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Original Article

Seroprevalence and Associated Risk Factors of *Toxoplasma gondii* Infections in Pregnant Women and Sheep in Meket District, North East Ethiopia

Mengistu Wodage[1], Biruhtesfa Asrade[2], Yechale Teshome[3], Araya Mengistu[4], Joel Bueso-Rodenas[5]

1-Meket Agriculture and Livestock Development Office, Ethiopia
2-College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia
3-Department of Veterinary Science, College of Agriculture and Environmental Sciences, Bahir Dar University, Ethiopia
4-Department of Veterinary Epidemiology and Public Health, CVMAS, University of Gondar, Ethiopia
5- Department Agro-food Technology, Catholic University of Valencia, Spain
***Corresponding author:** *armen.kassa@gmail.com*
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Abstract

Toxoplasmosis, caused by *Toxoplasma gondii*, is one of the most important zoonotic diseases in the world. The study aimed to estimate the seroprevalence and associated risk factors of *T. gondii* infections among sheep and pregnant women in Meket District, North-East Ethiopia. A cross-sectional study was conducted from October 2021 to June 2021. A simple random sampling strategy and a systematic random sampling strategy were used to select individual animal owners and pregnant women, respectively. A total of 530 blood samples (322 from sheep's jugular vein and 208 from the cephalic vein of pregnant women) were collected and examined using the latex agglutination test kit (LAT). A univariate and multivariate logistic regression test was applied to show the association between the dependent and independent variables, considering $p<0.05$ with a 95% confidence interval. The overall seroprevalence of *T. gondii* among individual sheep, flock levels, and pregnant women was found to be 31.4%, 72.5%, and 32.2%, respectively. Age, sex, water source, and cat ownership and cleaning of cat litter were significantly associated ($p<0.05$) with *T. gondii* infection in sheep and pregnant women, respectively. In conclusion, serological findings and the questionnaire survey indicated that *T. gondii* infection is highly prevalent in sheep and pregnant women in the study area, and hence appropriate control measures, including regular surveillance, health education, good hygiene, and management of cats, should be implemented to mitigate the problem.

Keywords: Meket, Pregnant women, Risk factors, Seroprevalence, Sheep, *Toxoplasma gondii*

Introduction

Toxoplasmosis is a neglected tropical zoonotic disease caused by *T. gondii* (Rahman et al., 2018). It is the most successful parasite due to its efficient transmission through the ingestion of infective oocysts with contaminated food and water or ingesting tissue cysts in undercooked meat (Smith, 2009; Ajzenberg, 2011). The rate of infection in animal hosts and humans has been reported differently in various parts of the world.

It causes abortion, stillbirth, neonatal mortality, encephalitis, and pneumonia, particularly in sheep (Radostits et al., 2007). Different studies revealed that the prevalence of Toxoplasmosis globally in sheep ranged from 0 to 100% (Olivier et al., 2007). The prevalence of Toxoplasmosis in sheep in Africa ranged from 5.6% to 67.9% (Vander et al., 2000; Hove et al., 2005; Sawadogo et al., 2005; Samra et al., 2007; Barakat et al., 2009); in Ethiopia, it ranged from 22.9% to 70.48% (Endrias and Daniel, 2014a; Birhanu et al., 2018). Toxoplasmosis seroprevalence positivity of various degrees were reported among pregnant women across the globe (Elnahas et al., 2003; Song et al., 2005; Pappas et al., 2009; Mosti et al., 2012; Abdel and Elbasheir, 2014; Cong et al., 2015; Dasilva et al., 2015; Alvarado et al., 2016; Ayi et al., 2016). In Ethiopia, a prevalence range of 64.8% to 85.4% for *T. gondii* infection in pregnant women was recorded in different parts of the country (Endalew et al., 2012; Birhan et al., 2015; Woinshet et al., 2015; Yohannes et al., 2017). Although the disease is an important public health concern, to our knowledge, no work has been done regarding Toxoplasmosis in Meket and its surroundings. Therefore, this study was initiated to fill the aforementioned gaps in Meket district to generate seroepidemiological information and public health significance of *T. gondii* infection in sheep and pregnant women in Meket district, North East Ethiopia.

Materials and methods

Descriptions of the Study Area

The study was conducted in Meket district, the Northeast part of Ethiopia. It is located 665 Km North of Addis Ababa capital of Ethiopia and 215 Km East of Bahir Dar capital of Amhara Regional State. The approximate geographical location of the area is between 11°73´-12°05´N latitude and 39°00´-39°02´ longitude. The altitude is from 1500 to 3300 meters above sea level, the maximum temperature is 25.5°C in March, and the minimum temperature is about 10.5°C during the month of July, whereas the average annual temperature of the study area is 18°C. The mean annual rainfall in the area ranges from 950 mm to 1800 mm (MDAO and MDA, 2019).

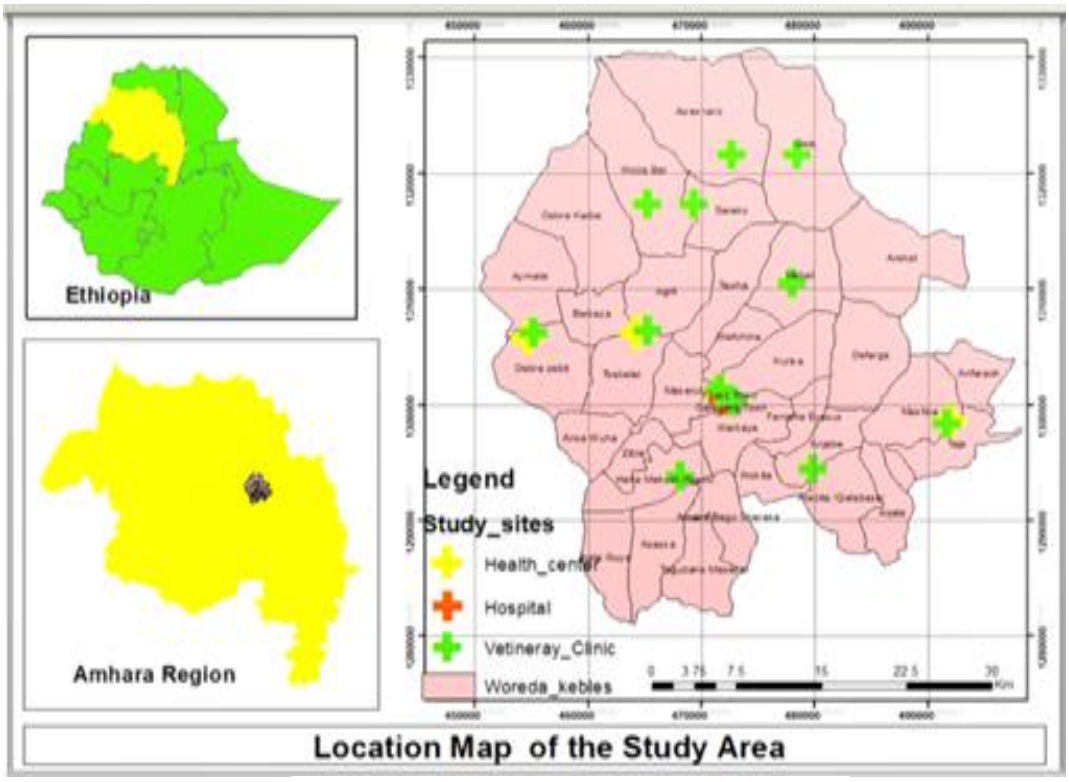

Fig. 1. Map of the study area (Source**:** GIS Software 2020).

Study population

In this study sheep kept under traditional smallholder farming systems were included. The study included 40 flocks and both sexes and age groups greater than six months. The study population also comprised all pregnant women aged ranged 15-49 years who visited health centers and hospital for antenatal care (ANC) in Meket district health institutions.

Study design

A cross-sectional study design was conducted from October 2021 to June 2021 to estimate the seroprevalence and associated risk factors of *T. gondii* infections in sheep and pregnant women. A Questionnaire survey and focus group discussion with the key informants were conducted to assess perception and risk factors towards the disease. At the beginning of the study a description of the study was done by the district animal health professions and medical laboratory experts and all data were collected accordingly with confidentiality.

Sampling

The sample size required for the seroprevalence study was calculated according to (Thrusfield, 2007) using the 95% confidence interval and an expected prevalence of 70.48% for sheep from East and West Shewa (Endrias *et al*., 2013) and pregnant women 83.6 % in Jimma Town (Endalew et al., 2012) with 5% absolute precision.

$$n=\frac{z^2*Pexp\,(1-Pexp)}{d^2}$$

Where: z = 1.96, Pexp = 0.7 or Pexp = 0.84 and d = 0.05 (the desired level of precision or accuracy). Based on these assumptions the required sample size of 322 and 208 for sheep and pregnant women, respectively were calculated. The samples were allocated proportionally to the selected kebeles based on agroecology. Twelve kebeles were randomly selected in the districts and sheep owners were selected with simple random techniques. Flock size was determined by the number of animals from 5-10 and 11-15 based on household level of sheep population and sheep greater than 6 months of age were included for the serological test. Similarly, sample size of pregnant women was allocated proportionally to health institutions. Pregnant women in the catchment of the health institution visiting antenatal care were used as sampling unit. Pregnant women in the age range of 15-49 years were included in the study. The selected institutions were Shedeho, Meket primary Hospital, DebreZebit health center, Agrit health center, Taja health center and Filakit health center by considering previous ANC giving record and health facilities. About 4500 pregnant women were expected to visit ANC annually in the institutions; 350 pregnant women of one-month data from the ANC registration book of the health institutions was obtained to approximate the total number of pregnant women attending ANC during the study period. Accordingly, 1050 pregnant women were expected to attend during the data collection period of 3 months; by using the formula for the K^n interval, $\boldsymbol{K = \frac{N}{n} = \frac{1050}{208} = 5.}$ Thus, systematic random sampling method was used with sampling interval of 5 to select the study participant's pregnant women who were the first visitor of ANC in the public health institute was sampled for this study (Teweldemedhin et al., 2019).

Questionnaire survey

A structured close-ended questionnaire was used to interview animal owners to collect risk factors such as animal age, sex, breed, flock size, management system, source of drinking water, agro-ecology, cat contact and socio-demography, behavioral and clinical characteristics to assess the perception and awareness of pregnant women towards the disease was including age, educational status, residence, occupation, tri-master pregnancy (gestation period), cat-owning, cleaning the cat litter, contact with soil, knowing about Toxoplasmosis, knowing about food-borne

diseases, eating habit of raw or undercooked mutton, eating raw or unwashed vegetables, source of drinking water were collected through questioner survey. Training was provided for data collectors and laboratory experts on the techniques, procedures and ways of expressing the questionnaires to collect the necessary information by the researcher. Questionnaire survey interviews and blood sample collections were undertaken in parallel.

Blood sample collection

Venous blood was collected aseptically in plain tubes from each of the study populations. At least 5 ml of blood was collected from the Jugular vein of sheep by veterinary experts and Cephalic vein in pregnant women by medical laboratory experts using labeled tubes. The collected animal blood was allowed to clot for up to 12 hours in the shade or centrifuged at 3000 rpm for 10 minutes and then the serum was collected using labeled cryovials. The serum was transported to Filakit health center laboratory in cooled containers with ice bags and it was stored at -20°C until a laboratory investigation performed. The serological test was carried out at Filakit health center and Shedeho Meket primary hospital.

Latex agglutination test (Toxo-Latex)

Examination of *T. gondii* antibodies [immunoglobulin G (IgG)] was done using a commercial Toxo-latex agglutination test kit (LAT; a slide agglutination test) (allowed for qualitative assessment of antibodies against *T. gondii*). It was performed according to the LAT manufacturer's instructions (SPINREACT, S. A/S.A. U Cira Santa Coloma, 7E-17176 SANT ESTEVE BAS (GI) SPAIN). The test kit was provided with a buffer, a freeze-dried positive and negative control that is latex suspension sensitized with a *T. gondii* antigen. Latex particles coated with soluble *T. gondii* antigen are agglutinated when mixed with samples containing antibodies anti-Toxoplasma. The test was considered positive when a layer of agglutinated materials formed.

Data qualitycontrol and precautions

The Toxo-Latex kit manufacturer's instructions were followed and precautions were taken to confirm the reliability of the Latex assay findings. For the laboratory works standard operating procedures and manufacturer's instructions were strictly followed. Positive and negative controls were used to check the quality of the latex agglutination kits for anti-*T.gondii.*

Data management and analysis

The raw data information and serum results were recorded in Microsoft Excel and analyzed using SPSS version 23. Descriptive Statistics proportion was performed to compute the number of serum samples positive for *T. gondii* infections. Univariate and multivariate logistic regression models were undertaken to assess the association of risk factors with the occurrence of seropositivity for Toxoplasmosis. *P*-values less than or equal to 2 in the univariate analysis were included in the multivariate analysis. In all the analyses, confidence levels were held at 95% and *p*-value less than 5% ($p<0.05$) were considered significant.

Results

Seroprevalence of Toxoplasmosis

The overall seroprevalence of T. gondii in sheep

The seroprevalence of 31.4 % (101/322) animal and 72.5% (29/40) flock levels were recorded. In this study, out of 40 flocks tested, 29 (72.5%) flocks were positive. From 29 flocks, 12 (30%), 3 (7.5%), 1 (2.5%), 3 (7.5%), 3 (7.5%), 1 (2.5%), 3 (7.5%), 2 (5%) and 1 (2.5%) flocks of sheep had one, two, three, four, five, six, seven, eight and ten seropositive animals, respectively. Flock level seroprevalence in highland (12/13, 92.3%),

midland (10/11, 91%) and lowland (7/16, 43.75%) were recorded.

Risk factors associated with T. gondii seropositivity in sheep

Out of 322 examined sheep, 35.6% and 20.2% seropositivity were recorded in adult and young aged groups. The univariable analysis showed that the risk of adults to be positive was 2.18 (COR = 2.18, CI: 1.22-2.91) times more compared to the young age groups. The seroprevalence of Toxoplasmosis was higher in female sheep (39%) compared to male sheep (21.2%). The probability of *T. gondii* infection in female sheep was (COR=2.37, CI: 1.43-3.93) times compared to males.

In the current study, highland (40.6%), midland (40.4%) and lowland (8.6%) were seropositive. The seroprevalence of Toxoplasmosis in this result was higher in cat contact (41%) compared to non-cat contact (22.8%). Flock size of 11-15 showed higher 46.6% seropositivity than flock size of 5-10 (12.5%). In the present study, higher seropositive was recorded in spring water (39%) followed by tap water (26.4%) and river water (21.7%).

Based on multivariable logistic regression, the probability of *T.gondii* infection in adults was 2.44 times higher than in younger once and the probability of infection in females was 3.15 times more compared to males. On the other hand, the probability of infection who drunk river water and spring water was (AOR= 0.05, 95% CI: 0.01-0.24) and (AOR= 0.10, 95% CI: 0.03-0.36**)** times (respectively**)** less likely to be infected compared with tap water (Table 1).

Table 1. Multivariable analysis and seroprevalence of *T. gondii* associated with risk factors in sheep in Meket district from October 2021-June 2021.

Risk factors	No of examined (N = 322)	No positive (%) (N = 101)	AOR* (95% CI**)	*p*-value***
Age				
Young	89 (27.6)	18 (20.2)	-	1
Adult	233(72.4)	83 (35.6)	2.44(1.27-4.69)	0.007*
Sex				
Male	137(42.5)	29 (21.16)	-	1
Female	185(57.5)	72 (39)	3.15(1.61-6.16)	0.001*
Agro-ecology				
Lowland	92(28.6)	8 (8.6)	-	1
Midland	104(32.3)	42 (40.4)	0.99(0.59-1.69)	0.205
Highland	126(39.1)	51 (40.5)	1.52(0.37- 1.46)	0.205
Cat contact				
Yes	151(46.9)	62(41)	-	1
No	171(53)	39(22.8)	0.65(0.38 -1.13)	0.131
Flock size				
5-10	144(44.7)	18 (12.5)	-	1
11-15	178(55.3)	83 (46.6)	0.75(0.23 -2.42)	0.632
Source of water				
Tape water	87(27)	23 (26.4)	-	1
Spring water	143(44)	56 (39)	0.10(0.03 – 0.36)	0.000*
River water	92(28.5)	20 (21.7)	0.05(0.01 - 0.24)	0.000*

*AOR = Adjusted odds ratio; **CI = Confidence interval; ***statistically significant at $p < 0.05$.

The overall seroprevalence of T. gondii in pregnant women

Of 208 samples, 67 (32.2%) sera samples were positive for *T. gondii* infection.

Risk factors associated with T. gondii seropositivity in pregnant women

Increment in age of the pregnant women had a direct relationship with the seropositivity of *T. gondii* in age groups ≥ 37 years (85%), 26-36 years (37.2%) and 15-25 years (15.9%). The educational status of the respondents was illiterate (61.4), grade 1-6 (19.1), grade 7-12 (26.5) and grade >12 (4.7). The probability of being infected by Toxoplasmosis in the educational status of grade >12 was 0.14 times less likely to be infected than grade 7-12 educated. Whereas grade 7-12 were 0.21 times less likely infected than grade 1-6 and also grade 1-6 were 0.31 times less likely infected than illiterates as compared with educated pregnant women.

Based on the current study, 39% of pregnant women's lived in rural followed by 22.7% in urban. The probability of urban infection by toxoplasmosis was 0.46 times less likely as compared with pregnant women who lived in rural areas. Although there was no significance difference in seropositivity, housewives contributed about 34.5%, while government employees (16%) and others (44.4%).

The seropositivity of pregnant women who owned a cat was 65.6%while it was lower (1.83%) for those who did not own a cat. *T. gondii* infections of pregnant women who had contact with soil were higher (37%) than no contact with soil (12.2%). It is 0.23 times more likely to be infected, if there is contact with soil and the cat litter is not cleaned (49.5%) compared to not cleaning the cat litter (9.8%).The probability of infection not cleaned the cat litter was 0.11 times less likely to be positive than pregnant women who cleaned the cat litter. The seroprevalence of Toxoplasma infection among those pregnant women who had drinking river water, spring water and tap water was (60%), (49.5%), and (8/9%) seropositive, respectively. Those who knew about food-borne disease showed 29.7% while those who did not know had 52% seropositivity. The probability of being infected by toxoplasmosis in pregnant women who were not knowing about food-borne disease was more likely to be infected (COR= 2.58, CI; 1.07-6.2) compared with knowing about food-borne disease.

Behavioral and clinical characteristics of pregnant women associated with seroprevalence of T. gondii in Meket district

The seroprevalence of Toxoplasma infection between raw meat eating habits (32.9%) and not eating habits (31.6%) resulted. Raw vegetable eating (31.5%) and not eating habits of raw vegetables (38.8%) resulted in *T.gondii* seropositive did not show a significant difference. The present study resulted in a higher seropositive third trimester of pregnancy was 60.5% and the next second-trimester pregnancy was 40% and the first-trimester pregnancy was 13.5% lower seropositive. The probability of *T. gondii* being infected in the third-trimester was 2.35 times more likely to be infected than the first-trimester and pregnant women in the second-trimester were 9.84 times more likely to be infected than the third and the first trimester gestation period. However, as trimester stage increased, higher seropositive results, which means the gestational age trimester at increasing seropositivity of *T.gondii* also increased. In multivariable logistic regression model analysis the variables only cat owning and cleaning the cat litter were significantly associated with *T. gondii* infections in pregnant women (Table 2).

Table 2. Multivariable analysis and risk factors of socio-demographic and clinical characteristics of pregnant women associated with Seroprevalence of *T. gondii* in Meket district from October 2021-June 2021.

Risk factors		No. Examined (N = 208)	No of positives N(%)67	AOR*(95% CI**)	*p*-value***
Age					
	15-25	94(45.2)	15 (15.9)	-	1
	26-36	94(45.2)	35 (37.2)	4.01(0.66- 24.24)	0.132
	≥37	20(9.6)	17 (85)	1.08(0.35- 9.15)	0.479
Educational status					
	Illiterate	57(27.4)	35 (61.4)	-	1
	Grade 1-6	47(22.6)	9 (19.14)	0.20(0. 01- 3.50)	0.404
	Grade 7-12	83(40)	22 (26.5)	0.19 (0. 01-3.59)	0.513
	Grade > 12	21(10)	1 (4.7)	0.23 (0.02 –3.16)	0.468
Residence					
	Rural	120(57.7)	47 (39)	-	1
	Urban	88(42.3)	20 (22.7)	1.11(0.36 -3.41)	0.858
Tri-master pregnancy					
	First	89(42.8)	12(13.5)	-	1
	Second	81(39)	32 (40)	3.25(0.75-	0.116
	Third	38(18.2)	23 (60.5)	2.26(0.61-	0. 220
Cat owning					
	Yes	99(47.6)	65 (65.6)	-	1
	No	109(52.4)	2 (1.83)	0.02(.0.03-0.06)	0.000*
Cleaning the cat litter					
	Yes	117(56.2)	58 (49.5)	-	1
	No	91(43.8)	9 (9.8)	0.21(0.05- 0.84)	0.027*
Contact with soil					
	Yes	167(80.3)	62 (37)	-	1
	No	41(19.7)	5 (12.2)	6.2(0.84- 45.69)	0.073
Source of water					
	Tap water	90(43.3)	8(8.8)	-	1
	Spring water	113(54.3)	56(49.5)	2.37(0.14-39.74)	0.548
	River water	5(2.4)	3(60)	0.37(0.27-5.15)	0.460

*AOR = Adjusted odds ratio; **CI = Confidence interval; ***statistically significant at $p < 0.05$.

Discussion

The current study was lower than the findings of 58.73% from Southwestern Ethiopia (Dechassa et al., 2016), 56% from Nazareth, Ethiopia (Negash *et al*., 2004), Zimbabwe (67.9%; Hove et al., 2005), and Egypt (47.5%; Barakat et al., 2009). Nevertheless, the current study was higher than reports from Central Ethiopia (20%; Endrias et al., 2014), Morocco (27.6%; Sawadogo et al., 2005), and South Africa (5.6%; Samra et al., 2007). The current result of flock seroprevalence (72.5%) was in line with the previous study of 70.48% (Endrias et al., 2013) from East and West Shewa zones and higher than 60.78% (Birhanu et al., 2018) from East Harergie, Ethiopia, and 58.89% in Algeria (Dechicha et al., 2015). The variation in the seroprevalence of *T. gondii* infection between the present study and the aforementioned studies might be due to differences in age, sex, agro-ecology, cat density, farm hygienic practices, management of animal production, and the type of serological tests used.

The association observed between infection with *T. gondii* and age group in sheep was agreed with previous reports made by different investigators (Endrias et al., 2013a; Mudassir et al., 2013; Dechassa et al., 2016). This could be attributed to the reality that the increment in disease prevalence in older animals is due to their exposure to the risk factors for a longer time than the younger

ones (Dubey, 2010; Anderlini et al., 2011; Pereira et al., 2012; Endrias et al., 2013; Dechassae et al., 2016). Sex had an association with the risk of *T. gondii* infection, which was in agreement with previous reports in Ethiopia (Endrias et al., 2013a; Mudassir *et al*., 2013; Berhanu, 2015), where higher infections were reported in females than males. The increased susceptibility of females might be associated with their lower immunologic resistance during certain periods of their lives (Guimares et al., 2013). On the contrary, sex has no significant association with the risk of *T. gondii* infection (Alvarado et al., 2013), and it has also been reported that there is no significant difference in seropositivity in sheep in relation to sex (Endrias and Daniel, 2014) in Ethiopia.

The lowest prevalence (8.6%) was recorded in lowland areas, where such areas are characterized by high temperatures, and a lower prevalence is found in hot and arid countries (Herwaldt, 2001). This variation could be described by the variation in temperature and moisture in these areas, and it's well known that the environment influences the epidemiology of Toxoplasmosis (Tenter et al., 2000; Dubey, 2004; Dechassa et al., 2016). Humidity increases the chance of oocyst survival, contributing to the higher seroprevalence, and a dry climate has an impact on the survival and epidemiological distribution of the parasite (Dubey, 2010; Dechassa et al., 2016).

Sheep that had contact with cats showed high toxoplasma seropositivity, which was consistent with some previous studies (Endalew et al., 2012; Endrias et al., 2013a). Similarly, the higher percentage of seropositivity in large flock sizes agreed with other findings (Guimares et al., 2013; Ahmad et al., 2015; Birhanus, 2015). Providing spring water to sheep resulted in a high positive percentage, which was in line with Endrias et al. (2013). This could be explained by possible contamination of the spring water by roaming infected cats (Silva et al., 2003; Tenter, 2009).

In the present study, an overall prevalence of 32.2% of seropositivity was recorded among pregnant women, which was close to reports done by Mebrahtu et al. (2019), Tamomh et al. (2016), and Iddawela et al. (2017). However, the current finding was lower compared to many author findings (Endalew et al., 2012 ;Endrias et al., 2013d; Endris et al., 2014; Birhan et al., 2015; Dasilva et al., 2015; Woyneshet et al., 2015; Abamecha and Hasen, 2016; Yohannes et al., 2017; Ayi et al., 2016). The current result was higher than the seroprevalence of *T. gondii* infection in pregnant women, which was reported by Yihenew (2012), Negash (2000), Cong et al. (2015), Alvarado et al. (2016), Song et al. (2005), and Elnahas et al. (2003). This high prevalence may be related to the presence of a large cat population and close contact with humans. In general, the variability could be attributed to differences in ecology, personal hygiene practices, feeding habits, socio-economic status, cat population, literacy status of the study subjects, laboratory diagnostic methods used, the sensitivity and specificity of serological diagnostic tests.

In the current study, an increase in seropositivity was observed as age increased (from nearly 16% in the age groups of 15–25 years old to 85% in the age groups of more than 37 years old), which was in line with previous studies (Nijem and Al-Amleh, 2009; Endalew et al., 2012; Birhan et al., 2015; Mebrahtu et al., 2019). The significant effect of age on *T. gondii* serostatus subjects may suggest that as age increases, there is a higher chance of a longer duration of exposure (Alsammani, 2016).

The educational status finding of the present study was in agreement with reports made by Endalew et al. (2012), Endrias et al. (2013), and Birhan et al. (2015). The finding regarding occupation was in agreement with results from Ethiopia by Endalew et al. (2012), Endrias et al. (2013), Woyneshet (2015) and Demissie (2019). High (37%) seropositivity was observed in pregnant women who had contact with soil as well as cleaning cat litter (9.8%), and these findings agree with other studies (Cook et al., 2000; Lopes et al., 2013). The result of the raw meat eating habit in the present study was consistent with studies conducted elsewhere where consumption of raw

meat was not associated with *T. gondii*seropositivity (Ertug et al., 2005; Hung et al., 2015). Although previous studies (Negash et al., 2008; Yibeltal, 2008; Dubey, 2010) show a significant association between eating raw vegetables and toxoplasmosis seropositivity, the current study did not show a significant difference. Given this, the absence of an association between consumption of raw meat and raw vegetables and toxoplasma infection may not be surprising and cannot rule out the role these food habits could have in Toxoplasma transmission (Woyneshet et al., 2015). A higher (60.5%) seropositive rate in the third trimester with a decreasing trend in the second and first trimesters of pregnancy was noticed, and this was consistent with the study reports in Southern Ethiopia (Demissie, 2019) and Ghana (Sefah-Boakye, 2015).

Conclusion

The study showed an overall seroprevalence of *T. gondii* among sheep (animal and flock level) and pregnant women of 31.4%, 72.5%, and 32.2%, respectively. In the assessment of the questionnaire's survey, all pregnant women had no knowledge of zoonotic toxoplasmosis in the study area. The serological finding of *T. gondii* infection was so prevalent as to be considered significant for public health significant. Potential risk factors such as age, sex, agro-ecology, cat contact, flock size, and water source were considered as risk factors associated with the occurrence of *T. gondii* infection in sheep, and age, educational status, residence, cat ownership, cleaning of cat litter, contact with soil, source of water, knowing about foodborne disease, and tri-master pregnancy were found to be potential risk factors for *T. gondii* infection in pregnant women. Among the potential risk factors for *T. gondii* infection in both sheep and pregnant women, age, water source, and cat ownership or contact were significantly associated with its occurrence. Treated water supervision for sheep, protection of access for cats not to be contaminated with oocysts, using treated or boiled water for pregnant women, avoidance of pregnant women from soil and contaminated litter, proper cleaning of cat litter, creation of awareness, regular surveillance, and further study as well as molecular isolation and characterization were suggested.

Ethical approval

Ethical clearance was obtained from Bahir Dar University and Amhara regional public health institute ethical review committee and permission was obtained from district agricultural and health offices. Written consent was taken from all study participants.

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Conflicts of Interest

No conflict of interest.

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