

SEROLOGICAL SURVEY OF CAPRINE TOXOPLASMOSIS IN ÉTHIOPIA: PREVALENCE AND RISK FACTORS

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Summary:

The study was conducted to determine the prevalence and risk factors of toxoplasmosis in goats in Southern and central Ethiopia between October 2005 and May 2006. A total of 641 goats sera were tested using Modified Direct Agglutination Test (MAT), of which 480 (74.8 % CI: 71.3, 78.2) were found to be positive. The highest prevalence was recorded in South Omo zone (82 %) while the lowest was observed in East Shewa zone (62.2 %). The study revealed that goats raised in southern Ethiopia are at a greater risk of acquiring *T. gondii* infection (OR = 2.55, CI: 1.726, 3.776; $p = 0.000$) than those which are raised in central Ethiopia. The prevalence of anti *T. gondii* antibody was significantly higher in older goats than in kids (OR = 2.33, CI: 1.490, 3.655; $p < 0.0002$) and in females than in males ($p < 0.0007$; OR = 0.68, CI: 0.542, 0.849). No significant difference was observed among goats kept under various husbandry practices. The high prevalence of toxoplasmosis in Ethiopian goats suggests a high risk of human infections. Further epidemiological investigation, isolation and genotyping of *T. gondii* are planned.

KEY WORDS : *Toxoplasma gondii*, goat, MAT, Ethiopia.

Résumé : ÉTUDE SÉROLOGIQUE DE LA TOXOPLASMOSE CAPRINE EN ÉTHIOPIE : PRÉVALENCES ET FACTEURS DE RISQUE

Cette étude a été réalisée pour déterminer la séroprévalence et les facteurs de risques de la toxoplasme caprine dans le sud et le centre de l'Éthiopie. Entre octobre 2005 et mai 2006, sur 641 chèvres testées par une technique d'agglutination directe haute sensibilité, 480 (74,8 % IC : 71,3, 78,2) ont été trouvées positives. La plus forte séroprévalence a été observée dans la partie sud de l'Omo (82 %) et la plus faible dans l'Est de Shewa (62,2 %). Les chèvres élevées dans le sud de l'Éthiopie sont plus à risque d'acquies une infection toxoplasmique que celles élevées en Éthiopie centrale. La prévalence des anticorps anti-*T. gondii* est significativement plus élevée chez les chèvres âgées que chez les chevreaux (OR = 2,33, IC : 1,490, 3,655; $p < 0,0002$) et chez les femelles que chez les mâles ($p < 0,0007$; OR = 0,68, IC : 0,542, 0,849). Aucune différence significative n'est observée en fonction des conditions d'élevage. La forte prévalence de la toxoplasme chez les chèvres en Éthiopie suggère un risque élevé de toxoplasme humaine. D'autres recherches épidémiologiques ainsi que des essais d'isolement et de génotypage des souches de toxoplasmes seront réalisés ultérieurement.

MOTS CLÉS : *Toxoplasma gondii*, chèvre, MAT, Éthiopie.

Toxoplasmosis is a true zoonotic disease caused by infection with an obligate intracellular protozoan parasite, *Toxoplasma gondii*. In most areas of the world, toxoplasmosis has been found to be highly prevalent in meat producing animals (Dubey, 1980; Tenter *et al.*, 2000). The seroprevalence of toxoplasmosis in goats varies among different countries and regions within a country, and up to 77 % prevalence has been reported (Tenter *et al.*, 2000). Several serological surveys have been carried out for antibodies to *T. gondii* in different species of animals in various parts of Africa (Deconinck *et al.*, 1996). In Ethiopia where over 18 million goats are

raised under various agro-ecological zones, however, only few studies have been carried out (Bekele & Kasali, 1989; Tamiru *et al.*, 2004; Tilaye & Getachew, 2002) that were geographically limited to central Ethiopia. Results of the previous studies have shown that prevalence of anti *T. gondii* antibodies ranged from 11.9 % to 35 %. In that country, the causes of most abortions, stillbirths and neonatal mortalities in flocks of goats remain unexplored and the relationship with seroprevalence of toxoplasmosis has not been investigated. The objectives of this study were, therefore, to determine seroprevalence and some risk factors of caprine toxoplasmosis in Ethiopia in specified study areas.

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MATERIALS AND METHODS

• Study areas

The study was conducted in southern Ethiopia (North Omo and South Omo) and central Ethiopia (East Shewa zone) from October 2005 to May 2006 (Table I and Fig. 1).

Region of sampling*	Altitude	Annual temperature	Rainfall	Climate	Number of goats tested
East Shewa ¹ 7° 9' N, 38° 7' E	1,650 m	12.7-27° C	760.9 mm	Semi-arid	209
North Omo ² 6° N, 36°-38° E	> 1,650 m		> 1,000mm	Warm and moist	176
South Omo ³ 4°-5° N, 36°-38° E	391-1,500 m	11.2-32° C	275.6 mm	Semi-arid	256

* : numbers refer to the place on Figure 1.

Table I. – Location and climatic conditions of the study areas.



1, 2 and 3: Study areas.

Fig. 1. – Map of Ethiopia showing study sites.

. Study design and sampling strategy

Cross-sectional study was conducted in different age and sex groups of goats. The goats included in this study were local breeds as Woyto-Guji and Boran-Arsi goats. Multistage and cluster sampling technique was employed to collect sera from study subjects.

. Serum Collection

Blood was collected by jugular vein puncture from 641 goats and the sera separated were stored at -20° until serological test was performed.

. Antigen preparation

The antigen for Modified Direct Agglutination Test (MAT) was produced in the peritoneal cavity of Swiss white albino mice as described by Desmonts & Remington (1980). Formalin fixed whole tachyzoites of *T. gondii* harvested from peritoneal cavity of mice co-infected with RH strain of *T. gondii* and TG 180 mouse sarcoma cells was employed as antigen. The final concentration of

antigen was adjusted to about 15,000 tachyzoites/ μ l based on the recommendation of OIE (2004).

. Serological analysis

The caprine sera were analyzed by MAT using "U" bottom micro plates. The sera were tested at dilutions of 1/20, 1/40 and 1/400 to avoid the prozone phenomenon. 0.2 M (14 ml/liter) β mercaptoethanol diluted in PBS was incorporated into the test system to inhibit non-specific agglutination due to IgM antibodies (Desmonts & Remington, 1980). Positive and negative controls were also tested alongside the test sera as references. Agglutination forming large carpet covering greater than 50 % of the bottom of the wells was considered positive. Sedimentation forming a point or a ring at the bottom of the wells of the plates was taken as negative reaction. Sera showing visible carpet of agglutination at dilution of 1/20 or higher were assumed positive to *Toxoplasma* infection (Dubey, 1983). Sera, which have shown doubtful reaction, were retested.

. Data Analysis

Seroprevalence of toxoplasmosis and confidence intervals were determined using standard epidemiological methods. Association between the outcome variable (prevalence of toxoplasmosis) and the explanatory variables (risk factors) was analyzed by chi-square (χ^2) tests. Backward Logistic regression (at significance level of 0.15) was used to analyze the significance of factors that were significant with univariate analysis.

RESULTS AND DISCUSSION

Out of 641 caprine sera tested 480 (74.9 %, CI: 71.3 %, 78.2 %) were found to be positive to anti *T. gondii* IgG antibodies. The seroprevalences were 82 %, 79.5 % and 62.2 % in South Omo, North Omo and East Shewa zones, respectively. The world seroprevalence of toxoplasmosis in goats has been shown to have great variability, Tenter *et al.*, 2000 indicated values from 0 % to 77 %. In Africa, seroprevalence ranges from 6.4 % (Djibouti) to 81.6 % (Nigeria) for goats (Chantal *et al.*, 1994; Arene, 1984). Geographical

Country	Test used	Number of animals tested	% positive	References
Africa				
Ethiopia	MAT	60	26.7	Tamiru <i>et al.</i> , 2004
Ethiopia	MAT	93	35	Tilaye & Getachew, 2002
Mali	DAT	103	45	Quilici <i>et al.</i> , 1976
Ethiopia	IHAT	753	11.6	Bekele & Kassali, 1989
Botswana	IHAT	1,799	30	Sharma <i>et al.</i> , 2003
Sudan	IHAT	134	63	Zain Eldin <i>et al.</i> , 1985
Tanzania	IHAT	79	14.2	Connor & Halliwell, 1985
Nigeria	DT	300	81.6	Arene, 1984
Tunisia	DT	85	60	BenRachid & Blaha, 1979
Uganda	ELISA	784	31	Bisson <i>et al.</i> , 2000
Ghana	ELISA	526	26.8	Van den Puije <i>et al.</i> , 2000
Zimbabwe	LAT	211	7.5	Pandey <i>et al.</i> , 1992
Djibouti	IFAT	1,081	6.4	Chantal <i>et al.</i> , 1994
Ivory coast	IFAT		68	Deconinck <i>et al.</i> , 1996
The Americas				
USA	MAT	99	65	Patton <i>et al.</i> , 1990
USA	IHAT	99	55	Patton <i>et al.</i> , 1990
Venezuela	IHAT	438	6.3	Nieto & Melendez, 1998
Brazil	ELISA	174	3.7-71.8	Figueiredo <i>et al.</i> , 2001
Brazil	IFAT	213	40.4	Silva <i>et al.</i> , 2003
Brazil	LAT	439	28.93	Pita Gondim <i>et al.</i> , 1999
Others				
Iran	LAT/IHAT	530	19.25	Hashemi-Fesharki, 1996
Iran	ELISA	200	15	Ghazaei, 2005
Italy	IFAT	2,445	12.3	Masala <i>et al.</i> , 2003
Czech	IFAT	129	66	Slosarkova <i>et al.</i> , 1999
Malaysia	MAT	400	35.2	Dorny <i>et al.</i> , 1993
Netherlands	MAT		5-90	Antonis <i>et al.</i> , 1998
Sri Lanka	MAT	139	22.3	Dorny & Van Aken, 1992
Afghanistan	IHAT	171	31.6	Kozojed <i>et al.</i> , 1976
New Zealand	LAT	298	37	Opel <i>et al.</i> , 1991
Indonesia	LAT	160	47.5	Matsuo <i>et al.</i> , 1996
Over 40 countries	Various tests		0-77	Tenter <i>et al.</i> , 2000

Table II. – Seroprevalence of *T. gondii* infections in goats from previous reports.

variation in prevalence occurs not only among different continents or countries but also within a given country. The current prevalence is higher than the prevalence of caprine toxoplasmosis reported in Ethiopia and elsewhere (Table II). The greater prevalence of toxoplasmosis in the current study could emanate from difference in the serological tests employed, the sample size and geographical variability. In Ethiopia, previous studies were performed on fewer numbers of animals and were limited to the cooler areas of central Ethiopian. Our study was based on relatively larger number of animals inhabiting various regions with different agro ecologies. In addition, we employed Modified Agglutination Test, which was found to be superior in performance to most other serological tests (Klun *et al.*, 2005). Previous workers reported seropositivity at dilutions > 1/32 but we reported starting from 1/20 dilutions. However, our result is comparable with the reports of Slosarkova *et al.* (1999); Arene (1984); Zain Eldin *et al.* (1985); Antonis *et al.* (1998); Patton *et al.* (1990); Figueiredo *et al.* (2001); Ben Rachid & Blaha (1979) and Klun *et al.* (2005).

In support of the mentioned local variations, our study showed that goats from southern Ethiopia are at higher risk of *T. gondii* infection than those in the Rift valley region. Southern Ethiopia has warm and moist weather while the central region is characterized by its hot and dry climate. Moreover, the selected study sites from southern Ethiopia are located at the vicinity of Mago and Nech Sar national parks where large variety of wild fauna including felids are found. Domestic animals share the same environment with these wild animals whenever there is shortage of grazing land. Both situations have positive impact on the persistence and dissemination of oocysts of *T. gondii*. The influence of the environment and wild animals on the epidemiology of toxoplasmosis has been well documented (Frenkel, 1990; Tenter *et al.*, 2000; Dubey, 2004).

The results of multivariate analysis using logistic regression model indicated that gender, study site and age have significant effect on the prevalence of toxoplasmosis. There was statistically significant difference ($\chi^2 = 56.1$, $p < 0.0002$) in the prevalence of anti *T. gondii* antibodies among different age groups of goats. The

prevalence was lower in the younger goats (< 1 year) (57.37 %, CI: 48.1 %, 66.3 %) than in the yearlings (> 1 year) (83.8 %, CI: 80 %, 87.1 %). Older goats were at higher odds (OR = 2.33) of *Toxoplasma* infection than kids. This difference in prevalence among age group is partly due to cumulative effect of age. Older goats as they lived longer are more likely to encounter oocysts of *T. gondii* from the environment.

The significantly higher ($\chi^2 = 14.6$; OR = 0.68; P = 0.0007) prevalence of *T. gondii* infection in young females (80.5 %, CI: 75.8 %, 84.6 %) than in young males (74.5 %, CI: 65.5 %, 82 %) was consistent with some previous reports (Okoh *et al.*, 1981; Tamiru *et al.*, 2004). But it was not found by other authors (Tilaye & Getachew, 2002). The fact that female goats are kept for longer period of time for breeding and milk production purposes than males may have contributed for the relatively higher prevalence in does. Male animals are the ones that are frequently sold and only fewer bucks are found per flock. On agro-ecological basis, it was shown that goats raised in southern Ethiopian region are at greater risk of *T. gondii* infections ($\chi^2 = 26.5$; OR = 2.55; p = 0.000) than goats raised in the Rift valley region. The seroprevalence was 81 % (CI: 77, 84.6) in southern Ethiopia while it was 62.2 % (CI: 55.3, 68.8) for Rift valley area. The absence of difference on the basis of management can be explained by the apparent absence of variation in husbandry practices that could have prevented access of cats to the grazing areas. The grazing areas in all the cases were open and can allow the pasture to be contaminated with cat feces. The high prevalence of toxoplasmosis reported in this study is a good marker of high risk for human infections since goats are important sources of meat in most parts of Ethiopia. Further epidemiological investigations and isolation and characterization of the genotypes of *T. gondii* are underway.

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