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Risk factors associated with the epidemiology of *Toxoplasma gondii* in cattle and buffaloes in the state of Pará, Brazil

Fatores de riscos associados à epidemiologia do *Toxoplasma gondii* em bovinos e bubalinos no Estado do Pará, Brasil.

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan that parasitizes several hosts worldwide, of which cats are definitive hosts. The objective of this study is to evaluate the risk factors associated with the epidemiology of *T. gondii* in cattle and buffaloes in the state of Pará, Brazil. A total of 2,070 bovine samples (1,749 cattle and 321 buffaloes) were evaluated in 100 rural properties distributed in 51 municipalities from all mesoregions of the state. The evaluated risk factors were animal gender, type of farm, and number of cats in the properties. The serological prevalence of *T. gondii* was evaluated using the indirect enzyme immunoabsorption assay (iELISA). The samples with a sample/positive (S/P) ratio ≥ 0.5 were considered positive and analyzed using the indirect immunofluorescence assay (IFA). The samples diluted at 1:64 were considered positive. The chi-square test with a level of significance of 5% and the odds ratio (OR) with a confidence interval of 95% was applied. The data were analyzed using BioEstat software version 5.0. The prevalence of *T. gondii* in bovines was 35.79% and 45.12% in ELISA and IFAT, respectively. The prevalence was 34.48% and 44.14% in cattle and 42.99% and 50.47% in buffaloes using ELISA and IFA, respectively. The mesoregion with the highest prevalence of infection was Marajó Island, corresponding to 42.86% (129) and 50.5% (152) in ELISA and IFA, respectively. In buffaloes, the prevalence of *T. gondii* using ELISA was higher in males ($p < 0.05$) in the two age groups. In contrast, the prevalence of *T. gondii* using IFA was higher in males in the age group 13 to 24 months ($p < 0.05$). The type of farm did not affect the prevalence of *T. gondii* in cattle ($p > 0.05$). However, the presence of cats strongly affected the occurrence of infection in cattle. These results demonstrate the high levels of anti-*T. gondii* antibodies in cattle and buffaloes in Pará and this may be a public health problem. Furthermore, the presence of cats is a risk factor for infection with *T. gondii* in these species in Pará.

Key words: Toxoplasmosis. Bovine. ELISA. IFA.

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Resumo

O *Toxoplasma gondii* é um protozoário intracelular obrigatório de ocorrência mundial e se encontra parasitando uma grande diversidade de hospedeiros, sendo os gatos de fundamental importância para o ciclo de vida do parasita. O presente estudo teve como objetivo avaliar os fatores de riscos associados a epidemiologia do *Toxoplasma gondii* em bovinos e bubalinos no Estado do Pará, Brasil. Foram avaliadas 2.070 amostras de bovídeos, sendo 1.749 bovinos e 321 bubalinos, em 100 propriedades rurais distribuídas em 51 municípios, envolvendo todas as mesorregiões do Estado. Os fatores de risco avaliados foram sexo, exploração pecuária e número de gatos existentes nas propriedades. A prevalência sorológica de *Toxoplasma gondii* foi avaliada pelo Ensaio de Imunoabsorção Enzimática Indireta (iELISA), considerando-se positivas as amostras que obtiveram razão S/P (sample/positive) $\geq 0,5$, bem como, submetidas a Reação de Imuno fluorescência Indireta (RIFI), sendo positivas as amostras regentes na diluição 1:64. Foi aplicado o teste do Qui-quadrado com 5% de significância e empregado cálculo da razão de chances (OR), com um intervalo de confiança de 95%. Todos os resultados foram tratados estatisticamente pelo *software* BioEstat 5.0. A prevalência de *T. gondii* nos bovídeos foi de 35,79% e 45,12% no ELISA e RIFI, respectivamente. Em bovinos a prevalência foi de 34,48% e 44,14% e em bubalinos de 42,99% e 50,47% no ELISA e RIFI, respectivamente. A mesorregião com maior prevalência foi a do Marajó com 42,86% (129) e 50,5% (152) no ELISA e RIFI respectivamente. Em relação aos bubalinos a prevalência de *T. gondii* foi mais elevada em macho ($P < 0,05$) nas duas faixas etárias avaliadas pelo ELISA. Já pelo RIFI a prevalência foi maior em machos somente na faixa etária de 13 a 24 meses ($P < 0,05$) não tendo influência do sexo nos bovinos. O tipo de exploração também não influenciou a prevalência de *T. gondii* em bovídeos ($P > 0,05$), porém a presença de gatos teve grande significância na ocorrência de infecção em bovídeos. Os resultados demonstram a elevada presença de anticorpos anti - *T. gondii* em bovinos e bubalinos no Estado do Pará, o que pode representar risco para saúde pública, além do que a presença de gatos é um fator de risco para a infecção por *T. gondii* nessas espécies no estado do Pará.

Palavras-chave: Toxoplasmose. Bovídeos. ELISA. RIFI.

Introduction

Toxoplasmosis is an important cosmopolitan zoonosis transmitted by the intracellular protozoan *Toxoplasma gondii*. Cats are definitive hosts and warm-blooded animals are intermediate hosts (PINTO et al., 2009).

Production animals are essential in the epidemiology of toxoplasmosis as a source of infection to humans. The animal species that are most sensitive to infection are goats, sheep, and swine, and the species that are least sensitive are cattle, horses, and birds (MILLAR et al., 2008).

In Brazil, studies conducted in Minas Gerais, Paraná, São Paulo, Bahia, and Rio Grande do Sul reported that the percentage of cattle with positive *T. gondii* antibodies varied from 1.03% to 49.17% (COSTA; COSTA, 1978; COSTA, 2001; DAGUER et al., 2004; SPAGNOL et al., 2009; SANTOS, 2012). Using the IFA, Gondim et al. (1999) reported

that the positivity rate in Bahia was 3.85% and Fuji et al. (2001) observed that the positivity rate in São Paulo was 3.2%. In the state of Pará, Silva et al. (2010) reported that the prevalence of infection in buffaloes was 1.1% whereas Silva et al. (2013) found a much higher prevalence in this species (41.6%).

The presence of definitive hosts for *T. gondii*, including domestic cats, in cattle properties, is a risk factor for the seroprevalence of infection (ALBUQUERQUE et al., 2011; SANTOS, 2012). The extensive grazing system used in Brazil also favors infection with *T. gondii* (MARANA et al., 1995; MILLAR et al., 2008).

The state of Pará has the fourth largest cattle herd and the largest buffalo herd in Brazil, with approximately 21 million and 500,000 animals, respectively (PARÁ, 2014). Moreover, Pará is the largest exporter of live animals, and many

slaughterhouses in this state are suitable for exporting beef. Therefore, knowing the distribution of infection with *T. gondii* in production animals is essential because of the economic, social, and public health importance of this activity to the state.

In addition, few studies to date evaluated the risk factors associated with *T. gondii* infection in bovine herds in the state of Pará. Therefore, the objective of this study is to evaluate the risk factors associated with the epidemiology of toxoplasmosis and the prevalence of anti-*T. gondii* antibodies in cattle and buffaloes reared in all mesoregions of the state of Pará, Brazil.

Materials and Methods

The municipalities were selected by the rate of animal movement. For this purpose, Animal Movement Guidelines (AMGs) issued in 2010 and 2011 by the Epidemiological Surveillance Service (ESS) of the Agriculture and Livestock Defense Agency of the state of Pará (Agência de Defesa Agropecuária do Estado do Pará-ADEPARÁ) were used. The data from the AMGs were grouped by municipality, microregion, and mesoregion, as previously established by IBGE. Network analysis (flow) was conducted using the Terra View software version 4.2 (PluginFlow). Furthermore, algorithms developed at the Laboratory of Epidemiology and Biostatistics of the School of Veterinary Medicine and Animal Science of the University of São Paulo (Laboratório de Epidemiologia e Bioestatística da Faculdade de Medicina Veterinária e Zootecnia da USP-LEB/FMVZ/USP) were used, allowing the selection of rural properties located in the municipalities with the highest inflow of cattle and buffaloes.

The FreeCalc software version 2, recommended by the OIE, was used to calculate the sample size using hypergeometric distribution and the formula proposed by Noordhuizen et al. (1998), $[1-(1-C)/1/(D*SENS)]*[M-(D*SENS-1)/2]$, where C is the level of confidence, M is the number of units

(animals/herds) at risk, D is the number of units with disease/infection, and SENS is the test sensitivity. The minimum sample size was 312 animals.

For the seroepidemiological survey, blood samples were collected in 2011 and 2012 from 2,070 bovines (1,749 cattle and 321 buffaloes) in 100 rural properties from 51 municipalities from all mesoregions of the state. The evaluated variables were animal species, gender, age, presence/number of domestic cats, and type of farm.

A random sampling method was used to select the animals in each chosen property. Blood samples were obtained by aseptic puncture of the jugular vein. The sera fractions were separated, labeled, and stored at -12 to -18 °C until serological analysis.

Indirect immunoenzymatic assay (iELISA), described by Cavalcante (2004), and the indirect immunofluorescence assay (IFA), described by Chiari et al. (1987) with some modifications, were performed in duplicate using commercially available Imunodot® kits for detecting *T. gondii*-specific IgG antibodies. In the IFA assay, the control samples at the dilution of 1:64 were considered positive.

In both techniques, the positive controls were sera of buffaloes and cattle with high antibody titers in iELISA and IFA, and the negative controls were sera of newborn buffaloes and cattle. The test sera and positive and negative controls were diluted 1:200. The absorbance was read at 405 nm using an iELISA reader.

The cut-off index (CI)—the mean optical density (OD) of negative-control sera multiplied by factor 2.5—was calculated. The samples with $OD \geq CI$ were considered positive for *T. gondii* whereas samples with $OD < CI$ were considered negative for *T. gondii*.

The results were analyzed using the chi-square test with a level of significance of 5% and the odds ratio (OR) with a 95% confidence interval. All results were statistically treated using BioEstat software version 5.0 (AYRES et al., 2007).

Results

In iELISA and IFA, the mean prevalence of antibodies to *T. gondii* in the evaluated animals was 35.79% and 45.12%, respectively, corresponding to 34.48% and 44.14% in cattle and 42.99% and

50.47% in buffaloes, respectively.

The mesoregion of Marajó had the highest prevalence of antibodies to *T. gondii*, corresponding to 42.86% and 50.50% in iELISA and IFA, respectively (Table 1).

Table 1. Distribution of *Toxoplasma gondii*-positive samples using ELISA and IFA by mesoregion in the state of Pará, 2015.

Mesoregion of Pará	Animals N* (%)	Animals with positive IgG	
		ELISA n** (%)	IFA n** (%)
Southeast	1177 (56.85%)	430 (36.53%)	538 (45.71%)
Northeast	392 (18.97%)	119 (30.35%)	161 (41.07%)
Marajó	301 (14.53%)	129 (42.86%)	152 (50.50%)
Lower Amazon	132 (6.37%)	42 (31.81%)	54 (40.91%)
Southwest	45 (2.17%)	17 (37.78%)	21 (46.67%)
Metropolitan area of Belém	23 (1.11%)	4 (17.39%)	8 (34.78%)
TOTAL	2070 (100%)	741 (35.79%)	934 (45.12%)

*N = total animals; **n = number of positive animals.

In the iELISA, the prevalence of antibodies to *T. gondii* in buffaloes aged 6 to 12 months was 48.33% (58/120) in males and 26.03% (19/73) in females ($p < 0.05$). In buffaloes aged 13 to 24 months, the prevalence of antibodies was 61.43% (43/70) in males and 31.03% (18/58) in females ($p < 0.05$).

In the IFA, the prevalence of antibodies to *T. gondii* in buffaloes aged 6 to 12 months was 51.66% (62/120) in males and 42.46% (31/73) in females ($p > 0.05$). In the age range 13 to 24 months, the prevalence was 65.71% (46/70) in males and 39.65% (23/58) in females ($p < 0.05$) (Table 2).

Table 2. Frequency of antibodies to *T. gondii* using ELISA and IFA by age group and gender of buffaloes raised in the state of Pará, Brazil.

Age (months)	Test	Gender	N*	Positive animals % (n**)	OR***	p****
6–12	ELISA	Male	120	48.33% (58)	2.6587	0.0035
		Female	73	26.03% (19)		
	IFA	Male	120	51.66% (62)	1.4483	0.2748
		Female	73	42.46% (31)		
13–24	ELISA	Male	70	61.43% (43)	3.5391	0.0012
		Female	58	31.03% (18)		
	IFA	Male	70	65.71% (46)	2.9167	0.0057
		Female	58	39.65% (23)		

*N = total animals; **n = number of positive animals; ***OR = odds ratio; ****the association was significant ($p < 0.05$) using the chi-square test.

In iELISA and IFA, there was no significant difference ($p > 0.05$) in the prevalence considering

the age group and animal gender (Table 3).

Table 3. Frequency of antibodies to *T. gondii* using ELISA and IFA by age group and gender of cattle raised in the state of Pará, Brazil.

Age (months)	Test	Gender	N*	Positive animals % (n**)	OR***	p****
6–12	ELISA	Male	470	38.71% (181)	0.9394	0.6826
		Female	500	40.00% (200)		
	IFA	Male	470	46.38% (218)	0.9076	0.4909
		Female	500	48.80% (244)		
13–24	ELISA	Male	434	29.72% (129)	1.1461	0.4413
		Female	345	26.96% (93)		
	IFA	Male	434	40.32% (175)	1.0511	0.7918
		Female	345	39.13% (135)		

*N = total animals; **n = number of positive animals; ***OR = odds ratio; ****the association was significant ($p < 0.05$) using the chi-square test.

With regard to the type of farm, the prevalence of antibodies using iELISA was 35.51%, 37.84%, 44.64% in production, dairy, and mixed properties, respectively, without significant differences ($p > 0.05$) whereas prevalence was 44.87%, 45.95%, and 53.57% in these three types of properties using IFA, respectively ($p > 0.05$) (Table 4).

Table 4. Frequency of antibodies to *T. gondii* using ELISA and IFA by the type of farm and species of bovine raised in the state of Pará, Brazil.

Test	Type of farm	N*	Positive animals % (n**)	OR***	p****
ELISA	Beef production	1977	35.51% (702)	0.6827	0.2059
	Mixed	56	44.64% (25)		
	Dairy	37	37.84% (14)		
	Beef production	1977	35.51% (702)	0.9045	0.9045
	Mixed	56	44.64% (25)		
	Dairy	37	37.84% (14)		
IFA	Beef production	1977	44.87% (887)	0.7053	0.2481
	Mixed	56	53.57% (30)		
	Dairy	37	45.95% (17)		
	Beef production	1977	44.87% (887)	1.0445	0.9713
	Mixed	56	53.57% (30)		
	Dairy	37	45.95% (17)		

*N = total animals; **n = number of positive animals; ***OR = odds ratio; ****the association was significant ($p < 0.05$) using the chi-square test.

With respect to the presence of cats in the properties, the seropositivity in iELISA was 20.32%, 33.64%, 44.02%, 39.47%, and 52.83% in properties with no cats, 1–3 cats, 4–6 cats, 7–9 cats, and >10 cats, respectively. There was a significant difference ($p < 0.05$) in seropositivity between properties with cats and those without cats (Table 5).

For IFA, the seropositivity was 35.29%, 43.28%, 50.54%, 46.49%, and 58.49% in properties with no cats, 1–3 cats, 4–6 cats, 7–9 cats, and >10 cats, respectively. There was a significant difference ($p < 0.05$) in seropositivity between the properties with cats and those without cats. This result indicates a positive correlation between the presence of cats and infection with *T. gondii*.

Table 5. Frequency of antibodies to *T. gondii* using ELISA and IFA according to the number of cats and bovines in the state of Pará, Brazil.

Test	Number of cats	Number of bovines	Positive animals % (n*)	OR**	p***
ELISA	0	374	20.32% (76)	0.5031	<0.0001
	1–3	871	33.64% (293)		
	0	374	20.32% (76)	0.3243	<0.0001
	4–6	552	44.02% (243)		
	0	374	20.32% (76)	0.3911	<0.0001
	7–9	114	39.47% (45)		
	0	374	20.32% (76)	0.2277	<0.0001
	>10	159	52.83% (84)		
IFA	0	374	35.29% (132)	0.7147	0.0103
	1–3	871	43.28% (377)		
	0	374	35.29% (132)	0.5337	<0.0001
	4–6	552	50.54% (279)		
	0	374	35.29% (132)	0.6278	0.0407
	7–9	114	46.49% (53)		
	0	374	35.29% (132)	0.3871	<0.0001
	>10	159	58.49% (93)		

*n = number of positive animals; **OR = odds ratio; ***Association was significant using the chi-square test ($p < 0.05$).

Discussion

These results indicate that the prevalence of antibodies to *T. gondii* in cattle in the state of Pará using iELISA and IFA was 34.48% and 44.14%, respectively. This percentage was similar to the mean value obtained in other regions of Brazil. The reported seropositivity was 48.51% (MARANA et al., 1995) and 41.4% (DAGUER et al., 2004) in Paraná, 17.4% in Rio Grande do Sul (SANTOS, 2012), and 11.83% in Bahia (SPAGNOL et al., 2009). In Pará, Carmo et al. (2017) observed that the prevalence of anti-*T. gondii* antibodies using IFA was 40.6% in beef cattle slaughtered for consumption in the metropolitan area of Belém.

The percentage of *T. gondii* antibodies in buffaloes was 42.99% using iELISA and 50.47% using IFA. Silva et al. (Prevalence) reported that the prevalence in Pará was 41.6% using iELISA and 36.0% using IFA. Souza et al. (2001) analyzed 411 serum samples of buffaloes for infection with *T. gondii* in São Paulo using IFA and found that 205 (49.9%) were seropositive. However, the seropositivity rates reported by studies on buffaloes in Brazil was lower

than those reported in the above studies. In this respect, the prevalence rates reported by Silva et al. (2010) in Pará, Gondim et al. (1999) in Bahia, and Fuji et al. (2001) in São Paulo were 1.1%, 3.85%, and 3.2% using IFA, respectively. Santos (2012) used IFA and found that the seropositivity was 27.2% in Rio Grande do Sul. Brasil et al. (2015) evaluated 136 buffaloes from 14 properties in Paraíba and found that the seropositivity was 12.5%. Guerra et al. (2014) hypothesized that variations in seroprevalence worldwide could be attributed to differences in ecosystems and the results of the serological tests used.

In the joint analysis of the variables, including species, sex, and age, there were no significant differences ($p > 0.05$) in the prevalence of antibodies in cattle. This result corroborates the study by Carmo et al. (2017) in Pará and Daguer et al. (2004) in Paraná, wherein there was no significant difference in prevalence between males and females ($p > 0.05$). However, El Fahal et al. (2013) reported that the prevalence of antibodies in cattle in Sudan was higher in males (30.8%) than in females (11.9%)

using ELISA whereas Daguer et al. (2004) found no significant differences in prevalence according to the age of the studied animals in Paraná.

Male buffaloes were significantly more affected, and the age group 13 to 24 months presented the highest seropositivity rate. In the state of São Paulo, female buffaloes aged 60 to 72 months carrying antibodies to *T. gondii* represented 62.5% of the positive animals, and 39.2% of the females aged 2 to 4 years contained antibodies to *T. gondii* (SOUZA et al., 2001). García and Vasquéz (1990) and Acha and Szyfres (1992) reported that the prevalence of toxoplasmosis was increased with age because there was a higher risk of exposure to the parasite as the age of the animals was increased.

With respect to the type of farm, the seroprevalence in production farms was higher than that in dairy and mixed farms (Table 3). This result may be because most of the beef cattle are reared under an extensive grazing system, which favors infection with *T. gondii* (MARANA et al., 1995; MILLAR et al., 2008).

There was a positive correlation ($p < 0.05$) between the number of cats and the prevalence of *T. gondii* antibodies in cattle farms in Pará. The higher was the number of cats in the properties, the higher was the seroprevalence. Santos (2012) observed that the presence of cats in the properties might indicate an environment contaminated with *T. gondii*. The results of this study corroborate with those of Albuquerque et al. (2011), wherein the number of cats and human contact with the animals were associated with the seroprevalence of *T. gondii* in cattle. However, the results differ from those of Santos (2008), who did not find a positive correlation between the prevalence of *T. gondii* and the number of cats.

Anderlini et al. (2001), Albuquerque et al. (2011), and Guerra et al. (2014) using cattle, and Brasil et al. (2015) using buffaloes, consider that the semi-intensive farming system is a risk factor

for toxoplasmosis, and justified that under these conditions, there was a higher probability of contact with oocysts because the animals remained part of the day in pastures and part of the day in confinement and consequently were exposed to both environments. Anderlini et al. (2001) observed that animals kept under an intensive farming system were at higher risk of contact with oocysts present in cat feces, which increased the risk of infection with *T. gondii*.

Although the transmission of toxoplasmosis via consumption of raw or undercooked beef is still unclear, Millar et al. (2008), Guerra et al. (2014), and Carmo et al. (2017) reported that the meat of these animals might contain viable cysts and be a source of infection for humans and domestic and carnivorous animals if consumed. This condition was confirmed by Lora et al. (2007) using PCR to detect genomic material of *T. gondii* in bovine meat, and 48.33% of the analyzed samples were positive for the parasite. The authors concluded that there was a potential risk for infection via consumption of raw or undercooked meat.

The prevalence of antibodies was higher in the mesoregion of Marajó. It should be emphasized that animal sanitation in Marajó Island is usually poor because of the lack of adequate animal management, and the physical characteristics of the region limit livestock productivity (BARBOSA, 2005). Ahmad and Qayyum (2014) observed that the number of animals positive for antibodies to *T. gondii* was significantly higher in farms with poor hygiene, and the presence of cats in the vicinity of the farm contributed to the spread of infection.

Conclusions

Our results indicated that infection with *T. gondii* was high in cattle raised in the state of Pará. The high prevalence of seropositive cattle and buffaloes suggests that these animals may be a source of infection to humans.

The high percentage of infection was correlated with the presence of cats in the studied properties, evidencing that cats are crucial in the epidemiology of toxoplasmosis.

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