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Research Article

Sero-prevalence of toxoplasmosis in Borana breed cattle in three selected district of Borana zone, Oromia regional state, southern Ethiopia

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Keywords

- *T gondii*
- Sero-prevalence
- Borana breed cattle
- Risk factors

Abstract

Objective: The objective of present study was to estimate sero-prevalence of toxoplasmosis and identify associated risk factors for the occurrence of the disease among Borana breed cattle in three selected districts of Borana zone of Oromia Regional state, southern Ethiopia.

Methods: A cross sectional (observational) study design was conducted from December, 2017 to April, 2018 to estimate the overall sero-prevalence of cattle toxoplasmosis in three selected districts of Borana zone. A totally 391 borana breed cattle from three selected districts (belonging to 50 herds) were tested for antibodies against Toxoplasma gondii by using Latex Agglutination Test (LAT). A questionnaire survey was made to assess associated risk factors and knowledge of pastoralists about toxoplasmosis.

Result: The overall sero-prevalence was 14.8% at animal level and 68% at herd-level. There was statistically significant variation ($P < 0.05$,) in *T.gondii* seroprevalence among the three districts. The slightly highest seroprevalence was recorded in Gomole district (100 %) followed by Yabello (83.3 %) and Elwaye (35%) districts at herd level and in Gomole (19.5%), followed by Yabello (16.9%) and Elwaye (8.7%) districts at animal level. There was significant association between herd-level seroprevalence of *T. gondii* infection and herd size, presence of domestic cats and hygiene at camp ($P<0.05$). About 66% of interviewed Pastoralists had cats in their home and almost all fed household leftovers, raw offal and were allowed to roam in the neighbor hoods fed on rodents and birds. Most (92%) interviewees were uneducated and all had no knowledge of toxoplasmosis.

Conclusion: Study districts, size of the herd, presence or vicinity of domestic cats and hygienic at camp are the main risk factors. The sero-prevalence of toxoplasmosis in studied districts of the Zone needs high attention in implementing the disease control and prevention strategies.

INTRODUCTION

Toxoplasmosis is one of the most common parasitic zoonosis, caused by the obligate intracellular protozoan *T. gondii,* which can infect almost all warm-blooded animals, including humans and domestic animals [1,2]. It is also one of the most prevalent parasitic infections of medical and veterinary importance due to its negative impacts on health and production. Its medical importance remained unknown until 1939 when *T. gondii* was identified conclusively in tissues of a congenitally-infected infant in New York City, USA [3], and its veterinary importance became known when it was found to cause abortion storms in sheep in 1957 in Australia [4].Cats and wild felids are the only definitive hosts that may pass oocysts with their faeces and play an important role in the epidemiology of Toxoplamosis. Domestic cats are most important the definitive host and are responsible for dissemination of infection through fecal contamination of pastures, food and water. The cat population is high in most areas of the world, and it has been estimated that there may be altogether as many as 200 million cats [5]. Millions of oocysts are then shed in the feces and spread in the environment. Similarly, *T. gondii* can also be found worldwide, in the environment and in the hosts. Consumption of raw and undercooked meat, transplacental transmissions is the other methods of dissemination of infection [6].

In animals the infection is usually subclinical although phenomenon of congenital transmission leading to abortion and neonatal mortality has been reported in animals including small ruminants [7]. Thus they remain as source of infection to human and carnivores through carnivorism. Even though, infection is subclinical; toxoplasmosis leads to great economic losses in ruminants especially in sheep, cattle and goats by causing embryonic death, foetal death, abortion, stillbirth and reduced flock milk production [8]. Worldwide prevalence of *T. gondii* infection in goat, sheep and cattle largely investigated. However,

in Africa most studies have been conducted on prevalence of toxoplasmosis in sheep and goat [9,10]. There was very few studies have been conducted on prevalence of toxoplasmosis in cattle particularyl in Ethiopia[11, 12]. There was only one report on prevalence of *T. gondii* in southern parts of Ethiopia particularyl in Borana [13]. Information on prevalence of this parasite in Borana breed cattle from southern parts of Ethiopia, particulary in Borana is not studied so far. Therefore, the objective of present study was to estimate sero-prevalence of toxoplasmosis and identify associated risk factors for the occurrence of the disease among Borana breed cattle in three selected districts of Borana zone of Oromia Regional state, southern Ethiopia.

MATERIALS AND METHODS

Study area

The study was carried out in three selected districts of Borana zone, namely Yaballo, Gomole and Elwaye. The capital city of the Borana zone is Yaballo, which is 575 km far from capital city Addis Ababa to south direction. The zone has latitude ranges between 943 and 2,400 meters above sea level with average annual rain fall of 400 to 1100 mm exhibiting a bimodal rainfall (long and short rainy seasons). Long rainy season (Ganna) extends from March–May while short rainy season (Hagayya) extends from September–November. The annual temperature varies 19–42 0C. The zone has an estimated population of 962,489 (male 487,024 and female 475,465) with 91.2% of population living in Rular area [14]. The milk is the main source of food in addition to being the source of income particularly during the rainy season when it is produced sufficiently.

Study population

Study animals were Borana breed cattle which managed under pastoral production system. The animals were from three selected districts namely, Yabello, Elwaye and Gomole with different age group, both sexes and body condition.

Sampling Frame and Sample size determination

The sampling frame consisted of three districts and associated cattle population in these areas. The sampling methods were multistage sampling in which the three districts were purposively selected from the existing 13 Districts of Borana zone considering agro ecology, ruminant population density, accessibility and availability of infrastructure. Then one Peasants Association (kebele) was randomly selected from two selected districts (Yabello and Elwaye) and two Peasants Associations (PA) (kebele) was randomly selected from Gomoledistrict by considering high ruminant population density in districts. Then two villages (locally known as‖ Olla) was randomly selected from each selected kebele. Herds were randomly selected from list of herds prepared together with PAs' development agents. Individual animals were randomly selected from herds using list of cattle names given by owners as sampling frame. Accordingly a total of 391 individual Borana breed cattle were sampled from selected study areas. The required sample size for study animals was determined by using Thrus field (15) assuming 95% of confidence interval and at 5% of desired precision. Since there was no previous expected prevalence in the area, thus 50% expected prevalence toxoplasmosis among cattle in study area. n = 1.962 x Pexp (1-Pexp)/ d2. Where: n= the required sample size, Pexp= expected prevalence/previous prevalence, d=desired absolute precision Therefore, the sample size required for this study was 384 by using above formula. However, total number of sampled animals was increased to 391 for better accuracy.

Study design

A cross sectional (observational) study design was conducted from December, 2017 to April, 2018 to estimate the overall sero-prevalence of cattle toxoplasmosis in three selected districts namely, Yaballo, Gomole and Elwaye of Borana zone of Oromia Regional State, Southern Ethiopia. The sero-prevalence was estimated in respect to the number of risk factors such as host factors (age, sex, and body condition of the animals), and the environmental factors included (origin of the animal such as district, herd size, hygienic at the barn and presence of cat).

Sample collection and transportation

Animals were restrained by owners and 8 ml of blood sample was collected from the jugular vein using disposable vacutainer tubes without anti-coagulant under aseptic condition. A blood sample was labelled properly on vacutainer tube and note book using pen and transported to Yaballo Regional Laboratory for serum separation, storage and test. A serum was separated by centrifugation of the tubes at 3200 RPM for 10 minutes. The sera sample was transferred to serum tubes (cryovial tube) and every animal's information that was recorded on vacutainer tube was recorded on cross ponding serum tubes (cryovial tube) using pencil. Sera was kept at -200c until serologically anti-*T. gondii* is performed by using Latex agglutination test Toxo-Latex ® (SPINRER EACT, S. A. Ctra. Santa coloma, Spain).

Study methodology

Serological testing by latex agglutination test (LAT): The serum samples and Toxoplasma antigen (Spinreact, S.A./ S.A.U., Ctra. Santa Coloma, Spain) were kept one hour in room temperature before beginning of the test. Then the vial of antigen was shacked gently and 25 µl of antigen was placed over the middle of area of each 6 cell and one drop of positive control serum (goat) in one of cell while the negative control serum was placed on the other cell. A total of 50 µl of test serum was placed on the rest 4 cells. The antigens and the serum were mixed on the plate with a stirrer and spread over the entire circle. Then the plate was placed on rotator after adjusting time and speed automatically for 4 minutes and the reading was taken immediately. Finally the result was observed for clamping (agglutination) by naked eye and in comparison with the two controls (positive and negative). The negative result was identified as negative control result which did not form agglutination (homogenous appearance) but distinct agglutination was indicator for positive toxo-Ab of at least 4 IU/ml similar with positive control.

Questionnaire survey: Additional information was collected by questionnaire survey of 50 herd man/ animal owner to evaluate awareness level of pastoralists about toxoplasmosis and to point out association between potential risk factors for the disease occurrence and sero-prevalence in study area. Risk factors like hygieneic at barn (boom) and it is surrounding, vicinity of cats and herd size; were obtained using a questionnaire on survey of

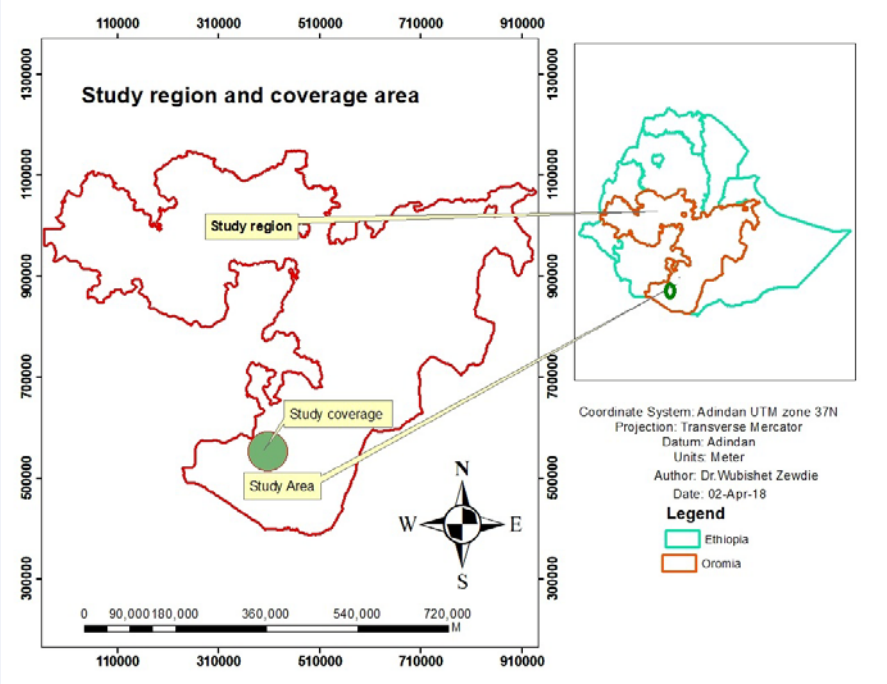

Figure 1 Map of Study Area.

cattle household/ownership or animals attendants interviews. Hygienic at barn and it is surrounding were categorized as high, (those cleaned daily), medium (those cleaned weekly) and poor (those cleaned monthly) and finally, herd size were categorized as small (those contained 1-10 cattle), medium (those contained 10-50 cattle) and large size (those contained ≥ 50 cattle). The abortion history and source of water of were not used as risk factor for occurrence for disease in present study; because based on toxoplasmosis studies it is determined that it is not an important cause of abortion in cattle. In case of water source the pastoralists use overlapping sources depending on the availability of water nearby and seasonal distribution of water sources therefore; it is not needed to as variable in this study.

Data Analysis

Data generated from the laboratory investigation and the questionnaire survey was recorded and coded on Microsoft Excel spread sheet (Microsoft Corporation) and analysed using STATA version 11.0 for windows (Stata corp. College Station, TX, USA). Descriptive statistics were utilized to summarize the data and the significant association between the prevalence of toxoplasmosis and explanatory variables was determined using Chi-square test (X^2).The explanatory variables included were host factors such as: sex, age, body condition and environment factors like, hygiene, herd size, vicinity of cats, and their associations with the level of prevalence were described. The difference was regarded as significant if P-value is <0.05 at 95% confidence interval.

RESULTS

Results of serological test by latex agglutination Test (LAT)

The overall animal- and herd-level seroprevalence of antibodies against *T. gondii* in Borana breed cattle of the current study area was 14.8% [95% CI 11.30%, 18.40%] and 68% [95% CI 54.60%, 81.4%], respectively. Both at animal- and herd-level seroprevalence of cattle toxoplasmosis was significantly different between districts (P<0.05). The highest animal level seroprevalence was recorded in Gomole district (Table 1)

Animal-level sero-prevalence

The anima level, sero-prevalence of *T. gondii* sero-positivity was significant in the origin of animals (P<0.05) (Table 1). while the age, sex and body condition of animals were not significant (P>0.05) (Table 2).

The Pearson's chi squared test analysis revealed that non-significant association was found between animal level seroprevalence and association risk factors (P>0.05)

Among the 50 cattle herds examined, 34 herds (68 %) had at least one seropositive cattle. Pearson's chi squared test analysis showed that the herd-level seroprevalence of *T. gondii* infection was significantly associated with districts (P=0.000). Herd-level seroprevalence of *T. gondii* infection was higher in Gomole district (100 %) followed by Yabello (83.30 %) and Elwaye (35%) districts (Table 3). The Pearson's chi squared test analysis revealed that significant association was found between herd level seroprevalence and presence of domestic cats, herd size and hygienic at boom (locally *"foora"*) (P<0.05) (Table 4).

The Pearson's chi squared test analysis revealed that significant association was found between herd level seroprevalence and association risk factor(P<0.05) (Table 4).

Results of questionnaire survey

A questionnaire survey was administrated to 50 herd man/

Table 1: Animal level sero-prevalence of toxoplasmosis in Borana breed cattle from three districts of Borana zone, Southern Oromia, Ethiopia.

District	animal tested	Seropositive (animals)	Prevalence% [95% , CI]	χ^2	p-value
Elwaye	138	12	8.7 [4.0, 13.50]	6.700	0.035
Gomole	123	24	19.5 [12.50, 26.60]		
Yaballo	130	22	16.90 [10.40, 23.40]		
Total	391	58	14.8 [11.30, 18.40]		

Table 2: Results of potential risk factors associated with *T gondii* sero-positivity in animal level.

Variable category		Number of tested	Number of positive	Prevalence% [95% , CI]	$_x2$	p-value
Sex	female	297	46	15.50 [11.40, 19.60]	0.419	0.518
	male	94	12	12.80 [6.0, 19.60]		
Age	young	127	16	12.60 [6.80, 18.40]	3.696	0.158
	adult	106	12	11.30 [5.20, 17.40]		
	old	158	30	18.0 [12.80, 25.14]		
Body condition score	good	172	22	12.80[7.80, 17.80]	1.665	0.435
	medium	146	10	13.70 [5.70, 21.70]		
	poor	73	26	17.81 [11.60, 24.01]		
Herd level seroprevalence						

Table 3: Herd level sero-prevalence of toxoplasmosis in Borana breed cattle from three districts of Borana zone, Southern Oromia, Ethiopia.

District	Herd tested	seropositive (herd)	Prevalence% [95% , CI]	$_x2$	p-value
Elwaye	20	7	35 [13.00, 57.0]	17.6011	0.000
Gomole	12	12	100 [73.5, 100*]		
Yaballo	18	15	83.3 [65.20, 100*]		
Total	50	34	68 [54.61, 81.40]		
*one-sided, 97.5% confidence interval					

Table 4: Results of potential risk factors associated with *T gondii* seropositivity in herd (n=50 herd).

Variable	category	seropositive (herd)	Prevalence% [95% , CI]	$_x2$	p-value
Presence of cat	No	17	34 [20, 47]	8.0745	0.004
	Yes	33	66 [52, 79.6]		
herd size	large	20	100 [83.157- 100*]	19.431	0.000
	medium	14	64.286 [37.580- 90.992]		
	small	16	31.25 [7.200-55.300]		
hygiene at camp (foora)	good	13	53.846 [24.926 -82.766]	8.4545	0.015
	medium	16.	50 [24.057- 75.944]		
	poor	21	1.476 77.286-100*]		
*one-sided, 97.5% confidence interval					

animals owner for evaluation of awareness level of pastoralist about toxoplasmosis in study area. Accordingly, among 50 pastoralists /herd man interviewed during sampling of blood from his/her herd all them were not completed university education. While 46 (92%) and 3(6%) were illiterate and completed Primary School respectively. Whereas (100%) study participants do not have the knowledge about Toxoplasmosis and the role of cats in transmitting zoonotic diseases to humans and animals. About 66% of interviewed individuals had cats in their home and almost all fed household leftovers, backyard slaughter raw offal and were allowed to roam in the neighbourhoods where they could feed on rodents and birds (Table 5).

DISCUSSION

The overall sero-prevalence of current study in Borana cattle breed was 14.8% [95% CI 11.30%, 18.40%]. The prevalence of *T. gondii* in this study was found to be higher than that report in Central Ethiopia (6.6%) by using an indirect haemagglutination assay [11], Somalia (7.1%) by using Latex Agglutination Test [16], Japan (7.3%) by using Modified Agglutination Test [17],

Table 5: Result of the questionnaire survey regarding possible risk factors and awareness level of pastoralists about toxoplasmosis in Borana cattle breed in three selected districts (n=50 herd man).

variable	categories	number of respondents	percentage
level of education	uneducated	46	92%
	Primary School	3	6%
	secondary School	1	4%
	University	0	0%
Presence of cat in home	no	17	34%
	Yes	33	66%
way of feeding meat to cat	raw	47	94%
	after cooking	0	0%
	both	5	6%
Knowledge of health risk of cat to cattle	Yes	0	0%
	No	50	100%
Knowledge of health risk of cat to human	Yes	0	0%
	No	50	100%
way of eating meat	raw	0	0%
	after cooking	49	98%
	both	1	2%
Knowledge of housing domestic cat and dog	Yes	0	0%
	No	50	100%
way of handling cat in home	using gloves	0	0%
	bare hand	50	100%
Salt supplementation	yes	50	100%
	no	0	0%
Knowledge about toxoplasmosis	Yes	0	0%
	No	50	100%

Portugal (7.5%) by using Modified Agglutination Test [18]. The higher prevalence of *T. gondii* in this present study may be due to the pastoralist habit domesticating of pets (cats) for control rodents within and outside the households. This finding was further strengthened by the recent report from Kenya where cat domesticated for rodents control within and outside the households as main source of infection [19]. Finally the management system of pastoralist was open grazing to common pasture that contaminated with oocysts. Which agree with finding of [20] who reported the prevalence was significantly higher in sheep and goat that raised outdoors as grazing animals and could thus have more contact with oocysts shed by cats in the environment.

The prevalence of *T gondii* in this study was found to be lower than that report in Central Ethiopia [21] who reported a prevalence of 25% by Slide Agglutination Test (SAT).

Nearly the high seroprevalence of *T. gondii* were observed cattle from Gomole district 19.50% [95% CI 12.40, 26.60] followed by Yaballo and Elwaye districts with seroprevalence of 16.90% [95% CI 10.40, 23.40] and 8.70% [95% CI 4.0, 13.40] respectively. This variation among the districts could be attributed to the differences in environmental temperatures and moistures [12]. Gomole mid low land characterized by slightly warmer and moister when compared to Yaballo mid low land and when compared with Elwaye low land that characterized by one of dry hot district of Borana zone.

The present study is showed a herd with poor hygienic conditions at camp (locally-Foora) a significantly higher number of seropositive animals. This may be due to the fact that proper cleaning at camp reduces the risk of contamination of feed and water with oocysts, which minimizes the risk of toxoplasmosis [22].

The current study showed a higher number of positive animals in herd where cats were present in the vicinity as compared to herd where no cats were present. These results are due to the fact that the presence and close contact with, cats is a very important factor in the epidemiology of toxoplasmosis. Cats shed millions of oocysts in the environment, which could be ingested by animals along with feed and water [7]. Similar findings study from Poland who reported that the presence of freeroaming cats is an important risk factor for the transmission of the infection in goats. Similar finding also from Ghana and Brazil [9] and [18] respectively, who reported that the same finding in goat and sheep.

Accordingly, cattle are evenly commonly exposed to *T.gondii* in medium, small and large herds; highest prevalence was recorded in the cattle that from large herd when compared with

those from medium and small herd. This may be due to fact that difficult in management system of large herd to clean their dug daily, and may be presence of cat in every household with large herd since at least one or two lactating cow present that able to fed their cats in every season of the years in Borana pastoralist.

In conclusion, Study districts, size of the herd, presence or vicinity of domestic cats and hygienic at camp are significant stood out as the main risk factors. The majority of interviewed pastoralists were uneducated and had cats in their home and almost all fed household leftovers, backyard slaughter raw offal and were allowed to roam in the neighborhoods where they could feed on rodents and birds resulting contamination of pasture. All interviewees had no awareness about toxoplasmosis, the role of cat the in transmission toxoplasmosis to domestic animals including human and housing domestic cat. The sero-prevalence of Toxoplasmosis in studied districts of the Zone needs high attention in implementing the disease control and prevention strategies. In order to control and prevention of disease in the herd and at animal cleaning barn and surrounding environment, preventing domestic cats from asses to pasture by housing the cat. Therefore, creation of public awareness on identified risk factor to herd man/owners and the role of cat in the transmission toxoplasmosis to domestic animals including human and housing domestic cat in order to control spread of disease to human and animals.

Annex: Description of body condition score

Score	Condition	Appearance
1	Emaciated	Shoulder, rib and back are visible
2	Very thin	Some muscle, no fat deposits
3	Thin	Some fat deposits, ribs visible
4	Borderline	Fore ribs not noticeable
5	Moderate	12th and 13th ribs not visible
6	Good	Ribs covered, sponginess
7	Very good	Abundant fat on tail head
8	Fat	Fat cover thick and spongy
9	Obese	Extremely fat throughout

(Source: (25)

From the above table;

- Number 1, 2 and 3 are poor body condition
- Number 4, 5 and 6 are medium body condition
- Number 7, 8 and 9 are good body condition

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