

Seroprevalence of *Toxoplasma gondii* in Ruminants in Morocco

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Abstract: Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii* (*T. gondii*). Toxoplasmosis diagnosis is critical especially in cattle and sheep flocks with a history of abortion outbreaks. Hence, *T. gondii* has a major economic impact in rural areas. Prevalence studies of *T. gondii* infection in livestock animals in Morocco are scarce hence, the objective of the current study is to determine *Toxoplasma gondii* seroprevalence in bovines and ovines slaughtered in Northern and Southern Morocco. We collected 357 blood samples from 226 bovines and 131 ovines in Meknes (Northern Morocco) and Settat (Southern Morocco) 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 similar in Northern and Southern Morocco establishing at 7.5 and 8.5%, respectively. In contrast, ovine *T. gondii* seroprevalence values were 2.43 times higher in Southern than in Northern Morocco with values of 18.0% in Settat versus 7.4% in Meknes. Additional epidemiologic studies are required to characterize the factors associated with this geographic discrepancy in sheep and to evaluate whether this translates into human toxoplasmosis.

Key words: *Toxoplasma gondii*, toxoplasmosis, cattle, sheep, Morocco, livestock animals, modified agglutination test

INTRODUCTION

Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii* (*T. gondii*) mediating unremitting infections in humans and animals (Butcher *et al.*, 2011; Dubey, 2009). The parasite has a worldwide distribution and is transmitted through species at various stages of its life-cycle; food and water contaminated with oocysts dispersed by cats and other felines which are the definitive hosts of the parasite (Lass *et al.*, 2012) unpasteurized milk containing the tachyzoite stages of the asexual reproductive cycle of the parasite (Dubey, 1996; Dubey *et al.*, 2014; Riemann *et al.*, 1975; Sukthana, 2006); undercooked meat containing tissue cysts (Guo *et al.*, 2015) because consumption of undercooked meat is one of the known risk factors for human toxoplasmosis a disease particularly threatening in immunosuppressed

patients and to the health of pregnant women and their fetuses transplacentally from the mother to her fetus causing congenital toxoplasmosis or abortion (Boyer *et al.*, 2011). Toxoplasmosis is also an abortifacient in livestock (Dubey and Welcome, 1988; Owen and Trees, 1999). Hence, *T. gondii* infections have major economic and public health impacts especially in the rural areas of Morocco (Sawadogo *et al.*, 2005). However, toxoplasmosis epidemiologic data in slaughtered animals intended for human consumption in Morocco are still scarce. The current study was undertaken to determine *T. gondii* seroprevalence in cattle and sheep from two regions of Morocco; in Meknes an area of Northern Morocco and Settat located in the southern areas of the Kingdom. We focused our study on cattle and sheep because both are the main meats consumed by local populations.

MATERIALS AND METHODS

Blood sample collection: During the study period spanning between September and November 2015, we collected blood samples of bovine and ovine origin. Venous blood from living animals or freshly slaughtered was drawn into dry blood collection tubes. Whole blood was centrifuged at 2000 rpm for 5 min and recovered serum was stored at +4°C. Two age groups were established based on dentition; young lambs and calves of less than a year of age without central permanent incisors; adult sheep and cattle older than a year with central permanent incisors.

The modified agglutination test: The serologic detection of anti-toxoplasma IgG antibodies was kindly carried out in Riems, France using a modified agglutination test (Dubey and Desmonts, 1987) routinely performed in Dr. Dominique Aubert laboratories. This method relies on the agglutination of infected sera incubated with whole-tachyzoites suspensions used as the antigen. Briefly, sera were treated with 2-mercaptoethanol to denature IgM and only detect IgG antibodies. Samples were loaded in a U-shaped well microtiter plate. All samples and controls were first diluted 1:6 and two-fold serial dilutions were performed for each sample on one row of the microplate. A suspension of formalin-fixed whole-tachyzoites prepared in reims was then added to the wells. Each microplate was homogenized, covered and allowed to incubate overnight at room temperature. The reading was performed the next morning. Sera agglutinating the antigen at a dilution 1:6 or higher were considered positive for *T. gondii*-IgG antibodies with a threshold of positivity of 3 IU/mL. Negative samples displayed precipitated tachyzoites at the bottom of the well.

Statistical analysis: All data were treated in Excel and processed using SPSS Version 13 Software for statistical analysis. The differences in prevalence were determined using the chi square test only for groups that could be compared. Risk associated with these parameters was expressed as an Odd Ratio (OR) with a 95% Confidence Interval (CI). The significance threshold was set at $p \leq 0.05$.

RESULTS

We undertook this study to investigate *T. gondii* seroprevalence in Northern and Southern Morocco Meknes and Settat, respectively. We collected 226

bovine: blood samples including 133 from Meknes and 93 from Settat and 131 ovine blood samples including 81 from Meknes and 50 from Settat.

T. gondii seroprevalence values were similar in young and adult bovines slaughtered in Settat and Meknes establishing at 8.6 and 7.5%, respectively. In Settat (Southern Morocco) where slaughtered bovines were mostly young, anti *T. gondii* antibodies were detected in the serum of 3 heifers out of 19 tested corresponding to a seroprevalence of 15.8% and in 5 bull calves out of 64 tested corresponding to a seroprevalence of 7.5%. Statistical analysis using the Chi square test revealed that although, the presence of anti *T. gondii* antibodies in young bovine blood was associated with the gender this association was not statistically significant ($p > 0.05$, OR = 2.2, 95% and CI = 0.47-10.26). In Meknes (Northern Morocco) where slaughtered bovines were mostly adult, anti *T. gondii* antibodies were detected in the blood of 9 cows out of 30 corresponding to a seroprevalence of 30.0% and in 1 bull out of 80 corresponding to a seroprevalence of 1.3%. Statistical analysis of our data using the Chi square test revealed that the presence of anti *T. gondii* antibodies in adult cattle was associated with gender and this association was statistically significant ($p < 0.001$, OR = 33.8% and CI = 4.05-282.4). Indeed when data were sex-matched, we noted that female cattle are more susceptible to contracting toxoplasmosis than male cattle both in Meknes and Settat with values reaching 30% in cows in Meknes and 13.6% in heifers in Settat. The 2.2 fold difference observed between Meknes and Settat is not surprising as older animals tested in Meknes have had more risk of being in contact with *T. gondii* than the younger animals tested in Settat. Indeed in Meknes when female cattle were slaughtered it consisted only of cows whereas in Settat mainly heifers were slaughtered during the time of our study and the 3 cows we tested in Settat did not present with toxoplasmosis. *T. gondii* seroprevalence in male cattle was 7.7 times higher in Settat than in Meknes with values establishing at 7.0 and 0.9%, respectively. When data were age-matched it appeared that bull calves slaughtered in Meknes mainly account for this discrepancy with seroprevalence values of 7.8% in bull calves compared to 0.0% in bulls tested in Settat. These data highlight the fact that young cattle, especially heifers, originating from Settat and its areas have higher odds of presenting with toxoplasmosis than young cattle raised in Meknes and its areas.

On the other hand, *T. gondii* seroprevalence in ovines was 2.4% higher in Settat than in Meknes with values reaching 18.0% in Settat versus 7.4% in Meknes. When data were age-matched and sex-matched it

appeared that ewe lambs were 3.14 more susceptible to contracting toxoplasmosis in Settlat than in Meknes with values of 18.2 and 5.8%, respectively. However, this difference was statistically weak ($p > 0.05$, OR = 0.27, 95%, and CI = 0.78-0.98). In adult ram lamb and adult sheep, data could not be used to draw conclusive affirmations due to the small size of the studied population. Altogether, these results reveal higher odds of toxoplasmosis in slaughtered animals of Settlat both in cattle and sheep when compared to those slaughtered in Meknes but these observations need to be confirmed by increasing the size of the studied population.

DISCUSSION

This study is the first investigating *T. gondii* seroprevalence both in cattle and sheep intended for human consumption in different regions of Morocco. The modified agglutination test used in this study was first proposed by Desmonts and Remington (1980) and is routinely used by many laboratories; it was chosen because it is the most sensitive interspecies comparative test while remaining specific for *T. gondii* infection (Dubey *et al.*, 1995). This test allows both detection and titration of anti *T. gondii* IgG. The test detects IgG presence in the serum collected from infected animals; anti *T. gondii* IgG will trigger a direct agglutination reaction in positive samples if incubated with whole-tachyzoite suspensions used as the antigen. Because the antigen used is complete, the test has the ability to detect all immunoglobulines produced in response to the parasite when sera are not pretreated with 2-mercaptoethanol. It must be noted that unlike in sheep, *T. gondii* seropositivity in cattle is usually lower due to innate resistance of bovines to the parasite (Esteban-Redondo and Innes, 1997).

Firstly, *T. gondii* seroprevalence values obtained from bovine blood samples collected in slaughterhouses in Northern and Southern Morocco did not show any significant geographical difference while they showed that toxoplasmosis seroprevalence in cows from Meknes was 23 folds higher than in bulls. Other groups have previously reported similar results highlighting the fact that female animals are more likely to get protozoan infections when compared to males (Sukthana, 2006). These differences may be attributed to a reduction in immunity in female animals during pregnancy and lactation. In the present study, we report bovine anti *T. gondii* antibodies with a prevalence of 8.6% a value similar to previously reported values originating from Japan estimating bovine *T. gondii* prevalence at 7.3% (Matsuo *et al.*, 2014) 6.6% in Central Ethiopia (Bekele and Kasali, 1989) and 7.4% in Indonesia (Ichikawa-Seki *et al.*,

2015). It seems that cattle *T. gondii* seropositivity in Morocco is similar to values reported in various parts of the globe.

Secondly, our data suggest that ovines slaughtered in Settlat are more likely to be infected with *T. gondii* than those slaughtered in Meknes although, the small size of the population we studied does not allow us to test the significance of this difference. Increasing the sample size will therefore be enforced in future studies. It is worth noting that the ovine seroprevalence values of *T. gondii* we report in Southern Morocco (18.0%) are lower than prevalence values recently reported in sheep from Brazil where values reach 53.3% (Cosendey-KezenLeite *et al.*, 2014) or found by Morocco's closest geographic neighbors in Southern Spain (49.3%) (Garcia-Bocanegra *et al.*, 2013). In Tunisia results are even more alarming with recently reported values of 38.2% in young sheep and 73.6% in adult sheep (Boughattas *et al.*, 2014). However, in Northern Morocco in Meknes, *T. gondii* seroprevalence values (7.4%) are closer to the 6.7% found in another African country Nigeria (Kamani *et al.*, 2010). Older studies conducted about 10 years ago in Southern Morocco in Marrakech's slaughterhouses have reported prevalence levels of 27.6% and 30% in sheep (Belbacha *et al.*, 2004; Sawadogo *et al.*, 2005). The national prevalence values reported by these two studies are higher than the ones we report in the current research: this could be explained by different age distribution of the analyzed population where older animals generally have higher prevalences. Beside, sensitivities of the tests used to detect the parasite could also account for these differences (Nunes *et al.*, 2015). Sawadogo *et al.* (2005) performed serological ELISA tests while Belbacha *et al.* (2004), directly detected tissue cysts in brain samples collected from slaughtered sheep and the virulence was confirmed by direct inoculation of infected the brain samples in mice. In addition other spatial and the temporal considerations discussed below could also the account for these differences.

Indeed, the differences in *T. gondii* seroprevalence levels observed in ovines from Northern and Southern Morocco raise questions on the factors associated with higher dissemination of the parasite in Southern Morocco. Recent studies deciphering the complex routes of toxoplasmosis infection suggest that factors like cat's promiscuity, species and meteorological conditions can also explain the variability in *T. gondii* seroprevalence levels across areas and chronologically (Afonso *et al.*, 2013; Gotteland *et al.*, 2014; Lelu *et al.*, 2012). Hence, Studies including more samples are necessary.

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 the 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*.

ACKNOWLEDGEMENTS

We acknowledge Dr. Dominique Aubert's team for their help performing the modified agglutination test. We would also like to thank the "Office National de Securite Sanitaire des produits Alimentaires" (ONSSA) for authorizing blood sample collection from Morocco's municipal slaughterhouses.

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