

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

Lorena C. Martínez-Rodríguez^a, Gabriel Andres Tafur-Gómez^{b,*}, Blanca Lisseth Guzman-Barragan^b

^a Grupo de Investigación Ciencia UDES, Programa de Medicina Veterinaria y Zootecnia, Facultad de Ciencias Agropecuarias, Universidad de Santander, campus Valledupar, Colombia

^b Universidad de Ciencias Aplicadas y Ambientales - U.D.C.A, Bogotá 111166, Colombia

ARTICLE INFO

Article history:

Received 21 June 2019

Received in revised form 21 February 2020

Accepted 28 March 2020

Keywords:

Goat

Sheep

Toxoplasma gondii

Seroprevalence

Risk factors

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.

© 2020 The Authors. Published by Elsevier Ltd on behalf of World Federation of Parasitologists.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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).

* Corresponding author at: Universidad de Ciencias Aplicadas y Ambientales – UDCA, Laboratorio de parasitología, Calle 222 # 55-37, Postal code: 111166 Bogotá D.C, Colombia.

E-mail address: gatafur@udca.edu.co. (G.A. Tafur-Gómez).

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 ($\geq 60\%$) 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 Epiinfo™ 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.

$$pp = \left[\frac{OD \text{ Sample} - OD \text{ NC}}{OD \text{ PC} - OD \text{ NC}} \right] \times 100$$

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 spread-sheet. 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, IL, 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% C.I: 21–26.2%). The seroprevalence identified in sheep was 25.1% (95% C.I: 22.4–28.6%) and the seroprevalence identified in goats was 18.4% (95% C.I: 22.4–28.6%). In the sheep population sampled, the seroprevalence in both females and males was 26% (95% C.I: 22.7–29.1%) and 17% (95% C.I: 8.3–28.5%), respectively. In the goat population sampled, the seroprevalence in both females and males was 18.1% (95% C.I: 5.6–13.7%) and 12% (95% C.I: 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 %	C.I (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

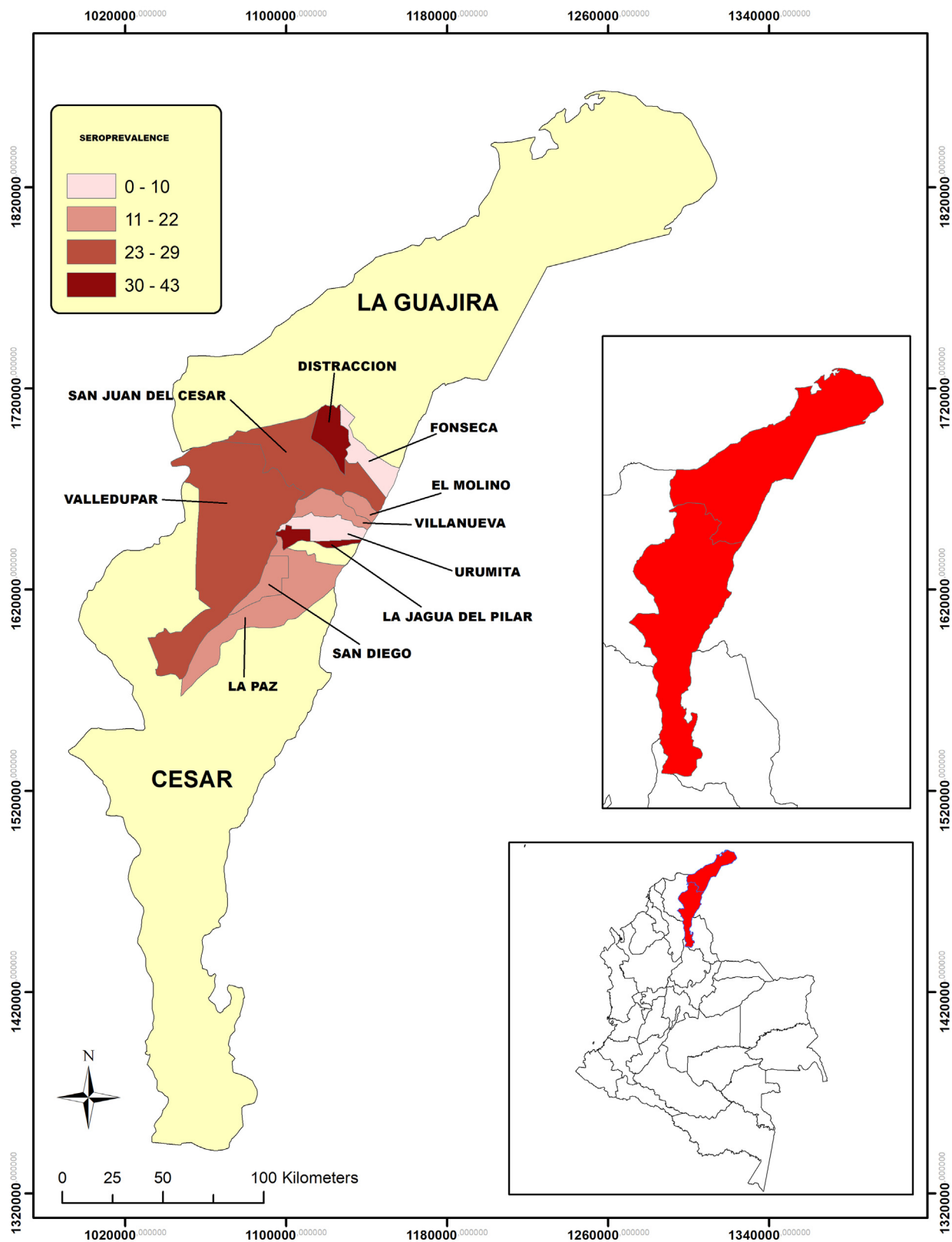


Fig. 1. Seroprevalence estimates of *Toxoplasma gondii* in sheep and goats.

3.2. Risk factors

Based on the univariable analysis, risk factors for sheep and goat *T. gondii* seropositivity were drinking water from local aqueducts (OR = 2.26 C.I: 1.674–3.059), having dogs and cats (OR = 2.9 C.I: 2.05–4.27), close contact with poultry (OR = 3.1 C.I: 2.11–4.820) and/or pigs (OR = 11.59 C.I: 6.21–21.65). However, the protective factors identified were the weaning over two months after birth (OR = 0.678 C.I: 0.498–0.919), having with equines (OR = 0.155 C.I: 0.048–0.500) and cattle (OR = 0.308 C.I 0.191–0.497), drinking water directly from small lagoons (OR = 0.452 C.I: 0.333–0.615) and cisterns (OR = 0.728 C.I: 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 variables by univariable analysis were analyzed by multivariable analysis. The risk factors identified by multivariable analyses were having pigs and 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). 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.

	OR	χ^2	p	CI
General variable				
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				
There are no management facilities.	1.578	9.609	0.002*	1.181–2.108
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
There are no records of the animal health.	1.777	9.751	0.002*	1.235–2.558
They do no disinfect or change clothes when handling animals belonging to groups with different health conditions.	2.155	6.527	0.011*	1.181–3.935
They do not have clean and supervised areas for the delivery of the females.	1.405	4.771	0.029*	1.035–1.909
Neonates do not have a clean, dry and well ventilated environment.	1.391	5.050	0.025	1.042–1.857

OR: odds ratio; C.I: confidence interval (95%).

* Statistically significant.

Table 3Multivariable logistic regression analysis of variables associated with *T. gondii* seroprevalence in small ruminants of northeastern areas of Colombia.

General variable	β	OR	<i>p</i>	C.I. (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; C.I: 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 oocyst, 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.

Acknowledgements

We express gratitude to VECOL laboratories, AGROSAVIA, ICA and Zoolab for financial, technical and operative support during the present research.

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.

References

- Alvarado, J., 1982. Prevalencia de toxoplasma en ovinos africanos del Tolima por inmunofluorescencia indirecta. Universidad del Tolima.
- Amdouni, Y., Rjeibi, M.R., Rouatbi, M., Amairia, S., Awadi, S., Gharbi, M., 2017. Molecular detection of *Toxoplasma gondii* infection in slaughtered ruminants (sheep, goats and cattle) in Northwest Tunisia. *Meat Sci.* 133, 180–184. <https://doi.org/10.1016/j.meatsci.2017.07.004>.
- Bezerra, M.J.G., Cruz, J.A.L.O., Kung, E.S., Silva, J.G., Santos, A.S., Moraes, É.P.B.X., Pinheiro Junior, J.W., et al., 2014. Occurrence of *Toxoplasma gondii* DNA in sheep naturally infected and slaughtered in abattoirs in Pernambuco, Brazil. *Pesqui Vet. Bras.* 34, 329–331. <https://doi.org/10.1590/S0100-736X2014000400005>.
- Blewett, D.A., Trees, A.J., 1987. The epidemiology of ovine toxoplasmosis with especial respect to control. *Br Vet J* 143, 128–135. [https://doi.org/10.1016/0007-1935\(87\)90004-2](https://doi.org/10.1016/0007-1935(87)90004-2).
- Buxton, D., Maley, S.W., Wright, S.E., Rodger, S., Bartley, P., Innes, E.A., 2007. *Toxoplasma gondii* and ovine toxoplasmosis: new aspects of an old story. *Vet. Parasitol.* 149, 25–28. <https://doi.org/10.1016/j.vetpar.2007.07.003>.
- Cañón-Franco, W.A., López-Orozco, N., Gómez-Marín, J.E., Dubey, J.P., 2014. An overview of seventy years of research (1944–2014) on toxoplasmosis in Colombia, South America. *Parasit. Vectors* 7, 427. <https://doi.org/10.1186/1756-3305-7-427>.
- Cavalcante, A.C.R., Carneiro, M., Gouveia, A.M.G., Pinheiro, R.R., Vitor, R.W.A., 2008. Risk factors for infection by *Toxoplasma gondii* in herds of goats in Ceará, Brazil. *Arq Bras Med Vet e Zootec* 60, 36–41. <https://doi.org/10.1590/S0102-09352008000100006>.
- Chiari, C.A., Neves, D.P., 1984. Toxoplasmose humana adquirida através da ingestão de leite de cabra. *Mem. Inst. Oswaldo Cruz* 79, 337–340.
- Cook, A.J., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenun, P.A., Foulon, W., et al., 2000. Sources of toxoplasma infection in pregnant women: European multicentre case-control study. *European Research Network on Congenital Toxoplasmosis. BMJ* 321, 142–147. <https://doi.org/10.1136/bmj.321.7254.142>.
- Dohoo, I., Martin, W., Stryhn, H., 2003. *Veterinary Epidemiologic Research*. first ed. Transcontinental Prince Edward Island, Charlottetown-Canada (Chapters 16 and 17).
- Dubey, J.P., 2009. Toxoplasmosis in sheep—the last 20 years. *Vet. Parasitol.* 163, 1–14. <https://doi.org/10.1016/j.vetpar.2009.02.026>.
- Dubey, J.P., Lappin, M.R., Thulliez, P., 1995. Long-term antibody responses of cats fed *Toxoplasma gondii* tissue cysts. *J. Parasitol.* 81, 887. <https://doi.org/10.2307/3284035>.
- Espinosa, A.C., Torres, L.C., Alzate, C., Lemus, E.J., Puyo, D.S., Ramírez, J.C., 2011. Prevalencia de *Toxoplasma gondii* en gatos domésticos del casco urbano del Municipio de Florencia-Caquetá. *Rev. Fac. Ciencias Agropecu.* 3, 27–30.
- Fortes, M.S., Lopes-Mori, F.M.R., Caldart, E.T., Constantino, C., Evers, F., Pagliari, S., de Almeida, J.C., et al., 2018. Caprine toxoplasmosis in Southern Brazil: a comparative seroepidemiological study between the indirect immunofluorescence assay, the enzyme-linked immunosorbent assay, and the modified agglutination test. *Trop. Anim. Health Prod.* 50, 413–419. <https://doi.org/10.1007/s11250-017-1450-1>.
- Frey, A., Di Canzio, J., Zurakowski, D., 1998. A statistically defined endpoint titer determination method for immunoassays. *J. Immunol. Methods* 221, 35–41. [https://doi.org/10.1016/S0022-1759\(98\)00170-7](https://doi.org/10.1016/S0022-1759(98)00170-7).
- Gómez Marín, J.E., 2002. Toxoplasmosis: Un problema de Salud Pública en Colombia. *Rev. Salud Pública* 4, 7–10.
- Gómez Moreno, L., Lara Rodríguez, D.G., Flórez Villamizar, H.M., 2014. Caracterización de la cadena ovina y caprina en Colombia. 1. *Rev. Innovando En La U*, pp. 75–83.
- ICA, 2016. Censo Pecuario Nacional. *Inst. Colomb. Agropecu.* 0. <https://www.ica.gov.co/Areas/Pecuaría/Servicios/Epidemiología-Veterinaria/Censos-2016/Censo-2017.aspx>, Accessed date: 10 March 2016.
- IDEAM, d. Atlas climatológico de Colombia. *Inst. Hidrol. Meteorología y Estud. Ambient.* 2014:0. <http://atlas.ideam.gov.co/presentacion/>, Accessed date: 10 March 2016.
- Innes, E.A., Bartley, P.M., Buxton, D., Katzer, F., 2009. Ovine toxoplasmosis. *Parasitology* 136, 1887–1894. <https://doi.org/10.1017/S0031182009991636>.
- Jácome, J., 2013. Prevalencia de infección por *Toxoplasma gondii* en mujeres embarazadas, en Valledupar. *Cesar, Rev. Colomb. Microbiol. Trop.* 3, 31–44.
- Jones, J.L., Dubey, J.P., 2010. Waterborne toxoplasmosis - recent developments. *Exp. Parasitol.* 124, 10–25. <https://doi.org/10.1016/j.exppara.2009.03.013>.
- Kijlstra, A., Eissen, O.A., Cornelissen, J., Munniksma, K., Eijck, I., Kortbeek, T., 2004. *Toxoplasma gondii* infection in animal-friendly pig production systems. *Investig. Ophthalmol. Vis. Sci.* 45, 3165–3169. <https://doi.org/10.1167/iov.04-0326>.
- López-Castillo, C.A., Díaz-Ramírez, J., Gómez-Marín, J.E., 2005. Risk factors for *Toxoplasma gondii* infection in pregnant women in Armenia, Colombia. *Rvista de Salud Pública* 7, 180–190.
- Machado-Torres, N.P., Manrique-Carrascal, E.E., Ruiz-Hoyos, B.M., Blanco-Turián, P.J., 2004. Alta frecuencia de seroconversión toxoplásmica en gestantes de Sincelajo-Sucre. *Infectio* 8, 263–267. <https://doi.org/10.22354/in.v8i4.261>.
- Munhoz, A.D., Mendonça, C.E.D., Barros, S.L.B., Guimarães, V.A.A., Ferraudo, A.S., 2014. Prevalence and risk factors associated to ovine toxoplasmosis in northeastern Brazil. *Rev. Bras. Parasitol. Vet.* 22, 230–234. <https://doi.org/10.1590/S1984-29612013000200042>.
- Nunes, A.C.B.T., da Silva, E.M.V., de Oliveira, J.A., Yamasaki, E.M., Kim, P. de C.P., de Almeida, J.C., Nunes, K.B., et al., 2015. Application of different techniques to detect *Toxoplasma gondii* in slaughtered sheep for human consumption. *Rev. Bras. Parasitol. Veterinária* 24, 416–421. <https://doi.org/10.1590/S1984-29612015076>.
- Pagmadulam, B., Myagmarsuren, P., Yokoyama, N., Battsetseg, B., Nishikawa, Y., 2020. Seroepidemiological study of *Toxoplasma gondii* in small ruminants (sheep and goat) in different provinces of Mongolia. *Parasitol. Int.* 74, 101996. <https://doi.org/10.1016/j.parint.2019.101996>.
- Perry, B.D., Grieve, A.S., Mogollon, J.D., de Galvis, A.L., 1978. Serological study of ovine toxoplasmosis in Colombia: prevalence of haemagglutinating antibodies to toxoplasma in sheep. *Vet. Rec.* 103, 584–585. <https://doi.org/10.1136/vr.103.26-27.584>.
- Saadatnia, G., Golkar, M., 2012. A review on human toxoplasmosis. *Scand. J. Infect. Dis.* 44, 805–814. <https://doi.org/10.3109/00365548.2012.693197>.
- Sacks, J.J., Roberto, R.R., F, N., 1982. Toxoplasmosis infection associated with raw goat's milk. *JAMA J. Am. Med. Assoc.* 248, 1728–1732. <https://doi.org/10.1001/jama.1982.03330140038029>.
- Santa, I.A.M., Valbuena, Y.A., Cortes, L.J., Sánchez, A.C.F., 2009. Seroprevalencia de la toxoplasmosis y factores relacionados con las enfermedades transmitidas por alimentos en trabajadores de plantas de beneficio animal en cinco ciudades capitales de Colombia, 2008. *Nova* 7, 66–70.
- Santos, K.R., Da Fonseca Lemos, J., Lopes, C.D., Sousa, R.A., De Oliveira, M.R.A., Luz, C.S.M., Júnior, S.C. de S., et al., 2018. Occurrence and risk factors for *Toxoplasma gondii* infection in goats from micro-regions of the state of Piauí. *Semin. Cienc. Agrar.* 2457–2464. <https://doi.org/10.5433/1679-0359.2018v39n6p2457>.
- Skinner, L.J., Timperley, A.C., Wightman, D., Chatterton, J.M.W., Ho-Yen, D.O., 1990. Simultaneous diagnosis of toxoplasmosis in goats and goatowner's family. *Scand. J. Infect. Dis.* 22, 359–361. <https://doi.org/10.3109/00365549009027060>.
- Tagwireyi, W.N., Etter, E., Neves, L., 2019. Seroprevalence and associated risk factors of *Toxoplasma gondii* infection in domestic animals in southeastern South Africa. *Onderstepoort J. Vet. Res.* 86, 1–6. <https://doi.org/10.4102/ojvr.v86i1.1688>.
- Tenter, A.M., 2009. *Toxoplasma gondii* in animals used for human consumption. *Mem. Inst. Oswaldo Cruz* 104, 364–369. <https://doi.org/10.1590/S0074-02762009000200033>.

- Tenter, A.M., Heckerroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. *Int. J. Parasitol.* 30, 1217–1258. [https://doi.org/10.1016/S0020-7519\(00\)00124-7](https://doi.org/10.1016/S0020-7519(00)00124-7).
- Triviño-Valencia, J., Lora, F., Zuluaga, J.D., Gomez-Marin, J.E., 2016. Detection by PCR of pathogenic protozoa in raw and drinkable water samples in Colombia. *Parasitol. Res.* 115, 1789–1797. <https://doi.org/10.1007/s00436-016-4917-5>.
- Tzanidakis, N., Maksimov, P., Conraths, F.J., Kiossis, E., Brozos, C., Sotiraki, S., Schares, G., 2012. *Toxoplasma gondii* in sheep and goats: seroprevalence and potential risk factors under dairy husbandry practices. *Vet. Parasitol.* 190, 340–348. <https://doi.org/10.1016/j.vetpar.2012.07.020>.
- Vesco, G., Buffolano, W., La Chiusa, S., Mancuso, G., Caracappa, S., Chianca, A., Villari, S., et al., 2007. *Toxoplasma gondii* infections in sheep in Sicily, southern Italy. *Vet. Parasitol.* 146, 3–8. <https://doi.org/10.1016/j.vetpar.2007.02.019>.