

Seroprevalence of *Toxoplasma gondii* in Ruminants: First Experience in Meknes, Morocco

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Abstract: Toxoplasmosis is a zoonosis of increasing concern in both human and veterinary medicine which is due to an obligate intracellular, protozoan parasite *Toxoplasma gondii*. Seroprevalence of *T. gondii* in animals intended for human consumption in the city of Meknes in Morocco has never been established. The aim of our study was to determine the seroprevalence in sheep, cattle and goats slaughtered in Meknes. We collected blood samples from 133 bovines, 81 ovines and 45 goats in Meknes slaughterhouses. We used a modified version of the direct agglutination test to detect anti *T. gondii* Igg antibodies in collected sera. Bovine *T. gondii* seroprevalence values were stabilising at 7.5%, ovine *T. gondii* seroprevalence values were 7.4% while goat seroprevalence were 2.2%. This study showed seroprevalence of bovine, ovine and goat *T. gondii* infection in Meknes. More comprehensive studies on livestock toxoplasmosis are required for further analysis of the parasite reservoir for human infection.

Key words: *Toxoplasma gondii*, Toxoplasmosis, cattle, sheep, goat, Meknes Morocco, livestock animals, modified agglutination test

INTRODUCTION

Toxoplasma gondii infection is widely prevalent in humans and animals worldwide (Dubey and Beattie, 1988). The major risk factor for humans is consumption of undercooked meat containing infective *T. gondii* tissue cysts, accounting for up to 63% of human infections (Cook *et al.*, 2000). Toxoplasmosis also causes heavy economic losses to sheep industry worldwide (Dubey *et al.*, 1995; Belbacha *et al.*, 2004).

Despite its ubiquity, the prevalence of toxoplasmosis in meat animals is not yet well known in Morocco and the national average is not yet established either in humans or in animals where the objective of this study to determine seroprevalence of *T. gondii* infection in the sheep, cattle and goat for slaughter in Meknes.

MATERIALS AND METHODS

Blood sample collection: During the study period between September and November 2015 we were able to collect 80 Sheep, 134 Cattle and 45 Goats venous blood samples into sec tubing from living animals for slaughter or freshly slaughtered in abattoirs in the city of Meknes. The samples were centrifuged (2000 rpm/5 min) and the recovered sera were stored at -4°C. Two age groups were established based on dentition: young lambs, calves and kids of less than a year of age without central permanent incisors; ii. adult sheep, cattle and goat older than a year with central permanent incisors.

Modified Agglutination Test (MAT): The detection of anti-Toxoplasma antibodies was carried out with the Modified Agglutination Test (MAT) which relies on the

agglutination of infected sera incubated with whole tachyzoites extracts used as the antigen. Sera were treated with 2-mercaptoethanol to remove IgM and only detect the IgG. Test samples and positive control were deposited in the first column of wells of the microplate, all samples and controls were first diluted to the sixth and serial dilutions to the half were performed for each sample on one row of the microplate. The same amount of antigen diluted figured was distributed to all the wells of the plate. Homogenized each plate, once covered was allowed to incubate overnight at room temperature. The reading was taken the next morning. Positive samples produced agglutination that was graded while negative samples displayed precipitated tachyzoites at the bottom of the well the cut-off precision titer threshold for positive samples has been set to a dilution to the 1/6th. All data were entered on Excel and processed by SPSS Version 10 Software.

RESULTS AND DISCUSSION

Our study was to determine the seroprevalence of toxoplasmosis in sheep, cattle and goats for the first time in the city of Meknes in Morocco. The present investigation showed that *T. gondii* specific Igg antibodies were detected in 6 (7.4%) of 81 Sheep, in 10 (7.5%) of 133 Cattle and in 1(2.2%) of 45 goats. The prevalence of *T. gondii* is shown in Table 1.

The seroprevalence in the adult cattle 9.1% were lower than adult sheep 25.0%. When compared seroprevalence of adult cattle by sex it was found that females had greater prevalence than males 30.0 and 1.3%, respectively. A seroprevalence of 6.5% was observed in young sheep while it was only 2.2% in young goats. In addition seroprevalence noted in young sheep males was higher than among young females with the respective values 12.5 and 5.8% (Table 1).

To the best of the knowledge of the researchers of the presented study, this is the first report about the occurrence of anti-*T. gondii* IgG antibodies in sheep, cattle and goats slaughtered in the slaughter houses of Meknes in Morocco and intended for human consumption.

The modified agglutination test proposed by Desmonts and Remington (1980) (Modified Agglutination Test for MAT) was chosen because it is the most sensitive of comparative tests (Dubey *et al.*, 1995) while remaining specific for *T. gondii* infection. This test allows the detection and titration of IgG anti-*T. gondii*. It consists in the detection of these antibodies in a disease produced by direct agglutination reaction integers Toxoplasma formalin (figured antigen). The antigen is complete the test detects all types of antibodies against the parasite.

The percentage of seropositivity for *T. gondii* on sheep slaughtered in the city of Meknes was 7.4%. Recent studies have reported similar results 6.7% in Nigeria (Kamani *et al.*, 2010) 3.0% in North Eastern China (Wang *et al.*, 2011) Siliana and Kasserine (10.8%) Tunis (Gharbi *et al.*, 2013) and different seropositivity prevalences in Marrakech (27.6% by Sawadogo *et al.* (2005) and 30% by Belbacha Morocco, Ben-Arous and Sidi-Bouزيد (20.3 %), this can be explained by the use of different detection techniques *T. gondii*.

Our results show increased seropositivity with age, indicating that horizontal transmission by sporulated oocyst ingestion takes place, results that are comparable to those obtained by Gorman *et al.* (1999) in Chile. In addition, differences were found in seroprevalence by sex with female sheep showing a significantly higher percentage of seropositivity than that of male sheep. In a previous study, it was suggested that female animals are more susceptible than males to *T. gondii* infections (Puije *et al.*, 2000).

Table 1: *T. gondii* seroprevalence in cattle, sheep and goat according to age group and sex

Age group (sex)	Cattle		Sheep		Goat	
	n, positive/total	Prevalence (%); 95% CI	n, positive/total	Prevalence (%); 95% CI	n, positive/total	Prevalence (%); 95% CI
Young	0/23	-	5/77	6.5 (2.1;14.5)	1/45	2.2 (0.7;11.8)
Female	0	-	4/69	5.8 (1.6;14.2)	0	-
Male	0/23	-	1/8	12.5 (0.3;52.7)	1/45	2.2 (0.7;11.8)
Adult	10/110	9.1 (4.6;16.1)	1/4	25.0 (0.6;80.6)	0	-
Female	9/30	30 (14.7;49.4)	1/3	33.3 (0.8;90.6)	0	-
Male	1/80	1.3 (0.03;6.8)	0/1	-	0	-
Total	10/133	7.5 (3.7;13.4)	6/81	7.4 (2.7;15.4)	1/45	2.2 (0.7;11.8)
Female	9/30	30 (14.7;49.4)	5/72	6.9 (2.3;15.5)	0	-
Male	1/103	0.9 (0.02;5.3)	1/9	11.1 (0.3;48.3)	1/45	2.2 (0.7;11.8)

N = Number of animals; CI = Confidence Interval

No study on the seroprevalence of toxoplasmosis in goats slaughtered has been carried out in Morocco and few were those made in Europe and that has given the values that ranged from some animals affected to >50% (Antonis *et al.*, 1996; Masala *et al.*, 2003). The present survey showed that the seroprevalence of *T. gondii* in young was of 2.2% in concordance with what was noted in the Northeastern China 2.4% (Wang *et al.*, 2011).

Seropositivity of *T. gondii* in cattle is not high and in this study the frequency of bovine antibodies to *T. gondii* was 7.5% similar to the value found in Japan 7.3% (Matsuo and Husin, 2014). Further, studies estimated the bovine prevalence at 9.7% in Czech Republic (Bartova *et al.*, 2015).

In our study, female cattle were more infected than male which opposite to what Nematollahi and Moghddam (2008) found that male cattle were more infected than females. This finding is similar to that of Samad *et al.* (1993).

CONCLUSION

In conclusion, *T. gondii* seroprevalence in ruminants in Morocco has not reached any alarming levels yet. However, if we pay attention to the surprisingly high values reported by our geographic neighbors, a better characterization of *T. gondii* geographic distribution and the risk factors associated with *T. gondii* dissemination in Morocco is needed if we are to prevent occurrence of higher levels of livestock infection. Investigating factors like cats vicinity, history of abortion outbreaks and availability of local sanitary facilities should be a priority. How this translates to human health is unknown as there are no epidemiologic studies of human toxoplasmosis in Morocco. In the light of this study, it appears that veterinary vaccination programs should be especially enforced in sheep flocks of Southern Morocco to alleviate the economic and public health burdens caused by *T. gondii*.

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