

Risk factors for *Toxoplasma gondii* infection in sheep in the northeastern region of Brazil

Fatores de risco para a infecção de *Toxoplasma gondii* em ovinos da região nordeste do Brasil

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Abstract

Toxoplasma gondii is an infective parasite that causes reproductive disorders such as abortion, fetal mummification, birth of weak offspring, and stillbirth, thereby causing economic losses to sheep production. The northeastern region of Brazil has approximately 171 million small ruminants, of which 5.4% are sheep. The present study aimed at determining the rate of occurrence of *T. gondii* in sheep flocks on 60 farms in 19 municipalities in the three mesoregions (eastern, semi-arid, and *sertão* or backlands) of the state of Sergipe, Brazil, and the risk factors associated with this infection. Serum samples were collected between 2011 and 2012, from 60 farms located in 19 municipalities in the three mesoregions: 680 in the eastern region, 280 in the semi-arid region, and 240 in the backlands, totaling 1,200 samples (990 females and 210 males). Anti-*T. gondii* antibodies were detected by means of the indirect fluorescence antibody test (IFAT \geq 64). The highest occurrence was detected in the eastern region (45.3%, $p = 0.001$). On farms with subsistence production, the risk of having animals infected by *T. gondii* was approximately twice as high as on breeding/rebreeding/fattening farms (OR: 3.03; CI: 1.97-4.68). There was a significant lack of sanitary care, such as absence of a dunghill ($p = 0.000$; OR: 1.60; CI: 1.26-2.03), quarantine ($p = 0.000$; OR: 1.87; CI: 1.45-2.41) and disinfection ($p = 0.003$; OR: 1.46; CI: 1.13-1.88). Regarding feeding, the risk of infection was 1.74 and 1.37 times higher in places that used a trough and/or that cats could access, respectively. The present study allows the conclusions that *T. gondii* is found on farms in the three mesoregions of the state of Sergipe and that environmental and management factors have an influence on sheep infection.

Keywords: Abortion. Oocyst. Sheep breeding. Parasitology. Toxoplasmosis.

Resumo

Toxoplasma gondii é um parasita cuja infecção leva a desordens reprodutivas como aborto, mumificação fetal, nascimento de cordeiros fracos e natimortos, provocando perdas econômicas na produção ovina. A região nordeste do Brasil possui aproximadamente 171 milhões de pequenos ruminantes, dos quais 5,4% são ovinos. Este estudo tem como objetivo determinar a ocorrência de *T. gondii* nos rebanhos ovinos de 60 propriedades de 19 municípios de três mesorregiões (leste, semiárido e sertão) do estado de Sergipe, Brasil, e os fatores de risco associados a essa infecção. Amostras de soro foram coletadas entre 2011 e 2012, em 60 propriedades localizadas em 19 municípios das três mesorregiões: 680 na região leste, 280 no semiárido e 240 no sertão, totalizando 1.200 amostras (990 fêmeas e 210 machos). Anticorpos anti-*T.gondii* foram detectados por reação de imunofluorescência indireta (RIF \geq 64). A maior ocorrência foi detectada na região leste (45,3%, $p = 0.001$). Em propriedades com produção de subsistência, o risco de animais infectados por *T. gondii* é aproximadamente duas vezes maior que nas de cria/recria/engorda (OR = 3.03/ IC: 1.97-4.68). A ausência de cuidados sanitários, como ausência de esterqueira ($p = 0.000$ / OR: 1.60; CI: 1.26-2.03); quarentena ($p = 0.000$ / OR: 1.87; CI: 1.45-2.41) e desinfecção ($p = 0.003$ / OR: 1.46; CI: 1.13-1.88) foram significantes. Em relação à alimentação, o risco de infecção aumenta 1.74 e 1.37 em locais que utilizam cocho ou com presença de gatos, respectivamente. Este estudo permite concluir que o *T. gondii* é encontrado em propriedades das mesorregiões do estado de Sergipe e fatores ambientais e de manejo estão influenciando nas infecções em ovinos.

Palavras-chave: Aborto. Oocisto. Criação de ovinos. Parasitologia. Toxoplasmose.

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Introduction

Toxoplasma gondii is a parasite in the phylum *Apicomplexa*. Felids are its definitive host and homoeothermic animals are its intermediate host. Transmission can occur through ingestion of food or water contaminated with oocysts, by eating raw or undercooked meat or by means of the transplacental route, in which tachyzoites can infect fetuses (FRENKELL, 1990; SILVA; LA RUE, 2006).

Infection by *T. gondii* is mostly present in areas with warm and wet weather (DUBEY, 2009). Sporulation of oocysts and their survival in the environment occur only under favorable conditions, such as proper aeration and suitable humidity and temperature. Under such conditions, oocysts become infective within five days (TENDER et al., 2000).

In relation to sheep production, *T. gondii* infection causes reproductive disorders (MILLAR et al., 2007), such as abortion, fetal mummification, birth of weak lambs, and stillbirth, thereby causing economic losses. One of the few studies that conducted an economic analysis of these losses, Freyre et al. (1999), reported losses of between 1.4 and 4.7 million dollars, in Uruguay.

In Brazil the reported prevalence of *T. gondii* among sheep has ranged from 25.75% to 80% (OGAWA et al., 2003; FIGLIUOLO et al., 2004; UENO et al., 2009; ROSSI et al., 2011). Minas Gerais State, Brazil, this study aimed to investigate the frequency of antibodies against these parasites in sheep sera from this region by using different serological methods. A total of 155 sheep serum samples were analyzed by the indirect fluorescence antibody test (IFAT; TESOLINI et al., 2012; MENDONÇA et al., 2013).

The northeastern region of Brazil has approximately 171 million small ruminants, of which 5.4% are sheep (IBGE, 2011). The seroprevalence in this area is between 11.11% and 48.4% (BISPO et al., 2011; MENDONÇA et al., 2013; CORREIA et al., 2015), and the risk factors associated with the infection have been water supply (BRANDÃO et al., 2009), sex and breed (SILVA et al., 2003), age, presence of cats, and water source (ANDRADE et al., 2013). The state of Sergipe has 1% of the total number of small ruminants

in the northeastern region, and its total sheep flock is approximately 168,801. Antibodies against *T. gondii* were only described in sheep flocks by Mendonça et al. (2013), whose study revealed 28.22% of serum-reactive samples. Regarding other species, evidence of *T. gondii* infection was detected in wild animals (53.1%) (PIMENTEL et al., 2009).

Since *T. gondii* infection is one of the most important causes of reproductive disorders in sheep flocks, its prevalence needs to be more thoroughly understood. Therefore, the present study aimed at determining the prevalence of *T. gondii* in sheep flocks of 60 farms in the three mesoregions of the state of Sergipe and the risk factors associated with infection.

Material and Methods

This study was conducted within the standards established by the Bioethics Committee of the “Pius X” School, in Aracajú, Sergipe, Brazil (approval no. 06/2014).

The state of Sergipe is located between the latitudes 9°30'49” and 11°34'05” and the longitudes 36°23'4” and 38°15'00”). Its territorial area is 21.915 km² and it is divided into three edaphoclimatic mesoregions: eastern region, semi-arid region, and backlands (SANTOS et al., 2014). Sergipe holds 1% of the small ruminant flock of northeastern Brazil, comprising a total of 170.547, of which 89.24% are sheep (SEBRAE, 2011).

Municipalities and farms were selected according to the state divisions and according to convenience, easiness of access and availability of producers. Animals were randomly selected. Healthy male and female sheep, with different husbandry standards (pure breed, half-breed, and mixed breed) older than six months of age were enrolled into this study.

Samples were collected between 2011 and 2012 from 60 farms located in 19 municipalities in the three mesoregions: 680 in the eastern region, 280 in semi-arid region, and 240 in the backlands – thus total of 1,200 serum samples (990 females and 210 males), 20 from each farm.

An epidemiological interview was conducted on each farm, in order to evaluate the risk factors associated with *T. gondii* infection. This interview consisted of closed questions related to the farm: mesoregion (eastern, semi-arid, or backland region), production system (extensive, intensive, or semi-extensive), terrain (rough, waterlogged, or flat), installations (pens with cemented, unpaved, or slatted floor), food storage accessibility to cats, availability of food (covered trough or uncovered

trough) and water source accessibility to cats. There was also one question related to herd features: purpose of the animal production (breeding/rebreeding, fattening, reproduction, or subsistence). There were also questions relating to management: water source (running, standing, or standing + running), water supply (from the source, containers inside the facility, containers inside and outside the facility, or containers outside the facility); disinfection of installations, use of a dunghill, food storage (uncovered place, covered place, or both), quarantine and presence of sheep with reproductive disorders.

Blood samples were collected via jugular vein puncture using a vacuum tube, without anticoagulant. The samples were centrifuged at 1,600 g for 10 minutes. The resultant serum samples were identified and stored at -20°C.

Samples were tested for antibodies to *T. gondii* by means of indirect fluorescence antibody test (IFAT), as described by Camargo (1974), with a cut-off point of 1:64 (GARCIA et al., 1999). Tachyzoites of *T. gondii* from the RH reference strain, cultured in Vero cells, were methanol fixed in slides as the antigen.

All the statistical calculations were performed using the EpiInfo 3.5.1 statistical package and a 95% confidence interval (CI). Descriptive analysis was performed to determine absolute and relative frequencies. Data were analyzed by means of Pearson's chi-square test or Fisher's exact test in the form of univariate analysis to study risk factors associated with infection by *T. gondii*. Logistic regression was used, taking the IFAT results (reactive or non-reactive) to be a dependent variable relating to the infection. In order to avoid exclusion of important risk factors, independent variables with statistical significance less than or equal to 20% were selected for the model (HOSMER; LEMESHOW, 1989).

Results

The rate of occurrence of antibodies against *T. gondii* was 93.3% at herd level and 40.1% at animal level. The highest prevalence was detected in the eastern region ($p = 0.001$), where the two municipalities with the highest frequencies were located: Divina Pastora and Arauá (73.3% and 71.7%, respectively). Table 1 presents the prevalence values for antibodies against *T. gondii* per municipality.

Table 1 – Occurrences of antibodies against *Toxoplasma gondii* detected by means of indirect fluorescence antibody test (≥ 64) on sheep serum samples, according to farm, animal, and region – Sergipe – 2011-2012

Municipality	Farms Animals	
	Positive/total (%)	Positive/total (%)
Aracaju	1/1 (100)	13/20 (65.0)
Arauá	3/3 (100)	43/60 (71.7)
Boquim	1/1 (100)	9/20 (45.0)
Divina Pastora	3/3 (100)	44/60 (73.3)
Estância	4/4 (100)	32/80 (40.0)
Itabaianinha	9/9 (100)	82/180 (45.6)
Itaporanga d'ajuda	4/5 (80)	38/100 (38.0)
Maruim	2/2 (100)	8/40 (20.0)
Nossa Senhora do Socorro	1/1 (100)	5/20 (25.0)
Salgado	3/3 (100)	23/60 (38.3)
São Cristóvão	1/2 (50)	11/40 (27.5)
Total for eastern region	32/34 (94.1)	308/680 (45.3)
Cumbe	1/1 (100)	4/20 (20.0)
Lagarto	6/6 (100)	44/120 (36.7)
Nossa Senhora das Dores	4/4 (100)	35/80 (43.8)
Simão Dias	2/3 (66.7)	12/60 (20.0)
Total for semi-arid region	13/14 (92.9)	95/280 (33.9)
Canindé de São Francisco	4/4 (100)	9/80 (11.3)
Feira Nova	2/2 (100)	20/40 (50.0)
Itabi	1/2 (50)	11/40 (27.5)
Nossa Senhora da Glória	4/4 (100)	38/80 (47.5)
Total for backlands region	11/12 (91.7)	78/240 (32.5)
Total	56/60 (93.3)	481/1200 (40.1)

The antibody titers detected were: 4096 (0.2%), 2048 (1.5%), 1024 (2.7%), 512 (3.3%), 128 (3.7%), 256 (5.2%), and 64 (83.4%). In the semi-arid region, only titers of 64 and 128 were detected (Table 2).

Table 3 presents the odds' ratio and chi-square analyses. The variables of mesoregion, purpose of production system, terrain, disinfection of installations, use of dunghill, availability of food, food storage accessibility to cats, and quarantine were selected for the multivariate analyses.

The statistical significance observed in multivariate logistic regression is described in Table 4. In subsistence farms, the risk of having animals infected by *T. gondii* was

approximately twice as high as in breeding/rebreeding/fattening farms ($p = 0.000$; OR: 3.03; CI: 1.97-4.68). Farms in the eastern region with waterlogged ($p = 0.005$; OR: 1.81; CI: 1.19-2.77) and semi-extensive production systems ($p = 0.012$; OR: 1.90; CI: 1.01-3.74) had the highest chance of having infected sheep. Lack of sanitary care, such as absence of a dunghill ($p = 0.000$; OR: 1.60; CI: 1.26-2.03), quarantine ($p = 0.000$; OR: 1.87; CI: 1.45-2.41) and disinfection ($p = 0.003$; OR: 1.46; CI: 1.13-1.88) were important for the infection. Regarding feeding, the risk of infection was 1.74 and 1.37 times higher in places that used a trough and/or which cats could access, respectively ($p < 0.05$).

Table 2 – Frequency of antibody titers against *Toxoplasma gondii* determined by means of indirect fluorescence antibody test on sheep serum samples, according to region – Sergipe – 2011-2012

Region	Positive samples (%)							Total (%)
	64	128	256	512	1024	2048	4096	
Eastern	251 (52.2)	13 (2.7)	17 (3.5)	11 (2.3)	8 (1.7)	7 (1.5)	1 (0.2)	308 (64.0)
Semi-arid	73 (15.2)	4 (0.8)	8 (1.7)	5 (1.04)	5 (1.04)	-	-	95 (19.8)
Backlands	77 (16.0)	1 (0.2)	-	-	-	-	-	78 (16.2)
Total	401 (83.4)	18 (3.7)	25 (5.2)	16 (3.3)	13 (2.7)	7 (1.5)	1 (0.2)	481 (100)

Table 3 – Risk factors associated with infection by *Toxoplasma gondii* in sheep flocks in the eastern, semi-arid and backlands regions – Sergipe – 2011-2012

Variable	Examined	Positive (%)	OR (95% CI)	p-value
Mesoregion				
Semi-arid	280	95 (33.9)	-	
Eastern	680	308 (45.3)	1.61 (1.20 – 2.18)	0.000
Backlands	240	78 (32.5)	0.58 (0.42 – 0.80)	
Sex				
Female	989	404 (40.8)		
Male	211	77 (36.5)	1.20 (0.87 – 1.65)	0.136
Breed				
Dorper	72	30 (41.7)	-	
Mixed	65	20 (30.8)	0.62 (0.29 – 1.33)	0.284
Santa Inês	1063	431 (40.5)	1.53 (0.87 – 2.78)	
Purpose of production				
Breeding/rebreeding/fattening	960	352 (36.7)	-	
Reproduction	140	64 (45.7)	1.45 (1.00 – 2.11)	0.000
Subsistence	100	65 (65.0)	2.21 (1.26 – 3.88)	
Production system				
Extensive	500	218 (43.6)	-	
Intensive	60	15 (25.0)	0.43 (0.22 – 0.81)	0.012
Semi-extensive	640	248 (38.8)	1.90 (1.01 – 3.74)	
Terrain				
Rough	120	36 (30.0)	-	
Waterlogged	580	254 (43.8)	1.82 (1.17 – 2.86)	0.010
Flat	500	191 (38.2)	0.79 (0.62 – 1.02)	
Installations				
Cemented pen floors	120	53 (44.2)	-	
Unpaved pen floors	540	196 (36.3)	0.72 (0.47 – 1.10)	0.077
Slatted pen floors	420	179 (42.6)	1.30 (1.00 – 1.71)	
Disinfection of installations				
Yes	580	205 (35.3)		
No	620	276 (44.5)	0.68 (0.53 – 0.85)	0.001

Table 3 – Continuation

Variable	Examined	Positive (%)	OR (95% CI)	p-value
Use of dunghill				
Yes	540	183 (33.9)	0.62 (0.49 – 0.78)	0.000
No	660	298 (45.2)		
Food storage				
Uncovered	100	32 (32.0)	-	0.345
Covered	920	354 (38.5)	1.33 (0.84 – 2.14)	
Uncovered and covered	20	6 (30.0)	0.69 (0.21 – 1.92)	
Cat access to food storage				
Yes	280	129 (46.1)	1.37 (1.05 – 1.80)	0.011
No	920	352 (38.3)		
Availability of food				
Covered trough	640	209 (32.7)	0.57 (0.43 – 0.74)	0.000
Uncovered trough	360	165 (45.8)		
Water supply				
From the source	440	181 (41.1)	-	0.458
Containers inside the facility	460	173 (37.6)		
Containers inside and outside the facility	220	96 (43.6)		
Containers outside the facility	80	31 (38.8)		
Cat access to water				
Yes	400	161 (40.3)	1.01 (0.78 – 1.29)	0.491
No	800	320 (40.0)		
Quarantine				
Yes	400	124 (31.0)	0.53 (0.41 – 0.68)	0.000
No	781	357 (45.7)		
Reproductive disorders				
Yes	680	276 (40.6)	1.04 (0.82 – 1.33)	0.363
No	520	205 (39.4)		

Table 4 – Logistic regression analysis on risk factors associated with infection by *T. gondii* in sheep flocks in the state of Sergipe – 2011-2012

Variable	p value	OR	95% CI
Eastern region	0.001	1.61	1.20 – 2.15
Subsistence system	0.000	3.03	1.97 – 4.68
Reproduction purpose	0.008	1.65	1.13 – 2.39
Semi-extensive system	0.012	1.90	1.01 – 3.74
Waterlogged	0.005	1.81	1.19 – 2.77
No disinfection	0.003	1.46	1.13 – 1.88
No dunghill	0.000	1.60	1.26 – 2.03
No quarantine	0.000	1.87	1.45 – 2.41
Food in uncovered place	0.000	1.74	1.33 – 2.27
Cat access in food storage	0.019	1.37	1.05 – 1.80

Discussion

In the present study, 93.3% of the herds had at least one sheep that was *T. gondii*-positive. The eastern region showed the highest number of foci ($p = 0.001$), and also held the municipalities with the highest percentage of reactive sheep (Divina Pastora, 73.3%; and Arauá, 71.7%). Mendonça et al. (2013) also detected higher percentage of positive samples in the eastern region of Sergipe (32.28%).

In addition to high temperatures, the eastern, semi-arid, and backland regions are characterized as having wet, semiarid, and dry weather, respectively. The

predominance of reactive sheep and foci in the eastern region could be related to its weather conditions. Places with low humidity and high temperatures are unfavorable for oocysts (DUBEY, 2010). Nonetheless, although the backlands had the least suitable environment for survival of the agent, cat feces (YILMAZ; HOPKINS, 1972) and soil (FRENKEL et al., 1975) both have the capacity to create microclimates suitable for oocyst development, thus enabling transmission of the agent and continuity of its life cycle.

IFAT showed antibodies titers of 64 in 83.2% of the sheep. The high number of sheep presenting the minimum titer

suggests that chronic infection by coccidia was present, as observed in similar studies on sheep in the states of Alagoas and Rio de Janeiro (PINHEIRO et al., 2009; LUCIANO et al., 2011). Mendonça et al. (2013), in the state of Sergipe (11.8%), and Brandão et al. (2009), in the state of Maranhão (40.5%), detected high percentages of seropositivity with a titer of 256, therefore slightly higher than in the present study (5.2%). In another study, titers higher than 1024, suggesting active infection (DUBEY; KIRKBRIDE, 1989), were detected in 21 (4.46%) out of 481 reagent sheep.

Farming under a subsistence system involved less technology and presented major deficiencies of hygiene, which made it more favorable towards maintenance of the agent ($p = 0.000$). Moreover, cats were commonly present in the farms studied, and this was an important risk factor for infection ($p = 0.000$).

Flock rearing for reproduction was also found to be a risk factor ($p = 0.008$). Transmission of *T. gondii* through semen has been reported amongst dogs (ARANTES et al., 2009) and sheep (DE MORAES et al., 2010). However, the epidemiological importance of this infection route is limited, since only males that are used in natural mating and that are in the acute phase of the disease, with tachyzoites multiplying, could have a chance of transmitting the parasite during copulation. Presence of infection showed associations with use of a semi-extensive system and with presence of waterlogged terrain, with risks of infection that were 1.9 and 1.81 times higher, respectively. Pinheiro et al. (2009) observed the same association in relation to the production system. In semi-extensive breeding, the animals are partially confined, thus increasing the risk

of contact with cats and sporulated oocysts of *T. gondii* (MAINAR et al., 1996).

Failure to disinfect the installations and absence of quarantine were also risk factors for infection by *T. gondii*. Places without proper hygiene presented higher risk of infection by oocysts. Silva et al., (2003) also reported the importance of hygiene with regard to sheep breeding in the state of Pernambuco. Absence of quarantine allows infected pregnant females be introduced into herds. Fetal membranes and aborted fetuses are potential sources of infection, since cats or other animals might eat them. Therefore, fetal membranes and aborted fetuses need to be incinerated, so as to prevent the spread of *T. gondii* to the herd.

The availability of feed in an uncovered place was a risk factor detected in this study, and it favored transmission of *T. gondii* to the herds, since feed in this situation can easily be contaminated with cat feces. The presence of cats was also a risk factor for infection in the present study, as previously observed in several other Brazilian studies (ROMANELLI et al., 2007; LOPES et al., 2010; LUCIANO et al., 2011; ANDRADE et al., 2013), including in the state of Sergipe (MENDONÇA et al., 2013).

The present study allows the conclusions that *T. gondii* is found in farms throughout the three mesoregions of the state of Sergipe and that environmental factors and management have an influence on sheep infection.

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