Molecular characterization and risk factors associated with abortions caused *by Toxoplasma gondii* in goats in Chittagong

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June, 2018

Authorization

The work presented in this thesis is entirely my own and I hereby declare that I am the sole author of the thesis entitled "Molecular characterization and risk factors associated with abortions caused by *Toxoplasma gondii* in goats in Chittagong. I also declare that it has not been previously submitted to any university for the award of a degree.

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee will be addressed

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DEDICATED TO MY RESPECTED AND BELOVED PARENTS AND TEACHERS

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The Author

Contents

List of tables

List of figures

List of abbreviations and symbols

AB-ELISA	Antibody- Enzyme Linked Immunosorbent Assay
bp	Base pairs
c-ELISA	Competitive- Enzyme Linked Immunosorbent Assay
CCCA	Chittagong city corporation area
CVASU	Chittagong Veterinary and Animal Sciences University
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic Acid
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
gp45	Glycoprotein 45
GRA	Granule Antigen
HE	Haematoxylin and Eosin
ID	identification
i-ELISA	Indirect Enzyme Linked Immunosorbent Assay
mg	miligram
MIC	Micronems Protein
ml	Mililitre
MLST	Microsatellite and Multilocus Sequence Typing

mM	Mili molar
MSP	Membrane Surface Protein
nPCR	Nested Polymerase Chain Reaction
OD	Optical density
OIE	Office International des Epizooties
OR	Odd ratio
PBS	Phosphate buffer saline
PCR	Polymerase Chain Reaction
ROP	Rhoptry Protein
rpm	Rotation per minute
RRA	RAP-1 related antigen
rRNA	Ribosomal Ribonucleic Acid
SAG	Surface Antigen
SAQTVH	Sahedul Alam Quadery Teaching Veterinary Hospital
Tg	*Toxoplasma gondii*
ul	Microlitre
uM	Micro molar
WASA	Water Supply & Sewerage Authority

Abstract

The current research was conducted to assess the seroprevalence, associated risk factors and molecular characterization of *Toxoplasma gondii* in goats in Chittagong City Corporation area (CCCA). 52 farms were selected on the basis of previous abortion history. Among these farms a total of 400 blood sample was collected to detect specific IgG against *Toxoplasma gondii* by ELIZA. For molecular characterization 20 aborted fetus of goat were collected those were admitted into SAQVTH, CVASU. The overall seroprevalence of toxoplasmosis in goat was 28.3%. The prevalence of infection was significantly higher in adults (OR=3.1, 95% CI:1.20,8.23; P=0.02) than in young goats, goats with poor BCS (OR=3.3, 95% CI: 1.2,8.8, P<0.01) than good BCS. Farm management like allowed to drink from stagnant water (OR=26.9, 95% CI:6.2,117.1, P<0.01) than those provided tap water, absence of feeding trough (OR=10.3, 95% CI:1.4,78.1, P<0.05) as compared to wooden and steel troughs, absence of drinking troughs (OR=3.2, 95% CI:1.2,8.7, P<0.05) than those use steel or plastic trough were statistically significant with *T. gondii* infection. Presence of cat in farm (OR=20.9, 95% CI:6.2,70.5, P<0.01) might play a vital role for the transmission of this infection. Goats of flooded areas had a significantly higher prevalence of toxoplasmosis than hilly and flat areas. In order to conduct histopatological examination and molecular characterization samples from placenta, fetal heart, lungs, liver, brain tissue was collected. Histopathological examination revealed bradyzoite cyst and tachyzoite cyst from some of brain and heart tissues. A nested PCR assay targeting B1 gene was applied and positive sample yield 197 bp amplification products consistent with *T. gondii*. In case of aborted fetus, the frequency of toxoplasmosis was 35%. Among the aborted cases, 50% abortion occurred at >3.5 months of gestation by *T. gondii*. In risk factor study, age of goats, presence of cat, BCS were not found as an influencing factors of toxoplasmosis in aborted does. The presence of *T. gondii* in fetal tissues along with characteristic histopathological changes strongly suggested active and or congenital toxoplasmosis. It can be concluded that exposure of goats to oocysts of T. gondii is widespread. Further studies to determine the genotype of the parasite, public health significance both in livestock and human are recommended.

Keywords: Toxoplasmosis, Goat, Seroprevalence, Risk factors, PCR, CCC.

Chapter I: Introduction

Goat is one of the major livestock species which plays an important role in agro-based economy of Bangladesh. Total goat population in Bangladesh is about 15 million (DLS, 2017). Most of the goats are reared by rural landless women. Annual production of meat from meat is 0.17 million and milk is 0.08 million mectric tons which is equivalent to the contribution of BDT. 5000 million to the national economy (DLS, 2017). Although goats contribute a lot to our national economy toxoplasmosis causes a great havoc by aborting the kids.

Abortion is one the most putative factors for goats in Bangladesh. Among different causal agents *Toxoplasma gondii* is significant one. *T. gondii* is an obligate intracellular protozoan that causes anthropozoonic diseases (Kim et al., 2004). This protozoan is capable of infecting all worm-blooded and some cold blooded animal. It has been estimated that one third people on the earth has been infected with *T. gondii* (Tenter et al., 2000). Human get infection through ingestion of cyst contaminated food (milk/meat) and oocyst contaminated water (Ferguson, 2009). Cats as a final host play important role in the spreading of the Diseases (Dubey, 2009). Poor hygienic management of farm, climate, presence of cat in farm, taking raw or uncooked meat and vegetables, inter-current diseases may act as potential risk factors for influencing this disease (Kijlstra et al., 2008). It is responsible both for abortion and stillbirth in animals. Moreover toxoplasmosis has detrimental effects on productive performance of goats (Radostits et al., 2006).

The prevalence of toxoplasmosis varies greatly among different countries or different geographical areas within same countries due to different farming practices and environmental condition (Tenter et al., 2000). In Bangladesh only a few studies were conducted by researcher especially in the Mymensingh area of Bangladesh based on MAT and LAT (Shahiduzzaman et al., 2011). In cattle, sheep and goat the prevalence of toxoplasmosis was recorded 16.10%, 17.60% and 12.0% respectively, in Bangladesh (Samad et al., 1993). Signs of toxoplasmosis vary depending on when the female gets exposed. *T. gondii* commonly invades the placenta and fetus

approximately two weeks after initial infection of the doe. Infection during the early stage of gestation can result in fetal death, resorption and abortion (Aitken, 2008).
The diagnosis of abortion due to toxoplasmosis is based on detection of antibodies in fetal fluid by serological test (Dubey et al., 1987), demonstration of parasites by immunohistochemistry (Uggla et al., 1987) and observation of characteristic histopathological change in placenta and brain tissue (Buxton and Finlayson, 1986). The isolation of *T. gondii* from aborted tissue sample is the gold standard method for diagnosis of toxoplasmosis (Ortega-Mora, 2007). Recently molecular assay such as PCR amplification of different gene to detect the *T. gondii* DNA in aborted sample is using as a valuable and reliable techniques for diagnosis of toxoplasmosis (Hurtado et al., 2001). Several PCR assay targeting gene have been developed for detection of Toxoplasma gondii such as B1 repetitive gene (Burg et al., 1989), P30 surface antigen (Savva et al., 1990), small ribosomal rRNA (Tenter et al., 1994) and 529 bp repeated element (Homan et al., 2000). Surveillance and monitoring of toxoplasmosis in human and animals is required for up to date information of toxoplasmosis in different countries, but unfortunately it was performed regularly only in few countries (Figueiredo et al., 2001).

Nowadays the popularity of commercial small ruminants farming business in Bangladesh is increasing and more and more people are engaging with this business. Despite of high economic return from goat farming, this enterprise faces several problem of which toxoplasmosis is crucial one. Though toxoplasmosis has huge impact on health of goats, it was not properly focused in Bangladesh especially in Chittagong area. Every year a bunch of aborted cases are admitted into SAQTVH, Chittagong. No one longs for confirmatory diagnosis. Treatment is performed based on presumptive diagnosis. However, confirmatory diagnosis is mandatory. Therefore, the study was designed with objectives:

1. To know the prevalence of abortion in goat in Chittagong city corporation area
2. To estimate sero-prevalence of toxoplsmosis in Chittagong city corporation area.
3. To find out risk factors associated with toxoplasmosis.
4. Molecular characterization of *T. gondii*.

Chapter-II: Review of Literature

2.1 Brief History

Toxoplasma gondii was first discovered by Nicolle and Manceaux (1908) in tissues of a hamster-like rodent, the gundi (*Ctenodactylus gundi*), which was being used for leishmaniasis research in the laboratory of Charles Nicolle at the Pasteur Institute in Tunis. The name *Toxoplasma gondii* was coined by Nicolle and Manceaux (1908) based on the crescent shape of the tachyzoites (In Greek: *toxo* = arc or bow, *plasma* = form or life). At about the same time, Splendore (1908) working in Sao Paulo, Brazil, discovered a similar parasite in rabbits also erroneously identifying it as *Leishmania*, but he did not name it (Dubey, 2010). The medical importance of *T. gondii* remained unknown until 1939 when it was identified in tissues of a congenitally infected infant presenting with the classic triad of symptoms, namely hydrocephalus, retinochoroiditis and intracranial calcification (Dubey, 2008; Innes, 2010b). The veterinary importance of *T. gondii* became known when it was found to cause abortion storms in sheep in 1957 (Dubey, 2008).

2.2. Etiology

Toxoplasmosis is caused by the obligate intracellular protozoan parasite *Toxoplasma gondii* (Dubey and Beattie, 1988; Tenter et al., 2000; Dubey, 2010). *Toxoplasma gondii* belongs to the Kingdom Animalia, Phylum Apicomplexa, Class Protozoa, Subclass Coccidian, Order Eucoccidia, Family Sarcocystidae and Genus *Toxoplasma* (Dubey, 2010).

2.3. Biology

2.3.1. Life cycle and transmission

The life cycle includes intestinal-epithelial (enteroepithelial) and extraintestinal stages in domestic cats and other felines but only extraintestinal stages in other hosts. Sexual reproduction occurs in the intestine of cats, and only asexual reproduction is seen in various tissues of intermediate hosts (all warm-blooded animals including most livestock and humans) (Figure 2.1) (Buxton, 1998; Marquardt et al., 2000; OIE, 2008; Tenter, 2009; Dubey, 2010). There are three

infectious stages, i.e. tachyzoites (rapidly multiplying stage), bradyzoites (contained in tissue cysts and slowly multiplying stage) and sporozoites (contained in sporulated oocysts) that are infectious for both intermediate and definitive hosts (Sibley et al., 2009; Tenter, 2009). Cats acquire infection by eating meat containing bradyzoites in tissue cysts of intermediate hosts, such as birds and rodents or by ingesting infective oocysts. Upon ingestion of tissue cysts proteolytic enzymes in the cat's stomach and intestine degrade the wall of the tissue cysts and bradyzoites are released. Bradyzoites then penetrate the intestinal epithelial cells of the cat where they initiate the development of numerous generations of *T. gondii* (Dubey, 2010).

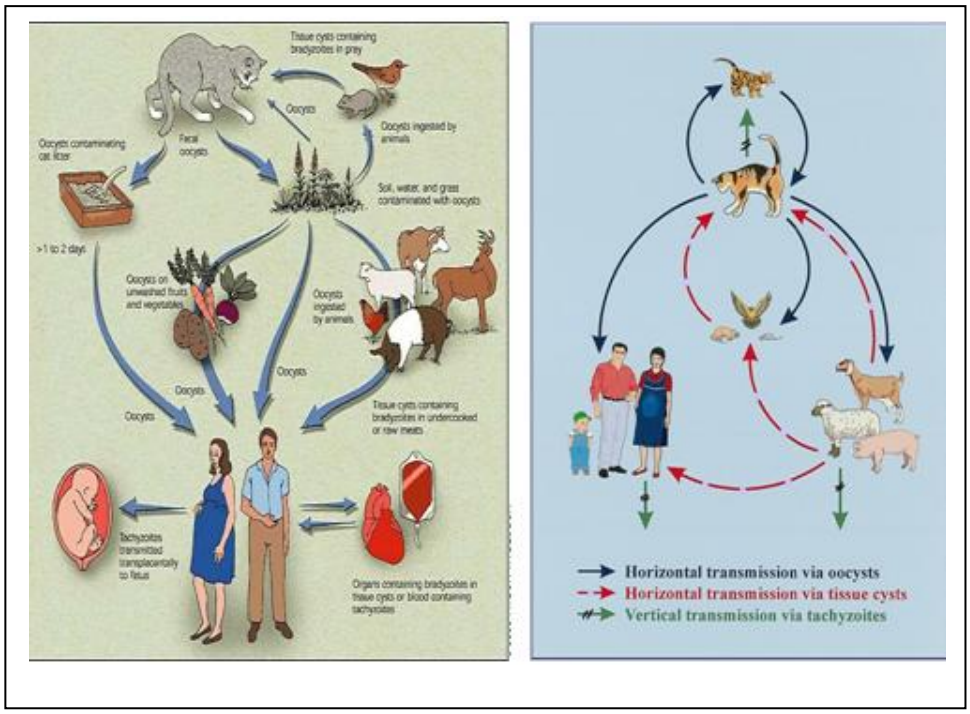

Figure 1.1. Pathways for *Toxoplasma gondii* infection [Adapted from: Jones et al., 2003 (A) and Tenter et al., 2000 (B)]

2.4. Pathogenicity and virulence

Pathogenicity of *T. gondii* is determined by the virulence of the strain and the susceptibility of the host species (Dubey and Lindsay, 2004). Genotypes might differ in migratory capacity and virulence, thus strains might differ in ability to cross placenta barrier to cause congenital disease as well as in their ability to cause toxoplasmic encephalitis. The higher virulence of type I in mice compared

with types II or III has been correlated with in vitro biological properties of migration, penetration and transmission. The host response is also essential for expression of virulence. Atypical and naturally recombinant strains are usually more virulent in mice than are types II or III (Dardé et al., 2014). There is a geographical variation in strains associated with congenital toxoplasmosis.

2.5. Economic significance

Toxoplasmosis is an economically important disease in animal husbandry globally as it is a major cause of reproductive failure by leading to early embryonic death and resorption, fetal death and mummification (Dubey, 2009), abortion, stillbirths, and neonatal death in small ruminants (Dubey and Kirkbride, 1989; Marquardt et al., 2000; Dubey, 2009, 2010). The severity of infection is associated with the stage of gestation at which the ewe becomes infected; the earlier in gestation, the more severe the consequences (Dubey, 2009). In Uruguay, Freyre et al. (1997) estimated annual losses due to toxoplasmosis in does during gestation to be 1.4 to 3.9% of the ewes investigated (n=1613), amounting to approximately US$ 1.4-4.7 million. The economic losses due to lamb mortality and missed lactation are estimated at 10 million Euros per year in Italy (Masala et al., 2003). Studies by investigators of Reading University estimated toxoplasmosis to cost to the sheep industry of UK between £12 million and £24 million each year (http://www.apd.rdg.ac.uk/AgEcon/livestockdisease/index.htm).Overall, toxoplasmosis results in increased production costs, diminished marketability of meat, fewer replacement animals, retardation of genetic progress and a major source of human infection (Leighty, 1990; Freyre et al., 1997). It has been estimated that one-third of the human population is chronically infected (Das, 1992; Dubey and Beattie, 1988; Tenter et al., 2000; Dubey, 2004, 2010).

2.6. Risk factor of Toxoplasma gondii in small ruminants

Limited epidemiological information regarding caprine toxoplasmosis risk factors is available in literature. However the risk factors that are identified by different researchers are given below:

2.6.1. Species

It has been reported that among small ruminants, sheep had relatively higher prevalence than goats (Gebremedhin and Gizaw, 2014). It may be due to the differences in feeding habits since sheep are more likely to get infection from the pasture as they graze close to the ground than goats which prefer browsing. It was also reported that sheep had higher prevalence that goat but had no significant association between them (Tzanidakis et al., 2012).

2.6.2. Age

It has been reported that age of the animals were positively associated with the seroprevalence of toxoplasmosis in goats. Adult animals had comparatively higher prevalence of toxoplasmosis infection compared to young animals (Ramzan et al., 2009; Rossi et al., 2011; Ahmad et al., 2015). It is due to exposure of adult animals to the risk factors for longer period of time than the younger ones (Van der Puije et al., 2000). But there is also exception that there was no significant difference between age group of less than one year of age and more than one year age in goat (Djokić et al., 2014).

2.6.3. Gender

It has been reported that female sheep and goats are more susceptible than males to toxoplasma infections (Alexander and Stimson, 1988; Ahmad et al., 2015). It may be explained by the fact that immunity in females is reduced by various factors such as pregnancy, nutrition and lactation (Messingham et al., 2001). Although there are so many published reports they reported that there were no significant correlation between toxoplasma infection and the gender of the animals (Cavalcante et al., 2008; Gebremedhin and Gizaw, 2014).

2.6.4. Cat presence

Cats are the definitive hosts of the parasite and play a vital role in infecting other animals by shedding oocysts in the environment (Lopes et al., 2010). Presence of cat specially the free roaming cats increase the risk of transmission of the infection in animals. Cats increase the oocysts load on nearby pastures resulting in contamination of environment. These oocysts when ingested along with food and water result in

postnatal infection. There were so many reports that presence of cat play vital role in transmission of toxoplasmosis in sheep and goat (Lopes et al., 2010; Ahmad et al., 2015). The number of cat presence in the farm premises also plays the role in epidemiology of toxoplasmosis. It was reported that Greater risk of infection was observed in farms with more than 10 cats was presence in farm (Cavalcante et al., 2008).

2.6.5. Raw or undercooked meat consumption

An association between raw meat consumption and *T. gondii* seropositivity (Mengesha et al., 1984; Masresha, 2012; Walle et al., 2013) was reported, but species of the animal used as source of raw meat was not mentioned. Negash et al. (2008) and Yibeltal (2008) reported consumption of raw or undercooked mutton as risk factors. Raw or undercooked beef consumption was also associated with a high IgG seroprevalence (96.77%) of *Toxoplasma* infection in a study on 279 abattoir personnel in Addis Ababa (Yimer et al., 2005).

2.6.6. Climate

A significantly higher seroprevalence of toxoplasmosis was reported in warm humid climatic zones as compared to drier areas (Guebre-Xabier et al., 1993).

2.6.8. Management system

The prevalence of toxoplasmosis was comparatively higher in animals that are reared in unhygienic condition in compared to that were reared in hygienic condition. If the feed and water were contaminated with cat feces it increases the risk of diseases. Gazzonis et al. (2015) identified that the farm that using river water had significantly higher prevalence than the farm using municipality water. Using of public supply water (Tzanidakis et al., 2012) and Usage of outdoor water source (Ahmad et al., 2015) in the farm is an important risk factor of toxoplasmosis. Types of feeding trough used in the farm were also an important factor in the epidemiology of toxoplasmosis. Using of wooden trough increasing the risk of Toxoplasma infection (Cavalcante et al., 2008).

2.7. Clinical toxoplasmosis

Toxoplasma gondii infection of intermediate hosts is typically subclinical; however, it can be an important cause of abortion in goat (Dubey, 2010) where vertical transmission between successive generations has been suggested (Hide et al., 2007). Among food animals, goats appear to be more susceptible to clinical toxoplasmosis, and adult goats were reported to have died of acute toxoplasmosis (Dubey and Beattie, 1988). Toxoplasmosis is a common cause of abortion (Tenter et al., 2000; Lindsay and Dubey, 2007; Dubey, 2010) and neonatal mortality in goats (Dubey, 2010). There have been reports of clinical toxoplasmosis in pigs with abortion, stillbirth, and even death in neonates. Fatal toxoplasmosis has also been reported in cats, dogs, avian species, primates, hares, marsupials (reviewed in Dubey, 2010) and sea otter (Miller et al., 2004).

2.8. *Toxoplasma gondii* detection methods

Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. The direct demonstration of *Toxoplasma* tachyzoites in cerebral tissues is the method of choice for a definitive diagnosis of cerebral toxoplasmosis (Pereira-Chioccola et al., 2009). Serodiagnosis is a useful and adequate tool to diagnose *Toxoplama* infection in both man and animals (Hashemi-Fesharki, 1996). Serological testing is the most commonly used technique for supporting the clinical diagnosis of toxoplasmosis. Several reliable serological tests are available for the detection of *T. gondii* antibodies. The Sabin-Feldman dye test (DT), and the complement fixation (CF), indirect fluorescent antibody test (IFAT), indirect hemagglutination (IHA), Enzyme linked immunosorbent assay (ELISA) and agglutination (AG) tests are some of the serological tests used for the diagnosis (Fraser et al., 1986). Bioassay involves the inoculation of animals or cell cultures with suspected T. gondii infected tissue (s) or suspected T. gondii oocysts (Dubey and Lappin, 1998). Both mice and cats are natural hosts of T. gondii and they can serve as a biological incubator to produce a large number of parasites to ease the identification of infection. Molecular methods rely on PCR for the specific detection or analysis of *T. gondii* DNA (Su et al., 2010). Molecular methods have become indispensable and reliable tools

(Williams and O'Donovan, 2009; Su and Dubey, 2010), not only in the diagnosis but also in the understanding of the epidemiology of *T. gondii* (Su and Dubey, 2010). Restriction Fragment Length Polymorphism (RFLP) analysis of specific genetic loci has been widely used for *T. gondii* genotyping. RFLP markers are amenable to high-throughput analysis using PCR amplification, followed by restriction digestion and gel electrophoresis (Sibley et al., 2009), which is extremely valuable for performing population surveys. All of the above methods sequence-based methods provide the best approach for detecting polymorphisms in new isolates or from previously unsampled populations (Sibley et al., 2009). The obvious disadvantage of sequence-based typing is its high cost and the need for access to sophisticated technology (Sibley et al., 2009).

2.9. Control methods

Control of *T. gondii* infection in animals depends mainly on management, legislation, hygiene and vaccination. During pregnancy a seronegative flock / herd, could be at risk if it was allowed access to an environment contaminated by cat faeces. Thus, all feed and water should be kept free from soiling as far as practically possible. Other measures to reduce environmental contamination by oocysts should be aimed at reducing the number of cats capable of shedding oocysts. These would include indoor keeping of cats to prevent hunting, feeding only canned or dried commercial food or well-cooked meats (Frenkel, 1974).

2.9.1. Vaccination

Vaccination of sheep with a live vaccine is an effective preventive measure (Buxton, 1998). A live vaccine (Toxovax1) is commercially marketed in the UK, France and New Zealand for reducing losses to the sheep industry from congenital toxoplasmosis (Buxton and Innes, 1995).

2.9.2. Legislation

Stray cats are apparently the key to the epidemiology of toxoplasmosis, and they would have to be controlled by enforced legislation in order to curb markedly the spread of the infection to other animals and to humans (Frenkel, 1974).

2.9.3. Hygien (Oocyst)

Pregnant women should not help in the lambing of ewes or in the care of newborn lambs. If untreated surface water is to be consumed or it is the main source of drinking water, filtering (absolute 1 µm filter) or boiling will eliminate *T. gondii*. Chlorine treatment will not inactivate *T. gondii*, however tincture of iodine (2%) will inactivate *T. gondii* with a long (3 h) exposure (Jones and Dubey, 2010).

2.9.4. Tissue cysts

Raw meat of any sort should be considered to be infected and be handled with great care. Hands, utensils, and cutting boards should all be washed with soap and water to prevent transferring a possible infection to the mouth. To avoid cross-contamination of other food sources with *T. gondii* tissue cysts, it is essential that a high standard of hygiene is maintained at slaughterhouses and in kitchens (Tenter, 2009).

2.9.5. Cooking, salting, freezing and irradiation

Food-borne horizontal transmission of *T. gondii* to humans can be prevented by thorough cooking (67^{o}C) of meat and other edible parts of animals before consumption (Tenter et al., 2000), and preferably at 70^{o}C, to kill the zoites (Marquardt et al., 2000). Salting and pickling procedures are also effective in killing tissue cysts. Pasteurization of milk kills all forms of the organism (Das, 1992).

2.10. Prevalence in Bangladesh

In Bangladesh different study was undertaken in different areas especially in Mymensing and Rajshahi area of Bangladesh. The overall highest prevalence of toxoplasmosis in sheep in Mymensingh area was recorded as 42% (Shahiduzzaman et al., 2011) and lowest prevalence was recorded as 17% (Samad et al., 1993). In a recent time a study was conducted in Rajshahi city area and the prevalence in sheep and goat were recorded as 69.9% and 61% respectively (Rahman et al., 2015b). In contrast, seroprevalence for young and adult sheep was similar (Rahman et al., 2014a).

2.11. Prevalence in other countries

In Pakistan, the prevalence of toxoplasmosis in sheep and goat were 42.28% and 44.13% respectively. In India, 25.3% in sheep, 30.3% goats (Chhabra et al., 1985). In Brazil, 31% seroprevalence was determined in goat (Oncel and Vural, 2007) and In Iraq, 25.4% sheep and 25 (28.4%) goats were seropositive. In Iran, antibodies against *T. gondii* were found in 18.3% serum samples. DNA of *T. gondii* was detected in 69% of fetal brains, 23% of cotyledons and 14.7% of blood samples (Moazeni Jula et al., 2013). In Saudi Arabia, A sero-prevalence survey of Toxoplasmosis found 43.5% in sheep flowed by 31.8% in goats and 24.4% in camels (Mosa et al., 2015). In Italy, the prevalence of toxoplasmosis was reveled 33.3% in goat (Cenci-Goga et al., 2013).

2.12. Molecular study of Toxoplasmosis in aborted animal

In Nigeria, a study was designed to detect *T.gondii* DNA from tissues of aborted caprine and ovine fetuses by single tube nested PCR. A total of 327 tissues from 45 and 31 caprine and ovine aborted fetuses were analyzed. All samples analyzed were negative for *T.gondii* DNA. The organism may not play important role in ovine and caprine abortions in the study area (Kamani et al., 2010a).

In Brazil, a survey was done to determine the contribution of *T. gondii* to reproductive failure using nested PCR and histopathological examination of fetuses, stillborns and placentas. The histopathological examination revealed no lesions characteristic of toxoplasmosis in the organs investigated. Nested PCR showed three aborted fetuses and two stillborns (14.3%) to test positive for *T. gondii*, with DNA amplification in all organs and the placenta, especially the heart and the placenta, which are the tissues of choice. This study substantiates the theory that *T. gondii* is involved in miscarriages and stillbirths and in the placentas of naturally infected sheep in Brazil. Such findings have not previously been described in the national literature (de Moraes et al., 2011).

In Egypt, a study was carried out on a flock of sheep and goats suffered from late abortion with incidences of 35.6 and 43.7%, respectively. (Ahmed et al., 2008).

In Italy, during the period 1999–2002, an experiment was conduct. From a total of 2471 ovine and 362 caprine fetal samples including muscle, liver, abomasum, spleen,

brain and placenta, 271 (11.1%) ovine and 23 (6.4%) caprine samples were *T. gondii* PCR-positive. Although *T. gondii* DNA was amplified from different types of tissues, placenta was the tissue with the highest detection rate. On the one hand, these results indicate that the seroprevalence of *T. gondii* infection in sheep and goats is relatively high; on the other PCR results demonstrate that *T. gondii* has a significant role in ovine and caprine abortion. Adequate management might be useful and essential to control the toxoplasmosis in the sheep and goats herds of Sardinia (Masala et al., 2003).

Chapter III: Materials and methods

3.1. Description of the study area

Chittagong is considered as the second largest financial capacity city of Bangladesh located 22°22'0"N and 91°48'0"E. The tropical monsoon climatic condition characterizes by annual average temperature of 13°C to 32°C, humidity of 70-85% and rainfall of 5.6 mm to 727.0 mm (Anon, 2016). In metropolitan city total goat population is 30320 (DLS, 2017).

3.2. Reference population, source population and study population

Cross sectional study design was followed to attain the study objectives. Goats of smallholder farms under Chittagong metropolitan city was considered as reference population. Farms having at least 5 goats per farm along with history of abortion was treated as source population. A total of 52 farms were chosen conveniently representing different subsides of CCCA (Chittagong City Corporation Area). There are 41 wards under 12 thanas in CCCA. All the wards were covered for sample collection. Distribution of sampled farms among different sub-sites of CCCA was as follows: Khulshi (7), Kotowali (6), Chandgaon (6), Double moring (5), Patenga (4), pahartali (6), Bandar (6), Bakalia (3), Bayzid (3), Halisohor (4) and some part of Hathazari (2). Goats more than 6 months of old of selected farms were considered for sample collection. A total of 400 blood sample was collected for risk factor analysis and 20 aborted fetuses were collected for histopathology and molecular characterization. This cross-sectional study was carried out from June, 2017 to December, 2017.

3.3. Questionnaire survey

A close ended questionnaire was used to collect data on farm demography, farm management and host's characteristics (Annex I). Age of animal was determined based on dentition (Shively, 1987) and herders' information. A separate questionnaire was made for aborted fetus samples using for collection of data regarding age of the animals, gestation age, presence or absence of cat, body condition of animals etc. The detailed information in questionnaire is given in Annex- I.

3.4. Blood collection and sera separation

Approximately 5 ml of whole blood was sampled from the jugular vein by using disposable plain vacutainer tubes and needles (BD Vacutainer Systems, Plymouth, UK). Samples were labeled and left at room temperature for a few hours to clot. The samples were then centrifuged at 4000 rpm for 5 minutes. The sera were collected in 1.5 ml eppendorf tubes (eppendorf-AG, Hamburg, Germany) and transported to the PRTC (Poultry Research and Training Centre), CVASU (Chittagong Veterinary and animal Sciences University) in an ice box and stored at -20°C until tested.

3.5. Serological examination (ELISA)

Serum sample were tested for the presence of IgG antibodies against *Toxoplasma gondii* using a commercial indirect ELISA kit (IDEXX toxo test, IDEXX Switzerland AG, Switzerland) according to manufacturer instruction. The kit was used for detection of antibodies against *T. gondii* in small ruminants.The test is validated if: - the mean value of the positive control O.D should not exceed 2.00 and the mean value of the negative control should not exceed. 0.500. The ratio of the mean O.D values of the positive and negative controls (ODPC and ODNC) is greater than equal to 0.300 would also criterion to validate the sample. All the serological tests were done in serological lab, PRTC, CVASU. For interpretation of the result S/P% was calculated as: S/P% = (OD 450 value of the sample - OD450 value of the negative control) / (mean OD 450 value of the positive control - OD450 value of the negative control) x 100. Any samples with an S/P less than or equal to 20% were considered as negative, the samples with an S/P between 20% and 30% were considered as suspected, the samples with an S/P between 30% and 100% were considered as weak positive and the samples with an S/P greater than or equal to 100% considered as positive.

3.6. Aborted fetus collection

Tissue samples were taken from the 20 aborted feti of goat that were admitted to SAQTVH, CVASU. Samples were divided into two portion- one portion for histopathological study and another portion for molecular study. The samples were then preserved into Bouin's solution (histopathological examination) and -20^{o}C temperature for further molecular examination.

Figure 2.1: Collected aborted feti of goat

3.6.1. Histopathology

Tissue samples were taken from placenta, brain, heart, lungs, and liver of aborted fetuses and were fixed in 10% bouin's solution. The fixed specimens were washed, dehydrated and embedded in paraffin wax. The tissues were sectioned at 4-5 thickness and stained with H & E as routine work for histopathological examinations according to Bancroft, (1996). The total detailed procedure has given in Annex-II.

3.6.2. DNA extraction

DNA was extracted from tissue samples. From Tissue samples DNA was extracted by using commercial DNA extraction kit (Favorgen®, Favorgen Biotech Corp, Taiwan) by following their instruction. Briefly, 25 mg tissue sample was taken into a sterilized mortar and pastle and then grinded it to make a homogenous mixture by using PBS (Phosphate Buffer Serum). Then the mixture was transferred into a centrifuge tube. 200 µl FATG1 Buffer and 20µl Proteinase K (10mg/ml) added to the sample mixture and mixed thoroughly by vortexing. The mixture was incubated at 60°C until the tissue is lysed completely and vortexing was done in every 10-15 min during incubation. To remove the drops from the inside of the lid, the tube was briefly spine. 4µl of RNase A (100 mg/ml) was added and incubated for 2 min at room temperature. After that 200µl FATG2 Buffer was added to the sample mixture, mixed thoroughly by pulse-vortexing and incubated at 70°C for 10 min. To remove the drops from the inside of the lid, the tube was briefly spine. 200 µl ethanol (96 ~ 100%) was added to the sample and mixed thoroughly by pulse-vortexing. To remove the drops from the

inside of the lid the tube was briefly spine. A FATG Mini Column was placed in a Collection Tube and then the sample mixture (including any precipitate) was transferred carefully to FATG Column. Centrifugation was done at 14000 rpm for 1 min and the flow-through was discarded. Then the same FATG Column placed into a new Collection Tube. FATG Column was washed with 500µl W1 Buffer by centrifugation for 1 min and then the flow-through was discarded. Again FATG Column washed with 750µl Wash Buffer by centrifugation for 1 min then discarded the flow-through. Centrifugation was done for an additional 3 min to dry the column. Finally FATG Column was placed to Elution Tube. 50 µl Elution Buffer was added to the membrane center of FATG Column and FATG Column was stand for 3 min. Final Centrifugation was done for 2 min to elute total DNA. DNA was stored at 4C or -20°C.

3.6.3. DNA amplification

PCR was performed in a 2720 thermal cycler (Applied Biosystem) in a total reaction volume of 25 µl containing 12.5 µl of GoTaq® G2 Hot Start Green Master Mix (2X Green GoTaq®Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 4mM MgCl2), 1.5 µl of each of the primers (10 pico mole) derived from B1 gene, 2 µl of template (sample DNA) and 7.5 µl nuclease free water. For the PCR amplification, initial denaturation was performed at 94ºC for 30 seconds, followed by 50 cycles of denaturation at 94ºC for 15 seconds, annealing at 45ºC for 30 seconds, extension at 72ºC for 45 seconds and final extension at 72ºC for 10 minutes. For the nested PCR, The PCR reaction was performed at similar temperature to the primary PCR, but the am-plification cycles was carried out for 35 cycles. The PCR amplified products were visualized by electrophoresis on agarose gel 1.5% stained with ethidium bromide.Under a transilluminator with a 50 bp DNA ladder, the product length of the positive result was 197 bp.

3.6.4. Primer used

The primer set both for the primary PCR and secondary PCR were derived from B1 gene.

a) For primary PCR

5′-TCA AGC AGC GTA TTG TCG AG3′

5′-CCG CAG CGA CTT CTA TCT CT-3′.

b) For secondary PCR

5′-GGA ACT GCA TCCGTT CAT GAG3′

5′-TCT TTA AAG CGTTCG TGG TG3′.

3.6.5. PCR products purification

Positive samples DNA were purified for sequencing. DNA was purified from PCR product using FavorPrepTM GEL/PCR Purification Kit (FAVORGEN® BIOTECH CORP) according to the instruction of manufacturer. Briefly, with 20 µl of PCR product 5 volumes of FADF buffer was added and mixed thoroughly by vortexing. The mixture was then transferred to a FADF column and centrifuged for 1 minute. The flow through was discarded. Again, 750 µl Wash Buffer was added to the column and centrifuged for 1 minute. After discarding the flow through the column was centrifuged again for 3 minutes to dry and placed on to a new micro centrifuge tube. 40 µl elution buffers was then added to the column and after standing for 2 minutes the column was centrifuged for 2 minutes to collect the eluted DNA.

3.7. Data management and analysis

The data generated were stored in a Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using STATA version 11.0 for Windows (Stata Corp. College Station, USA). Univariable and multivariable logistic regression models were used to compute the odds ratio of the potential risk factors. Potential risk factors included in the univariable models were selected based on the existing literature. Non-collinear variables that presented $P<0.2$ in univariable analysis were included into the multivariable regression model. Seroprevalence was calculated by dividing the total number of goats tested positive by ELISA by the total number of goats tested. $P\leq0.05$ in multivariable were considered significant.

Chapter IV: Results

4.1. Descriptive analysis

4.1.1. Demography of goat farmers

Goat farmers had a diverse profession in this study; such as self business, housewife, service, farmer, student etc. The educational qualification of goat farmers' was primary, secondary, higher secondary and graduate level (Table 4.1).

Table 4.1: Goat farmers' demographic information in Chittagong city corporation area

Variable	Category	Frequency number	Percentage (%)
Occupation	Farmer	32	8
	House wife	101	25.3
	Self-business	165	41.2
	Service	41	10.2
	Student	39	9.8
	Unemployed	22	5.5
Education	BA equivalent (Honors, engineering etc)	14	3.5
	HSC and equivalent (Diploma, Higher secondary)	49	12.2
	SSC and equivalent (vocational, secondary)	25	6.2
	Class VI-IX (Secondary)	63	15.8
	Class I-V (Primary)	91	22.8
	Illiterate	158	39.5

SSC: Secondary School Certificate; HSC: Higher Secondary Certificate; BA: Bachelor of Arts; MA: Master of Arts; MS: Master of Science.

4.2. Demography of study population

The sampled population was composed of adult does, bucks, preganat does, lactating does and goats of different age limit. (Table 4.2)

Table 4.2: Composition of study population

Categories	Frequency	Percentage (%)
No. of adult does	277	69.25
No. of adult bucks	123	30.75
No. of pregnant does	85	21.25
No. of lactating does	76	19
Goat under 12 months	88	22
13-24 months	148	37
23-36 months	95	23.75
37-48 months	69	17.25

4.3. Prevalence of toxoplasmosis

Among 400 blood samples, 113 were found positive for anti-*T. gondii* IgG. The overall prevalence of toxoplasmosis was 28.3% (95% CI: 23.9-32.9).

4.4. Risk factors

Results of univariable and multivariable logistic regression analysis of the potential risk factors related to altitude, host and farm characteristics are presented (Table 4.3 and 4.4).

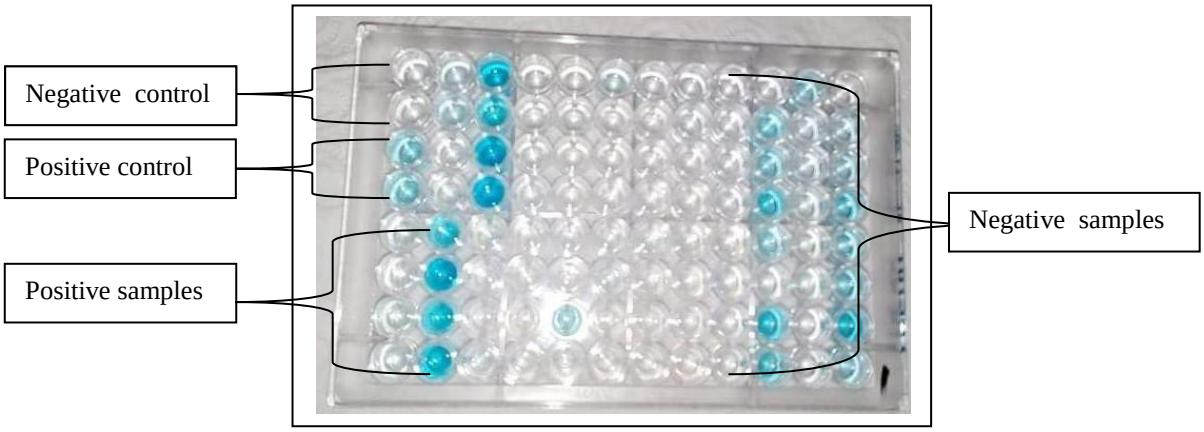

Figure 4.1: ELIZA PLATE

Table 4.3: Univariate logistic regression analysis on the predictors of *T. gondii* infection in goats of city corporation area, Chittagong

Variables	Categories (N)	Univariate analysis		
		Sample positive	95% CI	P value (χ^2 test)
Altitude				
Terrain type	plain (149)	24 (16.1)	10.6-23.0	<0.01
	Hilly (160)	54 (33.7)	26.5-41.6	
	Flooded (91)	35 (38.5)	28.5-49.2	
Farm characteristicts				
Source of the animal	Brought (205)	54 (26.3)	20.5-32.9	0.38
	Farm born (195)	59 (30.3)	23.9-37.2	
Types of breed reared	Meat (99)	22 (22.2)	14.5-31.7	0.25
	Milk (140)	40 (28.6)	21.3-36.8	
	Both (161)	51 (31.7)	24.6-39.5	
Housing system	Intensive (110)	15 (13.6)	7.8-21.5	<0.01
	Free range (64)	17 (26.6)	16.3-39.1	
	Semi-intensive (226)	81 (35.8)	29.6-42.5	
Grazing system	Cut-carry (152)	26 (17.1)	11.5-24.1	<0.01
	Free grazing (248)	87 (35.1)	29.1-41.4	
Hygienic condition	Good (118)	14 (11.8)	6.6-19.1	<0.01
	Moderate (122)	21 (17.2)	10.9-25.1	
	Poor (160)	78 (48.7)	40.8-56.8	
Presence of Cat in the farm	No (181)	14 (7.7)	4.3-12.6	<0.01
	Yes (219)	99 (45.2)	38.5-52.1	
Type of Pen	Raised (168)	31 (18.4)	12.9-25.2	<0.01
	Ground level (232)	82 (35.3)	29.2-41.8	
Pen flooring	Cement (157)	31 (19.7)	13.8-26.8	<0.01
	Ground level	82 (33.7)	27.8-40.1	

Variables	**Categories (N)**	**Univariate analysis**		
		Sample positive	**95% CI**	**P value (χ^2 test)**
	(243)			
Source of Water	WASA (tap) (103)	12 (11.6)	6.1-19.5	<0.01
	Running (river) (94)	15 (15.9)	9.2-24.9	
	Stagnant (pond) (203)	86 (42.4)	35.5-49.5	
Drinking trough	Plastic (168)	30 (17.8)	12.4-24.5	<0.01
	Steel (65)	12 (18.5)	9.9-30.0	
	Absent (167)	71 (42.5)	34.9-50.4	
Feeding trough	Steel (72)	5 (6.9)	2.3-15.5	<0.01
	wooden (117)	25 (21.4)	14.3-29.9	
	Absent (211)	83 (39.3)	32.7-46.3	
Vet service in house	Yes (146)	32 (21.9)	15.5-29.5	0.03
	No (254)	81 (31.9)	26.2-38.1	
Placenta disposal	Yes (170)	18 (10.6)	6.4-16.2	<0.01
	No (230)	95 (41.3)	34.8-47.9	
Host characteristics				
Age	1-12 months (88)	18 (20.5)	12.6-30.4	<0.01
	13-24 months (148)	35 (23.7)	17.1-31.3	
	25-36 months (95)	49 (51.6)	41.1-61.9	
	37-48 months (69)	11 (15.9)	8.2-26.7	
Sex	Male (123)	28 (22.7)	15.7-31.2	0.03
	Female (277)	85 (30.7)	25.3-36.5	
BCS	Good (92)	10 (10.9)	5.3-19.1	<0.01
	Fair (163)	33 (20.3)	14.3-27.2	

Variables	Categories (N)	Univariate analysis		
		Sample positive	**95% CI**	**P value (χ^2 test)**
	Poor (145)	70 (48.3)	39.9-56.7	
Breed	Black Bengal (88)	22 (25)	16.4-35.4	0.73
	Jamnapari (140)	40 (28.6)	21.2-36.8	
	Cross (172)	51 (29.7)	22.9-37.1	
Deworming history	Yes (151)	16 (10.6)	06.2-16.6	<0.01
	No (249)	97 (38.9)	32.8-45.3	
Vaccination history	Yes (115)	14 (12.2)	06.8-19.6	<0.01
	No (285)	99 (34.7)	29.2-40.6	
Type of breeding	No AI (Male) (115)	25 (21.7)	14.6-30.4	0.07
	AI (66)	16 (24.2)	14.5-36.4	
	Natural (219)	72 (32.9)	26.7-39.5	

OR= Odds Ratio, CI = Confidence Interval

Univariable logistic regression analysis revealed that, the prevalence of occurring *T. gondii* infection was significantly varied with housing system, feeding trough, type of pen, pen flooring, drinking trough, terrain type, presence of cat in the farm, placenta disposal system, grazing system, source of water, hygienic condition, BCS, age, deworming history and vaccination history (Table4.3). No significant effect was observed for sex, breed, type of breeding, source of animal, type of breed.

Table 4.4: Final multiplevariate logistic regression model to evaluate the effect of predictor variables on *T. gondii* infection in goats in the study area

Variables	Categories	Multiple logistic regression		
		OR	**95% CI**	**P Value**
Feeding trough	Steel (72)	1		
	wooden (117)	5.2	0.9-28.9	0.05
	Absent (211)	10.3	1.4-78.1	**0.02**
Type of Pen	Raised (168)	1		
	Ground level (232)	0.3	0.1-0.9	**0.03**
Drinking trough	Plastic (168)	1		
	Steel (65)	2.2	0.6-7.1	0.19
	Absent (167)	3.2	1.2-8.7	**0.02**
Terrain type	Flat (149)	1		
	Hilly (160)	5	2.1-12.5	**<0.01**
	Flooded (91)	5.1	1.9-13.8	**<0.01**
Presence of Cat in farm	No (181)	1		
	Yes (219)	20.9	6.2-70.4	**<0.01**
Grazing system	Cut-carry (152)	1		
	Free grazing (248)	0.2	0.1-0.7	**0.01**
Source of Water	WASA (tap) (103)	1		
	Running (river) (94)	2.8	0.7-11.5	0.14
	Stagnant (pond) (203)	26.9	6.2-117.1	**<0.01**
Vet service in house	Yes (146)	1		
	No (254)	2.2	1.0-4.7	**0.04**
Age	1-12 months (88)	1		
	13-24 months (148)	0.9	0.37-2.26	0.85
	25-36 months (95)	3.1	1.20-8.23	**0.02**
	37-48 months (69)	1.1	0.29-3.99	0.89
BCS	Good (92)	1		
	Fair (163)	1.5	0.5-4.1	0.38
	Poor (145)	3.3	1.2-8.8	**0.01**

OR= Odds Ratio, CI = Confidence Interval

Final multivariate logistic regression model showed that the odd ratio of determining *T. gondii* infection was 3.1 times higher in adult (25-36 months) goats than younger. Other statistically significant risk factors were poor BCS (OR=3.3, 95% CI:1.2, 8.8, P<0.01), goats that fed stagnant water (OR=26.9, 95% CI:6.2,117.1, P<0.01), presence of cat in farm (OR=20.9, 95% CI:6.2,70.5, P<0.01), opportunity of vet service in the farm (OR=0.03, 95% CI:1, 4.7, P<0.05), absent of feeding trough (OR=0.02, 95% CI:1.4,78.1, P<0.05), absent of drinking trough (OR=3.2, 95% CI:1.2,8.7, P<0.05), flooded areas (OR=5.1, 95% CI:1.9, 13.8, P<0.01) (Table 4.4). Besides, free grazing system (OR=0.2, 95% CI:0.1,0.7, P<0.01) and ground leveled type pen (OR=0.03, 95% CI:0.1,0.9, P<0.05), was observed as protective factors for toxoplamosis (Table 4.4).

4.5. Histopathological findings

For histopathological examination of brain, heart, liver, lung, placental tissue were collected from aborted feti samples. H & E stain was done for all the samples. In case of heart tachyzoite cyst (Figure 4.2) and in brain bradyzoite cyst (Figure 4.3) was found. Moreover, in lung chronic leukemia (Figure 4.4) was characterized by infiltration of lymphocyte within the lung tissue. Again, perivascular cuffng as well as accumulation of lymphocytes within the brain tissue (Figure 4.5) indicates the positivity of toxoplasmosis.

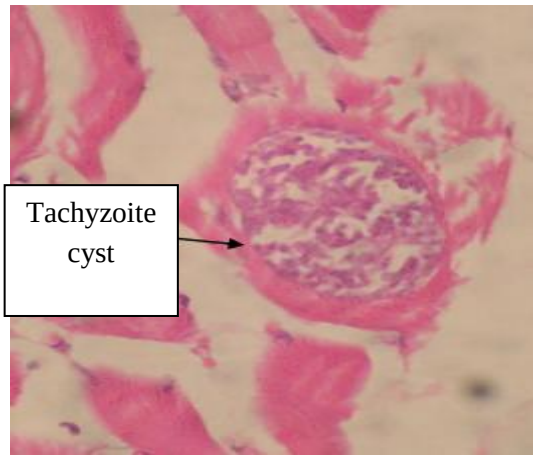

Figure 4.2: Dark purple tachyzoite in between muscle fibre (100X)

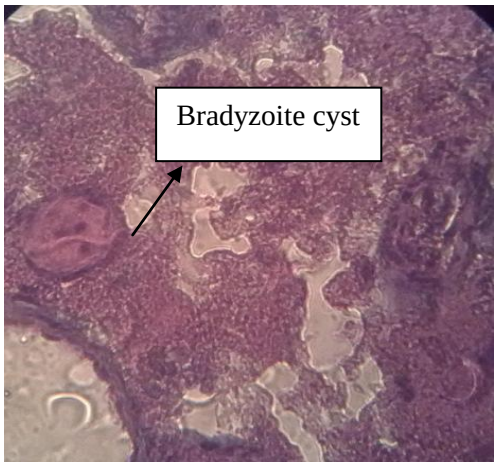

Figure 4.3. brain tissue characterized by presence of bradyzoite cyst (100X)

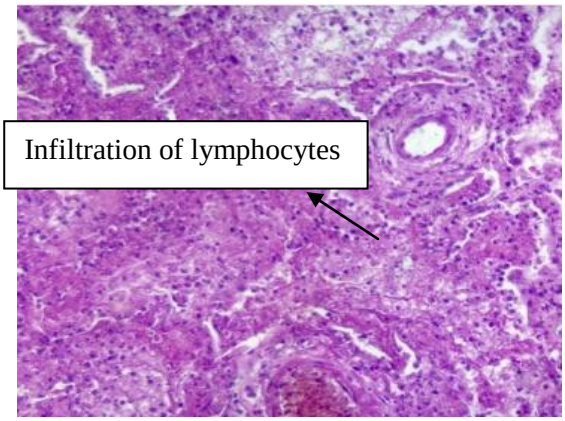

Figure 4.4: Chronic leukemia in lung (100X)

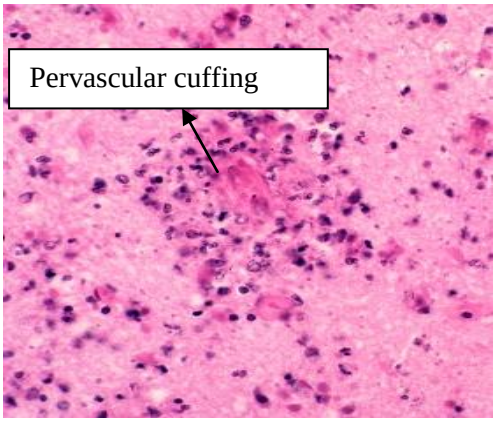

Figure 4.5. Chronic progressive encephalitis in brain tissue (100X)

4.6. Molecular examination:

DNA amplification of *Toxoplasma gondii* using B1 gene primer produced a 197bp fragment in nested PCR (4.5). A total of 87 sample were collected including heart tissue (20), liver tissue (20), lung tissue (20), brain sample (20) and placental sample (7).

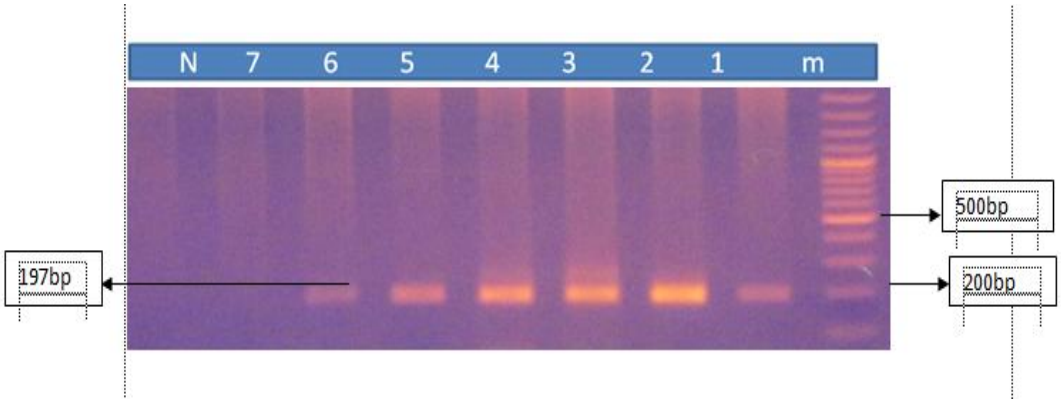

Figure 4.5: PCR products amplified using B1 gene. Lane M: 100 bp DNA ladder (Invitrogen), lane N: negative control, lane 1, 2, 3, 4, 5 and 6: positive 7 and 8; negative samples.

The prevalence of toxoplasmosis in aborted fetus was 44.28%. percentage of toxoplasmosis was found in brain sample 3 (15%). In addition, from heart muscle, liver, brain and placental tissue the number of positive cases were 2(10%), 1(5%), 3(15%) and 1(14.28%) (Table 4.5).

Table 4.5. Positive PCR result obtained from aborted fetus tissue

Tissue	No. of sample	Positive PCR
Heart muscle	20	2(10%)
Liver	20	1 (5%)
Lung	20	0 (0.00)
Brain	20	3 (15%)
Placenta	7	1 (14.28%)
Total	87	7(44.28%)

Table 4.6. Distribution of categorical variable among *T. gondii* positive aborted fetus

Factor	Factor level	No. of positive *Toxoplasma gondii*	Percentage (%)
Age category	2-3 years (13)	5	38.46
	>3 years (7)	2	15.38
Presence of cat	Yes (8)	5	62.5
	No (12)	2	16.67
Abortion at	<3.5 month (6)	1	12.5
	>3.5 month (12)	6	50

The overall prevalence of toxoplasmosis in aborted goats was 35%. Abortion rate was comparatively higher in goats aged between 2-3 years (38.46%) in compared to the goats aged greater than 3 years (10%) (Table 4.6). The percentage was higher in does that aborted at >3.5 months of age (50%) in compared to does that aborted at <3.5 month of age (12.5%). Presence of cat in the farm premises were not found as a significant factor of toxoplasmosis (Table 4.6).

Chapter V: Discussion

The overall seroprevalence of *Toxoplasma gondii* in goat in the study area was 28.3%. This finding is closely related to the findings of Akhoundi and Youssefi (2017) and Liu et al. (2010) who found 28.2% seroprevalence in goat in Iran as well as 29.8% prevalence rate in raw uncooked meat in chaina. Moreover, Samad et al. (1997) and Shahiduzzaman et al. (2011) the reported 12.88% and 32% seroprevalence of toxoplasmosis in mymensingh area of Bangladesh which is not similar with our present study. Again Yousefi et al. (2007) and Carneiro et al. (2009) showed 31% prevalence rate in sheep in Turkey and 31.1% in sheep in Brazil which is almost consistent with our present study. These dissimilarities in the seroprevalence could be due to differences in the relative cat densities and the access of cats to contaminated feed and water, the geographical variability, the serological tests used and the cut-off value reported (Zhao et al., 2011). According to the review of Dubey (2010) seroprevalence ranging from 3.2% in Mexico (by ELISA) to 90.9% in The Netherlands (by latex agglutination test) were reported. Also it differs because of difference in serodiagnostic tests used (Vesco et al., 2007).

T. gondii seroprevalence was significantly higher in adult goats (13-14 months) than in the young goats (<12 months) (11.4%). Increased risk of toxoplasmosis in adults is likely due to increased opportunities of exposure to several predisposing factors or sources of infections from the environment. Therefore, this difference in prevalence among age group can be explained by the cumulative effect of age (Hall et al., 2001; Dubey, 2010; Balea et al., 2012; Gebremedhin et al., 2014). The finding of this study is in conformity with other reports on caprine toxoplasmosis from Ethiopia (Teshale et al., 2007; Yibeltal, 2008) and from other countries (Jittapalapong et al., 2005; Carneiro et al., 2009; Chikweto et al., 2011).

The *T. gondii* seroprevalence was higher in females (30.7%) than in males (22.7%). This is consistent with previous reports from Ethiopia (Negash et al., 2004), Thailand (Jittapalapong et al., 2005) and Pakistan (Ramzan et al., 2009). The reasons behind this, physiological or hormonal differences between males and females might play a role in determining factors. Pregnancy and lactation

associated stresses might lower immunity leading to reactivation of tissue cysts (Zarnke et al., 2000). In contrast, Ragozo et al. (2009) reported that gender is not associated with *T. gondii* infection in goats. Researchers also reported that the susceptibility to infection from protozoan parasites was found greater in female animals compared to males (Alexander and Stinson, 1988).

Presence of cats might also significantly contribute in increasing the likelihood of infection in goats in present study. Cats are the definitive hosts of the parasite and play a vital role in infecting other animals by shedding oocysts in the environment (Lopes et al., 2010). A study from Poland reported that the presence of free-roaming cats is an important risk factor for the transmission of the infection in goats (Neto et al., 2008). Similar findings have also been reported in other studies in livestock animals (Puije et al., 2000; Chaudary et al. 2006; Lopes et al., 2010; Ahmad and Qayyum, 2014).

In this study seroprevalence was also higher in animals kept under poor hygienic conditions. Poor hygienic condition may favor the food and water to be contaminated with cat faeces, hence increase the likelihood of infection in livestock animals (Hove et al., 2005). Current study also found higher seroprevalence in livestock animals raised in extensive and semi-intensive management system.

As compared to extensive or semi-intensive management, animal raised intensively are usually caged and get little chance to ingest oocysts contaminated food and water (Anderlini et al., 2011). Similar findings have been reported in Ghanaian and Brazilian sheep raised extensively (Puije et al., 2000; Lopes et al., 2010). Increase in prevalence of toxoplasmosis in extensive management is also found more recently in sheep and goats from northeastern China (Wang et al., 2011).

Analysis of other risk factors showed outdoor water source was a putative risk factor for goats. This may be explained by the facts that goats are mostly infected by drinking contaminated water. Chances of getting infection through food are low as they usually browse the leaves which are far from the ground. On the other hand goat graze close to the ground and have better chances of getting infection via both food and water (Waldeland, 1976).

Sources of drinking water play important role in the epidemiology of toxoplasmosis. In this study it is observed that stagnant water (pond) is an important risk factor of toxoplasmosis in the study area. Tzanidakis et al. (2012); Andrade et al. (2013) also explained running water as an important factor of toxoplasmosis. The higher prevalence in animals that drank pond water is expected because of higher chance of contamination with oocyst. Water purification and chlorination processes are either ineffective against oocysts or non-existent (Bowie et al., 1997; Dubey, 2004). It could also be possible that some unknown confounding factors such as the poor sanitation of watering troughs can influence the probability of *T. gondii* seropositivity.

Use of drinking trough or wooden feeding trough or absent of any feeding trough has potential impact on occurence of toxoplasmosis. The capacity of these wooden troughs to retain moisture into the shady pens probably favors the survival of oocysts deposited together with the feces from cats frequently present in the facilities. The lack of feeding troughs or drinking trough also represented a high probability of infection. The absence of feeding trough on these farms was probably due to the fact that supplementary feeding was not performed. Thus, the animals would contract toxoplasmosis from pasture or water contaminated with sporulated oocysts. This finding is agreeing with Cavalcante et al. (2008).

Moreover, goats reared in hilly and flooded areas were more susceptible to *T. gondii* than that of flat areas. The influence of the environment on the epidemiology of toxoplasmosis has been well documented (Tenter et al., 2000; Dubey, 2004). The lowest prevalence (13.36%) was recorded in flat land area, which is characterized by a hot and arid climate compared to the tepid to cool sub moist agro-ecology in the flood affected lands and the moist agro climate in the highlands of the study area. Highland and flodded areas receive more rainfall; evaporation is relatively less and those areas have more forest canopies or vegetation. The resulting humidity is favorable to a higher chance of oocyst survival in the environment and infectivity to sheep, thereby contributing to the higher seroprevalence. 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., 2001b; Dubey, 2010). Furthermore, it is worth mentioning, that as compared to flat land, human

settlements and cat ownership are higher in highland. Many farmers in the highlands practice mixed crop-livestock farming. Grazing land is limited and some farmers' supplement goat with grains or commercial concentrate feeds that is often protected from rodent attack by keeping cats around. Similar to this findings, Kamani et al. (2010) also reported in Nigeria that a milder climate with higher rainfall and relative humidity favors a higher seroprevalence as compared to arid Sahel northern zones. However, a study from Mexico found that prevalence was higher at low altitudes (Caballero-Ortega et al., 2008). As during rainy seasons most of the low-lying areas of Chittagong City Corporation (CCC) submerge under water, it is very easy to contaminate water by cat feces resulting ingestaion of adulterate oocyst. Cavalcante et al. (2008) also mentioned different type of terrain in his study but which differs from our present study.

Abortion is a common problem in goat farming that can lead to significant health and economic issues. In this study the overall percentage of abortion due to toxoplasmosis in goat was 35% which is approximately similar to results of Ahmed et al. (2008) who recorded the incidence of late abortion in sheep was 35.6% during the breeding season. In this research the positivity of toxoplasmosis was higher in animals that aborted greater than 3.5 months of gestation in compared to animals that aborted less than 3.5 months of gestation. From this result it is clear that all the infection occurred between 40-120 days of gestation. It is already established that if infection occurred prior to 40 days of gestation causes embryonic death and fetal resorption, while infection between 40 and 120 results in fetal maceration, mummification or abortion and infection after 120 days produces stillbirth or birth of weak kids or lamb (Dubey et al., 1987; Menzies and Miller, 2007).

The prevalence of toxoplasmosis was significantly higher in poor body conditioned animals compared to healthy animals. It might be lower immunity of poor BCS animals than healthy one.

In case of histopathological examination the most common lesion observed in abortion by *T. gondii* is necrotizing placentitis exclusively in the cotyledonary areas and non suppurative enchepalitis (Caldeira et al., 2011). In this research there found bradyzoite cyst in brain tissue and tachyzoite cyst in heart tissue. In case of brain tissue there found chronic progressive encephalitis which is also described by Uggla et al. (1987) and Bari et al. (1993).

Limitations of the study

1. The study was conducted only in 52 smallholder goat farms. In aborted case, only 20fetus were collected. The sample size was small which might have introduced lower statistical power.

2. Due to financial limitation, larger sample size and random sampling was not possible. Sequencing of positive PCR samples were not possible.

3. There was no reliable population data base at livestock office which might help to conduct the study in more scientific and systemic ways.

Chapter VI: Conclusions, Recommendations and Future Directions

6.1. Conclusions

The overall seroprevalence of toxoplasmosis in CCCA was 28.3%. Age, BCS, grazing system, source of water, presence of cat in farm, feeding trough, drinking trough and terrain were significantly associated with toxoplasmosis in goat. The other management factors were not found as influencing component for toxoplasmosis. Brain and heart tissue were found as predilection site for cyst formation.

6.2. Recommendations

1. The management practice of farm is significantly related with higher prevalence of *Toxoplasma gondii* infection in goats. Try to keep away farm premises from cat along with proper disposal of placenta, use of feeding trough, drinking trough, feed oocyst free grass are recommended for as management of goat housing.
2. In the current study most of the goat farmers have the secondary level of education. They can easily be trained by providing simple leaflet about good management practice for goat farming.

6.3. Future Directions

The genotype of the parasite as well as public health significance should be studied.

Chapter VIII: References

Andrews, A.H., Blowey, R.W., Boyel, H., Eddy, R.G., 1992. Bovine Medicine: Diseases and Husbandry of Cattle. 1st ed. Blackwell Scientific Publication, London 9, 146–147.

Abu-Dalbouh, M. A., Ababneh, M. M., Giadinis, N. D., Lafi, S. Q., 2012. Ovine and caprine toxoplasmosis (*Toxoplasma gondii*) in aborted animals in Jordanian goat and sheep flocks. Tropical Animal Health and Production 44, 49-54.

ACIAR, 2007 (Australian Centre for International Agricultural Research).

AFSSA, 2005. Toxoplasmose: état des connaissances et évaluation du risqué lié à l'alimentation. In: Report of the Afssa working group ''*Toxoplasma gondii*".

Ahmad, N., Iqbal, Z., Mukhtar, M., Mushtaq, M., Mehmood, K., Qayyum, M., 2015. Seroprevalence and Associated Risk Factors of Toxoplasmosis in Sheep and Goats in Pothwar Region, Northern Punjab, Pakistan. Pakistan Journal of Zoology 47, 161-167.

Ahmed, Y. F., Sokkar, S. M., Desouky, H. M., Soror, A. H., 2008. Abortion due to toxoplasmosis in small ruminants. Global Veterinaria 2, 337-342.

Akhoundi, S., Youssefi. M. R., 2017. Seroprevalence of sheep toxoplasmosis in north of iran. Trakia Journal of Sciences 1, 79-82.

Aleme, H, Tilahun, G., Fekade, D., Berhe, N., Medhin, G., 2013. Sereoprevalence of Immunoglobulin-G and of Immunoglobulin-M Anti-*Toxoplasm gondii* Antibodies in Human Immunodeficiency Virus Infection/Acquired Immunodeficiency Syndrome Patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Journal of Infectious Diseases and Therapy 1, 119.

Alexander, J., Stimson, W., 1988. Sex hormones and the course of parasitic infection.Trends in Parasitology 4, 189-193.

Anderlini, G.A., Mota, R.A., Faria, E.B., Cavalcanti, E.F., Valenca, R.M.B., Junior, J.W.P., Albuquerque, P.P.F. and Neto, O.L.S., 2011. Occurrence and risk factors associated with infection by *Toxoplasma gondii* in goats in the state of Alagoas, Brazil. Revista da Sociedade Brasileira de Medicina Tropical 44: 157- 162.

Bancroft, J.D., A. Stevens and D.R. Turner, 1996. Theory and Practice of Histological Techniques. 4th ed., Churchill Livingstone, New York, Edinburgh, London, Melbourne, San Francisco, Tokyo.

Balea, A., Paştiu, A. I., Györke, A., Mircean, V., Cozma, V., 2012. The dynamics of anti-*Toxoplasma gondii* antibodies (IgG) in small ruminants and pigs from Cluj Country, Romania. Scientia Parasitologica 13, 163-168.

Burg, J. L., Grover, C. M., Pouletty, P., Boothroyd, J. C., 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. Journal of Clinical Microbiology 27, 1787-1792.

Buxton, D., 1998. Protozoan infection (*Toxoplasma gondii, Neospora caninum* and *Sarcocystis* spp.) in sheep and goats: recent advances (review article). Veterinary Research 29, 289–310.

Buxton, D., Innes, E.A., 1995. A commercial vaccine for ovine toxoplasmosis. Parasitology 110, 11–16.

Carme, B., Demar, M., Ajzenberg, D., Dardé, M.L., 2009. Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. Emerging Infectious Diseases 15, 656–658.

Cavalcante, A. C. R., Carneiro, M., Gouveia, A. M. G., Pinheiro, R. R., Vitor, R. W. A., 2008. Risk factors for infection by *Toxoplasma gondii* in herds of goats in Ceará, Brazil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 60, 36-41.

Cenci-Goga, B. T., Ciampelli, A., Sechi, P., Veronesi, F., Moretta, I., Cambiotti, V., Thompson, P. N., 2013. Seroprevalence and risk factors for *Toxoplasma gondii* in sheep in Grosseto district, Tuscany, Italy. BMC Veterinary Research 9, 25.

Chaudhary, Z.I., Ahmed, R.S., Hussain, S.M.I. and Shakoori, A.R., 2006. Detection of *Toxoplasma gonidi* infection in butchers and buffaloes by polymerase chain reaction and latex agglutination test. Pakistan Journal of Zoology 38: 333-336.

Chhabra, M. B., Gupta, S. L. and Gautam, O. P, 1985. Toxoplasma seroprevalence in animals in northern India. International Journal of Zoonoses 12, 136-142.

Chikweto, A., Kumthekar, S., Tiwari, K., Nyack, B., Deokar, M. S., Stratton, G., Macpherson, C. N., Sharma, R. N., Dubey, J. P., 2011. Seroprevalence of

Toxoplasma gondii in pigs, sheep, goats, and cattle from Grenada and Carriacou, West Indies. Journal of Parasitology 97, 950-951.

Dardé, M.L., Ajzenberg, D., Smith, J., 2014. Population structure and epidemiology of *Toxoplasma gondii In: Toxoplasma gondii:* The Model Apicomplexan: Perspectives and Methods, Edited by Louis M. Weiss and Kami Kim, second edition,, Lonson, Elsevier Ltd., 61-97.

Das, S.S., 1992. *Toxoplasma* in man, animals and in the environment. International Journal of Environmental Health and Research 2, 1-17.

de Moraes, É. P. B. X., da Costa, M. M., Dantas, A. F. M., da Silva, J. C. R., Mota, R. A., 2011. *Toxoplasma gondii* diagnosis in ovine aborted fetuses and stillborns in the State of Pernambuco, Brazil. Veterinary Parasitology 183, 152-155.

Djokić, V., Klun, I., Musella, V., Rinaldi, L., Cringoli, G., Sotiraki, S., Djurković-Djaković, O., 2014. Spatial epidemiology of *Toxoplasma gondii* infection in goats in Serbia. Geospatial Health 8, 479-488.

DLS (District Livestock Service), 2017. Personal communication.

Dubey, J.P., 2004. Toxoplasmosis- a waterborne zoonosis. Veterinary Parasitology 126, 57-72.

Dubey, J.P., 2008. The History of *Toxoplasma gondii*—The First 100 Years. Journal of Eukaryotic Microbiology 55, 467–475.

Dubey, J.P., 2009. Toxoplasmosis in sheep-The last 20 years. Veterinary Parasitology 163, 1-14.

Dubey, J.P., 2010. Toxoplasmosis of animals and humans. 2nd edition. CRC Press; Boca Raton, Florida, U.S.A, 1-313.

Dubey, J.P., Kirkbride, C.A., 1989. Economic and public health considerations of congenital toxoplasmosis in lambs.

Dubey, J.P., Lappin, M.R.,1998. Toxoplasmosis and Neosporosis. In: Infectious Diseases of the Dog and Cat. Greene, CE (ed.), Philadelphia, WB Saunders, 493–509.

Dubey, J.P., Lindsay, D.S., 2004. Biology of *Toxoplasma gondii* in cats and other animals. In Opportunistic Infections: *Toxoplasma*, *Sarcocystis*, and *Microsporidia*. Vol.9. Lindsay, D.S., Weiss, L.M. (Eds), Kluwer Academic Publishers, New York, 1-20.

Dubey, J.P., Welcome, F.L., 1988. *Toxoplasma gondii* induced abortions in sheep. Journal of the American Veterinary Medical Association 193, 698 – 700.

Frenkel, J.K., 1974. Breaking the Transmission Chain of *Toxoplasma*: A Program for the Prevention of Human Toxoplasmosis. Bulletin of the New York Academy of Medicine 50, 228 – 235.

Freyre, A., Bonino, J., Falcon, J., Castells, D., Correa, O., Casaretto, A., 1997. The incidence and economic significance of ovine toxoplasmosis in Uruguay. Veterinary Parasitology 73, 13-15.

Gazzonis, A. L., Veronesi, F., Di Cerbo, A. R., Zanzani, S. A., Molineri, G., Moretta, I., Moretti, A., Fioretti, D. P., Invernizzi, A., Manfredi, M. T., 2015. *Toxoplasma gondii* in small ruminants in Northern Italy–prevalence and risk factors. Annals of Agricultural and Environmental Medicine 22, 62-68.

Gebremedhin, E. Z., Gizaw, D., 2014. Seroprevalence of *Toxoplasma gondii* infection in sheep and goats in three districts of Southern Nations, Nationalities and Peoples' Region of Ethiopia. World Applied Sciences Journal 31, 1891-1896.

Górecki, M. T., Andrzejewska I., Steppa, R., 2005. Prevalence of Toxoplasma gondii in sheep and goats. Medycyna Weterynaryjna 61, 98−99.

Grigg, M.E., Ganatra, J., Boothroyd, J.C., Margolis, T.P., 2001. Unusual Abundance of Atypical Strains Associated with Human Ocular Toxoplasmosis. Journal of Infectious Diseases184, 633–639.

Guebre-xabier, M., Nurilign, A., Gebre-Hiwot, A., Hailu, A., Sissay, Y., Getachew, E., Frommel, D., 1993. Seroepidemiological survey of *Toxoplasma gondii* infection in Ethiopia. Ethiopian Medical Journal 31, 201– 208.

Hall, S.M., 1992. Congenital toxoplasmosis. British Medical Journal 305, 291-297.

Hashemi-Fesharki R., 1996. Seroprevalence of *Toxoplasma gondii* in Cattle, Sheep and Goats in Iran. Veterinary Parasitology 61, 1-3.

Hide, G., Gerwash, O., Morley, E.K., Williams, R.H., Hughes, J.M., Thomasson, D., Elmahaishi, M.S., Elmahaishi, K.H., Terry, R.S., Smith, J.E., 2007. Does vertical transmission contribute to the prevalence of toxoplasmosis? Parassitologia 49, 223–226.

Homan, W.L., Vercammen, M., De Braekeleer, J., Verschueren, H., 2000. Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. International Journal for Parasitology 30, 69-75.

Hurtado, A., Aduriz, G., Moreno, B., Barandika, J., García-Pérez, A.L., 2001. Single tube nested PCR for the detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes. Veterinary Parasitology 102, 17–27.

Innes, E.A., 2010a. Vaccination against *Toxoplasma gondii*: an increasing priority for collaborative research? Expert Reviews in Vaccines 9, 1117–1119.

Jones, J., Lopez, D., Wilson, M., 2003. Congenital Toxoplasmosis. American Family Physician 67, 2131-2138.

Jones, J.L., Dubey, J.P., 2010. Waterborne toxoplasmosis – Recent developments. Experimental Parasitology 124, 10-25.

Kamani, J., Mani, A.U., Godwin, O., Egwu, G.O., 2010a. Seroprevalence of *Toxoplasma gondii* infection in domestic sheep and goats in Borno state, Nigeria. Tropical Animal Health and Production 42, 793-797.

Kijlstra, A., Jongert, E., 2008a. Toxoplasma-safe meat: close to reality? Trends in Parasitology 25, 18-22.

Kijlstra, A., Meerburg, B., Cornelissen, J., De Craeye, S., Vereijken, P., Jongert, E., 2008b. The role of rodents and shrews in the transmission of *Toxoplasma gondii* to pigs. Veterinary Parasitology 156, 183-190.

Leighty, J.C., 1990. Strategies for control of toxoplasmosis. Journal of the American Veterinary Medical Association 196, 281-286.

Lindsay, D.S., Dubey, J.P., 2007. Toxoplasmosis in Wild and Domestic Animals In: Weiss, L.M., Kami, K., (Eds). *Toxoplasma gondii*. The Model Apicomplexan: Perspectives and Methods. Academic Press, London, UK, 133-152.

Lopes, W. D. Z., dos Santos, T. R., da Silva, R. d. S., Rossanese, W. M., de Souza, F. A., de Faria Rodrigues, J. D. A., de Mendonça, R. P., Soares, V. E., da Costa, A. J., 2010. Seroprevalence of and risk factors for *Toxoplasma gondii*

in sheep raised in the Jaboticabal microregion, São Paulo State, Brazil. Research in Veterinary Science 88, 104-106.

Marquardt, W.C, Demaree, R.S., Grieve, R.B., 2000. Chapter 13. The Extraintestinal Coccidia. In: Parasitology and Vector Biology. Second Edition, Academic Press, USA, 165-178.

Masala, G., Porcu, R., Madau, L., Tanda, A., Ibba, B., Satta, G., Tola, S., 2003. Survey of ovine and caprine toxoplasmosis by IFAT and PCR assays in Sardinia, Italy. Veterinary Parasitology 117, 15–21.

Masresha, A., 2012. Seroeprevalence of IgG and IgM Anti-*Toxoplasma gondii* antibodies among pregnant women attending anti-natal clinic at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. MSc thesis, Aklilu Lema Institue of Pathobiology, School of Graduate Studies, Addis Ababa University.

Mengesha, B., Tadese, T., Melamed, M.D., 1984. Seroepidemioloeical survey of *Toxoplasma gondii* infection in Addis Ababa, Ethiopia. Ethiopian Medical Journal 22, 214.

Mercier, A., Devillard, S., Ngoubangoye, B., Bonnabau, H., Banuls, A-L., Durands, P., Salle, B., Ajzenberg, D., Dardé, M.L., 2010. Additional Haplogroups of *Toxoplasma gondii* out of Africa: Population Structure and Mouse- Virulence of Strains from Gabon. PLoS Neglected Tropical Diseases 4, 11.

Messingham, K. A., Heinrich, S. A., Kovacs, E. J., 2001. Estrogen restores cellular immunity in injured male mice via suppression of interleukin-6 production. Journal of Leukocyte Biology 70, 887-895.

Miller, C.M., Boulter, N.R., Ikin, R.J., Smith, N.C., 2009. The immunobiology of the innate response to *Toxoplasma gondii*. International Journal for Parasitology 39, 23–39.

Moazeni Jula, F., Moazeni Jula, G., Nowzari, N., Kavari, A., Hashemzadeh, H., 2013. A Serological and Molecular study on *Toxoplasma gondii* infection in sheep and goat in Tabriz. Archives of Razi Inistitute 68, 29-35.

Mosa, M. B., El-Shahawy, I. S., Fawzi, E. M., 2015. Comparative Analysis of Toxoplasmosis in Farm Animals by Indirect Hemagglutination Assay and Enzyme linked Immunosorbent Assay. Alexandria Journal of Veterinary Sciences 46, 90-94.

Mosa, M. B., El-Shahawy, I. S., Fawzi, E. M., 2015. Comparative Analysis of Toxoplasmosis in Farm Animals by Indirect Hemagglutination Assay and Enzyme linked Immunosorbent Assay. Alexandria Journal of Veterinary Sciences 46, 90-94.

OIE, 2008. Toxoplasmosis. In: OIE Manual of Diagnostic tests and Vaccines for Terrestrial Animals 2, 1286.

Oncel, T., Vural, G., 2007. Occurrence of *Toxoplasma gondii* antibodies in sheep in Istanbul, Turkey. Veterinarski Arhiv 76, 547.

Ortega, Y.R., 2006. Toxoplasmosis. In Foodborne Parasites. Edited by Ortega YR, USA: Springer Science+Business Media, LLC, 109–125.

Pereira-Chioccola, V.L., Vidal, J.E., Su, C., 2009. *Toxoplasma gondii* infection and cerebral toxoplasmosis in HIV-infected patients. Future Microbiology 4, 1363–1379.

Plaut, M., Zimmerman, E.M., Goldstein, R.A., 1996. Health Hazards to Humans Associated with Domestic cats. Annual Review of Public Health 17, 221–245.

Puije, W.N.A., Bosompem, K.M., Canacoo, E.A., Wastling, J.M. and Akanmori, B.D., 2000. The prevalence of anti-Toxoplasma gondii antibodies in Ghanian sheep and goats. Acta Tropica, 76, 21-26.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., 2006. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th edn. Saunders, London, 1518-1522.

Rahman, M., Azad, M. T., Nahar, L., Rouf, S. M., Ohya, K., Chiou, S. P., Baba, M., Kitoh, K., Takashima, Y., 2014a. Age-Specificity of *Toxoplasma gondii* Seroprevalence in Sheep, Goats and Cattle on Subsistence Farms in Bangladesh. The Journal of Veterinary Medical Science 76, 1257-1259.

Ramzan, M., Akhtar, M., Muhammad, F., Hussain, I., Hiszczyńska-Sawicka, E., Haq, A.U., Mahmood, M.S., Hafeez, M.A., 2009. Seroprevalence of *Toxoplasma gondii* in sheep and goats in Rahim Yar Khan (Punjab), Pakistan. Tropical Animal Health and Producton 41, 1225–1229.

Rossi, G. F., Cabral, D. D., Ribeiro, D. P., Pajuaba, A. C. A. M., Corrêa, R. R., Moreira, R. Q., Mineo, T. W. P., Mineo, J. R., Silva, D. A.O., 2011. Evaluation of *Toxoplasma gondii* and *Neospora caninum* infections in sheep

from Uberlândia, Minas Gerais State, Brazil, by different serological methods. Veterinary 48, 987-992.

Samad, M. A., Rahman, K. B., Halder, A. K., 1993. Seroprevalence of *Toxoplasma gondii* in domestic ruminants in Bangladesh. Veterinary Parasitology 47, 157-159.

Sawadogo, P., Hafid, J., Bellete, B., Sung, R., T., Chakdi, M., Flori, P., Raberin, H., Hamouni, I., B., Chait A., Dalal, A., 2005. Seroprevalence of Toxoplasma gondii in sheep from Marrakesh, Morocco. Veterinary Parasitology 130, 89-92.

Sevgili, M., C. Babur, S. Nalbantoğlu, G. Karas & Z. Vatansever, 2005. Detection of seropositivity for Toxoplasma gondii in sheep in Şanlıurfa province. Turkish Journal of Veterinary and Animal Sciences 29, 107-111.

Shahiduzzaman, M., Islam, R., Khatun, M. M., Batanova, T. A., Kitoh, K., Takashima, Y., 2011. *Toxoplasma gondii* seroprevalence in domestic animals and humans in Mymensingh District, Bangladesh. Journal of Veterinary Medical Science 73, 1375-1376.

Sibley, L.D., Khan, A., Ajioka, J.W., Rosenthal, B.M., 2009. Genetic diversity of *Toxoplasma gondii* in animals and humans. Philosophical Transactions of Royal. Society B. Biological sciences 364, 2749–2761.

Shively, M.J., 1987. Veterinary Anatomy: Basic Comparative and Clinical. A and M Press, Texas, USA, 390-391.

Splendore, A., 1908. Un nuovo protozoa parassita de' conigli incontrato nelle lesioni anatomiche d'une malattia che ricorda in molti punti il Kala-azar dell'uomo. Nota preliminare pel. Revistas da Sociedade et Scientifique de Sao Paulo 3, 109–112.

Su, C., Shwab, E.K., Zhou, P., Zhu, X.Q., Dubey, J.P., 2010. Moving towards an integrated approach to molecular detection and identification of *Toxoplasma gondii*. Parasitology 137, 1–11.

Tembue, A. A. M., Alves, L. C., da Costa, A. J., Aparecidafaustino, M. d. G., de Sousa, T. R., Albuquerque, A. R., Ramos, R. d. N., María, I., Meunier, J. N., 2009. Prevalence of IgG antibodies anti-*Toxoplasma gondii* in goat and sheep from dry region of Pernambuco State, Brazil. Revista Ibero-Latinoamericana de Parasitología 68, 82-85.

Tenter, A.M., 2009. *Toxoplasma gondii* in animals used for human consumption. Memorias Do Instituto Oswaldo Cruz 104, 364-369.

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. International Journal for Parasitology 30, 1217-1258.

Teshale, S., Dumetre, A., Dardé, M. L., Merga, B., Dorchies, P., 2007. Serological survey of caprine toxoplasmosis in Ethiopia: prevalence and risk factors. Parasite 14, 155-159.

Tzanidakis, N., Maksimov, P., Conraths, F. J., Kiossis, E., Brozos, C., Sotiraki, S., Schares, G., 2012. *Toxoplasma gondii* in sheep and goats: Seroprevalence and potential risk factors under dairy husbandry practices. Veterinary Parasitology 190, 340-348.

Van der Puije, W.N.A., Bosompem, K.M., Canacoo, E.A., Wastling, J.M., Akanmori, B.D., 2000. The prevalence of anti-*Toxoplasma gondii* antibodies in Ghanaian sheep and goats. Acta Tropica 76, 21-26.

Velmurugan, G.V., Su, C., Dubey, J.P., 2009. Isolate designation and characterization of *Toxoplasma gondii* isolates from pigs in the United States. Journal of Parasitology 95, 95–99.

Waldeland, H., 1976. Toxoplasmosis in sheep. *Toxoplasma gondii* in muscular tissue, with particular reference to dye test titers and haemoglobin type. Acta Veterinaria Scandinavica 17, 403-411.

Walle, F., Kebede, N., Tsegaye, A., Kassa, T., 2013. Seroprevalence and risk factors for Toxoplasmosis in HIV infected and non-infected individuals in Bahir Dar, Northwest Ethiopia. Parasite and Vectors 6, 15.

Wang, C.R., Qiua, J.H., Gaoa, J.F., Liua, L.M., Wanga, C., Liuc, Q., Yand, C. and Zhua, X.Q., 2011. Seroprevalence of *Toxoplasma gondii* infection in sheep and goats in northeastern China. Small Ruminant Research 97, 130-133.

Williams, E.J., O'Donovan, J., 2009. Ovine abortion: an overview. Irish Veterinary Journal 62, 342–346.

Yai, L.E.O., Vianna, M.C.B., Soares, R.M., Cortez, A., Freire, R.L., Richtzenhain, L.J., Gennari, S.M., 2003. Evaluation of experimental *Toxoplasma gondii* (Nicolle and Manceaux, 1909) infection in pigs by bioassay in mice and polymerase chain reaction. Brazilian Journal of Veterinary Research and Animal Science 40, 227–234.

Yibeltal, M.M., 2008. Seroprevalence study of toxoplasmosis in small ruminants and humans (HIV/AIDS patient) in selected district of South Wollo, Ethiopia. MSc Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Debre-Zeit, Ethiopia.

Yimer, E., Abebe, P., Kassahun, J., Woldemical, T., Bekele, A., Zewudie, B., Beyene, M., 2005. Seroprevalence of human toxoplasmosis in Addis Ababa, Ethiopia. Ethiopian Veterinary Journal 9, 109-122.

Annex-I

A Questionnaire for Dedicating Toxoplasmosis

Farm level information

Farm ID no (FID):

Date:…/……../……

Goat ID no (GID): Sample ID no:

Name of Owner:

Occupation:

Monthly income from this source:

Who mainly rear and take care of this farm:

Education:

Family size:

Vill/ward:

Upazilla:

Dist:

Contact no:

Source of Animals: Farm brone/bought

Types of breed reared: Local/BBG/Cross

Farm size: No. of adult does: No. of adult bucks:

No. of goat kids: No of pregnant animal: No of lactating animal:

Housing system: Intensive/Semi-intensive/Extensive

Type of drinking trough:Cement /Tire/Absent

Type of feeding trough: Cement/mixed /wood/Absent

Type of pen: Raised/Ground level/Mixed

Pen flooring Cement/Stone/Ground floor Mixed

Type of terrain: Flat/Hilly, Hilly/periodically flooded

Presence of cat: Yes/No

Access of cats to water supplies: Yes/No

Access of cats to feeding facilities: Yes/No

Placenta disposal facilities: Yes/No

Grazing system: cut and carry/ Free grazing

Any history of abortion (Farm): Yes/No

If yes how much kid has aborted:

Type of abortion: Early (≤2m)/ late (≥2m)

Source of water: Stagnant water (pond), running water (river/lake)

Hygenic condition: High/Moderate/Poor

Access of vet to farm: Yes/No

Did they get vet service to their house: Yes/No

Occurance of any other disease for last 6 month: Yes/No

Individual animal information

Age: <12m, 13-24m, 25-36m, >36m

Sex: Male/Female

Body weight: BCS: 1/2/3/4/5

Breed: Color:

Dewormed: Yes/No Vaccination done: Yes/No

Type of breeding: Natural/AI

Date of parturition: No. of parity

Days in milk:

Rectal Temp:

Milking of doe:

Breed type: Meat/Milk/Dual

Reproductive status: Pregnant/Non-pregnant/ Lactating

Any history of abortion (Individual animal): Yes/No

If yes how much:

Previously any medication given: Yes/No

If yes which drug haven given

Annex-II

Preparation of histopathological slides

Tissue collection, identification and preservation (in smaller size; variable) in Bouin's Solution in a labeled plastic container.

↓

Made the tissue sample smaller after 3 days of collection (least 5 mm. thickness)

↓

Again fixation in Bouin's (10 folds of the tissue size and weight) and fixed for 3-5 days.

↓

Numbering and made garlanding (tissue string) of the tissue

↓

Overnight washing in running tape water to remove formalin.

↓

Dehydration in ascending ethanol series to prevent shrinkage of cells

50% Alcohol (2 hours)

70% Alcohol (2 hours)

80% Alcohol (1 hour)

95% Alcohol (1 hour)

100% Alcohol (3 changes one hour in each)

↓

Impregnation in melted paraffin (56°-60°c) for 3 changes (2 hours each)

↓

Embedding in paraffin to make block (Rest the cooked tissue overnight)

↓

Sectioning at 5-µm thickness until suitable ribboning.

↓

A small amount of gelatin was added to the water bath for better adhesion of the section to the slide.

↓

Water Bath: Section allowed spreading on warm water bath (55°c))

↓

Placing the section on grease free clear slides

↓

Air dry of the section and keeping in a rack.

↓

Routine Hematoxylin and Eosin staining procedure

↓

Deparafinization in 3 changes of Xylene (2 min. in each).

↓

Rehydration

In descending grades of alcohol

Absolute alcohol in 2 C (2 min. each)

95% alcohol in 1 C (2 min.)

80% alcohol in 1 C (2 min.)

70% alcohol in 1 Containers (2 min.)

↓

Running in distilled water (5 min.)

↓

Stain with Harris hematoxylin (10 min.)

↓

Wash in running tap water (10-15 min.)

↓

Dip in acid alcohol by 2 -4 dips

↓

Wash in tap water for (5 min.)

↓

Dip in ammonia water until sections become bright blue (2-4 dips)

↓

Wash in tap water for (10 min.)

↓

Stain with eosin for (1-2 min.)

↓

Dehydration in alcohol

70% alcohol in 1 Containers (30 second)

80% alcohol in 1 Containers (45 second)

95% alcohol 2 changes (2 min. each)

Absolute alcohol (2 min.)

↓

Cleaning

Absolute Xylene + Absolute Alcohol (2 min)

Xylene 1 (2 min.)

Xylene 2 (until mounting)

↓

Mounting of the stained slide with coverslip and DPX.