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Original Article

Factors associated with the distribution of natural *Toxoplasma gondii* infection among equids in Northeastern Brazil

Fatores associados à distribuição da infecção por *Toxoplasma gondii* em equídeos naturalmente infectados no Nordeste do Brasil

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Abstract

The aim of this study was to determine the frequency and factors associated with *Toxoplasma gondii* in naturally infected equids in northeastern Brazil. Serum samples from 569 equids (528 horses, 8 mules and 33 donkeys) were subjected to the indirect fluorescent antibody test. Generalized linear models were used to evaluate associated factors. Among the 569 animals sampled, 118 (30.6%) living in rural areas and 14 (26.42%) in urban areas were seropositive (p>0.05). Seropositive animals were observed on 95% of the farms and in all the municipalities. Donkeys/mules as the host, presence of domestic cats and rats on the farm, ingestion of lagoon water and goat rearing remained in the final model as factors associated with infection. Preventive measures such as avoiding the presence of domestic cats close to rearing areas, pastures and sources of water for the animals should be adopted. The wide-ranging distribution of positive animals also indicated that infection in other domestic animals and in humans, through the contaminated environment, was possible. It should be highlighted that there was the possibility that donkeys and mules would continue to have detectable titers for longer, thus explaining the prevalence found. Further studies are needed to confirm this possibility.

Keywords: Toxoplasmosis, horse, serology, risk factors.

Resumo

O objetivo deste estudo foi determinar a frequência e os fatores associados a *Toxoplasma gondii* em equídeos naturalmente infectados no Nordeste do Brasil. Amostras de soro de 569 equídeos (528 cavalos, 8 asnos e 33 muares) foram submetidas a reação de imunofluorescência indireta. Modelos lineares generalizados foram utilizados na avaliação dos fatores associados. Dos 569 animais amostrados, 118 (30,6%) soropositivos eram de área rural e 14 (26,42%) perteciam a áreas urbanas (p>0,05). Observaram-se animais soropositivos em 95% das fazendas e em todos os municípios. Asininos/muares como hospedeiro, presença de gatos domésticos e ratos na fazenda, ingestão de água de lagoa e criação de caprinos permaneceram no modelo final como fatores associados à infecção. Medidas de prevenção, como evitar a presença de gatos domésticos próximos aos locais de criação, de pastejo e fontes de água dos animais, devem ser adotadas. A ampla distribuição de animais positivos sinaliza a possibilidade de infecção também em outros animais domésticos, bem como em humanos pelo ambiente contaminado. Ressalta-se a possibilidade de que asininos e muares permaneçam com títulos detectáveis por mais tempo, justificando a prevalência encontrada, sendo necessários estudos para confirmar este possibilidade.

Palavras-chave: Toxoplasmose, cavalo, sorologia, fatores de risco.

Introduction

Toxoplasmosis is a zoonosis caused by the protozoon *Toxoplasma gondii*, which has cosmopolitan distribution. Infection by this agent affects 70 to 95% of the world's population (BRASIL, 2010). Herbivores mainly become infected while grazing, through ingestion of oocysts that have sporulated in the environment and, to a lesser extent, through vertical transmission (DUBEY, 2008, 2010).

In the European Union, the average consumption of horse meat is 110,000 tons per year (FAO, 2013). Although horses are

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considered to be resistant to toxoplasmosis, the possibility that horse meat can be a source of infection for cats and other animals in zoos, as well as for human beings, has already been proven (AL-KHALIDI & DUBEY, 1979; DUBEY & DESMONTS, 1987; MARQUES et al., 1998; SHAAPAN & GHAZY, 2007; POMARES et al., 2011; EVERS et al., 2013)

Seroepidemiological studies on horses have demonstrated that *T. gondii* infection has cosmopolitan distribution and that its prevalence may range from 1 to 71.2% (JAKUBEK et al., 2006; HAJIALILO et al., 2010; LOPES et al., 2013). Between different Brazilian states, large variations in the prevalence of *T. gondii* can be observed, with results ranging from 1.5 to 41.53%, regarding positivity among animals (VIDOTTO et al., 1997; MENDONÇA et al., 2001).

The differences in positivity that are found in different places may be associated with epidemiological factors such as different types of rearing, stable hygiene and different types of food (TENTER et al., 2000). These factors make it important to identify the possible risk factors associated with a given region. Furthermore, the sensitivity and specificity of serological tests, geographical areas and climatic conditions and the presence of cats close to rearing areas or sources of food or water may interfere with positivity for infection.

Because of the way in which infection occurs, positivity for *T. gondii* among horses can be seen as an indicator of environmental contamination by sporulated oocysts. This may therefore indirectly assist in characterizing areas as presenting greater or lesser contamination.

Thus, the present study had the objectives of ascertaining the distribution of *T. gondii* infection and evaluating factors associated with this, among equids that acquired this infection naturally, in the Ilhéus-Itabuna microregion of the state of Bahia, northeastern Brazil.

Materials and Methods

Study area and sample population

This study was conducted in accordance with the standards established by the Brazilian College of Animal Ethics and Welfare. The research proposal was approved by the Committee for Animal Research Ethics (protocol no. 002/2013) at Santa Cruz State University, Ilhéus, BA, Brazil. Data were collected between August 2013 and December 2014 in the microregion of Ilhéus-Itabuna, state of Bahia, northeastern Brazil. This geographical region is part of the mesoregion of Southern Bahia and has an estimated equid population of 90,974 animals.

The study area was located in the Atlantic forest. The annual average rainfall is 1445 mm, with relative humidity of 80%, and temperature of 24 °C (BRASIL, 2010). Five municipalities in this mainly rural microregion were selected for this study, and were ranked according to the size of their equid population, as follows: Itaju do Colônia (15°08' S; 39°43' W), Itapé (14°52' S; 39°25' W), Ibicaraí (14°51' S; 39°35' W), Santa Cruz da Vitória (14°57' S; 39°48' W), and Floresta Azul (14°50' S; 39°39' W). The municipality of Itabuna (15°8' S; 39°43' W), which is a mostly urban area, was also included in this study (Figure 1).

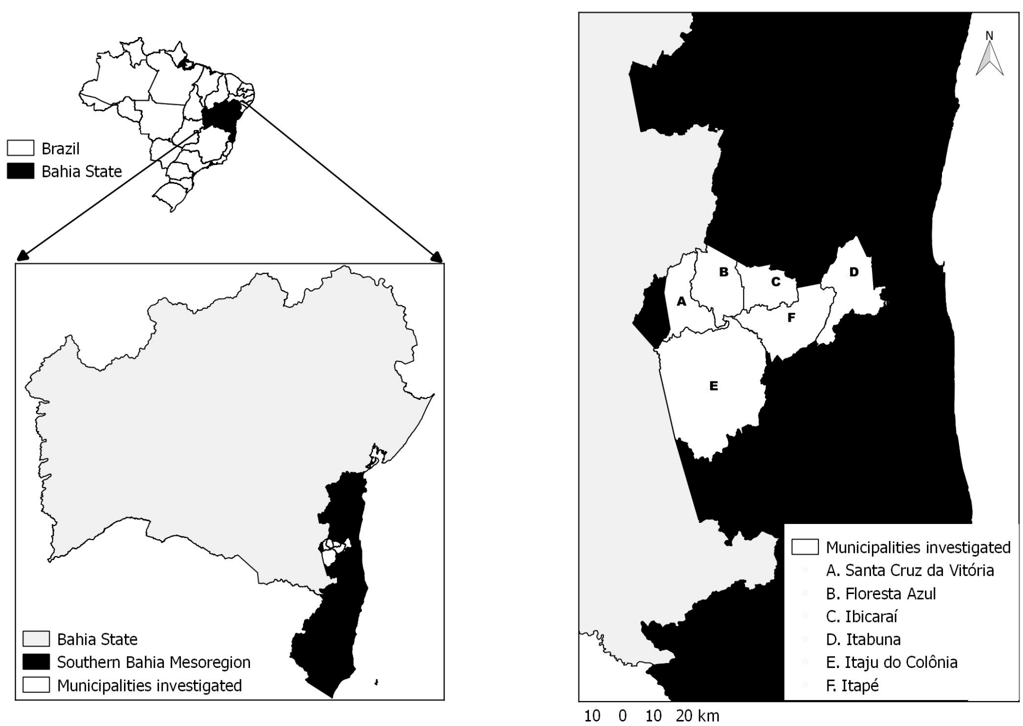

Figure 1. Location of the study area, which included the municipalities of Itaju do Colônia, Santa Cruz da Vitória, Itapé, Ibicaraí, Floresta Azul, and Itabuna, Bahia, Brazil.

Animals, farms and municipalities were selected based on their convenience. The numbers of animals per municipality were proportional to their equid populations. Blood samples were collected from 569 equids (528 horses, 8 mules and 33 donkeys); 516 out of these 569 equids were living on 20 rural farms; 53 horses out of these 569 equids were living in urban areas and were used by mounted police, or to draw coaches, or for horseback riding.

To evaluate potential risk factors, information regarding signalment (species, age and gender), farm characteristics and management (place where animal was kept, i.e. in a stable or elsewhere; and contact with other animal species) were obtained through semi-structured interviews with handlers (staff) or owners. The interviews were always conducted by the same researcher.

Sample collection and processing

A 10mL blood sample was collected from each animal by means of jugular venipuncture using a disposable needle (25 × 8mm) that was connected to a vacuum tube without anticoagulant. The tubes containing the samples were centrifuged for 10 minutes at 699*g* and the serum was poured into Eppendorf tubes and frozen at -20 °C for subsequent serological tests.

The serum samples were tested for antibodies to *T. gondii* by means of the indirect fluorescent antibody test using the RH parasite strain. Commercially available FITC-labeled anti-horse IgG (F-7759, Sigma-Aldrich St. Louis, MO, USA) was used as a secondary antibody at a dilution of 1:32. The serum dilution threshold for positivity was set at 1:64 (GARCIA et al., 1999; GHAZY et al., 2007; EVERS et al., 2013). The controls (positive and negative) were kindly provided by the Immunoparasitology Laboratory of the State University of São Paulo "Júlio Mesquita Filho", UNESP, in Jaboticabal, São Paulo.

Statistical analysis

The variables were categorized for the purposes of statistical modeling as follows: species (horse or donkey + mule); age, in the form of age ranges, i.e. young (≤3 years), adult (> 3 and <12 years) or senior (≥12 years); sex (male or female); presence of cats on the farm (yes or no); presence of wild felids on the farm (yes or no); equids kept in a stable (yes or no); equids had contact with sheep (yes or no); equids had contact with goats (yes or no); equids had contact with poultry (yes or no); equids had contact with cattle (yes or no); presence of rats on the farm (yes or no); presence of toxic plants in the pastures (yes or no); equids were drinking water from a weir (yes or no); equids were drinking water from a river (yes or no); equids were drinking water from a lagoon (yes or no); and equids were eating food that had been stored on the farm (yes or no). Positive results from serological tests for *T. gondii* (yes or no) were considered to be an outcome variable. Donkeys and mules were inserted into a single category due to the low number of animals.

Blood samples from animals in urban areas were only collected in the municipality of Itabuna. A total of 53 horses were sampled, including those used by the mounted police, or to draw coaches, or for horseback riding. These animals were excluded from the modeling because of the different management practices that had been applied to these horses and were utilized only to compare frequency distributions to toxoplasmosis between animals living in rural and urban areas.

Generalized linear models with binomial distribution were used to perform bivariate and multivariate analyses. Because of the possibility of clusters, intraclass correlation coefficients (ICCs) were calculated. Null models (STARKWEATHER, 2010) were estimated to obtain ICCs for the variables municipality and farm due to the possibility of observations of animals from the same municipality and or farm being correlated, forming clusters (DOHOO et al., 2003). In cases of cluster formation, the variables tested were considered random and generalized linear mixed models were used for the analyses.

The modeling strategy used in the multivariate analyses was backward, i.e. all variables were initially included in the model. Starting from this initial model, variables were selected at each step based on the Wald test, until the most parsimonious model that best explained the outcome was obtained. The significance level for variables to remain in the final model was set at 5%. The Akaike information criterion (AIC) was used to evaluate the fit of the models. Frequency distributions between animals living in rural areas and animals living in urban areas were compared using the chi-square test.

Odds ratios (OR) and their respective 95% confidence intervals (CI) were calculated based on the regression coefficients that had been estimated through the models. Statistical calculations were performed using the R software, version 3.2.5 for Windows (R DEVELOPMENT CORE TEAM, 2016), through the lmer4 package (BATES et al., 2015), version 1.1-12.

Results

A total of 30.62% (158/516; CI: 26.71-34.83%) of the horses in the rural area were seroreactive, with titers ranging from 64 to 4096 (Table 1). The horses presented positivity of 27.36% (130/475; CI: 23.45-31.66%), the donkeys 72.73% (24/33; CI: 54.48-86.7%) and the mules 50% (4/4; CI: 15.7-84.3%). At least one seroreactive animal was identified on 95% (19/20) of the farms, and the positivity on the farms ranged from 8.3 to 56.25%. Positive animals were identified in every municipality (Table 1). In table 2 are observed the results of the association between each of the explanatory variables with the outcome variable. The ICCs per municipality (5.7%) and per farm (6.2%) indicated that cluster formation existed, thus, generalized linear mixed models were used. The results of the associations in the full model are observed in Table 3. The donkey/mule species together, ingestion of lagoon water by the horses, presence of cats and rats and absence of goat rearing on the farm were identified as possible risk factors (Table 4). Among the horses in the urban area, seropositivity of 26.42% was observed (14/53; CI: 15.3-40.3%). This was not significantly difference from the positivity found in the rural area (p>0.05).

Table 1. Distribution of equidae serology results to *Toxoplasma gondii*, and their respective titrations, from the Ilhéus-Itabuna microregion, in the state of Bahia, Brazil.

Municipality	Total of animals	Serology							
		Negative	Titration						Positive
		%	64	128	256	512	1024	4096	%
Floresta Azul	98	78	10	2	4	2	2	0	20.4
Ibicaraí	80	44	13	12	8	2	1	0	45
Itaju do Colônia	186	140	26	15	4	1	0	0	24.7
Itapé	67	50	7	8	1	0	0	1	25.37
Santa Cruz da Vitória	85	46	16	14	8	1	0	0	45.88
Total	**516**	**358**	**72**	**51**	**25**	**6**	**3**	**1**	**30.62**

Table 2. Generalized linear mixed models for factors associated with *Toxoplasma gondii* infections in naturally infected equidae from the Ilhéus-Itabuna microregion, in the state of Bahia. Bivariate analysis.

Variable	Equids				Odds ratio	*P value*
	Positive		Negative			
	n	%	n	(%)	(95% CI*)	
Age range						
Young (ref)	27	31.4	59	68.6	-	
Adult	77	30.7	174	69.3	1.00(0.57-1.75)	0.99
Senior	54	30.2	125	69.8	1.13(0.62-2.07)	0.69
Species						
Equine (ref)	130	27.7	345	62.3	-	
Donkey or mule	28	68.3	13	31.7	4.85(2.20-10.74)	<0.001
Gender						
Female (ref)	114	30.4	261	69.6	-	
Male	44	31.2	97	68.8	0.67(0.33-1.34)	0.25
Equid drink water of weir						
Yes	144	3	321	69	1.55(0.61-3.93)	0.36
No (ref)	14	27.5	37	72.5	-	
Equid drink water of river						
Yes (ref)	58	29	142	71	-	
No	100	31.6	216	68.4	1.1(0.61-2.03)	0.73
Equid drink water of lagoon						
Yes	26	30.6	59	69.4	1.41(0.56-3.6)	
No (ref)	132	30.6	299	69.4	-	
Presence of cats in the farm						
Yes	134	33.8	263	66.2	2.4(1.05-5.48)	0.04
No (ref)	24	20.2	95	79.8	-	
Presence of wild felids in the farm						
Yes	44	29.7	104	70.3	1.55(0.71-3.38)	0.27
No (ref)	114	31	254	69	-	
Equid had contact with cattle						
Yes (ref)	133	30.4	304	69.6	-	
No	25	31.6	54	68.4	1.52(0.62-3.77)	0.36
Equid had contact with sheep						
Yes (ref)	35	33	71	67	-	
No	123	30	287	70	0.70(0.33-1.49)	0.35
Equid had contact with goat						
Yes (ref)	17	28.3	43	71.7	-	
No	141	30.9	315	69.1	0.88(0.26-2.95)	0.84
Equid had contact with poultry						
Yes (ref)	142	31.8	305	68.2	-	
No	16	23.2	53	76.8	0.58(0.25-1.34)	0.20

*CI: Confience Interval.

Table 2. Continued...

Variable	Equids				Odds ratio	P value
	Positive		Negative			
	n	%	n	(%)	(95% CI*)	
Presence of rats in the farm						
Yes	137	33.3	274	66.7	2.1(1.01-4.38)	0.05
No (ref)	21	20	84	80	-	
Equid kept in a stable						
Yes	10	20.8	38	79.2	0.71(0.32-1.61)	0.42
No (ref)	148	31.6	320	68.4	-	
Equid eat food stored on the farm						
Yes	107	26.9	291	73.1	0.57(0.25-1.27)	0.17
No (ref)	51	43.2	67	56.8	-	
Presence of toxic plants in the pasture						
Yes	73	31.6	158	68.4	1.18(0.65-2.15)	0.58
No (ref)	85	29.8	200	70.2	-	

*CI: Confience Interval.

Table 3. Generalized linear mixed model for factors associated with *Toxoplasma gondii* infections in equidae from the Ilhéus-Itabuna microregion, in the state of Bahia. Multivariate analysis, full model.

Variable	Category	Odds Ratio (95% CI*)	P value
Age range	Young (ref)		
	Adult	0.78 (0.44-1.40)	>0.05
	Senior	0.94 (0.50-1.74)	
Species	Equin (ref)		
	Donkey or mule	6.49 (2.68-15.74)	≤0.001
Gender	Fêmea (ref)		
	macho	1.12 (0.60-2.09)	>0.05
Equid drink water of weir	Yes	2.30 (0.50-10.52)	>0.05
	No (ref)		
Equid drink water of river	Yes (ref)		
	No	1.19 (0.54-2.65)	>0.05
Equid drink water of lagoon	Yes	8.07 (1.00-65.33)	0.05
	No (ref)		
Presence of cats in the farm	Yes	4.93 (1.60-15.15)	0.001
	No (ref)		
Presence of wild felids in the farm	Yes	0.53 (0.23-1.22)	>0.05
	No (ref)		
Equid had contact with cattle	Yes (ref)		
	No	1.24 (0.44-3.52)	>0.05
Equid had contact with sheep	Yes (ref)		
	No	1.77 (0.48-6.59)	>0.05
Equid had contact with goats	Yes (ref)		
	No	8.69 (1.17-64.30)	0.01
Equid had contact with poultry	Yes (ref)		
	No	0.69 (0.26-1.85)	>0.05
Presence of rats in the farm	Yes	3.03 (1.14-8.07)	0.01
	No (ref)		
Equid kept in a stable	Yes	1.21 (0.48-3.07)	>0.05
	No (ref)		
Equid eat food stored on the farm	Yes	1.90 (0.62-5.77)	>0.05
	No (ref)		
Presence of toxic plants in the pasture	Yes	0.62 (0.28-1.35)	>0.05
	No (ref)		

*CI: Confience Interval; AIC: 622.6.

Table 4. Generalized linear mixed model for factors associated with *Toxoplasma gondii* infections in equidae from the Ilhéus-Itabuna microregion, in the state of Bahia, Brazil. Multivariate analysis, final model.

Variable	Category	Odds ratio (95% CI*)	*P value*
Species	Equine (ref)		
	Donkey or mule	4.21 (2.07-8.57)	0.001
Equid had contact with goats	Yes (ref)	-	
	No	5.09 (1.5-17.24)	0.01
Presence of rats in the farm	Yes	2.00 (1.15-3.46)	0.05
	No (ref)	-	
Presence of cats in the farm	Yes	3.58 (1.76-7.28)	0.001
	No (ref)	-	
Equid drink water of lagoon	Yes	3.79 (1.32-10.87)	0.05
	No (Ref)	-	

*CI: Confience Interval; AIC: 604.2.

Discussion

The Ilhéus-Itabuna microregion has a hot humid climate, which may explain the high frequency and widespread distribution of seropositive horses found in the present study. According to Dubey (2010), herbivore infection is more prevalent in hot climates and humid areas because of better conditions for sporulation and maintenance of viable oocysts in the environment. This widespread distribution could also be seen in the present study through the similar positivity among animals living in rural areas and among those in urban areas. This result differed from what was found by Alvarado-Esquivel et al. (2012), who observed greater positivity among animals living in rural areas. Lastly, the importance of the region's climate can be proven by the low positivity found (1.5%) in the municipalities of Jacobina and Jequié (MENDONÇA et al., 2001). Although these municipalities are close to the area of the present study, they have a semi-arid climate with low relative humidity and low levels of rainfall.

Conflicting seropositivity results are found in the literature for *T. gondii* involving horses, mules and donkeys. Similar to the results from the present study, some previous studies demonstrated higher seropositivity among donkeys and mules than among horses (GARCÍA-BOCANEGRA et al., 2012; SAQIB et al., 2015), or elevated seropositivity in donkeys (EL-GHAYSH, 1998; HARIDY et al., 2010). However, the presence of similar prevalences between horses and donkeys has also been reported (YANG et al., 2013). Since horses are considered to be naturally resistant to *T. gondii* infection (DUBEY, 2010), it is possible that, with the evolution of infection, antibody titers fall below the cutoff point of the serological test, thus making the animals negative in this test (EVERS et al., 2013).

Presence of domestic cats in the horses' environment was identified as a risk factor associated with infection. This was expected, since cats are the definitive hosts of *T. gondii* and excrete oocysts into the environment. This forms the main means of infection for herbivores, given their dietary habits (AGANGA et al., 1983; TENTER et al., 2000; FERGUSON, 2009; DUBEY, 2010). The oocysts can be spread around the environment through wind, rain and surface water. Although hay, straw and grains were not evaluated as possible risk factors in the present study, these have already been identified as factors associated with infection among horses (ALVARADO-ESQUIVEL et al., 2012).

In the current study, presence of rats in the horses' environment was associated with higher positivity for *T. gondii*, since rats are natural hosts for *T. gondii* and are common prey for cats. Their presence in the environment may promote closure of the biological cycle, with excretion of millions of oocysts into the environment.

Consumption of lagoon water also appeared to be a risk factor in the present study. Lagoons are sources of stagnant water and this may explain why consumption of this water was a risk factor. This water may contain elevated concentrations of oocysts, given that they may remain viable for months or years in water or damp earth (TENTER et al., 2000). Oocysts resist temperature variations and a wide variety of disinfectants, which makes it impractical to attempt to destroy oocysts in large reservoirs of water (DUBEY, 2010).

Two large-scale outbreaks of human toxoplasmosis, in Canada and Brazil, were epidemiologically associated with oocyst contamination of drinking water (BOWIE et al., 1997; ARAMINI et al., 1999). This proves the efficiency of *T. gondii* transmission through contaminated water.

Age was not a factor associated with infection, and this finding corroborated what has been seen in the majority of studies carried out among horses (GARCIA et al., 1999; HAJIALILO et al., 2010; KARATEPE et al., 2010; ALVARADO-ESQUIVEL et al., 2012; GARCÍA-BOCANEGRA et al., 2012). In other species, studies have shown that age is a risk factor, but this generally does not occur with horses. One hypothesis for this is the difference in handling, given that horses start to be trained or mastered from an early age and frequent the same pastures and environments as adult horses, while cows, sheep, goats and pigs are separated into groups according to age for breeding, raising and finishing. Lastly, there was no explanation in the literature for finding that absence of goats was a possible risk factor for infection among horses.

In the light of the above, the high level of positivity that was found indicated that environmental contamination by oocysts was occurring. Therefore, there is a need for preventative measures such as avoidance of the presence of domestic cats close to the rearing areas, pastures and sources of water used by equids. The widespread distribution of positive animals also indicated that the possibility of infection among other domestic animals and in humans also exists, through the contaminated environment. Finally, it is probable that donkeys and mules continue to present detectable titers for longer. Further studies are needed to confirm this possibility.

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