



Short communication

Seroprevalence and correlates of *Toxoplasma gondii* infection in domestic sheep in Michoacán State, Mexico

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ABSTRACT

Toxoplasma gondii infection in sheep is of public health and economic importance. Seroprevalence of *T. gondii* infection and correlates were determined in 405 sheep from 7 farms in 4 geographical regions in Michoacán State, Mexico using the modified agglutination test (MAT). General sheep and environmental characteristics were obtained by a questionnaire. All sheep were raised in semi-extensive conditions in temperate climate. Antibodies to *T. gondii* were found in 121 (29.9%) of the 405 sheep with MAT titers of 1:25 in 46, 1:50 in 20, 1:100 in 7, 1:200 in 5, 1:400 in 7, 1:800 in 11, 1:1600 in 5, and 1:3200 or higher in 20. Seropositivity did not vary significantly with age, sex or breed. In contrast, seroprevalence varied among farms, geographic region, municipality, altitude, mean annual temperature, and mean annual rainfall. The median seroprevalence in farms was 32.6% (range 7.1–71.4%). Sheep raised in farms at ≥ 1900 m above sea level had a higher seroprevalence (44.1%) than those in farms at lower sea level (16.3%). Sheep raised in municipalities with $\leq 17.7^\circ\text{C}$ mean annual temperature had a higher seroprevalence (37.2%) than those in municipalities with higher mean annual temperature (14.1%). Sheep raised in a municipality with 600 mm of mean annual rainfall had a higher seroprevalence (71.4%) than municipalities with higher mean annual rainfall (29.1%). This is the first report on the seroprevalence and correlates of *T. gondii* infection in sheep in Michoacán State, Mexico. The role of environmental characteristics for *T. gondii* infection in sheep deserves further research.

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1. Introduction

Infections with *Toxoplasma gondii* in sheep (*Ovis aries*) occur worldwide (Dubey, 2009, 2010). Infection with *T. gondii* during pregnancy in sheep represents a serious

risk for congenital disease including embryonic or fetal death and mummification, abortion, stillbirth, and neonatal death (Dubey, 2009). Infection with *T. gondii* in sheep is of concern not only for sheep health but also for its impact in human health. Humans are at risk of infection with *T. gondii* by ingestion of raw or undercooked mutton from sheep infected with *T. gondii* (Dubey, 2009, 2010).

Little is known concerning *T. gondii* infection in sheep in Mexico in general and there is a lack of information on such infection in sheep in Michoacán State, Mexico in particular.

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Table 1
Seroprevalence of *T. gondii* infection and environmental characteristics in domestic sheep in Michoacán.

Region	Municipality	Meters above sea level	Mean annual temperature	Mean annual rainfall	Farms surveyed	Sheep tested	Seroprevalence of <i>T. gondii</i> infection	
						No.	No.	%
Bajío	Zacapu	1990	17 °C	800 mm	1	92	34	37
Cuitzeo	Charo	1900	17.7 °C	1145.5 mm	1	135	44	32.6
	Morelia	1920	18.7 °C	741.1 mm	2	79	10	12.7
	Sub-total				3	214	54	25.2
Lerma-Chapala	Briseñas	1520	18.3 °C	600 mm	1	7	5	71.4
Oriente	E. Huerta	2490	16.1 °C	1075.3 mm	1	50	25	50
	Jungapeo	1300	23.6 °C	1244.5 mm	1	42	3	7.1
	Sub-total					92	28	30.4
	All				7	405	121	29.9

In two previous studies on the seroprevalence of *T. gondii* infection in sheep in Mexico, we found a 15.1% seroprevalence in the northern state of Durango (Alvarado-Esquivel et al., 2012) and a 23.1% seroprevalence in the southern state of Oaxaca (Alvarado-Esquivel et al., 2013). Studying infections with *T. gondii* in sheep in Mexico is important since sheep meat is widely eaten in Mexico and an association of *T. gondii* infection with consumption of sheep meat has been reported in humans in Mexico (Alvarado-Esquivel et al., 2011). In the present study we sought to determine the seroprevalence and correlates of *T. gondii* infection in sheep raised in several municipalities in the west central Mexican state of Michoacán.

2. Materials and methods

2.1. Sheep surveyed

Through a cross-sectional study design, 405 domestic sheep were sampled from 7 farms in 6 municipalities of Michoacán State, Mexico from October 2011 to August 2012. Sheep were enrolled consecutively in the study when routinely monitored for brucellosis in farms in the region during the study period. The selection of farms was at random. Sample size was fixed for the brucellosis monitoring and sampling was performed by state veterinarians. All sheep on farms were included. General sheep and environmental characteristics were obtained by a questionnaire. Inclusion criterion was age 3 months and older. The number of sheep per farm ranged from 7 to 135 (median 48). Three farms (2 in the municipality of Morelia and 1 in the municipality of Charo) were located in the Cuitzeo region; 2 farms (1 in the municipality of Jungapeo and 1 in the municipality of Epitacio Huerta) were located in the Oriente region; 1 farm was located in the Zacapu municipality in the Bajío region; and 1 farm was located in the Briseñas municipality in the Lerma-Chapala region (Fig. 1). All surveyed regions had a temperate climate. However, mean annual temperature, and mean annual rainfall varied among regions (Table 1). Management of all sheep was under semi-extensive conditions (sheep were fed by grazing in communal natural grasslands during day time and kept in stables at nights). Sheep were born and raised

in the same farm they were tested. However, we cannot exclude the possibility that sheep were raised on other farms because we did not trace the origin of each sheep in an individual basis. General characteristics of the sheep including age, sex, and breed, and general characteristics of the environment of sheep raising including municipality, geographic region, altitude, management, and climate conditions were obtained. Age, sex and breed data were available for the majority of sheep. They were 3–72 months (median 40 months) old, and 43 were male and 309 were females. Breed was pure (Black belly 26, Katahdin 194, Dorper 2, and Pelibuey 2) in 224 sheep, and mixed in 176 sheep.

2.2. Serological examination

Sera were collected from whole blood by centrifugation and stored at -20°C until tested. Sheep sera were tested for *T. gondii* antibodies using 2-fold serial dilutions from 1:25 to 1:3200 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987). A titer of 1:25 was used as cut-off for seropositivity in MAT.

2.3. Statistical analysis

Statistical analysis was performed using Epi Info software version 3.5.4 (Centers for Disease Control and Prevention: <http://wwwn.cdc.gov/epiinfo/>) and SPSS version 15.0 (SPSS Inc., Chicago, IL). We used the Fisher exact test for comparison of the frequencies among groups. Multivariable analysis was used to assess the association between sheep characteristics and *T. gondii* seropositivity. The dependent variable was seropositivity by MAT for an individual animal. Independent variables included in the multivariable analysis were those with a *P* value ≤ 0.25 in the bivariate analysis: altitude, mean annual temperature, mean annual rainfall, farm, municipality, geographical region, and breed. Odds ratio (OR) and 95% confidence interval (CI) were calculated by multivariable analysis, using backward stepwise logistic regression analysis. A *P*-value of <0.05 was considered statistically significant. The Hosmer–Lemeshow goodness of fit test was used to assess the fitness of the regression model.

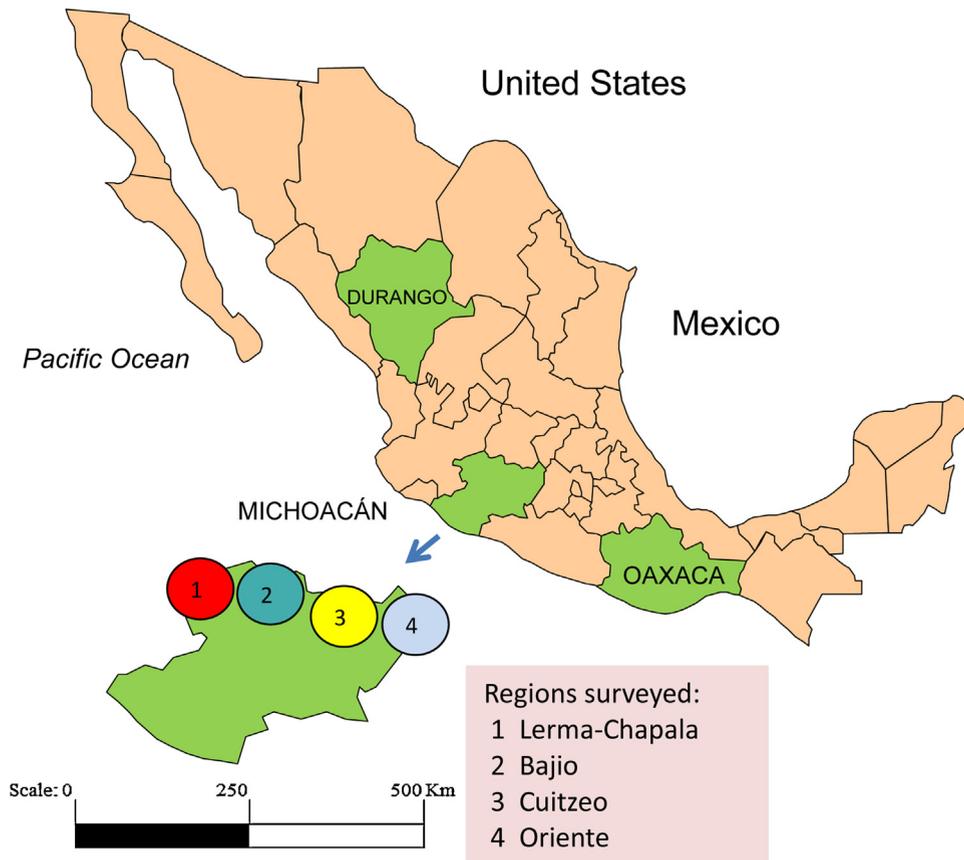


Fig. 1. Regions of Michoacán State where sheep were sampled for anti-*Toxoplasma gondii* antibodies. Michoacán State is located in central west Mexico. The map also shows other Mexican states where we have previously surveyed sheep for *Toxoplasma* seroprevalence (Durango and Oaxaca States).

3. Results

Antibodies to *T. gondii* were found in 121 (29.9%) of the 405 sheep with MAT titers of 1:25 in 46, 1:50 in 20, 1:100 in 7, 1:200 in 5, 1:400 in 7, 1:800 in 11, 1:1600 in 5, and 1:3200 or higher in 20. Seropositive sheep were found in all farms. Seroprevalence varied significantly by Fisher exact test among farms, geographic region, municipality, altitude, mean annual temperature, and mean annual rainfall (Table 1). The median seroprevalence in farms was 32.6% (range 7.1–71.4%). There was a statistically significant difference in seroprevalence of *T. gondii* infection in sheep among the 7 farms ($P < 0.001$) and 6 municipalities ($P < 0.001$) studied. Farms were divided into 2 categories for altitude, mean annual temperature and mean annual rainfall based on significant change points in the *T. gondii* seroprevalence. Thus, sheep raised in farms at ≥ 1900 m above sea level had a significantly ($P < 0.001$) higher seroprevalence (44.1%) than those in farms at lower altitude (16.3%). Sheep raised in municipalities with $\leq 17.7^\circ\text{C}$ mean annual temperature had a significantly ($P < 0.001$) higher seroprevalence (37.2%) than those in municipalities with higher mean annual temperature (14.1%). Seroprevalence varied significantly ($P < 0.001$) among the mean annual rainfall in the municipalities. Sheep raised in a municipality with 600 mm of mean annual rainfall had a significantly ($P = 0.02$) higher seroprevalence

(71.4%) than municipalities with higher mean annual rainfall (29.1%). Seropositivity did not vary significantly with age, sex or breed of sheep (Table 2).

Environmental and sheep characteristics with a *P* value equal to or less than 0.25 in the bivariate analysis included altitude ($P < 0.001$), mean annual temperature ($P < 0.001$), mean annual rainfall ($P < 0.001$), farm ($P < 0.001$), municipality ($P < 0.001$), geographical region ($P = 0.019$), and breed ($P = 0.10$). Multivariable analysis of such 7

Table 2
General characteristics of sheep and seroprevalence of *T. gondii* infection.

Characteristics	Sheep tested No. ^a	Seroprevalence		<i>P</i> value ^b	
		No.	%		
Age (months)					
	3–12	88	28	31.8	0.39
	13–24	34	10	29.4	
	25–36	77	32	41.6	
	37–72	61	18	29.5	
Sex					
	Male	43	15	34.9	0.44
	Female	309	101	32.7	
Breed					
	Pure	224	61	27.2	0.10
	Mixed	176	59	33.5	

^a Sums do not add up to 405 because of some missing values.

^b Obtained by Fisher exact test.

characteristics showed that *T. gondii* seropositivity was associated only with altitude (OR=1.68; 95% CI: 1.13–2.51; $P=0.01$), mean annual temperature (OR=0.43; 95% CI: 0.30–0.61; $P<0.001$), and mean annual rainfall (OR=0.74; 95% CI: 0.56–0.98; $P=0.03$). The result of the Hosmer–Lemeshow test was 2.88 ($P=0.409$), indicating an acceptable fit of our regression model.

4. Discussion

In the present work, we found a higher seroprevalence (29.9%) of *T. gondii* infection in sheep in the west central Mexican state of Michoacán than the 15.1% reported in sheep in the northern Mexican state of Durango (Alvarado-Esquivel et al., 2012) and the 23.1% found in sheep in the southern Mexican state of Oaxaca (Alvarado-Esquivel et al., 2013) by using the same MAT. Seroprevalence of *T. gondii* infection in sheep increases with age (Alvarado-Esquivel et al., 2012). Therefore, differences in the median age of sheep among the studies may explain the differences in the seroprevalence. The median age of sheep in the present study was 40 month old, while the median ages in sheep studied in Durango and Oaxaca were from 12 to 24 months old. Only a few other studies of *T. gondii* infection in sheep in Mexico have been reported. However, researchers have used different tests for detecting anti-*T. gondii* antibodies and comparison of the seroprevalence found in the present study with those in such studies should be taken with care. In two previous studies using indirect immunofluorescent test, García-Vázquez et al. (1990) found an overall 30% seroprevalence in 495 sheep from 3 states (Morelos, San Luis Potosí, Guanajuato) in central Mexico, and Cruz-Vázquez et al. (1992) reported a 37.9% seroprevalence in 702 sheep older than 1 year from Morelos State. In another study using indirect ELISA, Caballero-Ortega et al. (2008) reported seropositivity in 29.1% of 351 sheep from western Mexico (Colima State). Worldwide seroprevalence of *T. gondii* infection in sheep was reviewed by Dubey (2010).

In our present study, seropositivity to *T. gondii* infection was found in all farms in the 6 municipalities sampled indicating a wide parasite dissemination in sheep in the Michoacán regions explored. Michoacán State has about 220,000 sheep (<http://www.e-local.gob.mx/work/templatess/enciclo/michoacan/econ.htm>), and sheep meat produced in Mexico is consumed domestically. In addition, Mexico imports about 50% of the consumed sheep meat. The nearly one third of seropositive sheep found in the current study is of concern since consumption of sheep meat was recently associated with *T. gondii* infection in humans in Durango, Mexico (Alvarado-Esquivel et al., 2011).

We found that a number of factors correlated with the seroprevalence of *T. gondii* infection in sheep in Michoacán: municipality, geographical region, altitude, mean annual temperature and mean annual rainfall. Differences in the seroprevalence among the municipalities and geographical regions might be explained by differences in their altitudes, mean annual temperature and mean annual rainfall. It is likely that survival and transmission of *T. gondii* is better in the environment with higher seroprevalence (≥ 1900 m above sea level, $\leq 17.7^\circ\text{C}$ mean annual temperature, 600 mm of mean annual rainfall) than in those with

lower seroprevalence (≤ 1520 m above sea level, $\geq 18.3^\circ\text{C}$ mean annual temperature, ≥ 741.1 mm of mean annual rainfall). Altitude has been related with seroprevalence of *T. gondii* infection in sheep. In a previous study, we reported a significantly higher seroprevalence of *T. gondii* infection in sheep raised at 1560–1600 m above sea level than those raised at 1020–1080 m above sea level (Alvarado-Esquivel et al., 2013). In both studies, the seroprevalence increased with altitude. In another study in sheep in Mexico, seroprevalence was higher at sea level than at 1200 m above sea level (Caballero-Ortega et al., 2008). In contrast, in a study in sheep in Norway, researchers found an association of *T. gondii* seropositivity with farms situated at an altitude of >100 m above sea level (Skjerve et al., 1998). With respect to temperature, sheep raised in municipalities with $\leq 17.7^\circ\text{C}$ mean annual temperature had a significantly higher seroprevalence than those in municipalities with higher mean annual temperature. Little is known of the effect of environmental factors including temperature and rainfall on the seroprevalence of *T. gondii* infection in sheep. In a serological survey in sheep in South Australia, researchers found no correlation between seroprevalence of *T. gondii* infection and any climatic factors, namely, average annual rainfall, average annual evaporation, or mean temperature range (O'Donoghue et al., 1987). In contrast, in a study in sheep in South Africa, seroprevalence of *T. gondii* infection was associated with minimum average temperature in the provinces explored (Samra et al., 2007). We are not aware of previous reports about the association of *T. gondii* seroprevalence in sheep with the climatic parameters: 1560–1600 m above sea level, $\leq 17.7^\circ\text{C}$ mean annual temperature, and 600 mm of mean annual rainfall. Interestingly, sheep in the driest (600 mm of mean annual rainfall) municipality had the highest seroprevalence of *T. gondii* infection. It is possible that *T. gondii* oocysts remain longer in an environment with little rainfall because there is little wash or removal by the rain. The type of management of sheep correlated with the seroprevalence of *T. gondii* in a previous study in sheep in Mexico (Alvarado-Esquivel et al., 2013). However, in the present study all sheep were managed in the same semi-extensive conditions. Therefore, results of the present study suggest that environment climatic variables including mean annual temperature and mean annual rainfall are important factors correlating with the seroprevalence of *T. gondii* infection in sheep. Such results have epidemiological significance and point toward a limitation in reporting an overall seroprevalence of *T. gondii* infection in sheep in a given state. Thus, the seroprevalence in sheep in the northern region of Michoacán should not be extrapolated to other regions in the same state or another states in Mexico because of the wide differences in environmental conditions among regions or states. Similarly, interpretation of overall seroprevalences of *T. gondii* infection in sheep in previous studies without analyzing environmental conditions of the studied regions should be taken with care.

5. Conclusion

Results provide serological evidence of *T. gondii* infection in sheep in Michoacán, Mexico. Infected sheep

represent a potential source of infection for humans. Results on environmental factors associated with *T. gondii* infection in sheep warrant for further research for an optimal planning of prevention measures against *T. gondii* infection.

Conflict of interest statement

No conflict of interest.

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