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Sero Prevalence and Assessment of Risk Factors for *Toxoplasma gondii* In Small Ruminants in and Around Sebeta Town, Ethiopia

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Abstract: Sheep and goats play an important role in the epidemiology of toxoplasmosis by serving as intermediate hosts. The present cross-sectional study was undertaken in 388 small ruminants to estimate the sero prevalence of *Toxoplasma gondii* infection from December 2020 to July 2021 using Indirect Enzyme Linked Immunosorbent Assay (ELISA) as a diagnostic tool. Of 388 examined animals 197 (50.8%, 95% CI: 45.8–55.7) were seropositive for *T. gondii* antibody detection. Based on animal species 55.6% (155/279; 95% CI: 49.6-61.3) and 38.5% (42/109; 95% CI: 29.8–48.1%) sero prevalence was found in sheep and goats respectively, with statistical significance of (p=0.003). Geographical location also had a significant variation (p=0.001). *T. gondi* antibody Sero positivity was higher in female animals (53.89%) than in male (40.86%), which was also statistically significant (p=0.028). The association of Toxoplasma seropositivity with biological features of animals has shown that age, gender and species can influence small ruminant vulnerability to *T. gondii* infection. High values of seroprevalence both in sheep and in goats have been obtained in animals aged more than three years, in female animals and sheep than goats. The odds of *T. gondii* infection in small ruminants with double and triple abortion (AOR=1) were greatly exposed than with single abortion (AOR=1.88, 95%CI: 0.88-3.99) and those doesn't have abortion history with significant variation of (p=0.034) for the parasite. Therefore, the present study warranted to unravel the impact of the protozoa in food animals as well as the risk of transmission to humans.

Key words: Sero Prevalence · Toxoplasmosis · ELISA · Risk Factor

INTRODUCTION

Toxoplasmosis is zoonotic parasitic disease caused by *T. gondii* and most prevalent parasitic infections in human and veterinary medicine and has negative impacts on public health and animal production. *Toxoplasma gondii* is considered as the most successful parasitic pathogen worldwide. Cats (domestic and wild), the definitive hosts of *T. gondii*, are epidemiologically important animals because they shed environmentally resistant oocysts in the feces [1]. Warm-blooded vertebrates including humans, rodents, birds, livestock and marine mammals are intermediate hosts [2]. Transmission of *T. gondii* occurs by ingesting food or water contaminated with oocysts shed by cats or by eating undercooked or raw meat containing tissue cysts [3]. Although *T. gondii* infection in most people appears to be a symptomatic, it may result in life-threatening illness in some immune-compromised individuals [4].

Toxoplasmosis was an important cause of abortion, stillbirth and neonatal mortality in sheep and goats [5]. Usually it causes embryonic death and resorption, fetal death and mummification in animals. In goats and sheep abortion, stillbirth and neonatal death are common sequels of the infection [6]. It causes heavy losses through abortion, stillbirth, neonatal mortality, encephalitis and pneumonia particularly in sheep and goats [5]. The annual economic impact of toxoplasmosis in the United States is estimated to be $7.7 billion [7]. In Uruguay, 1.4 to 4.7 million US dollar lose per year has been reported due to sheep toxoplasmosis [8]. Masala *et al.* [9] in Italy accounted 10 million Euros

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economic loss per year due to lamb mortality and missed lactation caused by toxoplasmosis. The cost of toxoplasmosis to the UK sheep industry based on lost output (abortions/stillbirths), input costs and the cost of control measures was £11 million [10].

In Ethiopia, it is reported that abortions, stillbirth, prenatal lamb or kid mortality are the most common reproductive problems of small ruminants. Sheep and goats play an important role in the epidemiology of toxoplasmosis they have big potential to spread the tissue cysts of *T. gondii* to humans through consumption of raw or undercooked meat and /or offal [11]. In Ethiopia, the epidemiological status of *T. gondii* in sheep and in goats showed sero prevalence ranging from 22.9% to 56% and 11.6% to 82% [12- 17] respectively. Thus, information on the prevalence of toxoplasmosis in small ruminants is useful for assessment of the risk of meat of these animals to the public. Sero prevalence of *T. gondii* infection in small ruminants in Sebeta Hawas district of Oromia region was still not studied. This study was therefore, initiated with the objective of estimating the sero prevalence and associated risk factors in three geographical location of the district.

MATERIALS AND METHODS

Study Area: The study was conducted in three purposively selected Kebele of Sebeta Hawas district. Sebeta town was located in special zone of Oromia regional state in central highlands of Ethiopia at 24 km west of Addis Ababa on the main road to Jimma and lies at latitude and longitude of 8° 55-8.917°N and 38°37-38.617°E, respectively. The area receives an average annual rainfall of around 1100 mm, more than 85% of which falls in the main rainy season (June - September). The altitude of the area ranges from 2200–2600 meter above sea level and the average annual temperature ranges from 6–21°C. Crop production and livestock rearing are the main economic activities in areas [18].

Study Design, Animal Population and Sample Size: A cross-sectional sero epidemiological study was conducted from December, 2021 to July 2021 to determine the sero prevalence of *T. gondii* and risk factors for the infection in small ruminants (sheep's and goats). Animals included in this study were local breeds allowed to graze in grazing fields during day time. The inclusion criteria were domestic sheep and goat of any sex less than or equal to one year of age were considered as young while those above one year were categorized as adult. The sample size was determined at an expected prevalence of 50%, as there were no previous data concerning the occurrence of this infection in the study area, with 95% confidence interval and 5% absolute precision. Subsequently, the measured sample size using the formula described by Trusfield [19] for random sampling. Accordingly, the following formula was applied to determine the simple size:

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

where: n = required sample size
P_{exp} = expected prevalence
d = desired absolute precision (0.05)

Sampling Technique: Simple random sampling technique was carried out to collect sera from small ruminants. Indeed, animals less than 6 months were not included in the sampling criteria to prevent bias occurrence from maternal derived antibody. While sampling the essential individual animal data and presumed risk factors were recorded on the sample collection format.

Samples Collection: A total of 388 (sheep = 279; goats = 109) animals were bleed to collect the appropriate sera samples from the jugular vein of each animals. Blood was drawn using a 10 ml volume of non heparinized vacutainer tubes attached with 21G needle. Accordingly, about 5 ml bloods was collected from each animal and kept at room temprature to allow clotting for sera separation. As soon as a separate layer formed the serum part was decanted into a 1.8ml volume of cryovials having individual's labels and stored in ice packs and transported into National Animal Health Diagnostic and Investigation Center (NAHDIC) where it was stored at -20°C until laboratory work was conducted.

Diagnostic Procedures: Then after, all sera were tested for the presence of IgG antibodies against *T. gondii* using Indirect ELISA diagnostic kit (IDEXX Innovative Diagnostic, Switzerland) following the manufacturers recommendation. Briefly, Pre-dilute each samples and controls 1:400 in a tube using wash solution. Then, all reagents must be allowed to come to 18-26°C before use. Mix reagents by gentle inverting or swirling. Obtain antigen coated plates from foil bag which should be sufficient for the number of samples to be tested. Dispense 100µl of diluted positive control in to A1 and B1 wells. Dispense 100µl of diluted negative controls into C1 and D1 wells. Dispense 100µl of samples into the

remaining wells. Mix the contents of the micro-wells by gently tapping the plate or use a microplate shaker. Cover the plate tightly with sealant to avoid any evaporation and incubate for 1 hr (+5min) at +37°C (+3°C). Remove the solution and wash each well with approximately 300µl of wash solution 3 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tap each plate onto absorbent towel after the final wash to remove any residual wash fluid. Dispense 100µl the conjugate into each well. Cover the plate and incubate 1hr (+5min) at +37°C (+3°C). The plates should be tightly sealed. Then, Remove the solution and wash each well with approximately 300µl of wash solution 3 times. Avoid plate drying between plate washings and prior to the addition of the next reagent. Tap each plate onto absorbent towel after the final wash to remove any residual wash fluid. Dispense 100µl of TMB substrate N.12 into each well. Incubate at 18-26°C for 15minutes (+1 min.) in the dark. Dispense 100µl of stop solution N.3 into each well to cease the reaction. Lastly, read the reaction at a wavelength of 450nm.

Validity criteria:

- Mean of NC ≤ 0.500
- Mean of PC ≤ 2.00
- Mean of PC-Mean of NC ≥ 0.300.

Interpretation:

S/P%=100 (Sample A (450)-Mean NC/Mean PC-Mean NC)

- S/P%<20 = negative
- 20 ≤ S/P%<30 = Suspect
- 30< S/P%<100 = Weak positive
- S/P% ≥100 = positive

Data Management and Analysis: Data were recorded and coded using Microsoft Excel spreadsheet 2007 and analysed using STATA version 13.0 for MA Windows (STATACorp. LP, Texas USA). Sero-prevalence was calculated by dividing the number of animals possessing anti-*T .gondii* antibodies against the total number of animals tested. Relationship of risk factors with dependent variable was primarily assessed using cross tabulation. Univariable logistic regression analysis was performed to observe the strength of association between presumed risk factors with the response variable (*T. gondii* infection). For variables that showed co-linearity if any and variables with a p-value less than 0.25 upon a univariable logistic regression model, a further multivariable logistic regression analysis was built. P-value less than 0.05 were considered statistically significant for all statistical tools at a 95% CI.

RESULTS

Overall Sero-Prevalence: Out of 388 examined animals 197 (50.8%, 95% CI: 45.8–55.7) were sero positive for *T. gondii* antibody detection. Based on animal species 55.6% (155/279; 95% CI: 49.6-61.3) and 38.5% (42/109; 95% CI: 29.8–48.1%) sero prevalence was found in sheep and goats respectively with statistically significance of (p=0.003). The highest sero prevalence (70.46%) was recorded in Kebele 05, followed by Kebele 10 (43.75 %), while the lowest sero prevalence was found in Kebele 07 (34.96%) with significant variation (p=0.001). *T. gondii* antibody seropositivity was higher in female animals (53.89%) than in male (40.86%), which was also statistically significant (p=0.028). The prevalence of *T. gondii* between age categories of adult (53.09 %) and young (34.69%) animals were also statistically significant (p=0.016) as illustrated in Table 1.

Risk Factors: The odds of getting *T. gondii* infection was significantly higher in sheep (AOR = 1.34, 95% CI: 0.82-2.20) than in goats although statistically not significant (P = 0.233) but it was significant (P = 0.0.003) in univariable logistic regression. The likelihood of *T.gondi* infection, in Kebele 05 (AOR= 3.39, 95% CI: 1.92-5.99; P < 0.001) was higher than Kebele 07 (AOR=0.74, 95%CI: 0.43-1.28; P>0.05) and kebele 10. According to age factor, in adult animal (AOR = 2.22, 95% CI: 1.12-4.38) the infection rate of the parasite were higher than the youngest group with a statistical significance of (P = 0.021). The univariable logistic regression model revealed that the risk of getting the infection in female animals (COR = 1.69, 95% CI: 0.05-2.71) were higher than males. The strength of association for predictor variable over an outcome variable showed statistically significant (P =0.029) but insignificant for multivariable logistic regression analysis (p=0.103). The odds of *T. gondii* infection in small ruminants with double and triple abortion (AOR=1) were greatly affected than those affected with single abortion (AOR=1.88, 95%CI: 0.88-3.99) and those doesn't have abortion history which have significant variation based on the univariable logistic regression (0.034) although it is non significant on multivariable regression (0.100). Similarly, the univariable logistic regression model indicated that the probability of

Table 1: Relationship of presumed risk factors with the outcome variable using pearson chi square analysis

Variables	No of sample	No of positive	Percentage	χ^2	P-value
Kebele				39.3198	<0.001
10	96	42	43.75		
07	143	50	34.96		
05	149	105	70.46		
Sex				4.8091	0.028
Female	295	159	53.89		
male	93	38	40.86		
Age				5.8013	0.016
Young (< 1 year)	49	17	34.69		
Adult (>1year)	339	180	53.09		
Species				9.0878	0.003
Goat	109	42	38.53		
Sheep	279	155	55.55		
Abortion				8.5933	0.014
None	345	167	48.40		
Only one abortion	39	26	66.66		
Two and above	4	4	100		
Still birth				4.9111	0.086
None	375	187	49.86		
Only one Still birth	9	6	66.66		
Two and above	4	4	100		
Neonatal Mortality				3.9190	0.014
None	370	186	50.27		
One N. Mortality	14	7	50		
Two and above N. mortality	4	4	100		

Table 2: Association of presumed explanatory variables with response variable using logistic regression analysis

		Univariable		Multivariable	
Variable	Total positive (%)	COD(95% CI)	P-Value	AOD(95%CI)	P-value
Kebele					
10	43.75	*	-	*	-
07	34.96	0.69(0.41-1.17)	0.172	0.74(0.43-1.28)	0.285
05	70.46	3.07(1.8-5.24)	<0.001	3.39(1.92-5.99)	<0.001
Species					
Goat	38.53	*	-	*	-
Sheep	57.55	1.99(1.26-3.13)	0.003	1.34(0.82-2.20)	0.233
Sex					
Male	40.86	*	-	*	-
Female	53.89	1.69(0.05-2.71)	0.029	1.5(0.91-2.60)	0.103
Age					
Young (<1 year)	34.69	*	-	*	-
Adult (1 > year)	53.09	2.13(1.13-3.98)	0.018	2.22(1.12-4.38)	0.021
Abortion					
None	49.40	*	-	*	
Only one abortion	66.6	2.13 (1.06-4.28)	0.034	1.88(0.88-3.99)	0.100
Two and above	100	1		1	
Still birth					
None	49.86	*	-		
Only one Still birth	66.66	2.01(0.49-8.15)	0.328		
Two and above	100	1	-		
Neonatal Mortality					
None	50.27	*	-		
Only one N. Mortality	50	0.98(0.34-2.87)	0.984		
Two and above	100	1	-		

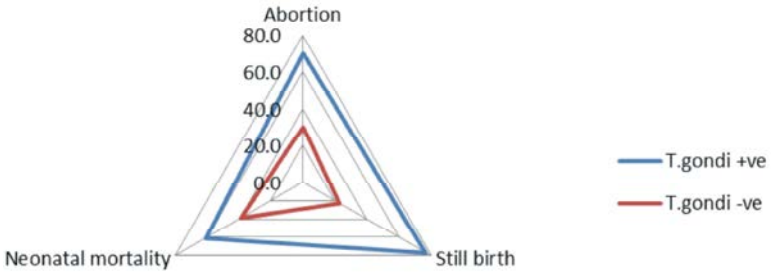

Fig. 1: *Toxoplasma gondii* effect proportion on reproductive disorders

acquiring *T. gondii* was insignificant in having one Still birth (P = 0.328) and two and above two still birth occurrences when compared to animals which are not exposed to still birth. In this study, neonatal mortality was not significantly associated with the probability of acquiring *T. gondii* infection (p>0.05) as showed in Table 2.

DISCUSSION

The present study stanch a widespread occurrence of *T. gondii* infection among small ruminants in the study area. The overall seroprevalence of *T. gondii* infection was 197 (50.8%, 95% CI: 45.8–55.7). This study was in agreement with the study of Dechassa *et al.* [20], (57.60%), in Jimma zone seka district, Southwestern Ethiopia. It might be due to the availability of the study areas on the same line with a relatively similar climatic condition. However, the current study was not in line with the findings of Gebremedhin and Daniel [21], (26.09%) in the three districts of Southern Nations, Nationalities and Peoples' region of Ethiopia. The difference might be attributed to geographical variation and the small number of samples processed by these authors.

The infection rate of the parasite in sheep and goats was 55.5% and 38.5% respectively. The prevalence in sheep was in agreement with previously reported studies in Jimma using Toxo Latex slide Agglutination test 58.8% [20] and in Nazareth 56% using ELISA [14]. But higher than the studies counducted by Gebremedhin and Daniel [21] which was 31.45% in the three districts of Southern Nations, Nationalities and Peoples' Region of Ethiopia. Various studies investigated and recorded that; the prevalence of the disease was also appeared 0 to 100% in different parts of the world [22]. This difference in prevalence is depending up on cat density, climate condition, age of the animals, species, sex, altitude, sample size and management of animal production [4, 5, 23].

In goats, the percentage of *T. gondii* infection was in consistent with the reported prevalence of 37.20% and 35% reported by Demissie and Tilahun [13] and Yibeltal [24] in South Wollo and in Debrebirhan respectively. However, the curent result was lower than the study reporeted by Teshale *et al.* [15] in south omo, Ethiopa with 74.8% prevalence which might be due to the reason of flock density/population is higher in Omo than in the current study area. However, it is in contrast with 24.1% using MDAT and 25.9% by ELISA test methods conducted in Nazareth, Ethiopia by Negash *et al.* [14] and 15.48% [25] in Central Ethiopia. This variations could be immaniated due to differences in the access of small ruminants to contaminated feed and water, the climatic variation and the diagnostic techniques used.

In this study, sheep, the odds of getting *T. gondii* infection was significantly higher in sheep (AOR = 1.34, 95% CI: 0.82-2.20) than goats although statistically not significant (P = 0.233) but it was statistically significant (P = 0.0.003) in univariable logistic regression. This finding is in accordance with the previous reports from Ethiopia [13, 15]. The highest rate of *T. gondii* infection detected in sheep may be due to the feeding habit. Unlike goats which prefer to browse, sheep graze close to the ground with the consequent high probability of ingestion of oocysts.

The likelihood of *T. gondii* infection, in Kebele 05 (AOR= 3.39, 95% CI: 1.92-5.99; P < 0.001) was higher than Kebele 07 (AOR=0.74, 95%CI: 0.43-1.28; P>0.05) and kebele 10. This variation among risk factors can be described by the variation in temperature and moisture in these areas. It is well known that the epidemiology of toxoplasmosis is influenced by the environment [5, 4]. Humidity increases, the chance of oocyst survival in the environment, thereby contributing to the higher sero-prevalence. A dry climate has an impact on the survival and epidemiological distribution of the parasite [1, 26].

The prevalence of *T. gondii* antibody detection was higher in adults (53.09 %) than in young's (34.69%).

In adult small ruminants (AOR = 2.22, 95% CI: 1.12-4.38) the infection rate of the parasite were higher than the youngest group with a statistical significance of (P = 0.021). This finding is relatively similar in adult than in young animals (OR = 2.93, 95% CI: 1.97, 4.35, P < 0.001) reported by Gebremedhi *et al.* [16]. This could be attributed to the reality that increment of the disease prevalence in older animals is due to exposure of animals to the risk factors for longer period of time than the younger ones [1, 16, 17]. This result is reasonable because infection is the function of age since animals of older age are more likely to ingest oocysts eliminated by definitive hosts (felids) from contaminated environments as compared to young age group. The finding also suggests that *T. gondii* infection of small ruminants is predominantly acquired postnatally [16].

In the current study, prevalence of anti-*T. gondii* antibody was higher in females (53.89%) than in males (40.86%). The risk of getting the infection in female animals (COR = 1.69, 95% CI: 0.05-2.71) were higher than males. The strength of association for predictor variable over outcome variable showed statistically significant (P =0.029). This finding is relatively in line with the reports of Gebremedhi *et al.* [16] (OR = 1.60, 95% CI: 1.04, 2.43, P = 0.033). This might be due to hormonal difference in relation to stress on lactating and pregnant animal leading to immunosuppression which increase susceptibility to toxoplasmosis in females [27]. Negash *et al.* [14] and Teshale *et al.* [15] also reported higher seroprevalence in female animal in Ethiopia than males.

The finding of epidemiological data revealed that in almost all farms and households included in this study, cats were present and their activities are not monitored and are allowed access to pastures, pens and barns. Oocyst shedding is thus widespread, which creates increased opportunity of exposure to *T. gondii*. The access of cats to feed storage units further increases the risk of contamination due to the cats' habit of burying their feces; these places are ideal for such unsavory feline activities. Even, the contamination of the pasture can be caused by the neighbors' cats or by feral cats [28].

The presence of abortions caused by *T. gondii* and access to raw meat seropositive animal poses a risk to cats themselves, which become infected after eating this organs or tissues, thus allowing the parasite to resume its life-cycle. The felines that consumed the aborted tissues with cysts of the parasite, can be also became an important way of transmitters [29].

In general, the association of Toxoplasma seropositivity with biological features of animals has shown that age, gender and species can influence small ruminant vulnerability to *T. gondii* infection. This research will benefit veterinarians and animal breeders because it will enable them to take adequate steps to protect these species, which are highly prone to *T. gondii* infection. It will also help specialists involved in food safety programs and finally.

CONCLUSION

This study brings good observation about the epidemiology of *T. gondii* infection in shoats. The uncontrolled movement of cats on pastures, in animal shelters, as well as in feed storage, contributes massively to the spread of oocysts and increases the oral infection rate of farm animals. The rearing systems and provision of some condemned organs from susceptible small ruminants to cats in the study areas seem to be favorable factors for unbroken chain transmission of this parasite from small ruminant to felines and vice versa. The higher seroprevalence encountered in these animal species used as a food source revealed the potential risk of *T. gondii* infection presented to people through consumption of an infected raw meat. So that, highlighting the high sero-prevalence and risk factors on transmission of toxoplasmosis in small ruminants in this study may trigger in raising farmers' awareness of the importance of the problem and the needs to implement control measures to prevent the transmission of the disease. Therefore, the presumed risk factors seems significantly indicative for the wide spread of the disease which needs further study through PCR and other sophisticated test methods instead of antibody detection using serological tools alone.

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