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Risk factors associated with *Toxoplasma gondii* seroprevalence in goats in the State of Paraíba, Brazil

Fatores de risco associados à soroprevalência de *Toxoplasma gondii* em caprinos do Estado da Paraíba, Brasil

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Abstract

A cross-sectional study based on planned sampling was carried out to determine flock-level risk factors associated with *Toxoplasma gondii* antibody prevalence in dairy goat flocks in a semiarid region of northeastern Brazil. Serum samples from 975 adult dairy goats from 110 flocks were examined by indirect immonufluorescent antibody test (IFAT), using cut-off point at 1:64 dilution. From the 110 flocks, 77 presented at least one seropositive animal, corresponding to a prevalence of 70% (95% CI: 60.5-78.4%). Out of the 975 animals, 177 (18.1%; 95% CI = 15.8-20.7%) tested positive. The presence of toxic plants (OR = 5.11; *P* = 0.045) and the fact that goat breeding is not the main activity on the farm (OR = 3.34; *P* = 0.014) were identified as risk factors. The results of the present study showed evidence of the presence of *T. gondii* infection in dairy goats from a semiarid region of northeastern Brazil using planned sampling. Further studies are needed to elucidate the importance of the identified risk factors in the epidemiology of the infection.

Keywords: *Toxoplasma gondii*, goats, risk factors, Brazil.

Resumo

Foi conduzido um estudo transversal baseado em amostragem planejada com o objetivo de determinar fatores de risco associados com a prevalência de anticorpos contra *Toxoplasma gondii*, em rebanhos de caprinos leiteiros, em uma região semiárida do Nordeste do Brasil. Amostras de soro de 975 caprinos leiteiros adultos, procedentes de 110 propriedades, foram examinadas pela reação de imunofluorescência indireta (RIFI), utilizando-se como ponto de corte a diluição 1:64. Das 110 propriedades, 77 apresentaram pelo menos um animal soropositivo, correspondendo a uma prevalência de 70% (IC 95%: 60,5-78,4%). Dos 975 animais, 177 (18,1%; IC 95% = 15,8-20,7%) foram positivos. Apesar da presença de plantas tóxicas (OR = 5,11; *P* = 0,045) e da caprinocultura não ser a principal atividade na propriedade (OR = 3,34; *P* = 0,014), foram identificados como fatores de risco. Os resultados do presente estudo mostraram uma evidência da presença da infecção por *T. gondii*, em caprinos leiteiros, em uma região semiárida do Nordeste do Brasil, utilizando-se uma amostragem planejada. Há necessidade de condução de estudos posteriores para elucidar a importância epidemiológica dos fatores de risco identificados.

Palavras-chave: *Toxoplasma gondii*, caprinos, fatores de risco, Brasil.

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Introduction

Toxoplasma gondii is a protozoan parasite found worldwide and it can infect a wide range of animal species, including goats; it has an indirect life cycle with felids as definitive hosts (DUBEY, 2010). In goats, *T. gondii* can cause abortion or neonatal mortalities (DUBEY; BEATTIE, 1988). The organism is estimated to infect 4-77% of the human population (TENTER et al., 2000). Although not normally a significant problem for healthy individuals, *T. gondii* infection can be life threatening to infants infected congenitally and pharmacologically immunosuppressed patients (CHINTANA et al., 1998). In animals, *T. gondii* infection not only results in significant reproduction and, hence, economic losses, but also has implications on public health, since the consumption of infected meat or milk can facilitate zoonotic transmission. *T. gondii* tachyzoites have been detected in milk of goats and some occurrences of human toxoplasmosis have been attributed to the consumption of non-pasteurized goat milk (SACKS et al., 1982; CHIARI; NEVES, 1984; SKINNER et al., 1990).

Goats are economically important in many countries, including Brazil, where this species is an important source of meat and milk for humans, particularly in the northeastern region, where 93.7% of the goat population is concentrated (IBGE, 2009). Dairy goat breeding is an increasing economic activity in Brazil and, in spite of the large number of animals – approximately 12 million, the country ranks only at the 18th position in terms of the amount of goat milk produced, mainly due to the low per goat milk productivity. Amongst other factors, infectious diseases such as toxoplasmosis may contribute to this problem, leading to reproduction failure (SKJERVE et al., 1998; FREYRE et al., 1999; BORDE et al., 2006).

The use of convenience sampling in epidemiological works to determine the occurrence of infectious diseases is very common and allows for the determination of important information; however, epidemiological inference should not be made based on this procedure because of the occurrence of biases. In Brazil, although many serological studies on *T. gondii* infection in goats are available, there are few works based on planned sampling. The aim of this research was to identify risk factors associated with flock-level prevalence of *T. gondii* infection in dairy goats from a semiarid region of the State of Paraíba, northeastern Brazil, based on planned sampling.

Materials and Methods

1. Study area and sampling

The present study was carried out from March 2009 to March 2010 in the municipality of Monteiro (7° 53' S and 37° 5' W), Ocidental Cariri microregion, semiarid region of the State of Paraíba, northeastern Brazil. The climate is classified as semiarid and temperatures range from 18 °C at night to 31 °C during the day, with mean temperature of 22 °C. The area is 599 m above sea level. Monteiro excels in the goat milk production not only in the State of Paraíba, but also in Brazil; it has the greatest number of goats in the state, in a total of 30,240 animals (IBGE, 2009).

The research was designed as a cross-sectional study of randomly selected dairy goat flocks. Blood samples were collected from female goats that were ≥12 months old. A two-stage sample design was followed. First, dairy goat flocks were randomly selected. The number of flocks to be sampled was determined considering the number of dairy goat flocks in the region (n = 180, according to data of the Center for Integrated Development of Goat Production of the State of Paraíba), expected flock prevalence of 50% (not considered a priori knowledge of the flock prevalence), and 10% desired accuracy for a 99% level of confidence (THRUSFIELD, 2007); resulting in 86 herds to be sampled. After that, the sample size of goats to be selected was individually determined for each flock in order to detect the presence of the infection. Calculations were made in accordance with the formula commonly applied in veterinary epidemiological investigations (THRUSFIELD, 2007) (Equation 1):

$$n = \left[1-\left(1-p\right)^{\frac{1}{d}}\right]\times\left(N-\frac{d}{2}\right)+1 \qquad (1)$$

where:
n – sample size;
p – probability of detection of at least one seropositive goat;
N – flock size;
d – number of seropositive goats in the flock.

The probability of detection of at least one seropositive goat in a flock was determined at 95% (p = 0.95), and the number of seropositive goats in each flock (d) was calculated assuming prevalence of 6.4% within the flock (FIGLIUOLO et al., 2004).

Finally, from five to 12 blood samples were collected in each flock, resulting in a total number of 975 samples in 110 flocks.

For the selection of goats to be sampled from each flock, animals were put in a crush pen and then selected using systematic random sampling (THRUSFIELD, 2007). In situations where there was no handling infrastructure, true random sampling was difficult to attain. In such situations, animals were put in a kraal and randomly captured.

2. Serum collection

A 10 mL blood sample was collected from each animal from the jugular vein using vacutainer tubes. Samples were allowed to clot and transferred in ice, as quickly as possible, to the Transmissible Diseases Laboratory of the Federal University of Campina Grande, Patos, State of Paraíba, Brazil. The sera were separated by centrifugation at 2000 rpm for 10 minutes, aspirated into Eppendorf tubes and stored at –20 °C until testing.

3. Epidemiological data collection

A structured questionnaire focusing on risk factors for toxoplasmosis was conducted with each farmer at the time of blood collection. Information was collected on a total of 22 flock-level factors including: management system; main activity in the farm; flock size; predominant goat breed; presence of cattle, swine, cats, dogs and wildlife; presence of toxic plants; availability of veterinary

services; animal purchasing; mineral supplementation; use of disposable syringes; lending of bucks for breeding; communal pasture grazing; use of disinfectants; use of maternity pens; and history of abortions, infertility, stillbirths and birth of weak animals.

4. Serological diagnosis

Indirect fluorescent antibody test (IFAT) was performed for detection of anti-*T. gondii* antibodies considering 1:64 dilution as cut-off point (GARCIA et al., 1999) and, according to the method by Camargo (1974), using RH strain tachyzoites as antigen. Positive and negative control goat sera were used. Anti-goat IgG (whole molecule; Sigma, St. Louis, MO, USA) was used as conjugate in a 1:400 dilution in sterile PBS (0.105 M Na_2HPO_4, 0.018 M KH_2PO_4, 1.37 M NaCl, 0.027 M KCl), pH 7.6.

5. Statistical analysis

Flocks that presented at least one seropositive animal were considered positive. Prevalence of positive flocks was estimated from the ratio of positive flocks to the total number of flocks investigated, with the exact binomial confidence interval of 95% (THRUSFIELD, 2007), using EpiInfo-6.04 software program.

Risk factor analysis was performed in two steps: univariate analysis and nonlinear logistic regression model. Univariate analysis was performed using the chi-square test or Fisher's exact test (ZAR, 1999), and the variables that presented $P \leq 0.20$ were used for multiple logistic regression. Nonlinear logistic regression model was then performed using the stepwise forward method (HOSMER; LEMESHOW, 2000), with significance level of 5%. Final model adjustment was verified by the Hosmer and Lemeshow test, and $P \geq 0.05$ was considered to indicate satisfactory fit. Collinearity between independent variables was assessed by correlation analysis, and when two variables were highly collinear (correlation coefficient > 0.90), only one variable was likely to enter the nonlinear logistic regression model; therefore, the selection of which collinear variable to enter the model was guided by biological plausibility (DOHOO et al., 1996). The tests were performed using SPSS for Windows – version 13.0 software package.

Results

From the 110 flocks, 77 presented at least one seropositive animal for *T. gondii*, corresponding to a prevalence of 70% (95% CI: 60.5-78.4%). Within-flock prevalence ranged from 8.3% to 85.7%. Out of the 975 animals, 177 (18.1%; 95% CI = 15.8-20.7%) tested positive for *T. gondii* antibodies.

In the univariate analysis for risk factors, the variables main activity in the farm; predominant goat breed; presence of cattle; presence of swine; presence of toxic plants; and history of abortions were associated ($P < 0.20$) with flock-level prevalence; and were then selected for multivariate analysis (Table 1). When these independent variables were subjected to nonlinear logistic regression model, presence of toxic plants (OR = 5.11; $P = 0.045$) and goat breeding not being the main activity in the farm (OR = 3.34; $P = 0.014$) were identified as risk factors (Table 2).

Table 1. Univariate analysis for flock-level risk factors associated with *Toxoplasma gondii* infection in dairy goat flocks in the municipality of Monteiro, State of Paraíba, northeastern Brazil, from March 2009 to March 2010.

Independent variables	Nº of flocks	Nº of positive flocks (%)	*P*
MANAGEMENT SYSTEM			
Intensive	2	2 (100)	
Semi-intensive	101	71 (70.3)	
Extensive	7	4 (57.1)	0.500
MAIN ACTIVITY IN THE FARM			
Goat breeding	76	48 (63.2)	
Other	34	29 (85.3)	0.019*
FLOCK SIZE			
≤25 goats	53	37 (69.8)	
>25 goats	57	40 (70.2)	1.000
PREDOMINANT GOAT BREED			
Purebred	7	3 (42.9)	
Mixed-bred	103	74 (71.8)	0.194*
PRESENCE OF CATTLE			
No	35	29 (82.9)	
Yes	75	48 (64)	0.074*
PRESENCE OF SWINE			
No	79	60 (75.9)	
Yes	31	17 (54.8)	0.052*
PRESENCE OF CATS			
No	80	59 (73.8)	
Yes	30	18 (60)	0.243

*Variables selected for the multivariate analysis ($P < 0.20$).

Table 1. Continued...

Independent variables	Nº of flocks	Nº of positive flocks (%)	*P*
PRESENCE OF DOGS			
No	37	26 (70.3)	
Yes	73	51 (69.6)	0.860
PRESENCE OF WILDLIFE			
No	81	56 (69.1)	
Yes	29	21 (72.4)	0.925
PRESENCE OF TOXIC PLANTS			
No	92	61 (66.3)	
Yes	18	16 (88.9)	0.103*
VETERINARY SERVICES			
No	104	73 (70.2)	
Yes	6	4 (66.7)	1.000
ANIMAL PURCHASING			
No	99	71 (71.7)	
Yes	11	6 (54.5)	0.300
MINERAL SUPPLEMENTATION			
No	48	32 (66.7)	
Yes	62	45 (72.6)	0.644
USE OF DISPOSABLE SYRINGES			
No	50	33 (66)	
Yes	60	44 (73.3)	0.531
BUCK LENDING FOR BREEDING			
No	67	48 (71.6)	
Yes	43	29 (67.4)	0.798
COMMUNAL PASTURE GRAZING			
No	105	74 (70.5)	
Yes	5	3 (60)	0.635
USE OF DISINFECTANTS			
No	62	45 (72.6)	
Yes	48	32 (66.7)	0.644
USE OF MATERNITY PENS			
No	101	72 (71.3)	
Yes	9	5 (55.6)	0.448
HISTORY OF ABORTIONS			
No	59	37 (62.7)	
Yes	51	40 (78.4)	0.113*
HISTORY OF INFERTILITY			
No	101	71 (70.3)	
Yes	9	6 (66.7)	1.000
STILLBIRTHS			
No	86	58 (67.4)	
Yes	24	19 (79.2)	0.392
BIRTHS OF WEAK ANIMALS			
No	84	56 (66.7)	
Yes	26	21 (80.8)	0.260

*Variables selected for the multivariate analysis ($P < 0.20$).

Discussion

Several researches on *T. gondii* seroprevalence in goats have been carried out in Brazil, especially in the northeast region. Frequencies of seropositive animals of 24.5%, 30.6%, 25.1% and 4.35% were reported in the states of Paraíba, Rio Grande do Norte, Ceará and Maranhão, respectively (FARIA et al., 2007; ARAÚJO NETO et al., 2008; CAVALCANTE et al., 2008; MORAES et al., 2011). All studies assessed animal-level frequency, not flock-level prevalence. In this study, the high flock-level prevalence (70%) and within-flock prevalence (8.3% to 85.7%) for *T. gondii* infection in dairy goats may be associated

Table 2. Flock-level risk factors associated with *Toxoplasma gondii* infection in dairy goat flocks in the municipality of Monteiro, semiarid region of the State of Paraíba, northeastern Brazil, from March 2009 to March 2010.

Risk factor	Odds ratio	95% CI	*P*
Presence of toxic plants	5.11	1.03-25.30	0.045
Goat breeding not being the main activity on the farm	3.34	1.28-8.68	0.014

Hosmer and Lemeshow test: $\chi^2 = 3.301$; $P = 0.509$.

with extensive management system and unlimited contact with free-roaming cats, both of which are important risk factors (ARAÚJO NETO et al., 2008; CZOPOWICZ et al., 2011). The municipality of Monteiro is an important area for goat milk production in northeastern Brazil, and given that unpasteurized goat milk poses an important risk factor for *T. gondii* infection in humans (JONES et al., 2009), the results provided background for further public health risk analysis (CZOPOWICZ et al., 2011).

The prevalence of toxoplasmosis in different regions may range, among other factors, according to climatic conditions, presence of cats and management system (DUBEY, 2010). In Brazil, frequencies of seropositive animals have ranged from 4.35% in the State of Maranhão (MORAES et al., 2011) to 68% in the State of Minas Gerais (BAHIA et al., 1993). The individual-level frequency of anti-*T. gondii* antibodies found in present work (18.1%) is considered low compared to other studies, which can be explained by the fact that these animals belong to dairy flocks, where management care is more appropriate. Another factor to be considered refers to environmental and climatic conditions. The region investigated in the present work is semiarid, where climate is characterized by low humidity and rainfall volume, which may have adverse effects on the viability and environmental spread of *T. gondii* oocysts (DUBEY, 2010).

In the risk factor analysis for *T. gondii*, the fact that goat breeding is not the main activity in the farm was associated to flock-level prevalence. This may be explained by the use of facilities, techniques and management unsuitable for dairy goat production in these farms, as well as by the low level of organization and lack of effective sanitary control.

Another factor associated to flock-level prevalence was the presence of toxic plants. According to Robert et al. (1981), high antibody titers may be found in acute infections and in the reactivation of infections due to immunosuppression. Some toxic substances can cause immunosuppression and impair the immunity of chronically infected hosts, which become unable to control, locally, the rupture of cysts and release of tachyzoites, allowing for the reactivation of acute infections (TENTER et al., 2000). Venturini et al. (1996) reported that low and repeated doses of mycotoxins, which are enough to induce subclinical intoxication, could increase the likelihood of toxoplasma cyst rupture and, hence, the recrudescence of chronic toxoplasmosis in persistently infected mice. In the State of Paraíba, according to Riet-Correa et al. (2006), the main toxic plants for goats are *Mascagnia rigida* (tinguí), *Prosopis juliflora* (algaroba), *Ipomoea asarifolia* (salsa), *Ipomoea carnea* subsp. *fistulosa* ('algodão bravo'), *Leucaena leucocephala* ('leucena'), *Manihot* spp. ('maniçobas'), *Mimosa tenuiflora* ('jurema preta') and *Aspidosperma pyrifolium* ('pereiro'). During the dry season, in areas with low fodder availability, toxic plants are often the only green plants found in pasture, and some of them present high palatability, which induces animals to eating. However, it is unclear which toxic plants from the region can cause immunosuppression and influence the prevalence of toxoplasmosis in goats. This probably happens because of the decreased capacity of intoxicated animals to control possible infections due to low infective parasitic load or less pathogenic *T. gondii* strains. This could be subject of further investigations.

Conclusions

The results of the present study showed evidence of the presence of *T. gondii* infection in goats from a semiarid region of northeastern Brazil using planned sampling. Further studies are needed to elucidate the importance of the identified risk factors in the epidemiology of the infection.

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