SHORT COMMUNICATION

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Seroprevalence and risk factors associated with *Toxoplasma gondii* infection in sheep of Veracruz State, southeast Mexico

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Abstract

Toxoplasma gondii is widely prevalent in sheep and their products pose a risk to public health. The aim of this study was to identify the seroprevalence and risk factors associated with *T. gondii* infection in sheep in Veracruz State, Mexico. The study was cross-sectional and it was carried out in thirteen municipalities distributed in three regions of Veracruz State. A total of 414 blood samples were collected from four districts of Veracruz State and analyzed for *T. gondii* antibodies using enzyme-linked immunosorbent assay. Total seroprevalence was 35.90% (149/414; 95.00% CI = 31.40-40.80). Seroprevalence by the municipality was 10.50% to 85.70% and for the district was 28.80% to 47.80%, respectively. Age, breed and productive status were identified as risk factors associated with *T. gondii* infection significantly. The infection by *T. gondii* is widely present in the districts of the Veracruz State with a high seroprevalence and risk factors associated with infection.

Introduction

Sheep production is affected by parasitic diseases such as toxoplasmosis caused by the intracellular protozoan *Toxoplasma gondii*.[1] Toxoplasmosis is widely prevalent in meat production animals and humans, is estimated that one-third of the worldwide human population has been exposed to *T. gondii*.[2,3] Transmission occurs by ingestion of food and water contaminated with oocysts excreted by infected cats that ingest meat with tissue cysts of *T. gondii*.[4] In ewes, toxoplasmosis causes embryo resorption, mummification, abortion and neonatal death, causing economic losses for sheep production.[1] Particularly, abortion is the principal clinical sign associating with high seroprevalence of *T. gondii* infection in flock affected.[5] The diagnosis of toxoplasmosis is made by laboratory tests such as the enzyme-linked immunosorbent assay (ELISA). This method is based on recognition of *T. gondii* surface antigens by host *T. gondii* specific immunoglobulins.[6] In Mexico, toxoplasmosis has been identified in domestic animal populations, but seroprevalence differs in each region due to environmental conditions diversity.[7] In this context, *T. gondii* seroprevalence of 15.10% has been notified in sheep of the Durango State, while Oaxaca and Michoacán were reported with 23.10% and 32.60%, respectively.[8-10] Furthermore, the ingestion of mutton meat has been associated with toxoplasmosis in the human population of Mexico.[11]

To our knowledge, there have been no surveys for toxoplasmosis in commercially raised sheep in Veracruz. Therefore, the aim of the present study was to determine the seroprevalence and risk factors associated with *T. gondii* infection in sheep from Veracruz districts.

Materials and Methods

Study area. The study was carried out in Veracruz, the southeast region of Mexico, located between 93°36' and 98°39' longitude west and 22°28' and 17°09' latitude north. The dominant climate is warm sub-humid with an

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average temperature of 23.00 ˚C and precipitation of 486 mm.[12] With the purpose of attending the agricultural producers, Veracruz is organized in 12 Rural Development Districts (RDD), each one consisting of different municipalities. This study included 13 municipalities from four RDD (Fig. 1) located and distributed according to the three livestock areas of Veracruz, North Zone: RDD 02 Tuxpan and RDD 03 Martinez de la Torre, having both the 6.10% of the ovine population. Center zone: RDD 04 Coatepec, it has 15.10% of the total ovine population in Veracruz. South Zone: RDD 09 San Andres Tuxtla with 2.10%. All these districts represent 23.00% of total flocks in Veracruz.[13]

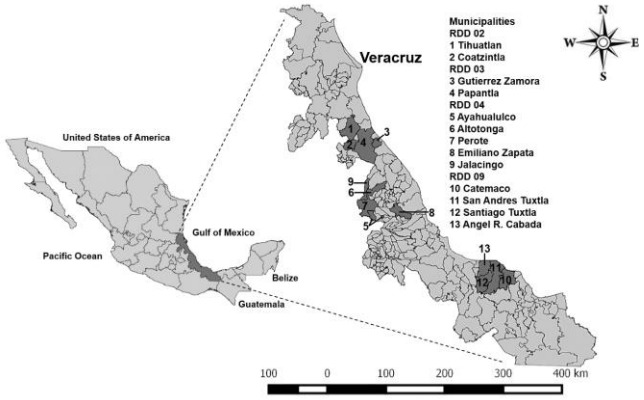

Fig. 1. Location and distribution of studied districts.

Study design and sample size. The study was an observational-cross multistage stratified type, where the sample size was calculated using the Win Episcope program (version 2.0; Clive, Edinburgh, UK)[14] under the modality of "estimate proportions" for an expected seroprevalence of 50.00%, 95.00% confidence and 5.00% error from a population of 664,532 sheep resulting in a minimum sample size of 385 animals. The 55 production units (PU) were randomly selected by clusters using the Canon and Roe tables.[15]

Sampling collection. Sampling was performed from August 2015 to May 2016. A total of 414 blood samples were collected were more than calculated to prevent sample losses. The samples were obtained by jugular vein puncture using vacuum tubes without anticoagulant. The samples were centrifuged at 1000 *g* for 15 min and serum was stored at – 20.00 ˚C until testing.

Data collection. Data were collected through surveys applied to owners or supervisors of PU to collect information about the flock and each animal sampled and included. Animal information consisted of sex, age, body condition (fatness degree), breed, feed, and production status. On the other hand, information collected from the flock consisted of the water source, presence of cats and rodents and production system. The animals included were ewes older than three months age and rams or males prospect to ram.

Serological analysis. Serum samples were analyzed to detect antibodies to *T. gondii* using a commercial ELISA kit (Toxotest Ab IDEXX® Laboratories, Basel, Switzerland) to a dilution of 1:400, then anti-ruminant IgG-peroxidase conjugates were added according to the methodology of the assay. Absorbance reading was at 450 nm using a plate reader (ELx800; BioTek Instruments Inc., Winooski, USA) and the interpretations of results were performed by the XChek® program provided by IDEXX ® Laboratories, too.

Statistical analysis. The seroprevalence and 95.00% confidence intervals (CIs) were calculated for each variable included in surveys. Association with seropositivity of *T. gondii* infection was estimated with Chi-square using online program Vassar Stats. The factors that were significant ($p < 0.05$) in the univariate analysis were subjected to logistic regression using the statistical program MINITAB (version 14.0; Minitab Inc., Boston, USA) to estimate interactions among independent and dependent variables.

Results

The general seroprevalence was 35.90% (149/414; 95.00% CI = 31.40-40.80). The seroprevalence according to district showed that RDD 02 Tuxpan had the highest value with 47.80% (11/23; 95.00% CI = 29.20-67.00), the factor RDD was not significantly associated as a possible risk factor ($p = 0.091$). The municipality of Coatzintla had the highest seroprevalence with 85.70% (6/7; 95.00% CI = 42.00-99.20), however, it was not considered as a risk factor due to the small sample size. In contrast, Perote had the lowest seroprevalence with 10.50% (4/41; 95.00% CI= 3.40-25.70), which can be considered as a factor of protection (Table 1).

According to sex, the origin of the animal, body condition, concentrated feed, mineral supplementation, water source, presence of cats and rodents and production system, a low seroprevalence variation among 30.00% to 51.10% was observed. Age, type of breed and productive status presented a wide variation of seroprevalence with a minimum value of 19.50% and 66.90% as a maximum value. In this group of variables, identified risk factors as the type of breed with crossbreed sheep (OR = 1.85, 95.00% CI = 1.21-2.82, $p = 0.005$) were significantly associated. Considering age, those animals between 25 to 36 months of age (OR = 1.80, 95.00% CI = 1.15-2.82, $p = 0.013$) and pregnant ewes (OR = 1.69, 95.00% CI = 1.11-2.57, $p = 0.018$) as productive status were associated as risk factors (Table 2).

According to the logistic regression analysis, those variables identified in univariate analysis as risk factors were included for the model; however, these were not significant interaction ($p = 0.425$).

Table 1. Seroprevalence and association as possible risk factors of *Toxoplasma gondii* infection in sheep by municipality and district of Veracruz State, southeast Mexico.

Municipalities and RDDs	No. tested	No. positive	Seroprevalence (%)	95.00%CI[a]	OR[b]	95.00%CI[a]	*p*-value[c]
RDD 02 Tuxpan	23	11	47.80	29.20-67.00	1.68	0.72-3.90	0.31
Coatzintla	7	6	85.70	42.00-99.20	11.07	1.32-92.91	NS
Tihuatlan	16	5	31.20	12.10-58.50	0.80	0.27-2.35	0.88
RDD 03 Martínez de la Torre	45	13	28.80	16.80-44.50	0.69	0.35-1.37	0.37
Gutiérrez Zamora	13	4	30.70	10.30-61.10	0.78	0.23-2.59	NS
Papantla	32	9	28.10	14.40-46.90	0.67	0.30-1.50	0.43
RDD 04 Coatepec	189	60	31.70	25.20-38.90	0.71	0.47-1.06	0.12
Altotonga	37	15	40.50	25.20-57.80	1.23	0.62-2.46	0.67
Ayahualulco	41	13	31.70	18.50-48.20	0.80	0.40-1.61	0.66
Emiliano Zapata	37	16	43.20	27.50-60.30	1.39	0.70-2.76	0.43
Jalacingo	36	12	33.30	19.10-51.00	0.87	0.42-1.81	0.86
Perote	38	4	10.50	3.40-25.70	0.18	0.06-0.53	0.00
RDD 09 San Andrés Tuxtla	157	65	41.40	33.60-49.50	1.45	0.96-2.19	0.09
Ángel R. Cabada	44	20	45.40	30.60-61.00	1.55	0.82-2.92	0.22
Catemaco	38	8	21.00	10.10-37.70	0.44	0.19-0.99	0.06
San Andrés Tuxtla	35	18	51.40	34.20-68.20	2.00	0.99-4.01	0.07
Santiago Tuxtla	40	19	47.50	31.80-63.60	1.69	0.88-3.27	0.15
Total	414	149	35.90	31.40-40.80	-	-	-

[a] Confidence Interval 95.00%; [b] Odds Ratio; [c] Yates-corrected Chi-square ($p < 0.05$). NS: Not significant.

Discussion

In the present study, a general mean seroprevalence of 35.90% was found which can be considered high compared to those reported in the states of Oaxaca (23.10%), Michoacán (29.90%), Durango (15.10%) and Colima (29.10%).[8-10,16] In relation to the municipalities, Coatzintla was the highest and Perote the lowest with 85.70% and 10.50%, respectively. The diversity of environmental conditions probably influenced the variation of seroprevalence among regions. However, conditions were not analyzed in this study. According to the study conducted in Michoacán, significant differences in seroprevalence among municipalities were found regarding differences in environmental conditions. In relation to sex, females and males showed low variation among seroprevalences with 35.70% and 36.90%, respectability, similar to those found in Michoacán State.[10] However, they differ with results from Durango where wide variations between males and females were identified.[8] Thus, they are equally likely to become infected with *T. gondii*.[1] On the other hand, females may show higher rates because of their larger population and a longer production period compared to males.[17] According to the origin, animals from other flocks showed higher seroprevalence (40.60%) than those born in the same herd (35.20%). This can be possible because sheep production is developed regionally depending on available resources and market demand, is considered a secondary livestock activity.[17] The semi-intensive production system presented the highest seroprevalence (37.30%), but it showed a lower variation in respect to intensive (30.00%) and extensive (36.20%) system. In contrast to that found for this same system in Oaxaca where it was significantly higher (33.20%) in comparison with semi-extensive (8.90%).[9] Sheep raised in semi-intensive systems showed higher consumption of stored feed; in other study conducted in goats in the municipality of Yecuatla, Veracruz, *T. gondii* seroprevalence has been associated with the risk of consuming stored feed.[18] Crossbred animals showed the highest seroprevalence (45.60%) and they were significantly associated as a risk factor, this was similar to that found in Oaxaca, where a seroprevalence for mixed breeds (37.50%) was found significantly higher than pure breeds (22.70%).[9] However, no differences were found among pure and crossbred sheep in Michoacán.[10] The association as a risk factor with crossbred sheep can be due to best cares for hair sheep and wool since these were confirmed by pure breeds and their commercial value is more important than crossbred.[9] In relation to age, in Michoacan, significant differences were not found significant differences among seroprevalences.[10] However, in Durango, the seroprevalence was increased significantly with age,[8] similar to the present study. Our results showed wide variation which is probably due to high exposure to *T. gondii* regarding sheep age since most sheep can become infected before four years generally; therefore the seroprevalence in a flock can increase up to 95.00% in sheep of six years old.[1] Also, it was identified that pregnant ewes had a higher probability of infection. This is important since *T. gondii* is associated with the presentation of abortion.[1] However; it depends on the stage of gestation, since when infection occurs before the last third pregnancy the risk of abortion increases.

Table 2. Seroprevalence and association of variables as possible risk factors for *Toxoplasma gondii* infection in sheep.

Variables and categories	No. tested	No. positive	Seroprevalence (%)	95.00%CI[a]	OR[b]	95.00%CI[a]	*p*-value[c]
Sex							
Female	330	118	35.70	30.60-41.20	0.95	0.57-1.56	1.00
Male	84	31	36.90	26.80-48.10	1.05	0.63-1.72	
Age (months)							
3-12	80	20	25.00	16.20-36.10	0.52	0.30-0.91	0.03
13-24	99	37	37.30	28.00-47.70	1.08	0.67-1.72	0.84
25-36	108	50	46.30	36.70-56.10	1.80	1.15-2.82	0.01
>37	127	42	66.90	57.90-74.80	0.83	0.53-1.29	0.47
Origin of animal							
Born in same flock	355	125	35.20	30.20-40.40	0.79	0.45-1.39	0.50
Bought from other flock	59	24	40.60	28.30-54.20	1.26	0.71-2.21	
Body condition							
Thin	211	85	40.20	33.60-47.20	1.46	0.97-2.19	0.07
Average	150	47	31.30	24.10-39.40	0.72	0.47-1.10	0.16
Fat	53	17	45.40	20.30-46.40	0.81	0.44-1.51	0.63
Type of breed							
Hair sheep	148	61	41.20	33.20-49.60	1.41	0.93-2.14	0.12
Wool sheep	128	25	19.50	13.20-27.60	0.31	0.19-0.52	< 0.001
Crossbreed	138	63	45.60	37.20-54.30	1.85	1.21-2.82	0.005
Concentrate feed							
Yes	185	76	41.00	33.90-48.50	1.49	0.99-2.23	0.06
No	229	73	31.80	25.90-38.40	0.67	0.44-1.00	
Mineral supplementation							
Yes	272	92	51.10	43.50-58.50	0.76	0.50-1.15	0.24
No	142	57	40.10	32.10-48.70	1.31	0.86-1.99	
Productive status							
First lambing	20	4	20.00	6.60-44.20	0.42	0.14-1.30	0.19
Pregnant	138	61	44.20	35.80-52.80	1.69	1.11-2.57	0.01
Lactating	27	11	40.70	23.00-60.90	1.24	0.56-2.74	0.74
Non pregnant	138	38	27.50	20.40-35.90	0.56	0.36-0.88	0.01
Ram	84	31	36.90	26.80-48.10	1.05	0.63-1.72	1.00
Weaned	7	4	57.10	20.20-88.10	2.40	0.53-10.91	NS
Water source							
River	117	42	35.90	27.30-45.30	0.99	0.63-1.55	0.92
Waterhole	107	34	31.70	23.30-41.50	0.77	0.48-1.24	0.34
Public network	143	52	36.30	28.60-44.80	1.02	0.67-1.56	1.00
Dam	47	21	44.60	30.40-59.70	1.50	0.81-2.78	0.24
Presence of cats and rodents							
Rodents	48	17	35.40	22.50-50.60	0.97	0.51-1.82	0.92
Cats	27	9	33.30	17.20-53.90	0.88	0.38-2.01	0.92
Both	339	123	36.20	31.20-41.60	1.07	0.63-1.81	0.88
Production system							
Intensive	40	12	30.00	17.00-46.70	0.74	0.36-1.50	0.51
Semi-intensive	134	50	37.30	29.20-46.10	1.08	0.70-1.66	0.77
Extensive	240	87	36.20	30.20-42.70	1.02	0.68-1.54	1.00

[a] Confidence Interval 95.00%; [b] Odds Ratio; [c] Yates-corrected Chi-square ($p < 0.05$). NS: Not significant.

In addition, about 4.00% of ewes are persistently infected and transmit the infection to their offspring.[1] It can be possible considering that pregnant females suffer hormonal and metabolic changes and are in immunosuppressive status due to continuous stress in comparison with non-pregnant females. The variables such as concentrated feed, mineral supplements, water source and presence of cat and rodents were not identified as risk factors due to a lower variation of the sero-prevalence among categories.

These results are important because Veracruz State is the third in sheep production since it has a flock estimated over 666,805 heads with an increase of 28.00% for the period from 2006 to 2015.[19] Furthermore, ingestion of mutton meat has been associated like an important source of *T. gondii* infection for humans, representing a public health risk.[1,11] Based on the results obtained in this study, it could be concluded that *T. gondii* infection is common in Veracruz State, southeast Mexico.

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Conflict of interest

The authors do not have any particular interest to declare.

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