risk to the fetus is when infection is acquired between the 10^{th} and 24^{th} weeks of gestation (Remington *et al.,* 2006). In pregnant women with AIDS and those on high dose immunosuppressive therapy like organ transplant, patients with malignancies and connective tissue disorders, there could be reactivation of *T. gondii* infection (Montoya and Remington, 2008). In a large percentage of congenitally infected patients, ocular disease may occur. It has been suggested that at least two thirds of ocular *toxoplasmosis* is caused by postnatal infections. The appearance of ocular lesions varies with duration of active retinal infection and intensity of inflammation which is predominantly characterized by necrotizing retinitis, satellite of an existing scar. Patients with concomitant *toxoplasmosis* and HIV infection manifested fever (63.5%), headache (44.7%), rashes (41.2%) and anorexia (34.1%) (Uneke *et al*., 2005). Studies from Brazil, United States and Poland suggest that human ocular *toxoplasmosis* may be associated predominantly with type I strains or with strains from a mixture of type I and III alleles (EFSA, 2007).

2.8. Diagnosis

T. gondii is confirmed by biological, serological, histological, or molecular methods, or by some combination of the above (Pal, 2007).

2.8.1. Serological tests

Serological tests used to diagnose *toxoplasmosis* in both animals and humans include, indirect fluorescent antibody test (IFAT), Modified agglutination test (MAT) (Shaapan *et al.,* 2008; Silva *et al.,* 2013) because it detects IgG with the additional advantage of not requiring a specific conjugate, and it also does not require complicated equipment for diagnosis (Dubey 2008; 2010a). The ELISA test has been widely used for the serological diagnosis of *T. gondii* in sheep (Andrade *et al.,* 2013; Endrias *et al.,* 2013a), the Sabin–Feldman dye test (SFDT), the indirect hemagglutination assay (IHA), the latex agglutination test (LAT) and complement fixation test (CFT) are also frequently used (Pal, 2007).

2.8.2. Bioassays of tissues in mice

The present trend, for obvious ethical reasons and animal welfare, it is limited or avoided to use biological testing for diagnosis. However, the bioassay in mice is one of the primary methods used to detect *T. gondii* cysts in tissue for confirming suspected cases of infection by the parasite. It has high sensitivity and specificity which make this biological testing the gold standard (Ueno *et al.,* 2009; Cenci-Goga *et al.*, 2011) but it is also very cost, difficult and slow. The choice of inoculums will depend upon the circumstances. Secretions, excretions, body fluids, and tissues taken by biopsy, such as lymph nodes or muscle tissue, are possible specimens from which to attempt isolation. Cerebral spinal fluid from a child with possible congenital infection and encephalitis or lymph node material from a person with lymphadenopathy are good sources of *T. gondii* (Dubey, 2010a).

2.8.3. Molecular detection of *T. gondii*

Molecular methods can be divided into 2 groups. The first group focuses on specific detection of *T. gondii* DNA in biological samples. The conventional PCR (c-PCR), nested PCR (n-PCR) and quantitative real-time PCR (qPCR) of repetitive DNA sequences belong to this group. The PCR molecular method focuses on a high resolution identification of *T. gondii* isolates. The multilocus PCR-RFLP, microsatellite, and multilocus sequence typing (MLST) of single copy DNA sequences belong to this group. The second group of molecular methods focuses on a high resolution identification of *T. gondii* isolates. The multilocus PCR, RFLP, microsatellite, and multilocus sequence typing (MLST) of single copy DNA sequences belong to this group (Su *et al*., 2010).

Molecular detection requires the use of Polymerase chain reaction (PCR) to isolate and amplify Deoxyribonucleic acid (DNA) from biologic samples as reported by Switaj *et al.* (2005). The use of PCR is most appropriate for patients with immune deficiencies. For this purpose 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. The most common use of PCR is for prenatal diagnosis of the congenital infection is using amniotic fluid (Remington *et al.,* 2004).

2.9. Treatment

The drugs which are used commonly used for treatment of toxoplasmosis are sulfadiazine (15-25 mg/kg) and pyrimethamine (0.44 mg/kg); which act synergistically. Although they cannot eradicate infection, the drugs are beneficial if given in the acute stage of the disease, when there is active multiplication of the parasite. These drugs are believed to have little effect on the bradyzoite stage. Sulfonamides, trimetoprim, pyrimethamine and clindamycin have been used to treat clinical *toxoplasmosis* (Elmore *et al.,* 2010). Combination of clindamycin and pyrimethamine may offer more effective therapy (Dubey, 2010b).

2.10. Prevention and Control of *Toxoplasmosis*

In Animals- Cats should be kept indoors and fed canned, cooked, or previously frozen food to keep them from hunting and catching infected rodents and birds and thus becoming infected. It has been shown in the laboratory that the addition of monensin to dry cat food can suppress the excretion of oocysts in feces (Acha and Szyfres, 2003). For some years, work has been under way to develop vaccines against *toxoplasmosis* for cats, sheep, and swine. So far, the only successful effort has been a modified live parasite vaccine for sheep, which is administered before impregnation to prevent congenital infections (Urquhart *et al*., 2005).

In humans- People handling raw meat, contact with cat stool, litter or litter box should wash thoroughly their hands with soap and water before they begin other tasks. All cutting boards sink tops, knives, and other materials coming in contact with uncooked meat should be washed with soap and water, meat should be frozen to –12ºC at least for 24 hours to kill *toxoplasma* tissue cysts, but sporulated oocysts can survive at –20 ºC for up to 28 days (Pal, 2007). Removal of cat feces daily from house, keeping cats out of sandboxes and other areas where children play to prevent the cats defecating there, unwashed fruits or vegetables as well as unpasteurized milk should not be eaten. Public education particularly the cat handler, women, butcher about the source of infection, mode of transmission, nature of disease, personal hygiene and hazards of eating raw or undercooked meat (Pal, 2007).

3. MATERIALS AND METHODS

3.1. Description of Study Area

Present study was conducted in three districts of Kafa Zone, SNNPRS; namely Mengiwo currently called, Adiyo kaka, Decha and Bonga (Figure 4). Adiyo Kaka district is found at a distance of 516 km and 86 km from Addis Ababa and Kafa zone respectively. The total livestock population of the district is 193,412 heads, out of which 88,116, 31,469, 13,234, 10,965 and 49,628 were cattle, sheep, goats, equines and poultry respectively. The district has a total human population of 123, 304 of which 59,906 are males and 63,393 are females. Decha district is located at a distance of 528 km and 98 km from Addis Ababa and Kafa zone respectively. The district has bimodal rainfall and the main rainy months are from July to September months and the second rainy months are from March to April months. Animal population of the district was estimated at 2, 75, 329 heads, of which 79,064, 48,750, 17,159, 4,642, 1, 25, 714 were cattle, sheep, goats, equines and poultry respectively. Decha district has a total human population of 148,918, of whom 74, 274 are males and 74,644 are females (CSA, 2015).

Bonga town/district is the administrative Centre of Kafa Zone and located at a distance of 430 km South-west of Addis Ababa. The area is characterized by a long rainy season that extends from March through October. Total animal population of the town was 11, 567 of which 3, 973, 595, 315, 422 and 6, 262 are cattle, sheep, goats, equines and poultry respectively. The estimated human population of the town was 37, 759, of which 18,656 are males and 19,103 are females (CSA, 2015). Currently, Bonga hospital gives services for 63,594 peoples and the major activities given by the hospital are major and minor surgery, inpatient and outpatient, ART, delivery and gynecology, family planning, dental, ophthalmology, pharmacy, laboratory and ANC services. The hospital is also used as a referral hospital for other health centers of Kafa Zone. The purposes of attending ANC were to find out the position of the baby, to receive a Tetanus Toxoid vaccination, checking of blood group and HIV status. The location, altitude, annual mean rainfall and the mean minimum and maximum temperature of study districts was shown in Table 1.

Table 1: Location, altitude, annual mean rainfall and the mean minimum and maximum temperature of study districts.

Variables	**Study districts**		
	Adiyo kaka	Decha	Bonga
Altitude (m.a.s.l)	500-3500	800-2500	1400-1650
Temperature (^{o}C) (min-max)	8-32	12.4-26.8	11.8-27.1
Rain fall (mm)	1075	1450	1584
Longitude	36 ^{o} 47’ E	36° 09' E	36°14′ E
Latitude	7 ^{o} 26 ’ N	6°49' N	7°16′ N

Source: Bureau of Planning and Economic Development (BPED, 2006)

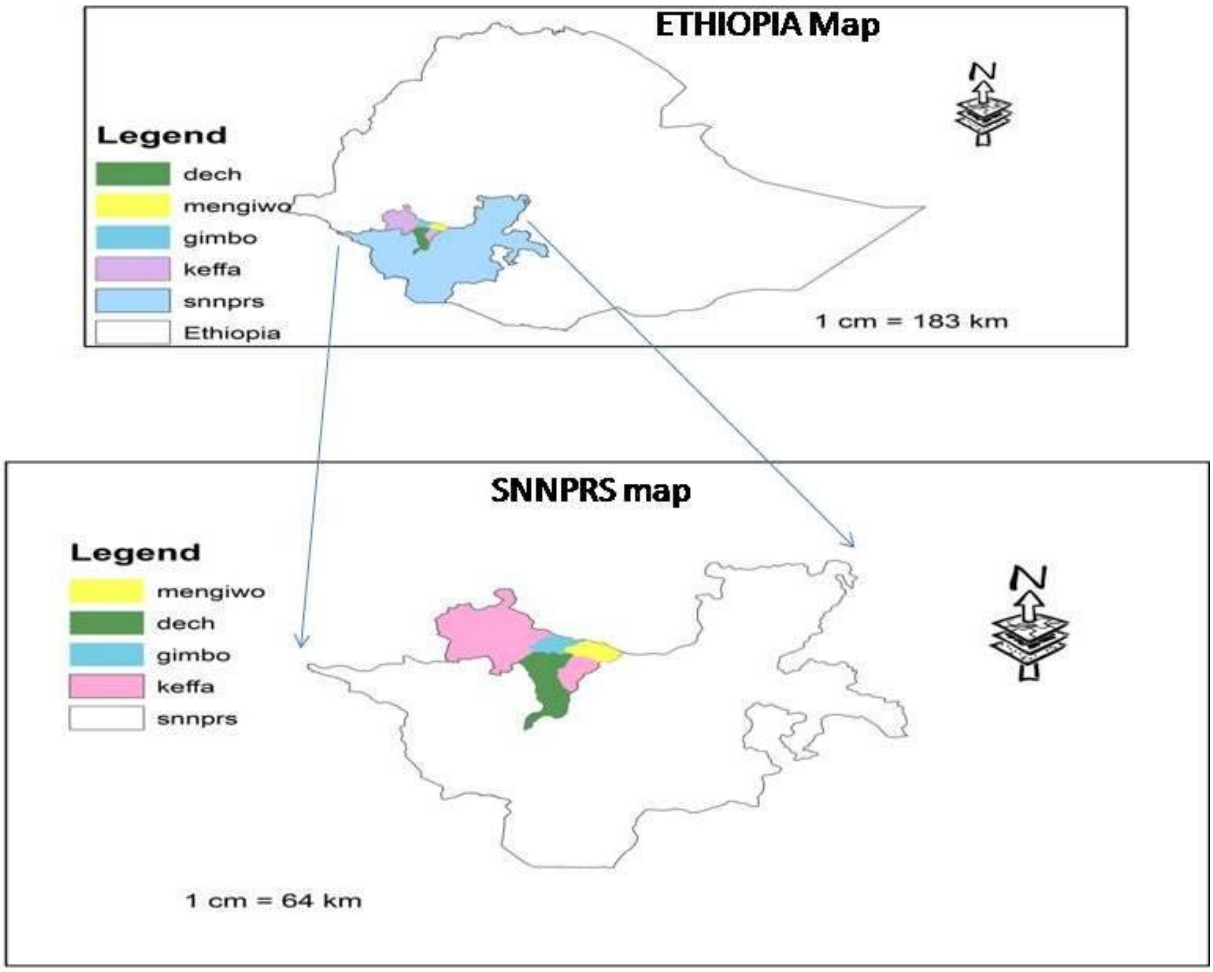

Figure 4. Map of study area.

3.2. Study Population and Study Period

Bonga breed of sheep raised by farmers in three study districts was the study population of the current study. Majority of sheep were kept under extensive management system in which few of them were feed with supplementary feed and other supportive managements including health care facilities.

Similarly, pregnant women aged between 15-44 years who visited the Bonga hospital for the purpose of follow up of ante natal care (ANC) services from February to May 2016 was also the study population of the present study. Each pregnant woman was included in the study upon signing the consent form for the study. Those pregnant women who failed to sign the consent form and unable to hear and talk were excluded from the study.

3.3. Study Design, Sampling Technique and Sample Size Determination

The study design in sheep was cross sectional using a multi stage sampling technique which was employed according to the following procedures. First, districts known for sheep population and undertaking community based breed improvement were identified and selected conveniently through discussion with district agricultural experts and Bonga Agricultural Research center. Then, from each selected district, the total of nine Peasant Associations (PA's) were selected conveniently based on existing population of sheep, proximity to road for transportation, practicing of community based breed improvement widely and this selected PA's were handled as a primary sampling unit. From each selected PA's, number of households owned sheep were identified and household flocks were randomly selected and taken as secondary sampling unit. Individual sheep of both sexes above six months of age found in a household flocks were taken as tertiary sampling unit and sampled randomly based on their ear tag ID number. For few numbers of sheep who have no ear tag ID number, randomization was done based on the name given to them by owners.

In sheep sample size was determined according to Thrusfield (2007) using an expected animal level prevalence of 50% and a desired absolute precision of 5% with 95% CI, since there was no previously expected prevalence in the study area. $n=1.96^2 \times Pexp\ (1-Pexp)/d^2$. Where, n =

required sample size; Pexp = expected prevalence and d = desired absolute precision. Based on the above formula, the estimated sample size was 384 but, 400 sheep were sampled. To reach the intended sample size, house hold flock and animals sampled were distributed to the three study districts proportionally allocated based on the existing house hold flock number and sheep population to respective PA's. Accordingly, 38, 30 and 12 flocks were selected randomly from Decha, Adiyo kaka and Bonga districts respectively. Totally, 80 flocks were selected from nine PA's. Similarly, 55% (n=220), 30% (n=120) and 15% (n=60) of sheep were sampled from selected flocks of Decha, Adiyo kaka and Bonga districts respectively. Flock size was decided by dividing a total number of sheep presented in 80 flocks (n=720) with selected flock number (80). Then, if number of sheep in one flock was greater than an average (i.e.9) it was taken as large flock size if not considered as small flock size. From each selected flocks 30% of sheep was selected randomly proportional to number of sheep presented in the flock.

In pregnant women, the study design was institutional based cross sectional study using systematic sampling technique. Sample size, for pregnant women was decided according to the statistical formula for sample size as the basis a Thrusfield (2007) with an expected prevalence of 83.6% from Jimma town (Endalew *et al*., 2012) and desired absolute precision of 5% with 95% CI. Accordingly, a total of 210 pregnant women were included in the study. To reach the decided sample size, one month data from the ANC registration book of the hospital was taken to approximate the total number of pregnant women attending ANC during the study period and averagely, it was estimated that 158 women attend ANC monthly and 632 women attend ANC during the specified study period. Then systematic sampling technique was used with sampling interval of 3 to select the study participants.

3.4. Sample Collection and Transportation

In sheep, approximately, 5 ml of blood was collected from Jagular vein aseptically into vacunator tubes which contained no anti-coagulants. In pregnant women, 4 ml of venous blood was drawn from each study participants using labeled test tube by trained medical laboratory technician. Blood collected from both sheep and pregnant women were put overnight at room temperature separately to allow clot and centrifuged for 10 minutes at 3000

rpm. Sera were collected in 1.5 Eppendorf tubes and stored at 4°C for 48–78 hours until transported in an ice box to Microbiology laboratory of College of Agriculture and Veterinary Medicine, Jimma University, where they were kept at –20 °C until tested.

3.5. Serological test

Toxo Latex Agglutination Slide Test (LAT)

Seroprevalence of *T. gondii* infection in sheep and pregnant women were determined by latex agglutination slide test (LAT). Serologically, serum was tested for the presence of anti *T. gondii* antibodies by LAT. The test was done according to the manufacturer's instruction (SPINREACT, S.A/S.A.U Ctra Santa Coloma, Spain) (Annex 8). Briefly, it is a slide agglutination test for the qualitative and semi quantitative detection of anti-*T. gondii* antibodies and the test reagent is standardized to detect more than 10 IU/ml anti-*T. gondii* antibodies. Latex particles coated with soluble *T. gondii* antigen are agglutinated when mixed with samples containing anti-*T. gondii* antibodies. The diagnostic sensitivity and specificity of the test is 96.1% and 89.6% respectively (SPINREACT, S.A/S.A.U Ctra Santa Coloma, Spain). A positive reaction is indicated by any observable agglutination in the reaction mixture. No agglutination within 4 minutes in case of negative reaction.

3.6. Questionnaire Survey

Assessment of risk factors associated with *T. gondii* infection in sheep was conducted through a written structured epidemiological questionnaire answered by sheep owners. Some of the potential risk factors that were included in the questionnaire were: district, flock size (large/ small), sex (female/male), age (Young ≤1 year /Adult >1 year), presence of cats in the house hold (Yes/No), occurrence of abortion in the flock, occurrence of still birth in the flock, source of drinking water (river/tap) and type of management system (Extensive/ semi intensive) (Annex 5).

Likewise, for assessment of risk factors associated with *T. gondii* infection in pregnant women pretested and organized questionnaire was used to collect information on socio-demographic data such as age, level of education, residential place, occupation, clinical and

obstetrical characteristics of pregnant women like gravidity, gestation period, history of abortion, history of blood transfusion, HIV status and behavioral characteristics of study participants like habit of eating of raw meat and vegetables, source of drinking water, contact with cat, handling of raw meat and contact with soil (Annex 6).

3.7. Data Management and Analysis

The data obtained from both questionnaire and laboratory tests were edited, classified accordingly, coded and entered into computer using Statistical Package for Social Science (SPSS) version 20. Descriptive analysis was used to describe the study population in relation to socio-demographic, behavioral, clinical and obstetrical characteristics in case of pregnant women and animal information and management type in case of sheep. Association of each independent variable with dependent variable was assessed by cross tabulation. Flocks containing at least one seropositive animal were considered as a positive. The prevalence of *T. gondii* infection was calculated as the number in study population testing positive to serological divided by the total population that were included in the study (Thrusfield, 2007).

Univariate logistic regression analysis was employed to test associations among the dependent and each independent variable in the logistic regression model. Non- collinear variables found to have a p-value of < 0.25 (Hosmer and Lemeshow, 2000) in univariate analysis were fitted into multivariate logistic regression model for controlling the possible effect of confounders. After selecting the final model of logistic regression, the beta (β) coefficients of each independent variable were observed to estimate odds ratio (OR) which is used for the assessing of strength of association. Then variables which had significant association were identified on the basis of 95% CI. A p-value ≤ 0.05 was considered statistically significant.

3.8. Ethical Considerations

The study was ethically approved by ethical review board of Jimma University, College of Agriculture and Veterinary Medicine, School of veterinary medicine (Ref. Vet/Med. 516/2016) and public health Sciences (No. RPGC/600/06).

Participants were informed about the study in their mother tongue and written informed consents were obtained from study subjects (Annex 4). Maximum effort was taken to minimize the pain and/or associated complications while collecting venous blood samples. All serum samples were collected using new disposable tubes, syringes and needles. Confidentiality and safety were assured all times. Information obtained from respondents was used solely for academic purposes.

3.9. Quality Control and Precautions

To assure the quality of the data, each day, the collected data was reviewed and checked for completeness by principal investigator. For laboratory investigations, standard operating procedures and manufacturer's instructions were strictly followed. The quality of latex agglutination test was checked by both positive and negative controls (Annex 8).

4. RESULTS

4.1. The overall Seroprevalence of *T. gondii* infection in Sheep

Among the 400 sheep tested for anti-*T. gondii* antibodies to estimate animal level *T. gondii* infection, 269 sheep were showed seropositivity and giving an overall animal level seroprevalence of 67.25% (95 confidence interval [CI]: 62.65%-71.85%). Out of eight (80) flocks tested for anti *T. gondii* antibodies, sixty nine (69) flocks were found to be seropositive. Therefore, the estimated flock level seroprevalence of *T. gondii* infection was 86.25% (95% CI: 78.7%-93.8%). Of sixty nine seropositive flocks, four flocks, seven flocks, twelve flocks, fifteen flocks and thirty one flocks had one, two, three, four and five seropositive sheep respectively. Highest animal level seroprevalence was observed in Decha district (163/220, 74%) followed by Adiyo district (72/120, 60%) and Bonga district (34/60, 56%). In the same way, highest flock level seroprevalence was also recorded in Decha district (35/38, 89.4%), followed by Adiyo district (25/30, 83.3%) and Bonga district (9/12, 75%) as illustrated in Table 2.

Table 2. Animal and flock level seroprevalence of *T. gondii* infection in sheep in three study districts.

District	**Animal level Seroprevalence (%)**			**Flock level seroprevalence (%)**		
	Tested	Positive	P (95% CI)	Tested	Positive	(95% CI)
Decha	220	163	74 (68.2-79.8)	38	35	89.4 (79.61-99.19)
Adiyo	120	72	60 (51.23-68.77)	30	25	83.3 (69.95-96.65)
Bonga	60	34	56 (43.44-68.56)	12	9	75 (50.5-99.5)
Total	400	269	67.25 (62.65-71.85)	80	69	86.25 (78.7-93.8)

Key to Abbreviation: P=Prevalence, CI= Confidence interval

4.2. Risk factors associated with *T. gondii* infection in sheep

4.2.1. Univariate logistic regression analysis

Univariate logistic regression analysis show that districts, age, sex, flock size, presence of cats in the house hold, occurrence of still birth and management system show statistically significant association with *T. gondii* infection ($P<0.05$). However, occurrence of abortions and source of drinking water were not significantly associated with *T. gondii* seropositivity ($P>0.05$) as shown in Table 3. The variables with the p-value of less than 0.25 in univariate analysis were taken to multivariate analysis to control confounders and to see their independent effect on *T. gondii* seropositivity. Accordingly, districts, age, sex, flock size, presence of cats in the house hold, occurrence of still births and management systems were subjected to multivariate analysis.

4.2.2. Multivariate logistic regression analysis

In multivariate logistic regression analysis, age ($P=0.050$), sex ($P=0.035$) and presence of cats in the household ($P=0.041$) were remained independent predictors of *T. gondii* seropositivity. The model containing age, sex and presence of cats were selected as the final model. The odds of being seropositive for anti *T. gondii* antibodies was 1.69 (95% CI: 1.01-2.85) times higher in adult sheep compared to young. The probabilities of being seropositive for anti *T. gondii* antibodies was 1.77 (95% CI: 1.04-2.99) times higher in female compared to male. The likelihood of acquiring *T. gondii* infection was 1.74 (95% CI: 1.02-2.95) times in sheep with close contact with cats compared to those didn't have. Nevertheless, origin of animals, flock size, occurrence of still birth and management system were not significantly associated with *T. gondii* seropositivity ($P>0.05$) after multivariate analysis (Table 3). Multicollinearity among independent variables was checked by variance inflation factor (VIF) (Schwarz, 2007). However, none of the variables were found to be collinear variables. Therefore, the model was found to be stable. The goodness of fit of the model was assessed by Hosmer and Lemeshow goodness fit test. There was insignificant difference between the observed and predicted values. The value of Hosmer and Lemeshow test for the last model was chi square (X^2) =13.39, $P= 0.063$. Hence, the model was fitted well with the data.

Table 3. Risk Factors associated with *T. gondii* infection in sheep by univariate and multivariate logistic regression

Risk factors	Category	No. tested	Seropositive (%)	Univariate		Multivariate	
				COR (95% CI)	P- value	AOR (95% CI)	P-value
	Decha	220	163 (74)	2.18 (1.02-3.96)	0.010	1.29 (0.59-2.51)	0.594
Districts	Adiyo kaka	120	72 (60)	1.15 (0.61-2.15)	0.668	0.81 (0.40-1.60)	0.538
	Bonga	60	34 (56)	1.00		1.00	-
Flock size	Small	285	203 (71.2)	1.83 (1.17-2.88)	0.008	1.66 (0.98-2.81)	0.059
	Large	115	66 (57.4)	1.00		1.00	-
Sex	Female	274	198 (72.2)	2.02 (1.3- 3.14)	0.002	1.77 (1.04-2.99)	0.035
	Male	126	71 (56.3)	1.00		1.00	-
Age	Adult	283	204 (72)	2.07 (1.32-3.24)	0.001	1.69 (1.01-2.85)	0.050
	Young	117	65 (55.5)	1.00		1.00	-
Presence of cats	Yes	271	197 (72.6)	2.1 (1.36-3.26)	0.001	1.74 (1.02-2.95)	0.041
	No	129	72 (55.8)	1.00		1.00	
Occurrence of abortion	Yes	104	70 (67.3)	0.82 (0.49-1.39)	0.462	-	-
	No	170	128 (75.3)	1.00		-	-
Occurrence of still birth	Yes	185	136 (73.5)	1.62 (0.97-2.70)	0.014	1.26 (0.71-2.26)	0.433
	No	89	62 (69.7)	1.00		-	-
Management system	Extensive	300	192 (64)	0.53 (0.32-0.89)	0.017	1.04 (0.47-2.33)	0.915
	Semi intensive	100	77 (77)	1.00	-	-	-
Source of drinking water	River	340	235 (69)	1.7 (0.98-2.99)	0.6	-	-
	Tap	60	34 (56)	1.00		-	-

Key to Abbreviation: COR= Crude Odds Ratio, AOR= Adjusted Odds Ratio, CI= Confidence Interval, 1.00 = reference group

4.3. The Overall Seroprevalence of *T. gondii* infection in Pregnant Women

Out of the total of 210 pregnant women sera studied for the presence or absence of anti *T. gondii* antibodies, 159 (75.7%) (95% CI: 69.9%-81.5%) were found to be seropositives.

4.3.1. Socio-demographic characteristics of pregnant women and *T. gondii* seropositivity

The mean age ± SD (Standard Deviation) of the study participants was 30.58 ± 8.31 years. The age range of the studied populations was found between 15–44 years, of which the highest number of participants, 77 (36.6%) were found between the age of 36–44 years and 65 (84.4%) of them were found to be seropositive. More than half of study subjects, 131 (62.4%) were urban residents and 79 (37.4%) were living in rural areas. Being seropositive, 67 (84.8%) for anti *T. gondii* antibodies was highly found in rural women. In level of education, 46 (21.9%), 63 (30%) and 101 (48.1%) were illiterate, elementary and high school and above respectively of which highest seroprevalence was noted in women who have elementary education 51(81%). In occupation, 156 (74.3%) were house wives followed by government employee's 37 (17.6%), merchants 14 (6.7%) and private practitioners' 3 (1.4%) respectively, of which merchants were highly infected by *T. gondii*, 12 (85.72%) (Table 4).

Table 4. Socio-demographic characteristics of pregnant women and *T. gondii* seropositivity.

Socio-demographics	Category	Frequency (%)	No. positive (%)
Age (Years)	15-25	68 (32.4)	47 (69.12)
	26-35	65 (31)	47 (72.3)
	36-44	77 (36.6)	65 (84.4)
Residence	Urban	131 (62.4)	92 (70.2)
	Rural	79 (37.6)	67 (84.8)
Educational status	Illiterate	46 (21.9)	34 (73.9)
	Elementary	63 (30)	51 (81)
	≥High school	101 (48.1)	74 (73.3)
Occupation	House wife	156 (74.3)	119 (76.3)
	Merchant	14 (6.7)	12 (85.72)
	Private	3 (1.4)	2 (66.67)
	Government employ's	37 (17.6)	26 (70.3)

4.3.2. Behavioral characteristics of pregnant women and *T. gondii* seropositivity

One hundred and thirty four (63.8%) of pregnant women had the habit of eating raw meat, in which 117 (87.3%) of them were found to be seropositive for anti *T. gondii* antibodies. Majority of study populations had the habit of consuming raw vegetables, 201 (95.7%) and out of this, 155 (77.1%) of them were found to be seropositive for anti *T. gondii* antibodies. One hundred fifty-eight (75.2%) pregnant women had the habit of drinking raw milk of which 121 (76.6%) contain anti *T. gondii* antibodies in their sera. From the total of 108 (51.4%) of the pregnant women who had cat in their home, 89 (82.4%) of them were found to be seropositive. Ninety-three (44.3%), 44 (21%) and 73 (34.8%) of participants were use wells, tap and streams as a source of drinking water respectively, in which being seropositive for anti *T. gondii* antibodies was more observed in individuals who use streams, 64 (87.7%). Out of 185 (88.1%) study participants who had history of contact with soil, 143 (77.3%) were found to be seropositive. Out of 102 (48.6%) study subjects who don't wash their hands after handling of raw meat, 89 (87.3%) were found to be seropositive (Table 5).

Table 5. Behavioral characteristics of pregnant women and *T. gondii* seropositivity

Behavioral characteristics	**Category**	**Frequency (%)**	**No. positive (%)**
Habit of eating raw meat	Yes	134 (63.8)	117 (87.3)
	No	76 (36.2)	42 (55.2)
Habit of eating raw vegetables	Yes	201 (95.7)	155 (77.1)
	No	9 (4.3)	4 (44.4)
Habit of drinking raw milk	Yes	158 (75.2)	121 (76.6)
	No	52 (24.8)	38 (73.1)
Presence of cat at home	Yes	108 (51.4)	89 (82.4)
	No	102 (48.6)	70 (68.6)
Source of drinking water	Tap	44 (21)	27 (61.4)
	Well	93 (44.3)	68 (73.1)
	River	73 (34.7)	64 (87.7)
Contact with soil	Yes	185 (88.1)	143 (77.3)
	No	25 (11.9)	16 (64)
Washing of hands after handling of raw meat	Yes	108 (51.4)	70 (64.8)
	No	102 (48.6)	89 (87.3)

4.3.3. Clinical and obstetrical characteristics of pregnant women and *T. gondii* seropositivity

From the study participants, about 9 (4.3%) of respondents had history of blood transfusion, of which 8 (88.8%) of them were found to be seropositive for anti *T. gondii* antibodies. From the study population 13 (6.2%) were found to be HIV positive and 11 (84.6%) of them were seropositive for anti *T. gondii* antibodies. Regarding the gestational period, 27(12.9%) of the pregnant women attended ANC during their first trimester, 80 (80.1%) their second trimester and 103 (49%) their third trimester of gestational period. Among the tested sera, highest proportion of seropositivity was observed in second trimester of pregnancy, 62 (77.5%). Majority of study subjects, 126 (60%) were multigravidae and 103 (81.7%) of them were sero- positive for anti *T. gondii* antibodies. Of the total study subjects, 34 (16.2%) had history of abortion, 31 (91.2%) were also contain anti *T. gondii* antibody in their sera (Table 6).

Table 6. Clinical and obstetrical characteristics of pregnant women and *T.gondii* seropositivity

Clinical and obstetrical	Category	Frequency (%)	No. positive (%)
Gravidity	Primigravidae	84 (40)	56 (66.67)
	Multigravidae	126 (60)	103 (81.75)
Gestation period	1st trimester	27 (12.9)	20 (74)
	2nd trimester	80 (38.1)	62 (77.5)
	3rd trimester	103 (49)	77 (74.8)
History of abortion	Yes	34 (16.2)	31 (91.2)
	No	176 (83.8)	128 (72.8)
Blood transfusion	Yes	9 (4.3)	8 (88.9)
	No	201 (95.7)	151 (75.1)
HIV status	Negative	197 (93.8)	148 (75.1)
	Positive	13 (6.2)	11 (84.6)

4.4. Risk factors associated with *T. gondii* infection in pregnant women

Analysis of risk factors to get *T. gondii* infection among study populations was done using univariate and multivariate logistic regression analysis.

4.4.1. Univariate logistic regression analysis

The results of univariate analysis show that some socio-demographic, behavior and clinical and obstetrical characteristics of the respondents were found to have positive association with *T. gondii* seropositivity. Independent variables like age, residence, gravidity, history of abortion, presence of cats at home, eating of raw meat, eating of raw vegetables, drinking water from river/wells and failure to wash hands after handling of raw meat were significantly associated with seropositivity of *T. gondii* infection ($P<0.05$). On the other hand, level of education, occupation, gestation period, HIV status, habit of drinking raw milk, blood transfusion and contact with soil were not significantly associated with *T. gondii* seropositivity ($P>0.05$) as shown in (Table 7). The variables with the *P*-value of less than 0.25 in univariate analysis were taken to multivariate analysis to control confounders and to see their independent effect on *T. gondii* seropositivity.

4.4.2. Multivariate logistic regression analysis

In multivariate analysis, age ($P= 0.046$), gravidity ($P= 0.009$), history of abortion ($P= 0.036$), habit of eating raw meat ($P\leq0.001$), habit of eating raw vegetables ($P= 0.046$), source of drinking water ($P= 0.004$) and failure to wash hands after handling raw meat ($P= 0.049$) were identified as the independent predictors of *T. gondii* infection. The relative risk of acquiring *T. gondii* infection was 1.17 (95% CI: 0.44-3.08) and 2.82 (95% CI: 1.02-7.82) times more likely to occur in pregnant women within the age category between 26-35 and 36-44 years respectively, compared to pregnant women within the category of age between 15-25 years. The probability of contracting *T. gondii* infection was 3.3 (95% CI: 1.36-8.04) times greater in multigravidae than primigravidae.

Pregnant women who had history of abortion in their life were 4.4 (95% CI: 1.10-10.49) times more likely to be seropositive for anti *T. gondii* antibodies compared to those who didn't had abortion. Likewise, the probability of acquiring *T. gondii* infection was 5 (95% CI: 2.22-11.68) times more likely to occur in individuals who had the habit of eating raw meat compared to those who consume well cooked meat. The odds of being seropositive for anti *T. gondii* antibodies were 5 (95% CI: 1.67-15.44) and 2.34 (95% CI: 0.82-6.62) times high in those study subjects who used stream/rivers and wells as a source of drinking water respectively, than those who used tap water as a source of drinking water. Study subjects who had the habit of eating raw vegetables were 5.5 (95% CI: 1.03-29.50) times more likely to acquire *T. gondii* infection than those who didn't eat raw vegetables. The probability of getting *T. gondii* infection was 2.4 (95% CI: 1.00-5.56) times higher in those study participants who didn't wash their hands after handling raw meat than those who wash hands properly.

Multivariate logistic regression analysis also indicated that, residence, presence of cats and contact with soil were not identified as the independent predictors of *T. gondii* infection (P>0.05). Nevertheless, the probability of acquiring *T. gondii* infection was 1.27 (95% CI: 0.51-3.17) greater in participants who live in rural areas as compared to urban setting, 1.65 (95% CI: 0.72-3.74) times higher in cat owning as compared to not cat owning and 2.4 (95% CI: 0.46-8.72) times higher in those who had contact with soil than those who didn't have contact with soil (Table 7). Multicollinearity among independent variables was checked by tolerance or variance inflation factor (VIF) (Schwarz, 2007). However, none of the variables were found to be collinear. Hence, the model was found to be stable. The goodness of fit of the model was assessed by Hosmer and Lemeshow goodness fit test. There was insignificant difference between the observed and predicted values. The value of Hosmer and Lemeshow test for the last model was chi square (X^2) = 10.354, P= 0.241. Thus, the model was fitted well with the data.

Table 7: Risk factors associated with *T. gondii* infection in pregnant women by univariate and multivariate logistic regression

Variables	**Sero Status (%)**		**Univariate**		**Multivariate**	
	Positive	**Negative**	**COR (95% CI)**	**P-value**	**AOR (95%CI)**	**P-value**
Age (year)						
15-25	47 (69.12)	21 (30.88)	1.00	-	1.00	-
26-35	47 (72.3)	18 (27.7)	1.17 (0.56-2.47)	0.686	1.17 (0.44-3.08)	0.757
36-44	65 (84.4)	12 (15.6)	2.42 (1.09-5.40)	0.031	2.82 (1.02-7.82)	0.046
Residence						
Urban	92 (70.2)	39 (29.8)	1.00	-	1.00	-
Rural	67 (84.8)	12 (15.2)	2.37 (1.16-4.86)	0.019	1.27 (0.51-3.17)	0.610
Educational status						
Illiterate	34 (73.9)	12 (26.1)	1.03 (0.47-2.28)	0.934	-	-
Elementary	51 (81)	12 (19)	1.55 (0.72-3.34)	0.263	-	-
≥High school	74 (73.3)	27 (26.7)	1.00	-	-	-
Occupation						
House wife	119 (76.3)	37 (23.7)	1.36 (0.61-3.02)	0.448	-	-
Merchant	12 (85.72)	2 (14.28)	2.54 (0.49-13.28)	0.270	-	-
Private	2 (66.67)	1 (33.33)	0.85 (0.06-10.33)	0.896	-	-
Government employs	26 (70.3)	11 (29.7)	1.00		-	-
Gravidity						
Primigravidae	56 (66.67)	28 (33.33)	1.00		1.00	-
Multigravidae	103 (81.75)	23 (18.25)	2.24 (1.18-4.25)	0.014	3.3 (1.36-8.04)	0.009

Key to Abbreviation: COR=Crude Odd Ratio, AOR= Adjusted Odd Ratio, CI= Confidence Interval, 1.00= reference group

Table 7 cont…,

Variables	Sero Status (%)		Univariate		Multivariate	
	Positive	**Negative**	**COR (95% CI)**	**P-value**	**AOR (95% CI)**	**P- value**
Gestation period						
1st trimester	20 (74)	7 (26)	1.00		-	-
2nd trimester	62 (77.5)	18 (22.5)	1.20 (0.44-3.30)	0.716	-	-
3rd trimester	77 (74.8)	26 (25.2)	1.04 (0.39-2.73)	0.942	-	-
History of abortion						
No	128 (72.8)	48 (27.2)	1.00		1.00	-
Yes	31 (91.2)	3 (8.8)	3.88 (1.14-13.27)	0.031	4.4 (1.10-17.49)	0.036
HIV status						
Negative	148 (75.1)	49 (24.9)	1.00		-	-
Positive	11 (84.6)	2 (15.4)	1.82 (0.39-8.50)	0.446	-	-
Blood transfusion						
No	151 (75.1)	50 (24.9)	1.00		-	-
Yes	8 (88.9)	1 (11.1)	2.65 (0.32-21.7)	0.364	-	-
Presence of cats						
No	70 (68.6)	32 (31.4)	1.00		1.00	-
Yes	89 (82.4)	19 (17.6)	2.14 (1.12-4.01)	0.021	1.65 (0.72-3.74)	0.236
Habit of eating raw meat						
No	42 (55.2)	34 (44.8)	1.00		1.00	-
Yes	117 (87.3)	17 (12.7)	5.57 (2.82-11.68)	≤0.001	5.1 (2.22-11.68)	≤0.001

Key to Abbreviation: COR= Crude Odd Ratio, AOR= Adjusted Odd Ratio, CI= Confidence Interval, 1.00 = reference group

Table 7 cont…,

Variables	Sero Status (%)		Univariate		Multivariate	
	Positive	Negative	COR (95% CI)	P-value	AOR (95%CI)	P-value
Habit of eating raw vegetables						
No	4 (44.4)	5 (55.6)	1.00		1.00	-
Yes	155 (77)	46 (23)	4.2 (1.08-16.33)	0.038	5.5 (1.03-29.50)	0.046
Habit of drinking raw milk						
No	38 (73)	14 (27)	1.00		-	-
Yes	121 (76.6)	37 (23.4)	1.2 (0.59-2.46)	0.609	-	-
Source of drinking water						
Tap	27 (61.4)	17 (38.6)	1.00		1.00	-
Well	68 (73.1)	25 (26.9)	1.71 (0.80-3.66)	0.166	2.34 (0.82-6.62)	0.110
River/streams	64 (87.7)	9 (12.3)	4.48 (1.77-11.28)	0.001	5 (1.67-15.44)	0.004
Hand washing after handling of raw meat						
Yes	70 (64.8)	38 (35.2)	1.00		1.00	-
No	89 (87.3)	13 (12.7)	3.7 (1.84-7.51)	≤0.001	2.4 (1.00-5.56)	0.049
Contact with soil						
No	16 (64)	9 (36)	1.00		1.00	-
Yes	143 (77.3)	42 (22.7)	1.92 (0.79-4.65)	0.151	2.4 (0.46-8.72)	0.197

Key to abbreviation: COR= Crude Odd Ratio, AOR= Adjusted Odd Ratio, CI= Confidence Interval, 1.00= reference group

5. DISCUSSION

5.1. The overall seroprevalence of *T. gondii* infection in sheep

There has been increasing interest to estimate the prevalence of *T. gondii* infection in food animals because of their role in the transmission of the infection to humans through the ingestion of undercooked meat and unpasteurized milk (Cenci-Goga *et al.*, 2011). The overall animal level seroprevalence of anti *T. gondii* antibodies in sheep in the present study was 67.3% (95% CI: 62.65-71.85) which is similar with previously reported prevalence elsewhere: 67.7% from Zimbabwe (Hove *et al.*, 2005), 71% from Grenada, West Indies (Sharma *et al.*, 2015). In contrast, it was higher than the reports of some scholars, 52.6% from Natharet, Ethiopia (Tamiru *et al.*, 2004), 31.6% and 20% from Central Ethiopia (Endrias *et al*., 2013a; Endrias *et al.*, 2014) respectively, 20.3% from North western China (Yin *et al.*, 2015), 17.9% from North Eastern China (Xu *et al.*, 2015), 8% from South Africa (Arye *et al*., 2015), 11.1% from Brazil (Correia *et al.*, 2015). On the other hand, this result was lower than the other reports (Klun *et al.*, 2006; Mor and Arslan, 2007) who reported 84.5% and 95.7% from Serbia and Turkey respectively.

The variability of the overall seroprevalence observed in different studies may be associated with the differences in the age and management system, the climatic conditions, serological techniques used (Cenci-Goga *et al.*, 2011), differences in the relative cat densities, the access of sheep to contaminated feed and water and variation of sample size (Innes *et al*., 2009). Free grazing is common practice in the present study area in which sheep set free during the day time and are gathered into home at night, there is also deficient basic infrastructure, with insufficient hygienic practices. The absence of regular cleaning and reproductive management during the postpartum period may facilitate the contact with *T. gondii* oocysts present in the environment (Correia *et al*., 2015). It is also worth mentioning that the study area was well known for the abundance of vegetation that cover and protect *T. gondii* oocysts released by domestic or stray cats against desiccation which promotes their survival and sporulation in the environment; results high seropositivity. It is well recognized that climate plays a major role in the survival, distribution and transmission of *T. gondii* infection (Tenter *et al.*, 2000).

5.2. Risk Factors Associated with *T. gondii* infection in Sheep

Multivariate logistic regression analysis indicated that adult sheep were (AOR=1.69, P=0.050) times more likely to be infected by *T. gondii* compared to young sheep which is similar with some earlier and recent studies (Chikweto *et al.,* 2011; Andrade *et al.,* 2013; Endrias *et al*., 2013a; Ahmad *et al.,* 2015). On the contrary, other researchers reported absence of significant association between age of animal and *T. gondii* infection (Yin *et al.,* 2015; Arye *et al.,* 2015). The higher seroprevalence of the disease distinguished in adult sheep might be due to the effect of exposure to *T. gondii* oocysts with increased age which increases their chance to get infection (Dubey, 2010b), or it suggest that most animals acquire infections post natally (Dubey, 2009) or due to heavy environmental contamination with oocysts shed from stray cats, or due to less immune to parasitic infections in adult animals (Roberts *et al*., 2001).

Present study also showed statistically significant association between *T. gondii* infection and sex of animals. Female sheep were more likely infected (AOR= 1.77, P= 0.035) compared to males which is consistent with the different findings (Endrias *et al.,* 2013a; Ahmad *et al.,* 2015). On the other hand, (Ueno *et al.,* 2009; Cosendey Kezen Leite *et al.,* 2014) were reported higher seroprevalence of *T. gondii* infection in male sheep. High seropositivity noted in female sheep may be explained by the fact that immunity in female is suppressed by pregnancy and lactation related stresses (Kelly *et al.,* 2001), low level of progesterone during pregnancy could be contributing to the susceptibility to *T. gondii* infection (Ramirez *et al.,* 2014) or it could be due to management system that ewes are retained in the farm for longer periods for breeding purposes than males (Endrias *et al.,* 2013a).

The study also revealed that presence of domestic cats in the household was significantly contributed in increasing the likelihood of infection in sheep (AOR=1.74, P= 0.041) compared to their absence. This result is in accordance with some previous reports of Sechi *et al*. (2013) and Ahmad *et al*. (2015). Positive association observed between presence of cats in the household and *T. gondii* seropositivity could be due to the truth that cats act as the final host in the life cycle of *T. gondii* and excrete oocysts in their feces that can be a source of infection for humans and other animals (Dubey, 2008; Lopes *et al*., 2010). Oocysts excreted by cats remain infective for

months to years in warm and moist ecological conditions (Dubey and Jones, 2010). On the contrary, the finding of Endrias *et al.* (2013a) and Cosendey Kezen Leite *et al.* (2014) did not show significant association between presence of cats in the household and *T. gondii* infection.

Even though, the study did not show statistically positive association between flock size and *T. gondii* seropositivity, relatively high seroprevalence of *T. gondii* infection was observed in sheep sampled from small flock size. Absence of statistically significant association between seroprevalence of *T. gondii* infection and flock size may be due to equal exposure of sheep to *T. gondii* oocysts through free grazing system since they have communal grazing lands and water points. Absence of statistically, significant difference between the study districts indicates the similarity of climatic conditions and management practices of study area. The study also did not show significant association between seroprevalence of *T. gondii* infection and management system which might be partly explained by the absence of difference in husbandry practices.

5.3. The overall seroprevalence of *T. gondii* infection in Pregnant Women

The significance of monitoring of *T. gondii* infection in humans is of great importance because of the risk of the infection to pregnant women, immunocompromised hosts and newborns (Ahmad and Qayyum, 2014). The current study was among hospital based few studies in Ethiopia and the first study to be conducted among pregnant women attending ANC at Bonga hospital of Kafa Zone. The overall seroprevalence 75.7% (95% CI: 69.9-81.5) found in pregnant women in the present study was close to the result of Endrias *et al.* (2013b) who reported 81.4% seroprevalence of *T. gondii* infection from central Ethiopia among women of child bearing age. However, the overall seroprevalence of *T. gondii* infection in pregnant women in this study was lower than that observed formerly in Jimma town, south western Ethiopia (Endalew *et al.*, 2012), Addis Ababa city (Woyneshet *et al.*, 2015) and Gondar town, North western Ethiopia (Mengistu *et al.*, 2014) who reported 83.6%, 85.4%, 88.6% of seroprevalence of anti *T. gondii* antibodies in pregnant women respectively. The present result was higher than the reports of Birihan *et al.* (2015) and Kefale *et al.* (2015) who reported 68.4% and 18.5% of seroprevalence of anti *T. gondii* antibodies in pregnant women from Debra Tabor and Bahirdar town north western

Ethiopia respectively. It was also quite higher than the result reported by different scholars among pregnant women from different countries of Africa and Asia; 20.2% from Sudan (Rauff and Elbasheir, 2014), 12.2% from Rwanda (Esperance, 2014), 18.1% from South Africa (Bessong and Mathomu, 2010), 41.6% from Tanzania (Shao *et al.,* 2015), 48.9% from Nigeria (Nasir *et al.,* 2015), 58.7% from Ivory Coast (Koffi *et al.,* 2015), 49.52% from India (Sarkar *et al.,* 2012) and 45.4% from Yemen (Al- Eryani *et al.,* 2016).

The difference of seroprevalence results of *T. gondii* infection in pregnant women in different studies could be attributed to variation in climatic conditions and living standards of the people in the definite areas. Furthermore, sampled populations, the difference of sensitivity specificity serological tests, genetic back ground of the parasite and host and the type of immune response elicited by the parasite (Ferreira *et al.,* 2014; Abbas *et al*., 2014), economic status, culture of the society, presence or absence of certain risk factors (Dubey, 2010a; Akhlaghi *et al*., 2014) are some factors contributing for the variation of seroprevalence *T. gondii* infection across the globe.

The high overall seroprevalence of *T. gondii* infection among pregnant women in present finding might be associated with suitability of climatic conditions for the survival and stability of *T. gondii* oocysts in the environment, feeding habit and insufficient hygienic condition of study subjects, and high seroprevalence of *T. gondii* infection in sheep (67.25%) (Unpublished raw data) as *T. gondii* can be transmitted to humans through ingestion of mutton infected by *T. gondii* tissue cysts. In addition, in the current study area, large numbers of stray cats roam streets and community places. Environment is not only contaminated accidentally by stray cats but also many owners allow indoor cats to defecate outside home which increase the probability of infection. Less attention given to the infection and low level of awareness about its transmission ways are the other factors that contributed to the high prevalence of infection.

5.4. Risk factors associated with *T. gondii* infection in pregnant women

In multivariate analysis, assessment of risk factors for *T. gondii* infection in pregnant women revealed that age, gravidity, history of abortion, consumption of raw meat, consumption of raw vegetables, source of drinking water and failure to wash hands after handling of raw meat were

found to be independent predictors of *T. gondii* infection ($P<0.05$). The probability of being seropositive for anti- *T. gondii* antibodies were 2.8 (95% CI: 1.02-7.82) times higher in pregnant women found between the age category of 36-44 years compared to those pregnant women of age range between 15–25 years which is in line with the reports of some studies (Birihan *et al.,* 2015; Birihanu *et al.,* 2016). However, different studies reported the absence of significant association between age and *T. gondii* seropositivity (Woyneshet *et al*., 2015; Dechassa *et al.,* 2016).

Statistically significant association observed between the age and *T. gondii* seropositivity may be due to the fact that the likelihood of an individual coming into contact with oocyst either in one or more of the transmission routes increases as age increases (Spalding *et al.,* 2005). The relative risk of acquiring *T. gondii* infection were 3.3 (95% CI: 1.36, 8.04) times higher in multigravidae than primigravidae. Similarly, a study from Brazil has also found statistically significant increase in seroprevalence in multigravidae women (Barbosa *et al*., 2009). However, Kefale *et al.* (2015) reported the absence of significant association between *T. gondii* infection and gravidity. The observed significant association between gravidity and *T. gondii* seropositivity might be due to the probability of acquiring *T. gondii* infection is the cumulative effect of age; as the age and number of pregnancy increases, exposure to infection also increased.

Similar to the previous reports (Kefale *et al*., 2015; Kasim and Zinabu, 2015; Dechasa *et al.,* 2016) in the current study, statistically strong significant association was observed between consumption of raw meat and *T. gondii* seropositivity. The likelihood of acquiring *T. gondii* infection among pregnant women who consume raw meat were 5.1 (95% CI: 2.22-11.68) times more than those didn't consume. In contrast, other studies reported the absence of significant association between seropositivity of *T. gondii* and ingestion of raw meat (Endalew *et al.,* 2012; Endrias *et al*., 2013b; Woyneshet *et al.,* 2015). This difference in different study does not mean raw meat is not source of *T. gondii* infection, but it depends on cultural and eating habits in different human populations (Abu *et al.,* 2015) and difference of seroprevalence of *T. gondii* infection in meat producing animals in different countries. Like recent reports of Ayi *et al.* (2016), in this study, neglecting of hand washing after handling of raw meat had statistically significant association with anti-*T. gondii* seropositivity in which those pregnant women who

didn't wash their hands after handling of raw meat were 2.4 (95% CI: 1-5.56) times more infected by *T. gondii* than those who wash their hands. That is why *Toxoplasma* tissue cysts present in meat or meat-derived products have been shown to serve as important sources of infection for humans (Abu *et al*., 2015).

In this study, there was significant difference between source of drinking water and *T. gondii* seropositivity in which participants who used streams/rivers for drinking purpose were 5 times (95% CI: 1.67-15.44) more likely contracting *T. gondii* infection compared to those who used tap water. This is consistent with some previous reports (Endrias 2013b; Esperance, 2014; Andiappan *et al.,* 2014; Kasim and Zinabu, 2015). This observed relationship indicates that contamination of rivers and well waters by *T. gondii* oocysts from felids feces and inadequate water management (Peterson *et al.,* 2010) which could serve as the source of *T. gondii* infection. However, the finding contradicts with the reports of Gyang *et al.* (2015) which did not show significant association between *T. gondii* seropositivity and sources of drinking water.

The study also showed significant association between *T. gondii* seropositivity and habit of eating raw vegetables. The odds of acquiring *T. gondii* infection among pregnant women who consume raw vegetables was 5.5 (95% CI: 1.03-29.5) times more than those who didn't consume it. Similarly, the importance of raw vegetables in the transmission of the disease has been reported by other studies (Mohammad *et al.,* 2013; Kefale *et al.,* 2015). Insufficient hygienic method for transportation and selling of vegetables together with the poor quality water for washing of vegetables might have contributions for the contamination of vegetables by *T. gondii* oocysts (Endrias *et al*., 2013a; Anteneh *et al.,* 2014). On the other hand, it contradicts with the finding of Woyneshet *et al.* (2015) and Cong *et al.* (2015) who reported the absence of significant association between *T. gondii* seropositivity and eating of raw vegetables.

Strong significant relationship was also found between *T. gondii* infection and history of abortion. The likelihood of having anti- *T. gondii* antibodies in their serum were 4.4 (95% CI: 1.10-17.49) times higher in pregnant women who had the history of abortion in their life compared to those who didn't have which is consistent with different reports (Li *et al.,* 2014; Kamal *et al.,* 2015; Kefale *et al.,* 2015) indicating the presence of *Toxoplasma* cysts in

chronically infected uteri lead to infection of the fetus in the first trimester and frequently to repeated miscarriage (Ajayi and Omilabu, 2010). Nonetheless, the present result is contradictory with other studies of Ertug *et al.* (2005) and Endalew *et al.* (2012) that did not show significant association between *T. gondii* seropositivity and history of abortion.

The study did not show significant relationship between *T. gondii* infection and presence of cats in the house hold. This is similar with other studies (Mwambe *et al.*, 2013; Raouff and Elbasheir, 2014; Shao *et al.*, 2015), but it disagree with some studies (Kefale *et al.*, 2015; Birihanu *et al.*, 2016 Dechassa *et al.*, 2016). Absence of significant association between *T. gondii* seropositivity and presence of cats at home indicates that probably cats in some households are not infected at all or presence of cats in the house hold may not necessarily be a risk factor, rather, frequent exposure to feline feces or neglect of preventive measures are of more importance to develop risk.

Despite the absence of significant association between *T. gondii* infection and the residential place, relatively higher seroprevalence was observed in rural pregnant women than urban dwellers which are in harmony with some investigations (Babaie *et al.*, 2013; Cong *et al.*, 2015). This indicates habitual of the residents in rural areas, the presence of domestic animals and favorable environmental conditions for survival and sporulation of *T. gondii* oocysts. In dissimilarity, studies of different scholars showed high seropositivity of *T. gondii* infection in urban pregnant women (Sucilathangam *et al.*, 2013; Kudakwashe and Yesuf, 2014). Although relative risk of infection was more in pregnant women who have contact with contaminated soil, no significant association was observed between contact with soil and *T. gondii* seropositivity. This result agrees with the findings of different authors (Doudou *et al.*, 2014; Puccio *et al.*, 2014) and disagrees with previous studies of Jafari *et al.* (2012) and Kudakwashe and Yesuf, (2014).

6. CONCLUSION AND RECOMMENDATIONS

This study was the first study to be conducted to estimate the seroprevalence and associated risk factors of *T. gondii* infection in sheep and pregnant women in Kafa Zone. The seroprevalence of *T. gondii* infection in sheep and pregnant women were found to be high. Age, sex and presence of cats in the house hold were found to be the independent predictors of *T. gondii* infection in sheep whereas, age, gravidity, consumption of raw meat, consumption of raw vegetables, source of drinking water, history of abortion and failure to wash hand after handling of raw meat were identified as the independent predictors of *T. gondii* infection in pregnant women. The high seroprevalence of *T. gondii* infection observed in sheep indicates that the infection is widely distributed in the study area and may cause economic losses in livestock sector through morbidity and mortality and it is also a good indicator for human infection as consumption of raw mutton is popular among Ethiopian peoples. Based on this conclusion, the following recommendations were given.

- Awareness creation for public in general and pregnant women in particular about adequate cooking of meat, washing of fruits and vegetables, washing of hands with soap and water after handling raw meat, cat litter and soil and drinking of water after boiling.

- A routine *T. gondii* screening program for pregnant women during attending ante natal care must be initiated in every Ethiopian health institutions.

- Improvement of management system and sufficient sanitary measures for food-producing animals are paramount, because, this not only to protect animals from the disease burden but also to minimize the risk of its transmission to humans.

- Further epidemiological studies should be undertaken using serological tests like MAT and ELISA to identify the type of antibody initiated against *T. gondii* infection in both sheep and pregnant women and bioassay to isolate, quantify and characterize genotypes of *T. gondii* in animal tissue.

- Large scale investigations must be carried out by increasing sample size and including other species of animals to define the economic and health impacts of this zoonosis and to formulate guidelines and policies aimed at justifying its potentially vicious outcomes.

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8. ANNEXES

Annex 1: English version information of the investigator

Principal investigator: Jalel Negero (DVM, MSc candidates)
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Annex 2: kafinoonoo language version information of investigator

Heereche shuunechoochi qihoo

Shuunech shigo: Jalel Negero (DVM, MSc doyeechoo)
Shuunee xaa,oo/Addresso: Jimma University, College of Agriculture and Veterinary Medicine (JUCAVM), school of Veterinary Medicine
W. S. H. 307 Jimma, Ethiopia; keke haddoo = +251 910 275 458, e-mailee addressoo: negerojalel@gmail.com

Annex 3: English version written informed consent form

1. Title of the study: Seroprevalence and associated risk factors of *T. gondii* infection in sheep in selected districts of Kafa Zone and pregnant women attending Ante Natal Care at Bonga Hospital South Western Ethiopia

2. Risks: the risks of this study will be minimal. You may decline to answer any or all questions and you may terminate your involvement at any time if you choose

3. Benefits: There will be no direct benefit to you for your participation in this study.

4. **Confidentiality:** Your responses will be anonymous. Participant data will be kept confidentially.

5. Voluntary Participation: Your participation in this study is voluntary. If you do decide to take part in this study, you will be asked to sign a consent form.

6. Costs to Subject: There are no costs to you for your participation in this study.

7. Compensation: There is no monetary compensation to you for your participation in this study.

8. Consent: By signing this consent form, I confirm that I have heard or read and understood the information and have had the opportunity to ask questions. I understand that my participation is voluntary. I voluntarily agree to take part in this study.

Name of participant _____

Signature _____Date _____

Annex 4: Kafinoonoo language version (kooreeti qihee yechi qitso/foormo)

1. **Heereche shuunee shimboo**: Seroprevalence and associated risk factors of *toxoplasmosis* in sheep in selected districts of Kafa Zone and pregnant women attending Ante Natal Care at Bonga Hospital south western Ethiopia.

2. **Hayiboo**: Hini heerechiyee shuunoochi ne toommoch shaggemi hayiboo aalloone. Ebichi qoodooch hiniyee deshi beeti eechena,ochi wochoon ubba,qatoon woyee ne qaawit gooroch neechoo hakkiyin.

3. **Gaacoo**: Neechi danemmi gaacoo amonoona aallone tunebaani guudoonaa shuunee shuuraaroonaa heereche shuunoocheniye bi danemo.

4. **Mullibeshiiyoo/xiishittino**: neechi wochoo woyee imade,eeyoo arichiyaache. Shuurareechi na,ochi qihoo xiishoonaa quyeeyoo qaawihe.

5**. Xu,ii aalee shuuraaroo**: neechi shuuraaroo hin heerechoochee shuni woyee niiyoonaane. Hini heerechoochee qoodeeyoo woyee neechi ga,oo beeyemooch ne qaawigaata qaaniti qitsochi woyee saanduuqqoch dukkoo qaawihe.

6. **Shuunoochi gijjoo/ gattiyoo**: Hini heerechiyoochi ne shuuraaroochi qocheemmi gijjoo aalloone.

7. **Keree imo:** neechi shuuraarooyichi immiyeemmi keree imoo aalloone.

8. **Mashaamee/ xiishiiyoot**: taachii dukkoonaa shigoon hini koorichee toommooch kooreeti qihee na,oon shemmeti, waayeeti shalligite ubba qiheena,oochi echoon echeeyoochinaa wochee imochi mallee echeenaon echehoo.

Shuuraarechoochi shigoo _____Dukkoo _____Decoo _____

Annex 5: **Questionnaire format for animal information**

1. Name of the owner_____Date_____Code_____
2. Origin of animal (Address)_____
3. Flock size ☐ large: ≥ 9 animals ☐ small: <9 animals
4. Sex ☐ Male ☐ Female
5. Age ☐ Young: ≤1year ☐ Adult: > 1 year
6. Presence of cats in the household ☐ Yes ☐ No
7. Occurrence of abortion (only for female sheep) ☐ Yes ☐ No
8. Occurrence of still birth (Only for female sheep) ☐ Yes ☐ No
9. Management system ☐ Extensive: receive no supplementary concentrate feed
   ☐ Semi-intensive: receive supplementary feed
10. Source of drinking water: ☐ River ☐ tap/piped

Annex 6- English version Questionnaire format for the study of pregnant women *T. gondii* infection risk factors

1. Name (optional)_____ Code_____ Date_____
2. Age (in years): ☐ 15-25 ☐ 26-35 ☐ 36-44
3. Residence: ☐ Urban ☐ Rural
4. Level of education: ☐ Illiterate ☐ Elementary ☐ High school & above
5. Occupation ☐ House wife ☐ Merchants ☐ private ☐ government worker
6. Gravidity: ☐ Primigravidae ☐ Multigravidae
7. Gestation period: ☐ 1st trimester ☐ 2nd trimester ☐ 3rd trimester
8. Have you had history of abortion before? ☐ Yes ☐ No
9. HIV status: ☐ Yes ☐ No
10. Do you have cats at home? ☐ Yes ☐ No
11. Have you ever eaten raw meat? ☐ Yes ☐ No
12. Do you consume raw vegetables? ☐ Yes ☐ No
13. What is the source of your drinking water? ☐ River/stream ☐ Well ☐ piped/tap
14. Do you drink raw milk? ☐ Yes ☐ No
15. Do you wash your hands after handling of raw meat? ☐ Yes ☐ No

16. Have you received blood from anybody? ☐ Yes ☐ No

17. Do you have exposure or contact with soil? ☐ Yes ☐ No

18. If question No. 17 is yes, tick one ☐ House cleaning ☐ Farming ☐ Garden

Annex 7: Kafinoonoo language version of questionnaire format for pregnant women

1. Shigoo _____koodoo _____ Decoo_____

2. Eanoo(Natonaa) ☐ 15-25 ☐ 26-35 ☐ 36-44

3. Bi beetii xaa,oo ☐ katammo ☐ Ballagaroo

4. Doyyee daqqo ☐ Doyaaneena'o ☐ ikkine daqqo ☐ Guttinee daqqona dambba

5. Shuunoo ☐ kechii endde ☐ Giixxachoo ☐ Biqelli shuunabeto ☐ Taatee shuunaachoo

6. Yeqqefe hinnoo 1^{nnee} bushee shimichoo 2^{nnee} bushee shimichoo

7. Yeqafee gooro ☐ 1^{nno} trimester ☐ $2^{nno\ trimester}$ ☐ 3^{nno} trimester

8. Eabiya Aafii yeqafa kicha hinno beete? ☐ Beete ☐ Alloonee

9. HIV daqqo ☐ poozetivoo ☐ Negaativoo

10. Kexochi kularee beete? ☐ Beete ☐ Alloonee

11. Gaaree menoo maatin? ☐ Beete ☐ Alloonee

12. Gaaree daaddebechoo maatin? ☐ Beete ☐ Alloonee

13. Uchi aacoo aabicheeniye itoo uchii beetoo? ☐ Woce aaco ☐ Biiree ☐ Boombee aaco

14. Gufaani ejjoo uichabeetin? ☐ Beete ☐ Alloonee

15. Kechii gijjonaa tokkaa danebeetin? ☐ Beete ☐ Alloonee

16. Ashi waani damo deqqa ariyine? ☐ Beete ☐ Alloonee

17. Shawona tokkee yesho beete? ☐ Beete ☐ Alloonee

18. Shawoonaa tokki yeshee boochoo? ☐ Kechii hidoo ☐ Goyonaa ☐ Daaddee bechoni

Annex 8: procedures of Toxo Latex Agglutination Slide Test

Principle of the test

Toxo-Latex Test is a rapid slide agglutination procedure, developed for the direct detection of anti *toxoplasma* antibodies in human serum. The assay is performed by testing a suspension of latex particles coated with antigenic extract of *T. gondii* against unknown samples.

The presence or absence of a visible agglutination indicates the presence or absence of anti *toxoplasma* antibodies in the sample tested.

Reagent composition

Toxo-Latex reagent is a suspension of polystyrene latex particles coated with antigenic extract of *T. gondii* in a buffered saline solution. Contains 0.95g/L of sodium azide. Positive control is human serum with an anti-*toxoplasm*a antibody concentration > 10 iu/ml. contains 0.95g/L of sodium azide. Negative control is animal serum. Contains 0.95g/L sodium azide.

Procedure Qualitative test

Before using the kits; the test reagents and samples were brought to room temperature. The antigen was resuspended gently to disperse the latex particles. One drop (50ul) of the sample under test was placed into one of the circles on the card. One drop of positive control and one drop of negative control were dispensed into two additional circles. One drop (25ul) of Toxo-Latex Reagent was added to each circle next to the sample to be tested. The contents of each circle were mixed with a disposable pipette while spreading over the entire area enclosed by the ring. Use separate pipettes for each sample. The card was rotated slowly by means of a mechanical rotator (80-100 r. p. m) for a period of 5 minutes. The degree of agglutination was observed immediately; non-reactive: smooth suspension with no visible agglutination, as shown by negative control and reactive: any degree agglutination visible macroscopically.