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Prevalence and risk factors associated to ovine toxoplasmosis in northeastern Brazil

Prevalência e fatores de risco associados à toxoplasmose ovina no Nordeste do Brasil

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Abstract

In this study, we aimed to determine the prevalence and risk factors associated to *Toxoplasma gondii* infection in sheep from northeastern Brazil. A total of 932 ovine serum samples from 54 properties in 19 municipalities of the state of Sergipe were collected and assayed using indirect fluorescent antibody test. The assay used antibodies against *Toxoplasma gondii* (IFAT-IgG) with a cutoff point of 1:64. We observed that 28.22% (263/932) of the ovine samples were serum-reactive. In a logistic regression, factors such as consumption of water directly from the source, consumption of water from a deep well, and age below 12 months were associated with protection; whereas factors such as presence of cats on the property, presence of slatted floor, and use of exchanged or borrowed breeding males were associated with infection. The studied area can be considered endemic for toxoplasmosis, so it is necessary to adopt preventive and control measures because this zoonotic infection poses risks to public health.

Keywords: *Toxoplasma gondii*, sheep, seroepidemiology, risk factors, Brazil.

Resumo

Objetivou-se determinar a prevalência e os fatores associados à infecção por *Toxoplasma gondii* em ovinos do Nordeste brasileiro. Foram processadas 932 amostras de soro de ovinos procedentes de 54 propriedades de 19 municípios do Estado de Sergipe e analisadas pela reação de imunofluorescência indireta para pesquisa de anticorpos contra *Toxoplasma gondii* (RIFI-IgG), com ponto de corte de 1:64. Observou-se que 28,22% (263/932) dos ovinos foram sororeagentes. Na regressão logística não condicional, ingestão de água direto da fonte, ingestão de água de poço profundo e idade inferior a 12 meses foram fatores associados à proteção; enquanto que a presença de gato na propriedade, presença de aprisco ripado e troca ou empréstimo de machos reprodutores foram identificados como fatores associados à infecção. Ao considerar a região de estudo como endêmica para a doença, cabe a adoção de medidas preventivas e de controle, uma vez que por apresentar caráter zoonótico a infecção destes animais representa riscos à saúde pública.

Keywords: *Toxoplasma gondii*, ovino, seroepidemiologia, fatores de risco, Brasil.

Introduction

Toxoplasmosis is a zoonosis with a worldwide distribution and is caused by the coccidian protozoan *Toxoplasma gondii* (DUBEY; BEATTIE, 1988). *Toxoplasma gondii* is an obligate intracellular parasite (DUBEY, 1994) that infects warm-blooded animals,

including humans (TENTER et al., 2000). Domestic cats are important in the transmission of the disease (DUBEY et al., 1995) to ruminants because felids are the only animals that eliminate oocysts in their feces (FRENKEL et al., 1970).

In sheep, toxoplasmosis may give rise to various disorders and lead to lower reproductive yields, especially during pregnancy (DUBEY; KIRKBRIDE, 1990). Serological studies have been carried out worldwide, showing significant variation in prevalence of infection (DUBEY, 2009). These differences may occur due to

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the study area, associated factors, and criteria for animal selection, as well as the technique used (ROSSI et al., 2011). Furthermore, the established cutoff point for serum dilution during antibody analysis is an important factor because, even when the same technique is applied, the use of different cutoff points can interfere with results (DUBEY, 2009).

Some of the risk factors associated with infection include age, gender, water source, presence of cats, type of production system (MASALA et al., 2003; VESCO et al., 2007), capacity of grazing areas, genotypes of parasite and host, infection period, and duration of infection before pregnancy (SILVA; DE LA RUE, 2006).

Considering the seriousness of infection by *T. gondii* in sheep worldwide and the lack of data in the state of Sergipe, this study aimed to determine the prevalence of *T. gondii* infection in sheep flocks and any factors associated with infection.

Materials and Methods

Location of the study

This study was conducted in northeastern Brazil, state of Sergipe (9° 30' 49" and 11° 34' 05" latitude; 36° 23' 40" and 38° 15' 00" longitude). The state of Sergipe holds an ovine flock of 247,703 animals and it is divided in three mesoregions: the "Sertão Sergipano" (backwoods of Sergipe state) is home to 41.19% of this population, the "Agreste Sergipano" (dry area) holds 44.12%, and the "Leste Sergipano" (eastern region of the state) contains 14.69% of the flocks (Sergipe, 2007).

The backwoods of Sergipe state mesoregion presents hot semi-arid tropical climate. This climate occurs across the western part of the state and it is characterized mainly by presenting seven to eleven dry months. The annual average rainfall ranges from 400 to 700 mm and it is irregularly and poorly distributed throughout the year, mainly through thunderstorms and heavy rainfall. Additionally, it presents periodic droughts and long droughts, high temperatures - over 40 °C during the day, long days even in winter, sharp evaporation, and temporary rivers with salt or brackish water mostly (BRASIL, 2012).

The dry area of Sergipe state mesoregion presents hot and humid tropical climate. It rains throughout the year, mainly from April to August. It presents only one to three dry months, with total annual rainfall ranging from 1000 to 1400 mm. The temperature remains high, around 25 °C, and it varies little over the month because of the proximity of the ocean (BRASIL, 2012).

The eastern region of the state presents hot semi-humid tropical climate with rainfall around 700-900 mm per year. With four to six dry months (October to March) a year, it is sensitive to the effects of droughts. Rainfall is erratic and poorly distributed, but there is a certain concentration from April to August. Thunderstorms are frequent during the months of December and January. Day temperatures over 30 °C drop during the night (BRASIL, 2012).

Sampling design

The sampling universe was defined based on the largest sheep herds from the municipalities in each region of the state. Nineteen municipalities were studied: six in the mesoregion of "Sertão

Sergipano" (83,728 animals), six in the "Agreste Sergipano" (89,688 animals), and seven in "Leste Sergipano" (20,373 animals), comprising a total of 193,789 animals, i.e., 78.23% of the ovine population of the state.

The minimum number of animals to be sampled (383) was calculated using the statistical software EPI-INFO, version 3.5.1 (DEAN; ARNER, 2009). For this calculation, we used expected prevalence of 50%, sampling error rate of 5%, and 95% confidence level for a population of 193,789 animals. The number of properties to be visited and the number of minimum samples to be collected in each municipality were determined as a percentage of the municipal herd.

Non-probability sampling was used to select producers. This was the method of choice since there is no representative list of sheep producers in the state, which made randomized design impossible. The prevalence of *T. gondii* in the farms was categorized as low, medium, or high, based on the results.

Sample collection

This study was conducted within the standards established by the Brazilian Society of Ethics and Animal Welfare, under the protocol number (CEUA/UESC-39/2009). Blood samples were collected via jugular vein puncture. Samples were centrifuged at 1600 × g for 10 minutes and sera were separated, placed in microtubes, and frozen at -20 °C. Semi-structured interviews were conducted with the owner or head caretaker during the visits, covering data on the animal, the breeder, the property, the production system, and the food and health management of each farm.

Ultimately, 932 samples were collected from 54 properties located in 19 municipalities. Of these, 38.20% (356/932) were in the 'Sertão Sergipano', 44.85% (418/932) in the 'Agreste Sergipano', and 16.95% (158/932) in the 'Leste Sergipano'. Based on the level of improved breeding and the diet, the production systems were classified as extensive (19/54), semi-intensive (33/54), and intensive (02/54).

Serology

The sera were tested for antibodies to *T. gondii* by indirect fluorescent antibody test using the RH parasite strain. Commercially available FITC-labeled anti-ovine IgG (F-7634, Sigma-Aldrich St. Louis, MO, USA) was used as secondary antibody. Serum dilution threshold for positivity was set at 1:64 (FIGLIUOLO et al., 2004). Negative and positive controls consisted of pre and post infection sera from sheep inoculated with tachyzoites. The samples were four-fold titrated (ROMANELLI et al., 2007).

Statistical analysis

Data obtained from the interview were tabulated in the statistical package EPI INFO 3.5.1 (DEAN; ARNER, 2009) and then analyzed by chi-squared test along with Yates correction or Fisher's exact test (SAMPALIO, 1998).

The odds ratio (OR) of the bivariate analysis was calculated using measures of association and 95% confidence interval (CI). The

variables with p value lower or equal to 20% and showing biological plausibility were selected and subject to Spearman correlation test to determine collinearity ($p \geq 0.8$), using the BIOESTAT® 5.0 software. Finally, multivariate analysis with non-conditional logistic regression was performed, with the final model created through the input and output variables of the system (*backward*).

We performed a multiple correspondence analysis to evaluate the possible influence of the variables in the final model of logistic regression on the level of prevalence of *T. gondii* at the properties. This analysis is a multivariate exploratory technique that uses data processed with Statistica® software.

Results

Antibodies against *T. gondii* were detected in 28.22% (263/932) of the sheep investigated (Table 1). The percentages of positive animals on the farms ranged from 6.25% to 61.11%, and 94.44% (51/54) of the properties presented at least one positive animal. Considering each mesoregion, 97 (27.25%) of the animals in the 'Sertão Sergipano', 115 (27.51%) in the 'Agreste Sergipano' and 51 (32.28%) in the 'Leste Sergipano' were positive. The statistical difference between mesoregions was not regarded as significant ($p > 0.05$). Infection was observed in 94.74% (18/19) of the municipalities, with positive results ranging from 6.25% to 47.62% (Table 1).

The variables age below 12 months, consumption of water from a deep well, exchanged or borrowed breeding males, presence of cats on the property, presence of sheepfold fitted with slatted floor, presence of tamped earth floor stalls, consumption of water from a cistern, gender, consumption of water directly from the source, and controlled mating showed p -value below 0.20; In addition, there was no collinearity among these variables, which fits the primary model of non-conditional logistic regression.

The final logistic regression model showed that consumption of water from a deep well, consumption of water directly from the source, and age below 12 months were protective factors, whereas factors such as presence of cats on the property, use of slatted floor, and use of exchanged or borrowed breeding males were associated with risks of infection (Table 2).

In the multiple correspondence analyses, higher chi-square values (where the value was higher than the expected one) were observed for the following variables: consumption of water from a deep well (1.68), absence of cats on the property (1.55), and consumption of water directly from the source (1.14) in properties with low prevalence to *T. gondii*.

Discussion

This is the first report of *T. gondii* infection seroprevalence in sheep from Sergipe state, Brazil. This study sampled from the largest number of animals of any study conducted in northeastern Brazil, which is the region with the largest amount (56.9%) of sheep in the country (BRASIL, 2011). Considering that *T. gondii* infection is not eliminated by the immune system of affected sheep, the antibody titer results in this study indicate the presence of tissue cysts (SHKAP et al., 1992), making the intake of raw or undercooked meat an important route of transmission (DUBEY; KIRKBRIDE, 1989).

There was no significant difference in the number of positive results between different mesoregions ($p > 0.05$). These mesoregions present distinct climates; higher rates of infection should be expected in the 'Leste Sergipano', due to its humid climate, therefore, favoring the viability of oocysts (DUBEY; BEATTIE, 1988); in turn, the 'Agreste' features semi-arid climate and it is regarded as a transition area, whereas the 'Sertão' presents drier semi-arid climate with low and irregular rainfall indexes and periodic droughts. Hence, results showing no significant difference in infection prevalence

Table 1. Distribution of seropositivity for *Toxoplasma gondii* in sheep from the state of Sergipe, northeastern Brazil, 2008.

Mesoregion	Municipalities (Positive/Total)	Properties (Positive/Total)	Total animals	Titration					Positive Animals (%) ³
				Negative	64	256	1024	4096	
Leste (East)	(6/7 ²)	(6/8 ²)	158	107	9	19	14	9	32.28
Agreste (Dry area)	(6/6)	(23/24)	418	303	35	51	20	9	27.51
Sertão (Backwoods)	(6/6)	(22/22)	356	259	28	40	26	3	27.25
Total	(18/19)	(51/54)	932	669	72	110	60	21	28.22

¹ Positive. ² Total. ³ $p > 0.05$.

Table 2. Final model of non-conditional logistic regression of factors associated with *Toxoplasma gondii* infection in sheep from the state of Sergipe, northeastern Brazil, 2008.

Variables	Odds ratio	CI (95%)	P value
Consumption of water from a deep well	0.3187	0.1609	0.631
Slatted floor	16.092	11.501	22.517
Consumption of water directly from the source	0.7319	0.5368	0.9979
Age < 12 months	0.321	0.2221	0.464
Cats on the property	16.615	11.833	23.330
Exchanged/borrowed breeding males	14.956	10.220	21.889

$P < 0.0001$, likelihood=766.311; CI - confidence interval.

among the regions indicate that, despite the fact that there are regions with adverse climatic conditions for parasite maintenance, *T. gondii* remains present and has the potential to infect animals and, possibly, humans (DUBEY, 1998).

We noted that 61.11% (32/54) of the properties had domestic cats. The presence of cats is a factor associated with infection, as shown by Vesco et al. (2007) and Romanelli et al. (2007). The presence of cats is of fundamental importance in the epidemiology of *T. gondii* since, depending on the environmental conditions, oocysts shed in the feces of cats can survive for months or even years (FRENKEL et al., 1975). These oocysts can then become a main source of infection for sheep. We found correspondence between the variable absence of cats on the property and low prevalence of toxoplasmosis in sheep.

The use of exchanged or borrowed breeding males was also identified as a factor associated with infection. It is likely that these animals disseminate the parasite among herds because the presence of DNA from the parasite in the semen of sheep has been detected in natural (MORAES et al., 2010) and experimental infections (LOPES et al., 2009). Additionally, Lopes et al. (2011), using immunohistochemistry, identified *T. gondii* in the epididymis, seminal vesicles, and prostate of experimentally infected animals. The presence of *T. gondii* in the reproductive tract of males indicates the possibility of venereal transmission, which may partly explain the results of studies that suggest a form of infection other than oocysts, once the presence of cats was not characterized as a risk factor (FIGLIUOLO et al., 2004; SOARES et al., 2009).

The use of slatted floor on a property was associated with infection. Despite the lack of information in the literature, slatted floor promotes the accumulation of organic matter in a shaded spot that is subject to rare or improper cleaning. Therefore, it is possible to relate the use of slatted floor to environmental conditions that favor oocyst development (DUBEY; BEATTIE, 1988; RODRÍGUEZ-PONCE et al., 1995).

Our results show that animals under the age of 12 months were less likely to become infected. The low prevalence of infection in young animals is probably due to lower parasite exposure, suggesting that the chance of contact with sporulated oocysts increases with age (FIGLIUOLO et al., 2004; VESCO et al., 2007; BAHRIENI et al., 2008; ROSSI et al., 2011). These observations corroborate the fact that 76.19% (16/21) of the sheep older than 12 months presented higher titrations. Our findings confirm Bahrieni et al. (2008), who suggested that adult animals are more exposed to *T. gondii* transmission and that this increased exposure results in greater chances of becoming infected or reinfected.

When examining the possible association between the sources of water provided to animals and infection by *T. gondii*, we found that animals that consumed water directly from the source or from a deep well were less likely to become infected; therefore, these were regarded as protective factors. Possibly, this occurs due to the inaccessibility of cats to these types of water sources, thus hindering water contamination by oocysts. When analyzing the association of positive animals with various sources of drinking water, Romanelli et al. (2007) observed an increase in positive results across the following sources: fountain, artesian well, and rainwater reservoirs. The observed increase confirms our findings, as does the work by Vesco et al. (2007), who observed that animals that drink

surface water have a greater chance of becoming infected. Water source and the chance for transmission through contamination with oocysts excreted by felids also presented association with the low prevalence of toxoplasmosis in the properties, indicating that water source might be a viable mechanism for possible infection control.

Our results indicate that *T. gondii* infection has spread across the ovine flocks in the state of Sergipe and that the studied region presents endemic infection that poses risk of transmission to humans (DUBEY; KIRKBRIDE, 1989). It is necessary to instruct the residents and producers of Sergipe in order to raise awareness about the importance of this zoonosis and to establish infection control through preventive care. Furthermore, producers should be encouraged to supply water – if possible – from sources that are less likely to be contaminated by oocysts, protect water sources from the contact with cats and their feces, and avoid the introduction of untested breeding males due to the possibility of *T. gondii* transmission.

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