

## Seroprevalence of *Toxoplasma gondii* Infection in Sheep and Goats in Three Districts of Southern Nations, Nationalities and Peoples' Region of Ethiopia

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**Abstract:** Toxoplasmosis is an important parasitic zoonosis distributed globally. 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 184 sheep and goats to estimate the seroprevalence of *Toxoplasma gondii* infection using Indirect Enzyme Linked Immunosorbent Assay (ELISA) as a diagnostic tool. The overall seroprevalence was 26.09% (95% confidence interval [CI]: 19.90–33.06). The highest seroprevalence (40.54%) was recorded in Doyogena, followed by Boloso sore (18 %) districts, while the lowest seroprevalence was found in Bena Tsemay (15%) district (P=0.001). The odds of acquiring *T. gondii* infection was significantly higher in sheep (Odds Ratio [OR] = 2.6, 95% CI: 1.16-5.81; P = 0.028) than goats, in high land altitude (OR= 4.85, 95% CI: 1.76-6.92; P < 0.001) than mid and low land altitudes and in adult (OR = 8.55, 95% CI: 2.79-26.15; P < 0.001) than young sheep. The seroprevalence of toxoplasmosis in small ruminants is high. Altitude, species and age are the potential risk factors for *T. gondii* infection. Further epidemiological studies are warranted to unravel the impact in food animals as well as the risk of transmission to humans.

**Key words:** Seroprevalence • Toxoplasmosis • ELISA • Risk Factor • Ethiopia

### INTRODUCTION

*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 faeces [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, 4].

Although *T. gondii* infection in most people appears to be asymptomatic, it may result in life-threatening illness in some immuno-compromised individuals [4]. The disease has also been reported as the second largest cause of

death due to foodborne illness in the United States [5]. In Ethiopia, toxoplasmosis is the most common disease complication next to tuberculosis among HIV seropositive admissions and deaths [6]. However, despite having such an adverse health effect similar to salmonellosis and campylobacteriosis, toxoplasmosis is still a neglected and underreported disease [7].

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. 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. There is scarce knowledge about the epidemiology of *T. gondii* infection in animals and humans in Africa in general and in Ethiopia in particular.

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Although earlier studies in Ethiopia on toxoplasmosis in sheep [8-12] and in goats [8-10,13-15] showed seroprevalence ranging from 22.9% to 56% and 11.6% to 82% respectively, the seroprevalence in Doyogena, Boloso sore and Bena Tsemay districts of Southern Nations Nationalities and Peoples' Region (SNNPR) of Ethiopia is still unknown. This study was initiated with the objective of estimating the seroprevalence associated risk factors in these districts of SNNPR.

## MATERIALS AND METHODS

**Study Area and Population:** The population of interest were sheep (*Ovis iris*) and goats (*Capra hircus*) living in Boloso Sore, Bena Tsemay and Doyogena districts of SNNPR of Ethiopia (Figure 1). Small ruminant production systems are mainly agropastoral in Bena Tsemay district and sedentary in Boloso sore and Doyogena districts.

Bena Tsemay district (5°01' - 5°70' N and 36°20' - 37°06' E) is found in South Omo Zone and has semi-arid and arid climatic conditions, with mean annual rainfall increasing from the extreme south lower part, with some 350 mm, to the upper part where it ranges to 1400 mm. The district has a high ambient temperature ranging from 26 to 35°C [16]. The altitude ranges from 500- 2160 m above sea level (masl) (average 1330 m).

Boloso Sore (6°98' - 7°17' N and 37°63' - 37°84' E), is one of the 12 districts found in Wolayita Zone. The altitude in the district ranges from 1540-2020 masl (average 1780 masl). There are two ecological zones in Boloso Sore, namely midland (86.4%) and highland (13.6%). With rainfall dispersed throughout the year into two main rainy seasons and one small season. The area receives an annual rainfall of 1,551 mm and the mean maximum and minimum daily temperature are 25.4 and 13.4°C [17]. Boloso sore is one of the populated places in the SNNPR.

Doyogena (7°28' - 7°48' N and 37°74' - 37°89' E) is one of the 7 districts in Kembata Zone. Topographically, the Zone lies between an elevation ranges of 501-3000 masl (average 2300 masl). The zone has three agroecologies, sharing highland 13.7%, midland 71.17% and lowland 11.14%. The annual average temperature of the zone ranges from 26-27.5°C and the annual average rainfall ranges between 1001-1400 mm.

The agro-ecologies of the study area were classified as lowland (altitude range of 1000-1500 masl), midland (altitude 1500-2500 masl) and highland (altitude 2500-3360 masl) [18]. Sheep and goats = one year of age were

considered as adult while those below one year were categorized as young.

The inclusion criteria were domestic sheep and goat of any sex older than 6 months of age.

**Study Design:** This cross-sectional study was carried out from September, 2013 to January 2014. From the three purposively sampled study districts, study animals were sampled using systematic random sampling technique.

Age was determined by observation of the erupted permanent incisors [19]. Animals' = 1 year were considered as young while those above one year were considered adult.

**Collection of Blood:** About 5 ml of blood from 184 animals (124 sheep and 60 goats) was collected from jugular vein, using vacutainer tubes without anticoagulant, properly labeled and the sera were separated by centrifugation at 3200 RPM for 10 minutes. The extracted sera were transferred to other sterile vials and kept at -20°C until serologically assayed.

**Indirect ELISA Test:** Serum samples were tested for the presence of IgG antibodies against *T. gondii* using a commercial indirect ELISA kit (ID VET, Montpellier, France) according to the manufacturer's instructions. The wells of the kit are coated with *T. gondii* P30 surface antigen and it uses non-species specific protein conjugates and it can be used in several animal species. For interpretation of the result S/P% was calculated as:  $S/P\% = \frac{(\text{OD } 450 \text{ value of the sample} - \text{OD } 450 \text{ value of the negative control})}{(\text{mean OD } 450 \text{ value of the positive control} - \text{OD } 450 \text{ value of the negative control})} \times 100$ . Any samples with an S/P less than or equal to 40% were considered as negative, the samples with an S/P between 40% and 50% were considered as doubtful, the samples with an S/P greater than or equal to 50% considered as positive. The doubtful sera were retested.

**Data Analyses:** The data generated from field and laboratory investigations were entered and coded using Microsoft Excel® 2007. Statistical analyses were performed using SPSS 18.0 (Statistical Package for Social Sciences (SPSS) Inc., Chicago, IL, USA). Descriptive statistics was used to summarize the data. Mantel-Haenszel Chi-square with corresponding odds ratio (OR) and 95% confidence interval (CI) was used to assess the potential risk factors. The differences were considered statistically significant when  $P \leq 0.05$ .

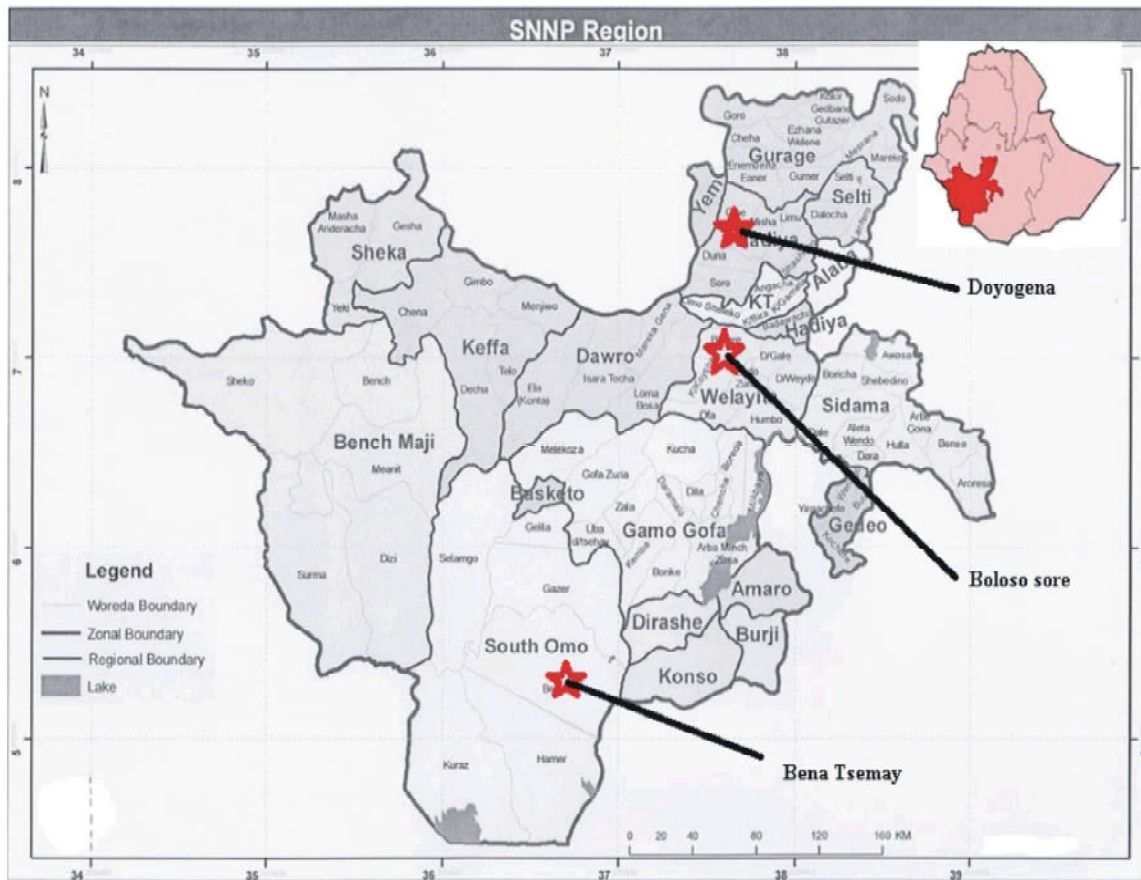


Fig. 1: Map of Southern Nation Nationalities and Peoples' Region (SNNPR) of Ethiopia showing the study districts

**Ethical Considerations:** This research project was approved by the animal ethical committee of the College of Veterinary Medicine and Agriculture, Addis Ababa University. All efforts were made to minimize animal suffering during the course of the study. Informed written consents were obtained from all sheep and goats owners who participated in the study.

## RESULTS

**Seroprevalence:** An overall seroprevalence of 26.09% (95% confidence interval [CI]: 19.90–33.06) was recorded. There was a significant difference in *T. gondii* IgG seropositivity between study districts ( $P < 0.05$ ) (Table 1).

**Risk Factors:** The odds of acquiring *T. gondii* infection was significantly higher in sheep (Odds Ratio [OR] = 2.6, 95% CI: 1.16-5.81;  $P = 0.028$ ) than goats, in high land altitude (OR = 4.85, 95% CI: 1.76-6.92;  $P < 0.001$ ) than mid and low land altitudes and in adult sheep (OR = 8.55, 95% CI: 2.79-26.15;  $P < 0.001$ ) than young sheep. However,

*T. gondii* seropositivity was not significantly different with respect to gender in both sheep and goats. In goats, the seroprevalence was not significantly different between adult and young ( $P > 0.05$ ) (Table 2).

## DISCUSSION

In this study, data on seroprevalence and some risk factors of small ruminant toxoplasmosis in Boloso sore, Doyogena and Bena Tsemay districts of SNNPR of Ethiopia were reported. In present study, 31.45% of sheep and 15.0% of goats were seropositive for *T. gondii* and hence are considered to be in the chronic stage of the infection. The high rate of *T. gondii* infection suggests a high environmental contamination with oocysts.

The seroprevalence demonstrated in sheep in this study (31.45%) is consistent with the previous reports from Ethiopia (22.9% - 34%) [8, 9, 11, 12, 15] and other African countries such as, Ghana (33.2%) [20] and Morocco (27.6%) [21], but lower than that reported from Ethiopia (56%) [10], Zimbabwe (67.9%) [22] and Egypt

Table 1: Overall seroprevalence of *T. gondii* infection in three districts of SNNPR

District	No. tested	No. positive	% seroprevalence	95% CI	Chi-square	P-value
Boloso sore	50	9	18	7.17-28.83	13.5384	0.001
Bena Tsemay	60	9	15	5.83-24.17		
Doyogena	74	30	40.54	29.20-51.88		
Total	184	48	26.09	19.90-33.06		

Table 2: Association of seroprevalence of *T. gondii* infection with potential risk factors

Risk factor	No. tested	No. pos.	% seropre.	OR (95% CI)	Mantel-Haenszel X <sup>2</sup>	P
<b>Species</b>						
Goat	60	9	15	1.00	4.828	0.028
Sheep	124	39	31.45	2.6 (1.16-5.81)		
<b>Altitude</b>						
Low & midland	110	18	16.36	1.00	12.120	<0.001
Highland	74	30	40.54	4.85 (1.76-6.92)		
<b>Sex: Sheep</b>						
Male	24	6	25	1.00	0.261	0.609
Female	100	33	33	1.48 (0.54-4.07)		
<b>Sex: Goat</b>						
Male	7	0	0.0		0.377	0.539
Female	53	9	16.98			
<b>Age: Goat</b>						
Young (<1 year)	22	2	9.09	1.00	0.354	0.552
Adult (≥ 1 year)	38	7	18.42	2.26(0.43-11.98)		
<b>Age: Sheep</b>						
Young (<1 year)	46	4	8.70	1.00	15.798	<0.001
Adult (≥ 1 year)	78	35	44.87	8.55(2.79-26.15)		

(47.5%) [23] and (98.4%) [24]. Seroprevalence lower than the current study was also reported from South Africa (5.6%) [25]. Similarly, the seroprevalence reported in goats (15%) was in accord with the 11.6% [8] and 19.7% [14] seroprevalence reported in Ethiopia and 19.3% in Tanzania [26], but lower than the 25.9% [10], 35% [9] and 74.8% [13] seroprevalence reported in Ethiopia as well as the 26.8% reported in Ghana [20], 31% in Uganda [27] and 41.7% [24] and 59.4% [23] in Egypt. Compared to the current result, much lower seroprevalence of 4.6% from goats in Nigeria [28] has been reported. The wide variation in the seroprevalence of *T. gondii* infection seen between the present study and aforementioned studies might be due to difference in sample size, agro-ecology, climate, cat density, farm hygienic practices, animal management, type of serological tests used and the cut-off value used [1, 29].

Previously, there were no reports on seroprevalence of *T. gondii* infection of animals in this region, except the reports of Teshale *et al.* [13] who reported much higher seroprevalence (82%) in goats of South Omo Zone of SNNPR using in house MAT which

appears to be highly sensitive as compared to the presently used commercial ELISA which detected only IgG antibodies in 15% of goats studied from Bena Tsemay district of South Omo Zone. However, in their study they used 1:20 as a cut-off add in-house between for and MAT.

In this study, sheep is the most abundant species studied (124/184) and highest prevalence of *T. gondii* infection (31.45%, 39/124) was detected in this species. The odds of acquiring *T. gondii* infection in sheep is 2.6 times higher as compared to goats (P<0.028). This finding is in accordance with the previous reports from Ethiopia [9, 10, 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.

*T. gondii* seroprevalence was significantly higher in small ruminants from the highlands (40.54%) as compared to small ruminants of midlands and lowlands areas (16.36%). This might be attributed to the warm and moist conditions of highland tending to favor the survival of

oocysts in the environment. Indeed, Boloso sore and portions of Doyagera are located in humid or sub-humid zone with an average annual rainfall of 1551 mm and 1001-1400 mm, respectively, while Bena Tsemay district is found in lowland area with semi-arid and arid climatic conditions with high ambient temperature (26 to 35°C) and relatively low and variable rainfall (350-1400 mm), less favorable for prolonged survival of the oocysts. The influence of altitude on the prevalence of *Toxoplasma* infection has been previously reported from Ethiopia [11, 14]. In Mexico, sheep raised in farms at  $\geq 1900$  m above sea level were reported to have a higher seroprevalence (44.1%) than those in farms at lower sea level (16.3%) [30].

The significant association observed between infection with *T. gondii* and adult age group in sheep was in agreement with previous reports by different investigators [11]. This observation is plausible 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.

Contradictory to what was published recently in Ethiopia [11, 14], which indicated the role of gender as an important variable for infection by *T. gondii* (high infection in females than males), in this study, gender has no significant association with the risk of *T. gondii* infection. Alvarado-Esquivel *et al.* [30] also reported absence of significant difference in seropositivity in sheep with respect to gender.

The widely practiced habit of consumption of raw or undercooked small ruminant meat among Ethiopians coupled with the high *T. gondii* infection rate presently estimated are believed to contribute to the spread of the parasite to humans' leading often to asymptomatic persistent infection but also to clinical disease notably in immunocompromised people and congenitally infections.

It was concluded that the seroprevalence of toxoplasmosis in small ruminants is high. This study provides the evidence that sheep are more likely to acquire *T. gondii* infection than goats, adult age group of sheep are highly affected than young sheep and sheep and goats from highland are more affected than those from mid and low land areas. The high seroprevalence of toxoplasmosis suggest that humans might get the parasite from sheep and goats. Further large scale epidemiological studies are essential to elucidate the impact of the disease for eventual control of the disease.

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