Toxoplasma gondii in small ruminants in northeastern areas of Colombia: Seroprevalence and risk factors

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Abstract
Sheep and goats are susceptible to infections with Toxoplasma gondii and could play an important role in the transmission of the zoonotic parasite to human. We conducted a cross sectional study to estimate the seroprevalence and to assess the risk factors for T. gondii seropositivity in small ruminants under traditional husbandry systems. This study was carried out from November 2015 to April 2016 in randomly selected small ruminants (n = 1038) from 48 farms located in Colombia, in the departments of northern Cesar in the north and La Guajira in the south. An indirect ELISA was used to detect IgG antibodies to T. gondii in the animals. A standardized questionnaire was used to obtain information on putative risk factors. We conducted the association analyses by using univariable and multivariate logistic regression and report odds ratios (OR) with 95% confidence interval (C.I). The overall seroprevalence in small ruminants was 23.5% (C.I: 21–26.2%). Sheep showed a higher seroprevalence (25.1% C.I: 22.4–28.6%) than goats (18.4% C.I: 22.4–28.6%). The association analysis recognized as risk factors for T. gondii seropositivity farming pigs in addition to small ruminants (OR = 1.96 C.I: 1.414–2.743), the inexistence of manure heap (OR = 2.254 C.I: 1.480–3.433) and drinking water from locally aqueducts (OR = 1.489 C.I: 1.006–2.204). The results of the study confirmed that exposure to T. gondii is common in sheep and goats in dry Caribbean regions of Colombia.

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1. Introduction

Toxoplasma gondii is a protozoan parasite that affects sheep and goat production in several countries. The Toxoplasmosis in these species can cause damages including abortion, stillbirth, deficits in milk production, emaciation, and pneumonia (Buxton et al., 2007; Blewett and Trees, 1987). In fact, the infection in these species likely occurs by drinking water or feed consumption contaminated with feces of infected cats (Vesco et al., 2007). The shedding oocysts could survive in terrestrial and aquatic reservoirs for extended periods. In this sense, T. gondii is an important water borne disease that could be influenced by changes in water flow, poor quality of water and environmental fragmentation (Jones and Dubey, 2010).
Meat and milk from infected sheep and goats have been recognized as a source of infection in humans. Viable bradyzoites have been recovered from muscle in persistently infected sheep and the *T. gondii* DNA has been identified in slaughtered sheep and goats in many parts of the world (Amdouni et al., 2017; Dubey, 2009; Nunes et al., 2015; Santa et al., 2009). Seropositive sheep and goats can be assumed to harbor tissue cysts in their muscle (Tenter, 2009) and the undercooked consumption of this meat is considered an important source of human infection, as well as an important risk factor for *T. gondii* infection in pregnant women (Cook et al., 2000; Skinner et al., 1990). Similarly, humans could acquire the infective tachyzoites by drinking raw milk from infected goats (Skinner et al., 1990; Chiari and Neves, 1984).

The Toxoplasmosis infection is an important zoonosis. Prevalence of the human infection varies in different parts of the world reaching rates up to 75% (Saadatnia and Golkar, 2012). The people at higher risk for toxoplasmosis are pregnant women (especially the fetus), children, immunocompromised individuals located principally in developing countries (Tenter et al., 2000). In Colombia, this infection is a major health concern for humans and is present in several areas of the country. In pregnant women the seroprevalence was identified between 50 and 60% and the incidence of congenital infection was present in one of 1000 newborns (Cañón-Franco et al., 2014). In the northeastern areas of country, *T. gondii* infection in pregnant women showed a high seroprevalence (≥60%) in comparison to the rest of the country (Gómez Marín, 2002; Machado-Torres et al., 2004; Jácome, 2013).

Furthermore, sheep and goat population in Colombia are growing, 65% of these animals are raised for meat and 35% for milk production. The animals are located mainly in the northeastern areas of country (Gómez Moreno et al., 2014). However, there are limited studies conducted to determine the seroprevalence of *T. gondii* in small ruminants and no control programs exist. Therefore, the current study investigated the risk factors associated with seroprevalence of *T. gondii* in sheep and goats located in the northeastern areas of Colombia. The final reach of this study is to create health profiles to encourage the design of strategies, in order to control the infection in Colombia.

2. Materials and methods

2.1. Study area

This study was conducted in the northeastern areas of Colombia, specifically in the south municipalities La Guajira department (San Juan del Cesar, Distracción, Fonseca and EL Molino) and the north of Cesar (La Jagua del Pilar, Urumita, Villanueva, La Paz, San Diego and Valledupar). In these municipalities, the ecological characterization is similar and the rain patterns are predominantly bimodal with a prominent dry season during the first months of the year, moderate rains during July – August, decreasing until the end of August until September – November making up the principal rainy season, with total rainfall pluviometry reaching 1500 mm per year (IDEAM, n.d.).

2.2. Study population

This study was focused on small ruminants under traditional husbandry systems located in the northeastern areas of Colombia. Traditionally, the sheep and goats were reared together with other farm animals. The small ruminants shared the same grazing areas with animals of different sex and age range. The population census of small ruminant registered for the study area was 57,163 (ICA, 2016).

2.3. Study design and sampling

We conducted a cross sectional study involving a total sample of 1038 small ruminants comprised by 793 sheep and 245 goats from 48 farms. The median, minimum and maximum number of animals sampled per farm were 20, 3 and 120, respectively. The sample size was determined considering a previous study (Perry et al., 1978) in which the total *T. gondii* seroprevalence was 58% in 1655 ovine from 6 departments of Colombia. According to known population of animals, the sample size was estimate by a probabilistic method sampled using Epinfo™ version 7 (CDC) software, with 60% of expected prevalence, 95% confidence interval and a design effect of 1.5 (Dohoo et al., 2003). Animals were randomly selected by each municipality from the population census registered.

2.4. Sample collection

All procedures involving the collection of samples followed the national and international protocols for research in veterinary medicine. The collection of blood samples from animals was carried out from November 2015 to April 2016. These samples were collected from the jugular vein of animals in tubes without anticoagulant. Later, kept refrigerated storage at 4 °C until arrival at the laboratory. Then, the serum was separated by centrifugation at 500g for 10 min and preserved at −20 °C until analyses were conducted.
2.5. Serology

For this assay, we employed a commercial Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (PrioCHECK® Toxoplasma Ab SR, Prionics, Schlieren-Zurich, Switzerland) that includes the plates coated with tachyzoite-antigen of *T. gondii* derived from cell culture, a peroxidase-labelled anti-small ruminant secondary antibody, tetramethyl benzidine (TMB) as a chromogenic substrate, control sera and buffer solutions. This test was done following manufacturer’s instructions. Briefly, the serum of animals and control was diluted in 1:100 completing a volume to 100 μl/well. The diluted samples were put in duplicate to coated plates and incubate for 1 h at room temperature. Then, the plates were washed, and the conjugate was added following by incubating for 1 h at room temperature. Finally, the plates were washed and the TMB substrate was added. This reacted in 15 min and the absorbance of plates was measured using the ELISA lector at filter of 450 nm. We calculated the mean value of optical density (OD) absorbance of negative and positive controls including in ELISA kit. The percentage of positivity (PP) of the controls and test sera was calculated according to the formula below.

\[ PP = \left( \frac{OD_{Sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \right) \times 100 \]

The OD values of all samples were expressed as PP and this value was determined individually for each plate as suggested by the manufacturer. We calculated the cut-off considering the mean OD value and two units of Standard Deviation of negative controls (Frey et al., 1998). The sera test above or equal to the cut-off of 20 PP was positive and the results below to the cut-off of 20 PP was negative.

2.6. Statistical and association analyses

The seroprevalence was found by dividing the number of seropositive animals with sampled animals using Epiinfo™ version 7 (CDC) software to analyze the data. The 95% of confidence intervals (CI) for the prevalence values were calculated using the SAS® software (SAS INSTITUTE 9.0). Then, the prevalence data was introduced in the software Arc Gis 10. 1 (ESRI) using the shape from web site https://sites.google.com/site/seriescol/shapes for epidemiologic map design.

To evaluate the presence of risk factors associated with seropositivity, we employed a standardized questionnaire with closed and dichotomic answers. This questionnaire followed the parameters of national animal health authority (Instituto Colombiano Agropecuario - ICA). The data obtained from this questionnaire and results of positivity were stored in a Microsoft Excel spreadsheet. We evaluated 90 variables of plausible risk factors including sex of the animals, having also other animal species, the water source and quality, the management practices and biosecurity inside of the farms. The univariable analysis was performed using Pearson’s Chi-square test to assess the relationship between *T. gondii* seropositivity and variables, followed by a multivariable logistic regression model. Variables with \( p \leq 0.05 \) in the univariable analysis were included in the multivariable logistic model. Statistical analyses were performed using SPSS software version 20 (SPSS Inc., Chicago, II, USA) (Dohoo et al., 2003).

3. Results

3.1. Seroprevalence

In this study, the total sheep and goat population sampled comprised 1038 animals and the total seroprevalence was 23.5% (95% CI: 21.26–26.2%). The seroprevalence identified in sheep was 25.1% (95% CI: 22.4–28.6%) and the seroprevalence identified in goats was 18.4% (95% CI: 22.4–28.6%). In the sheep population sampled, the seroprevalence in both females and males was 26% (95% CI: 22.7–29.1%) and 17% (95% CI: 8.3–28.5%), respectively. In the goat population sampled, the seroprevalence in both females and males was 18.1% (95% CI: 5.6–13.7%) and 12% (95% CI: 2.5–31.2%), respectively. The seroprevalence varied by municipality between 0 and 42.9 detailed in Table 1 and illustrated in Fig. 1.

### Table 1

Seroprevalence of *Toxoplasma gondii* in small ruminants by counties of northwestern region of Colombia.

| Counties         | Number of animals sampled | Number of positive animals | Seroprevalence % | CI (95%)     |
|------------------|---------------------------|---------------------------|------------------|--------------|
| Distracción      | 35                        | 15                        | 42.9             | 26.3–60.7    |
| El Molino        | 12                        | 2                         | 16.7             | 26.3–60.7    |
| Fonseca          | 77                        | 8                         | 10.4             | 4.6–19.4     |
| La Jagua del Pilar| 14                       | 6                         | 42.9             | 17.7–71.1    |
| San Juan del Cesar| 144                      | 41                        | 28.5             | 21.3–36.6    |
| Urumita          | 15                        | 0                         | 0.0              | 0–21.8       |
| Villanueva       | 10                        | 2                         | 20.0             | 2.5–55.6     |
| La Paz           | 98                        | 22                        | 22.4             | 14.6–32      |
| San Diego        | 81                        | 12                        | 14.8             | 7.8–24.5     |
| Valledupar       | 552                       | 136                       | 24.6             | 21.1–28.4    |
| Total            | 1038                      | 244                       | 23.5             | 21–26.2      |
Fig. 1. Seroprevalence estimates of Toxoplasma gondii in sheep and goats.
3.2. Risk factors

Based on the univariable analysis, risk factors for sheep and goat *Toxoplasma gondii* seropositivity were drinking water from local aqueducts (OR = 2.26 CI: 1.674–3.059), having dogs and cats (OR = 2.9 CI: 2.05–4.27), close contact with poultry (OR = 3.1 CI: 2.11–4.820) and/or pigs (OR = 11.59 CI: 6.21–21.65). However, the protective factors identified were the weaning over two months after birth (OR = 0.678 CI: 0.498–0.919), having with equines (OR = 0.155 CI: 0.048–0.500) and cattle (OR = 0.308 CI 0.191–0.497), drinking water directly from small lagoons (OR = 0.452 CI: 0.333–0.615) and cisterns (OR = 0.728 CI: 0.546–0.972). Some variables associated to management and biosecurity conditions were statistically not significant and this was identified as possible risk factors (Table 2). The gender was statistically not significant as risk factor for *T. gondii* seroprevalence.

The significant factors by univariable analysis were analyzed by multivariable analysis. The risk factors identified by multivariable analyses were having pigs and small ruminants (OR = 1.96 CI: 1.414–2.743), the inexistence of manure heap (OR = 2.254 CI: 1.480–3.433) and drinking water from locally aqueducts (OR = 1.489 CI: 1.006–2.204). While having cattle and having other drinking water sources appeared as protective factors. However, having dogs and cats, having birds, management and biosecurity conditions variables were no significant statistically and irrelevant for the model (Table 3).

4. Discussion

This is the first report of *T. gondii* seroprevalence and risk factors association in sheep and goats from the northeastern areas of Colombia. The combined seroprevalence (23.5%) identified in this study was lower than to seroprevalence reported in sheep in Cesar (65%) and Guajira (59%) localities obtained by indirect Hemagglutination test (Perry et al., 1978). These differences may be due to tests used in both studies, because ELISA in relation to indirect Hemagglutination test is more sensitive. This situation makes the comparison between studies difficult. The evaluation of serologic methods used for diagnostic of *T. gondii* in goats showed substantial concordance between modified agglutination test (MAT) and ELISA, but almost perfect concordance between immunofluorescence assay (IFA) and ELISA (Fortes et al., 2018).

In comparison, the total seroprevalence of *T. gondii* in sheep and goats in this study was lower than the seroprevalence in sheep (74.3) reported in the template humid areas of Tolima department obtained by IFA (Alvarado, 1982). However, the total seroprevalence in sheep and goats obtained in this study was similar to sheep seroprevalence (28.22%) and goat seroprevalence (22%) reported in the arid and semiarid areas of the Brazilian northeast obtained by IFA and ELISA, respectively (Munhoz et al., 2014; Santos et al., 2018). These results suggest that the seroprevalence found in this study may be associated with suitable climatic conditions, because the Cesar and La Guajira departments have similar ecological patterns characterized by a dry period during most of the year.

The results of this study add to the worldwide knowledge on the role of sheep and goats in the epidemiology of *T. gondii*. Several studies have investigated seroprevalence and risk factors in traditional husbandry system in different parts of the world. In Mongolia the seroprevalence in goats (32%) and sheep (34.8%) had no correlated between age and sex of animals.

### Table 2

Univariable analysis of risk factors associated with *Toxoplasma gondii* seroprevalence in small ruminants in northeastern areas of Colombia.

| General variable | OR    | χ²   | p    | CI     |
|------------------|-------|------|------|--------|
| Goats and sheep mixed with equines | 0.155 | 12.756 | 0.000* | 0.048–0.500 |
| Goats and sheep mixed with bovines | 0.308 | 25.597 | 0.000* | 0.191–0.497 |
| Goats and sheep mixed with canines and felines. | 2.962 | 35.635 | 0.000* | 2.050–4.279 |
| Goats and sheep mixed with pigs | 11.599 | 87.419 | 0.000* | 6.213–21.655 |
| Goats and sheep mixed with birds | 3.193 | 33.042 | 0.000* | 2.115–4.820 |
| Drinking water from aqueduct water | 2.263 | 29.011 | 0.000* | 1.674–3.059 |
| Drinking water from small lagoons | 0.452 | 26.291 | 0.000* | 0.333–0.615 |
| Drinking water from cisterns | 0.728 | 4.641 | 0.031* | 0.546–0.972 |
| Weaning over two months | 0.678 | 6.242 | 0.006* | 0.498–0.919 |

| Variable of managed practices and biosecurity | OR    | χ²   | p    | CI     |
|-----------------------------------------------|-------|------|------|--------|
| There are no management facilities. | 1.578 | 9.609 | 0.002* | 1.811–2.108 |
| There are no corrals or appropriate enclosures for the management. | 1.536 | 8.325 | 0.004* | 1.146–2.058 |
| There are no facilities for handling females and neonates at the time of delivery. | 1.653 | 11.057 | 0.001* | 1.227–2.227 |
| There are no appropriate feeders and drinkers for handling. | 1.389 | 4.812 | 0.028* | 1.035–1.864 |
| There is no manure heap. | 1.389 | 4.812 | 0.028* | 1.035–1.864 |
| There are no records of the animal movements. | 1.707 | 12.867 | 0.000* | 1.272–2.290 |
| They do not disinfect or change clothes when handling animals belonging to groups with different health conditions. | 1.777 | 9.751 | 0.002* | 1.235–2.558 |
| They do not have clean and supervised areas for the delivery of the females. | 2.155 | 6.527 | 0.011* | 1.811–3.935 |
| Neonates do not have a clean, dry and well ventilated environment. | 1.405 | 4.771 | 0.029* | 1.035–1.909 |
| General variable | OR    | χ²   | p    | CI     |
|------------------|-------|------|------|--------|
| Goats and sheep mixed with equines | 0.155 | 12.756 | 0.000* | 0.048–0.500 |
| Goats and sheep mixed with bovines | 0.308 | 25.597 | 0.000* | 0.191–0.497 |
| Goats and sheep mixed with canines and felines. | 2.962 | 35.635 | 0.000* | 2.050–4.279 |
| Goats and sheep mixed with pigs | 11.599 | 87.419 | 0.000* | 6.213–21.655 |
| Goats and sheep mixed with birds | 3.193 | 33.042 | 0.000* | 2.115–4.820 |
| Drinking water from aqueduct water | 2.263 | 29.011 | 0.000* | 1.674–3.059 |
| Drinking water from small lagoons | 0.452 | 26.291 | 0.000* | 0.333–0.615 |
| Drinking water from cisterns | 0.728 | 4.641 | 0.031* | 0.546–0.972 |
| Weaning over two months | 0.678 | 6.242 | 0.006* | 0.498–0.919 |

OR: odds ratio; CI: confidence interval (95%).
* Statistically significant.
Table 3
Multivariable logistic regression analysis of variables associated with *T. gondii* seroprevalence in small ruminants of northeastern areas of Colombia.

| General variable                                      | β     | OR    | p      | CI (95%) |
|-------------------------------------------------------|-------|-------|--------|----------|
| Goats and sheep mixed with bovines                    | −0.647| 0.524 | 0.000* | 0.370    | 0.740    |
| Goats and sheep mixed with pigs                       | 0.678 | 1.969 | 0.000* | 1.414    | 2.743    |
| Drinking water from aqueduct water                    | 0.398 | 1.489 | 0.046* | 1.006    | 2.204    |
| Drinking water from small lagoons                     | −0.931| 0.394 | 0.000* | 0.270    | 0.576    |
| Drinking water from cisterns                          | −0.545| 0.580 | 0.005* | 0.398    | 0.845    |
| Weaning over two months                               | −0.711| 0.491 | 0.019* | 0.271    | 0.891    |
| Inexistence of manure heap                            | 0.813 | 2.254 | 0.000* | 1.480    | 3.433    |

Potential risk factors (*P < 0.05*) were selected for inclusion in the multivariable model.
OR: odds ratio; CI: confidence interval (95%), *statistically significant.
Likelihood ratio chi-square: 98.869; *P: 0.000*; number of observations = 1038.

(Pagmadulam et al., 2020). In Northern Greece the sheep showed higher seroprevalence (48.6%) than in goats (30.7%) associated to intensive and semi-intensive managements (Tzanidakis et al., 2012). In South Africa the seroprevalence was higher in sheep (64.46%) and goats (53.91) than pigs (36.96%), cats (32.11%) and chickens (33.58%) when having other farm animals associated to infected cats (Tagwireyi et al., 2019). These variation in the seropositivity can related to differences in management practices, biosecurity and climate variation at each small ruminant farms (Tenter et al., 2000).

Having also pigs on the farms was a risk factor, perhaps linked to typical outdoor management and on farm slaughter practices which allow transmission cycle of the parasite on the farm (Kijlstra et al., 2004). The small ruminants are also often slaughtered on the farms, and this practice may be linked to the success of the parasite (Bezerra et al., 2014; Dubey et al., 1995) and could explain the high prevalence identified in women from these areas (Jácome, 2013).

Drinking water from locally aqueduct was a risk factor for *T. gondii* seropositivity in this study. Furthermore, water standing longer periods in drinking bowls could be a risk, as it could become contaminated with oocysts, but this not directly evaluated in this study. Similarly, the consumption of raw water from locally aqueduct was considered an important risk factor for *T. gondii* infection in pregnant women in an studied area of Colombia (López-Castillo et al., 2005). Additionally, the raw water samples collected in an area of Colombia showed 58.6% of positivity to *T. gondii* oocysts by PCR (Triviño-Valencia et al., 2016).

The inexistence of manure heap as a risk factor for *T. gondii* seropositivity in small ruminants may be related to high concentration of animals in the grazing area, since animals reared with the extensive conditions of arid zones are pastured in small grazing areas. This overgrazing may increase the urine and feces per grazing area and the humidity provides by them may contribute to oocyst survival in the soil. *T. gondii* seroprevalence has been associated with higher density of animals in both indoor and outdoor husbandry systems (Tzanidakis et al., 2012).

Weaning more than two months after birth was identified as a protective factor and could be due to milk being the main source of water. This avoid the consumption of contaminated water from drinking bowls. That the parasite can be transmitted by milk has been demonstrated for some host species (Vesco et al., 2007; Sacks et al., 1982).

As the presence of cats was not identified as a potential risk factor in this study, the *T. gondii* infection in small ruminant farms could be influenced by contact with cats (Innes et al., 2009). For example, in one study showed that the probability of infection was higher in farms with >10 cats present (Cavalcante et al., 2008). However, the presence of cats not associated with seropositivity in small ruminants under intensive systems (Tzanidakis et al., 2012). However, the absence of feline population control programs in both urban and rural areas could result in more cases of infection in small ruminant farms (Espinosa et al., 2011). A reason for the presence of cats are not appearing a risk factor in our study could be the combination of this variable with that of canines in the questionnaire.

5. Conclusions

This cross-sectional study confirms that *T. gondii* infection is common in sheep and goats under traditional husbandry systems in Colombia. Having also pigs, lack of manure heap in high density grazing areas, as well as the aqueduct system at farms were associated with seropositivity in small ruminants. Consequently, the design of strategies for control of infection are needed. This should include the access of technical assistance programs to farm owners, in order to improve husbandry practices in these farms, along with epidemiological surveillance and evaluation of the zoonotic impact of infection on the population.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.
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Ethics statement

The animals used in this study received handling and treatment under qualified veterinary supervision in accordance with the animal experimentation rules described in the International Guiding Principles for Biomedical Research Involving Animals.

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