

## Sero-Epidemiology of Camel Toxoplasmosis and Public Awareness on its Zoonotic Importance in Central Afar Region, North East Ethiopia

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**Abstract:** Toxoplasmosis is one of the most important camel diseases endemic in the pastoral areas. A cross-sectional study was carried out to study sero-epidemiology of camel toxoplasmosis and public awareness on its zoonotic importance in central Afar region of Northeast Ethiopia. A total of 384 sera sample were collected from 56 camel herds and 56 individuals have interviewed for the questionnaire survey. Out of 384 tested samples, 262 (68.2%, 95% CI: 63.5% to 72.9%) were found positive by Modified Agglutination Test/MAT. The Chi-square analysis revealed that it was only presence of cat ( $\chi^2=8.698$ ,  $P=0.003$ ) and presence of wild felids ( $\chi^2=5.225$ ,  $P=0.022$ ) that showed statistically significant ( $P<0.05$ ) association with seropositivity of camel toxoplasmosis. According to both univariable and multivariable logistic regression analysis, it is only exposure to cat has statistically significant association ( $P=0.014$ ) with seropositivity of camel toxoplasmosis. Out of the 56 herds in which blood sample is collected, 54 herds (96.4%, 95% CI: 91.5% to 100%) were found at least with one seropositive animal. The questionnaire survey revealed that all (100%) of the respondents had no knowledge about toxoplasmosis and its method of transmission to human. Finally, this study demonstrated that camel toxoplasmosis is highly prevalent in the study area. Hence, it is suggested that there is a need for prevention and control of the disease as well as raising public health awareness in decreasing the public health implication of the disease in the area.

**Key words:** Afar • Camel • Epidemiology • Ethiopia • Public awareness • Toxoplasmosis

### INTRODUCTION

Toxoplasmosis is a disease caused by *Toxoplasma gondii* which is an intracellular protozoan organism with large number of intermediate hosts including humans [1]. It is a global parasite with no known geographic boundaries [2]. This parasite causes high infection rates because of its broad host range and benign co-existence with the host. Felids, particularly the domestic cat, are its definitive host and the only animal species in which oocyst develop [3].

This parasite has a great importance in food safety, human health and animal husbandry [4]. *T. gondii* cause Toxoplasmosis which is an important zoonotic disease

and responsible for major economic losses in all classes of livestock through abortion, stillbirth and neonatal losses [5-7].

Camels are one of the important livestock resources in Ethiopia with the population estimated to be over one million. The arid and semi-arid areas of the country that constitutes more than 60% of the total area and home of 7.8 million pastoral and agro-pastoral communities [8] are suitable for camel production. The eastern and southern parts of the country, namely; Afar, Somali and Borena are the major areas where camel husbandry is widely practiced. In these areas, the livelihood of the pastoral communities is certainly ensured by dromedaries [9,10].

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Camels acquire *T. gondii* infection through ingestion or inhalation of sporulated oocysts that are shed by cats or wild felids in the environment [11]. The prevalence of *T. gondii* infection in camels varies widely from area to area depending on the different localities of the globe [12] ranging from 3.12% in Iran [13] to 90.9% in Turkey [14].

Even though a number of investigations have been carried out in many countries of the world, little is known on the epidemiology and public health importance of camel toxoplasmosis in Ethiopia.

The objective of this study was to determine epidemiology of camel toxoplasmosis and assess awareness and practice of the community towards zoonotic importance of the disease in central zone of Afar region, Ethiopia.

### Specific Objectives:

- To determine sero-prevalence of toxoplasmosis in camels in the study area,
- To identify risk factors conducive for transmission and distribution of camel toxoplasmosis in the study area,
- To assess awareness and practice of the community towards zoonotic importance of the disease

### Methods

**Description of Study Area:** Afar regional state is located in the Great Rift Valley, comprising semi-arid range land in north eastern Ethiopia. According to regional estimates the livestock population of Afar is about 10.12 million Tropical Livestock Unit/TLU and out of this about 859,580 (8.5%) are camels. The Afar Regional State has five administrative zones, which are further subdivided into 32 districts. Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. The population of the region is estimated to be about 1.2 million of which 90% are pastoralists and 10% agro-pastoral [19]. This study was conducted in Asayita district of central zone of Afar region of north eastern Ethiopia.

**Study Population:** The approximate camel population in Asayita district is 11981 which were considered as study population for the sero survey and 56 individuals were interviewed for the questionnaire survey. Camels which are above six months of age with no history of vaccination against toxoplasmosis were included in the study. Individual sampled camel's history such as sex, age, production system, body condition score etc were recorded.

**Study Design:** A cross-sectional study design was conducted to determine the prevalence of toxoplasma infection in camels in the selected district and the potential risk factors associated with the sero-positivity. The study was conducted in the pastoral and agro-pastoral areas of Asayita district. Pastoralist association (PA) is the lowest administrative unit within the district that was considered during the study. Accordingly, four PAs from the district were randomly selected.

**Sampling Methods:** There are 13 PAs in Asayita district in which about 30% of the PAs in the district were included in the study on the basis of feasibility and affordability. Hence, four PAs were selected randomly. Multi-stage cluster sampling technique was used in this study by considering PAs as primary units, camel herds found in each PAs as secondary units and selected camel herds as tertiary units. Cluster sampling is the suitable method for this study as constructing sample frame for random sampling is not possible in pastoral production system. The formula to determine cluster size is as follows [20]:

$$g = \frac{1.962 (n \times Vc + p (1-p))}{nd^2} \text{ then total sample size (TS) } = g \times n$$

Where g = Number of cluster, p = prevalence, n = number of animal per cluster or herd, Vc = variation between cluster and d = precision level. However, to apply the formula there is no information about the variation between clusters (Vc) in the study areas. Therefore, it is necessary to look for other alternatives. Since there is no previous year's prevalence of toxoplasmosis in the districts, the average expected prevalence is assumed to be 50% for the areas within 95% Confidence Interval (CI) at 5% desired accuracy. Hence, the formula used by Thrusfield [20] to calculate sample size (n) is as follows:

$$n = \frac{1.962 \times P_{ex} \times (1-P_{ex})}{d^2}$$

Where n = sample size, d = desired absolute precision (0.05),  $P_{ex}$  = expected prevalence (50%), thus the desired sample size for  $P_{ex} = 0.5$  is  $n = 384$ . Therefore, 384 serum samples were collected from Asayita district. A study by Demeke [21] reported the average herd size in the pastoralist area to be 14 animals, hence,  $384/14 \sim 28$  herds (Clusters) are required in which all camels in a given herd are expected to be sampled. However, in this study a

two-stage cluster sampling at herd level is planned and the number of herds is doubled to get the required sample size. Hence, a total of 56 (28x2) herds were randomly selected to get the required sample size.

Since there is no recorded data of the camel population in each PAs of the district, the total sample size were distributed equally to all selected PAs. Finally, individual camels from each randomly selected herd were sampled proportionally based on the number of camels found in that herd using the systematic random sampling technique on the spot.

**Study Methodology:** A sample collection format was also prepared to record the history of the individual camels such as age, sex, parity, body condition score, physiological status, contact with cats and other herd characteristics while taking blood. Age groups of camels were classified as young (6 months-4 years), adult (Above 4 years). Herd size was classified into three categories as small (Up to 10 camels in a herd), medium (11-20 camels) and large (More than 20 camels in the herd) by considering both the minimum and maximum herd size presented in the study areas. Parity was also be categorized into three classes as no parturition, single parity (Primiparous) and greater than one (Pluriparous). Moreover, body condition score of the camels during sampling were evaluated and recorded based on the characteristics set by Faye and Bengoumi [22]. According to these authors, body condition score of dromedaries range from 5 (Excellent) to 0 (Very poor).

**Laboratory Work:** About 6-8 ml blood sample were collected from jugular vein of each camel of selected herds using plain vacutainer tubes. The collected blood samples were allowed to clot at room temperature. Then, serum was separated from clotted blood by decanting to plastic criovials. Separated sera were stored at - 20 °C until laboratory test is performed by using MAT (Modified Agglutination test).

**MAT Laboratory Procedure:** *Toxoplasma gondii*-specific IgG antibodies in camel sera were detected by the modified agglutination test (MAT, Toxo screen DA, bioMérieux® SA, Leon, France) following the procedure described by Dubey and Desmonts [23]. Briefly, the serum samples were diluted 1:40 and 1:4000 using phosphate buffer saline (PBS, PH=7.2) and 25µl of diluted sera were placed in U- bottom well of microtitre plate. Sera samples were treated with 0.2 M 2-mercaptoethanol to remove non-specific IgM or IgM-like substances. Clear agglutination

above half of the well and sedimentation of antigen at the bottom of the well were recorded as positive and negative results, respectively.

**Questionnaire Survey:** One individual from each sampled herd was interviewed to assess the awareness and practice of the community towards zoonotic importance of the disease. Issues such as knowledge about the disease and its transmission methods, camel products consumption behavior, birth and/or abortion materials handling and disposing mechanisms were interviewed.

**Data Management and Statistical Analysis:** The data were summarized, cleaned and compiled after coded data are stored in Microsoft Excel 2007 spread sheet and transferred to SPSS® Version 20 for statistical analysis. Descriptive and analytic statistics were computed using software SPSS® Version 20. Logistic regression and Chi-square test ( $\chi^2$ ) were employed to see the association of risk factors with that of seropositivity to *Toxoplasma* antibody. The degree of association were computed using Odds ratio (OR) and 95% confidence interval (CI). Odds ratio (OR) is used to indicate the degree of risk factor association with the disease occurrence signified by 95% confidence intervals. Odds ratio is the ratio of the odds of disease occurring among individuals exposed to a variable and the odds of the disease occurring among individuals not so exposed [20].

## RESULTS

**Animal Level Seroprevalence:** A total of 384 sera sample were collected from 56 camel herds with no previous history of vaccination against toxoplasmosis. Out of 384 tested samples, 262 (68.2%, 95% CI: 63.5% to 72.9%) were found positive by MAT. The Chi-square analysis revealed that it was only presence of cat ( $\chi^2=8.698$ ,  $P=0.003$ ) and presence of wild felids ( $\chi^2=5.225$ ,  $P=0.022$ ) that showed statistically significant ( $P<0.05$ ) association with seropositivity of camel toxoplasmosis than the other risk factors considered during the study (Table 1).

According to both univariable (OR: 0.56, 95% CI: 0.35-0.89) and multivariable (OR: 1.78, 95% CI: 1.12-2.81) logistic regression analysis, it is only exposure to cat that has statistically significant association ( $P=0.014$ ) with seropositivity of camel toxoplasmosis (Table 2).

**Herd Level Seroprevalence:** Out of the 56 herds in which blood sample is collected, 54 herds (96.4%, 95% CI: 91.5% to 100%) were found at least with one seropositive animal.

Table 1: Animal level seroprevalence of camel toxoplasmosis in Asayita district

Risk factor	Category	No tested	Positive (%)	Chi-square	P-value
Production system	Pastoral	268	189 (70.5)		
	Agro- pastoral	116	73 (62.9)	2.152	0.142
Sex	Male	72	49 (68.0)		
	Female	312	213 (68.3)	.001	0.972
Age	≤4 years	96	65 (67.7)		
	>4years	288	197 (68.4)	0.160	0.899
Parity*	No parturition	42	27 (64.3)		
	Primiparous	68	47 (69.1)	0.454	0.797
	Pluriparous	202	131 (64.8)		
Herd size	≤10	98	67 (68.4)		
	11-20	156	106 (68.0)	0.010	0.995
	>20	130	89 (68.5)		
Physiological status*	Heifer	36	23 (63.8)		
	Dry	32	23 (71.9)	1.108	0.775
	Lactating	162	108 (66.7)		
	Pregnant	82	51 (62.2)		
Reproductive problems*	No problems	75	49 (65.4)		
	Abortion	89	62 (69.7)	2.771	0.428
	Still birth	71	49 (69.0)		
	Neonatal mortality	77	45 (58.4)		
Presence of cat	Yes	209	156 (74.6)		
	No	175	106 (60.6)	8.698	0.003
Presence of wild felids	Yes	278	199 (71.6)		
	No	106	63 (59.4)	5.225	0.022
Body condition score	Good	92	66 (71.7)		
	Fair	178	111 (62.4)	5.461	0.065
	Poor	114	85 (74.6)		

\*Some of the values does not sum to 384 because some of the variables are only for she camels; Variables with P<0.05 are highlighted in bold

Table 2: Logistic regression analysis of risk factors associated with seropositivity

Risk factor	Category	Univariable		Multivariable	
		OR (95% CI)	P-value	OR (95% CI)	P-value
Production system	Pastoral	-			
	Agro- pastoral	0.75 (0.46-1.21)	0.377	1.36 (0.85-2.19)	0.201
Sex	Male	-			
	Female	1.19 (0.67-2.10)	0.555	0.85 (0.48-1.50)	0.571
Age	≤4 years	-			
	>4years	0.96 (0.57-1.60)	0.865	1.04 (0.62-1.73)	0.885
Parity	No parturition	-			
	Primiparous	1.09 (0.17-7.06)	0.931	0.92 (0.60-1.42)	0.721
	Pluriparous	0.85 (0.47-1.54)	0.585		
Herd size	≤10	-			
	11-20	1.05 (0.59-1.88)	0.871	0.98 (0.73-1.30)	0.886
	>20	1.00 (0.60-1.68)	0.999		
Physiological status	Heifer	-			
	Dry	0.85 (0.12-5.74)	0.864	1.05 (0.75-1.47)	0.777
	Lactating	0.67 (0.27-1.67)	0.391		
	Pregnant	0.79 (0.45-1.38)	0.409		
Reproductive problems	No problems	-			
	Abortion	0.69 (0.29-1.64)	0.405	1.11 (0.88-1.41)	0.385
	Still birth	0.62 (0.32-1.19)	0.150		
	Neonatal mortality	0.63 (0.32-1.24)	0.179		
Presence of cat	Yes	-			
	No	0.56 (0.35-0.89)	0.014	1.78 (1.12-2.81)	0.014
Presence of wild felids	Yes	-			
	No	0.69 (0.42-1.14)	0.144	1.48 (0.90-2.42)	0.118
Body condition score	Good	-			
	Fair	1.16 (0.61-2.20)	0.659	0.92 (0.68-1.25)	0.583
	Poor	1.71 (1.00-2.93)	0.051		

Table 3: Herd level seroprevalence of camel toxoplasmosis in Asayita district

Risk factor	Category	No tested	Positive (%)	Chi-square	P-value
Production system	Pastoral	39	39 (100)	4.758	0.029
	Agro pastoral	17	15 (88.2)		
Herd size	≤10	14	13 (92.9)	1.262	0.532
	11-20	23	22 (95.6)		
	>20	19	19 (100)		
Reproductive problems	Yes	54	53 (98.2)	12.982	0.000
	No	2	1 (50.0)		
Presence of cat	Yes	37	37 (100)	4.039	0.044
	No	19	17 (89.5)		
Presence of wild felids	Yes	40	40 (100)	5.185	0.023
	No	16	14 (87.5)		
Water	Clean	4	3 (75.0)	5.744	0.017
	Contaminated	52	51 (98.0)		
Exposure to flood	Yes	53	52 (98.1)	8.153	0.004
	No	3	2 (66.7)		

Variables with P<0.05 are highlighted in bold

According to the chi-square test, herd level risk factors such as production system, reproductive problems, presence of cat, presence of other wild felids, drinking water for camels and exposure to floods have statistically significant association with the herd level seropositivity (Table 3).

**Questionnaire Survey:** In addition to the serological survey, awareness and practice of the community towards zoonotic importance of the disease was assessed using a structured questionnaire. A total of 56 respondents (One individual from each sampled herd) were interviewed. Out of the 56 respondents, 49 (87.5%) were male, 7 (12.5%) were female and again 47 (83.9%) of them were illiterate and 9 (16.1%) were literate and 39 (69.6%) were pastoralists and 17(30.4%) were agro pastoralists.

According to the questionnaire survey, all respondents encountered reproductive problems such as abortion, still birth and neonatal mortality cases at least once per year in their herd. All (100%) of the respondents had no knowledge about toxoplasmosis and its method of transmission to human. In addition, only 67.8% of the respondents had cat in their home, around 82.1% drink untreated water, 37.5% had ever consumed raw meat and all (100%) consume raw milk, handle aborted fetus with bare hands and dispose birth or aborted materials by throwing it in the field.

## DISCUSSION

A total of 384 sera sample were collected from 56 camel herds with no previous history of vaccination against toxoplasmosis.

**Animal Level Seroprevalence:** Out of 384 tested samples, 262 (68.2%, 95% CI: 63.5% to 72.9%) were found positive by Modified Agglutination Test/MAT. This finding is in agreement with 67% seroprevalence using LAT in Sudan [11] and 66.7% using ELISA in Egypt [24]. When compared with this study finding, much lower seroprevalence were reported earlier in which 3.12% in Iran [13] 6.5%, 16% and 13.1% in Saudi Arabia [25-27] 20% in Sudan [28] 22.4% in United Arab Emirates [29] and (17.4 - 31.4%) in Egypt [12, 30,31] and also relatively lower seroprevalence were recorded in Sudan [32] which is 51.3% and in Egypt [33,34] 46%-54.2%. However, much higher seroprevalence was recorded in Turkey (90.9%) by Utuk *et al.* [14]. The variation in seroprevalence between the present study and the above reports might be due to the difference in climatic conditions [35] farming and management practices [36] sample size [28] and sensitivity difference in the serological tests used [25, 35].

Moreover, the present finding is higher than the previous report of 49.6% using DAT and 40.5% using ELISA tests [18] in Ethiopia. This difference could be due to the presence of high number of cats and other wild felids in the present study area than the aforementioned one. In the present study area, there are 2-3 cats per house hold including inside restaurants and a number of stray cats in the towns; there is a tendency of disposing wastes including the cat feces, food leftovers, slaughter byproducts to rivers and flood lines by the residents which have the potential to contaminate water bodies, ponds, vegetations found around the river basins and flood lines. In this area, there is continuous movement of camels from one area to another area searching for feed

and water. The camels in the present study inhabit the areas following river basins and flood lines which are already soiled by the contaminated flood and use it as a source of drinking water and feed. Even though camels are browsers (Lower chance of acquiring oocyst of the parasite), most of the time they consume whatever they found green around the river basin as far as it is palatable by them and due to serious shortage of water in the area, camels drink whatever type of water along their way which increases the probability of exposure to the parasite. Hence, a collective effect of all this conditions and the sensitivity difference of the type of tests employed made the variation of seroprevalence in both studies.

Risk factors such as production system, sex, age, herd size, parity, physiological status, reproductive problems, presence of cat, presence of wild felids and body condition score were considered during the statistical analysis. Chi-square analysis revealed that it was only presence of cat ( $\chi^2=8.698$ ,  $P=0.003$ ) and presence of wild felids ( $\chi^2=5.225$ ,  $P=0.022$ ) that showed statistically significant ( $P<0.05$ ) association with seropositivity of camel toxoplasmosis than the other risk factors considered during the study. Exposure to cats and wild felids had statistically significant association with seropositivity in this study which is in agreement with the previous finding in Ethiopia [18]. Even though it was not a statistically significant association, relatively higher seroprevalence was recorded in pastoral production system (70.5%), she-camels (68.3%), adult ( $>4$  years) (68.4%), primiparous (69.1%), large ( $>20$ ) herd size (68.5%), dry she-camels (71.9%), abortive camels (69.7%) and camels with poor body condition (74.6%) than the other categories of risk factors.

According to univariable logistic regression analysis, it is only exposure to cat that has statistically significant association (OR: 0.56, 95% CI: 0.35-0.89,  $P=0.014$ ) with seropositivity of camel toxoplasmosis. The univariable logistic regression analysis showed that those camels exposed to cat have 0.56 times more chance of acquiring the parasite than the camels of not exposed. In addition, the multivariable logistic regression analysis revealed that it is presence of cat (OR: 1.78, 95% CI: 1.12-2.81,  $P=0.014$ ) that has significant association with seropositivity than the other risk factors compared during the analysis.

**Herd Level Seroprevalence:** Out of the 56 herds in which blood sample is collected, 39 (69.6%) were from pastoral and the other 17 (30.4%) were from agro-pastoral production system in which 100% of the pastoral and

88.2% of the agro-pastoral herds were found at least with one seropositive animal. This 100% possibility of seropositivity of camels in pastoral production system but 88.2% in agro-pastoral is due to some of the herd level risk factors such as source of water and exposure to flood are limited in the agro-pastoral production system than the pastoral. Totally, out of the 56 herds sampled, 54 herds (96.4%, 95% CI: 91.5% to 100%) were found at least with one seropositive animal in their group.

According to the chi-square test, herd level risk factors such as production system ( $\chi^2=4.768$ ,  $P=0.029$ ), reproductive problems ( $\chi^2=12.982$ ,  $P=0.000$ ), presence of cat ( $\chi^2=4.039$ ,  $P=0.044$ ), presence of other wild felids ( $\chi^2=5.185$ ,  $P=0.023$ ), drinking water for camels ( $\chi^2=5.744$ ,  $P=0.017$ ) and exposure to floods ( $\chi^2=8.153$ ,  $P=0.004$ ) have statistically significant association with the herd level seropositivity. This chi-square test revealed that the camels has a possibility to acquire the parasite as a herd when they are drinking contaminated water by cat feces and other wastes and when exposed to floods coming from different contaminated areas.

**Questionnaire Survey:** In addition to the serological survey, awareness and practice of the community towards zoonotic importance of the disease was assessed using a structured questionnaire. A total of 56 respondents (One individual from each sampled herd) were interviewed. Out of the 56 respondents, 49 (87.5%) were male, 7 (12.5%) were female and again 47 (83.9%) of them were illiterate and 9 (16.1%) were literate and 39 (69.6%) were pastoralists and 17(30.4%) were agro pastoralists.

According to the questionnaire survey, all respondents encountered reproductive problems such as abortion, still birth and neonatal mortality cases at least once per year in their herd. All (100%) of the respondents had no knowledge about toxoplasmosis and its method of transmission to human. In addition, only 67.8% of the respondents had cat in their home, around 82.1% drink untreated water, 37.5% had ever consumed raw meat and all (100%) consume raw milk, handle aborted fetus with bare hands and dispose birth or aborted materials by throwing it in the field. This questionnaire survey showed that, the community has no awareness about the disease in animals and its zoonotic importance in the study area. In addition, the community is highly exposed to the disease because of high probability of acquiring the disease from different sources such as untreated water, raw meat, unpasteurized milk and handling birth materials in bare hand [13, 35] which are the major means of transmission of the disease to human being.

## CONCLUSION

Exposure to cat and other wild felids was found as the major means of acquiring the parasite and conditions such as production system, drinking contaminated water and exposure to flood were also source of infection of camels at herd level. The questionnaire survey showed that, the community has no awareness about the disease in animals and its zoonotic importance. In addition, the community is highly exposed to the disease because of high probability of acquiring the disease from different sources such as untreated water, raw meat, unpasteurized milk and handling birth materials in bare hand. Therefore, prevention and control measures should be employed in camels to decrease the impact of the disease by limiting the associated risk factors and sources of infection and transmission. Awareness creation to the community about preventing the disease risk factors and means of transmission as well as imparting health education to prevent the zoonotic implication of the disease is important.

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