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Seroprevalence of *Toxoplasma gondii* in dogs of riverside communities of Mato Grosso Pantanal, Brazil

Soroprevalência de *Toxoplasma gondii* em cães de comunidades ribeirinhas do Pantanal de Mato Grosso, Brasil

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Abstract

Toxoplasma gondii is an intracellular protozoan with worldwide distribution and dogs act as sentinels of human infection. This search aimed to determine the occurrence of antibodies against *T. gondii* in dogs of the communities on the Cuiabá River, Mato Grosso and variables associated with infection. The dogs of the riverside communities in Cuiabá River, which includes Barranco Alto, Praia do Poço, Engenho Velho, Varginha, Bom Sucesso, Passagem da Conceição and São Gonçalo Beira Rio, were evaluated for the presence of *T. gondii* antibodies by indirect immunofluorescence antibody test (IFAT). The prevalence and factors associated with infection were calculated by chi-squared test (χ^2) or Fisher's exact test, and univariate and multiple analysis. Of the 248 dogs surveyed, 107 (43.1%) were seropositive for *T. gondii*. The seroprevalence ranged from 25.6% to 64.3%. There was no statistically significant difference between the communities studied ($p > 0.05$). As for the associated factors, the only statistically significant factor was that of dogs living with cats ($p = 0.02$), with approximately twice the risk of acquiring infection. In conclusion, the seroprevalence in dogs of riverside communities in the Baixada Cuiabana demonstrated that high rates of infection, being the factor associated with infection, contact with domestic cats.

Keywords: *Toxoplasma gondii*, dogs, associated factors, Cuiabá River, Pantanal, Brazil.

Resumo

Toxoplasma gondii é um protozoário intracelular com distribuição mundial e o cão atua como sentinela para infecção humana. Esta pesquisa teve por objetivo determinar a ocorrência de anticorpos contra *T. gondii* em cães de comunidades ribeirinhas ao Rio Cuiabá, Mato Grosso e as variáveis associadas à infecção. Os cães das comunidades ribeirinhas do Rio Cuiabá, que inclui Barranco Alto, Praia do Poço, Engenho Velho, Varginha, Bom Sucesso, Passagem da Conceição e São Gonçalo Beira Rio, foram avaliados para a presença de anticorpos para *T. gondii* pela reação de imunofluorescência indireta (IFI). A prevalência e fatores associados com a infecção foram calculados pelo teste de qui-quadrado (χ^2) ou exato de Fisher, e análise univariada e multivariada. Dos 248 cães estudados, 107 (43,1%) foram soropositivos para *T. gondii*. A prevalência variou de 25,6% a 64,3%. Não houve diferença estatisticamente significativa entre as comunidades estudadas ($p > 0,05$). Quanto aos fatores associados, o único fator estatisticamente significante o convívio com gatos ($p = 0,02$), com cerca de duas vezes mais risco de adquirir a infecção. Em conclusão, a soroprevalência em cães de comunidades ribeirinhas da Baixada Cuiabana demonstram altas taxas de infecção, sendo o fator associado à infecção, o contato com gatos domésticos.

Palavras-chave: *Toxoplasma gondii*, cães, fatores associados, Rio Cuiabá, Pantanal, Brasil.

Toxoplasma gondii is an intracellular protozoan with worldwide distribution that can infect several species of warm-blooded animals (LANGONI et al., 2013). The seroprevalence of *T. gondii* in Brazil is often high (SOUZA et al., 2003), ranging from 3.1% to 91% of the canine population in studies in several states, such as Minas Gerais, Santa Catarina, São Paulo, Rio de Janeiro, Bahia and Para (DUBEY et al., 2012; DREER et al., 2013).

Although cats are the only known hosts that can excrete oocysts into the environment, dogs are considered sentinels for infection of *T. gondii* in humans because they live in close contact with humans and livestock (SOUZA et al., 2003; MILLÁN et al., 2013).

The high prevalence worldwide is related to environmental and socioeconomic factors because the transmission is associated with raw or undercooked meat intake containing protozoan cysts or

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contact with oocysts in the environment and vertical transmission (DUBEY, 2007; FERREIRA et al., 2014). It is estimated that one-third of the human population is infected by the parasite. The majority of infections are asymptomatic; however, *T. gondii* infection in pregnant women and immunocompromised patients can cause serious illnesses and even death (ZHANG et al., 2015). Similarly, infection in dogs may be asymptomatic, but clinical signs are variable and may include respiratory, digestive, ocular, and neuromuscular symptoms (HOSSEININEJAD et al., 2011; LANGONI et al., 2013).

Toxoplasma gondii infection has acquired special importance as a waterborne disease (VITALIANO et al., 2015). The importance of the dog as a sentinel for infection and improper sanitation conditions in the riverside communities of the Cuiabá River, the wetland basin trainer of Mato Grosso, motivated this work for determining the occurrence of antibodies against *T. gondii* in dogs and to determine the variables associated with infection.

A sectional study was conducted in the coastal communities in Cuiabá River: Barranco Alto, Praia do Poço, Engenho Velho and Varginha, located in the municipality of Santo Antonio do Leverger (15°51'57"S and 56°04'37"O); Bom Sucesso and Passagem da Conceição, in the municipality of Várzea Grande (15°38'49"S and 56°07'58"O) and the community of São Gonçalo Beira Rio, in the city of Cuiabá (15°35'45"S and 56°05'49"O). The main sources of financial income of these communities are fishing and tourism.

The dogs were sampled during home visits by census form. Each dog was represented by an epidemiological form containing information on breed, gender, age, diet, contact with cats, access to the street, presence of livestock and types of floor to detect possible factors associated with infection.

After consent of the owners, the dogs were contained, submitted to general clinical evaluation and approximately 5 mL of blood was collected by cephalic or jugular puncture after sterilization. The blood samples were transferred to a tube without anticoagulant, centrifuged at 5000 rpm for 5 minutes and the serum obtained was stored at –20 °C until serological analysis.

The presence of antibodies against *T. gondii* was assessed by immunofluorescence reaction (IFAT) using as antigen tachyzoites of *T. gondii* (RH strain) at a concentration of 1×10^7 Taq/mL and conjugated anti-dog IgG (Sigma Aldrich®) (CAMARGO, 1964). Dog sera were tested in geometric progression, and were considered reagents for titration ≥ 1:16 (MOURA et al., 2009) compared with the positive and negative controls on each slide.

The prevalence values were calculated at the 95% confidence intervals, and a chi-squared test (χ^2) with Yate's correction or Fischer's exact test was used to test for associations between the independent variable and the seroprevalence of anti-*T. gondii* antibodies in dogs using the EpiInfo 3.3.2 programme (CDC, Atlanta, USA). The variables with a $p \leq 0.20$ in the univariate analysis were selected for the multiple analysis. The χ^2 goodness-of-fit test was performed using the Hosmer and Lemeshow statistic, and $p \leq 0.05$ was considered significant.

This work is in agreement with the Animal Experimentation Ethical Principles adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL) and was approved by the Ethics Committee on Animal Use (CEUA) -UFMT under the Protocol N° 23108.014950/11- 5.

Of the 248 surveyed dogs, 107 (43.1% [CI 37.1 to 48.8]) were seropositive for *T. gondii*. The seroprevalence found in the municipalities studied ranged from 25.6% to 64.3%. The riverside community with the highest seroprevalence was Praia do Poço in Santo Antônio do Leverger with 64.3% seropositive dogs. When analysing the prevalence by municipality, Várzea Grande had the highest prevalence with 47.1% (CI 36.8 to 57.5), followed by Santo Antônio do Leverger with 43.6% (CI 35.3 to 51.9) and Cuiaba with 25% (CI 14.3 to 48.8) (Figure 1). However, there was no statistically significant difference between them ($p > 0.05$).

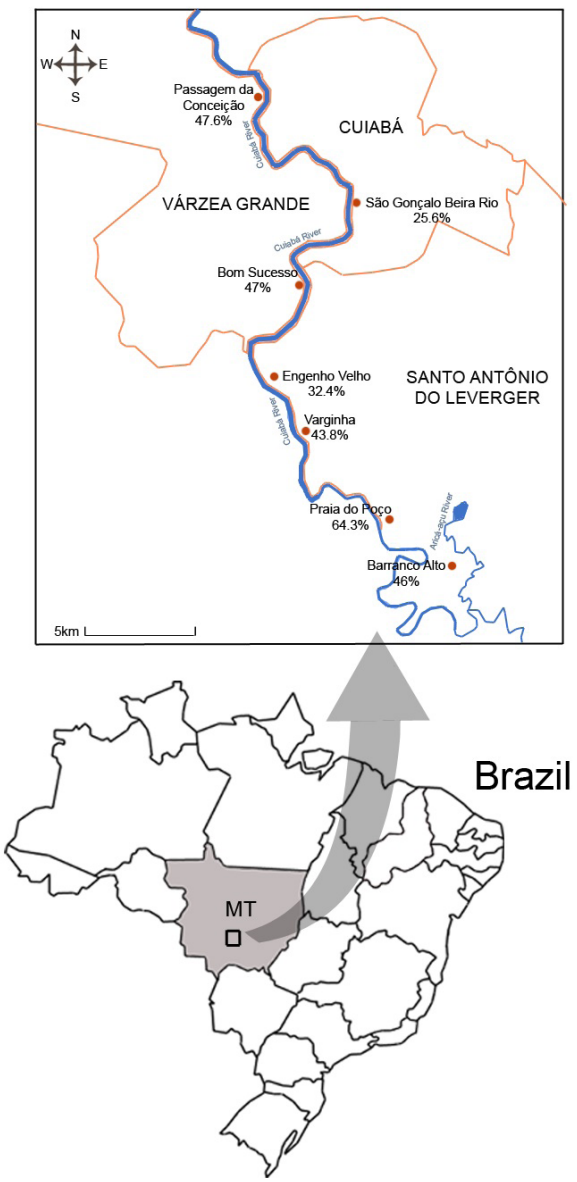

Figure 1. Location of riverside communities Pantanal surveyed. Seroprevalence for *Toxoplasma gondii* in dogs and location of Riverside Communities of São Gonçalo Beira Rio in the city of Cuiabá, Passagem da Conceição and Bom Sucesso in the municipality of Várzea Grande and Barranco Alto, Engenho Velho, Praia do Poço and Varginha in the municipality of Santo Antônio de Leverger, Mato Grosso.

The antibody titres presented by seropositive dogs were 17 (1:16), 40 (1:64), 38 (1:256), 9 (1:1024) and 3 (1:4096). Of the 248 surveyed dogs, 101 (40.72%) showed some clinical signs, of which 54 (53.46%) had antibodies anti-*T. gondii*. The main clinical changes in the seropositive dogs consisted: lymphadenopathy, dermatopathy, ophthalmopathy, weight loss, anorexia and splenomegaly.

Among the surveyed dogs, the most common characteristics were undefined breed (88.7%), males (64.1%) and adults (64.1%) (from one to six years), but there was no statistically significant difference in relating these variables and seroreactivity for *T. gondii* (Table 1). Concerning the possible environmental habits and aspects associated with infection in the univariate and multiple analysis, only the association with cats showed a statistically significant difference (p = 0.02) (Table 1).

The prevalence of infection by *T. gondii* in dogs is important because the dog is sentinel and determines the magnitude of environmental contamination by oocysts (MIRÓ et al., 2004). In this study, the prevalence of canine *T. gondii* infection was 43.1%. However, prevalences greater than 60% have been detected in Brazil and other countries (DREER et al., 2013; DAVOUST et al., 2015). In all surveyed communities, the prevalence was above 25%, which was considered high. Although not statistically significant, the observation of different prevalence between communities can be explained by differences in the environmental characteristics (ALVARADO-ESQUIVEL et al., 2014). Furthermore, serological technique and used cut off explain different prevalences in Brazil and worldwide (BOA-SORTE et al., 2015).

The riverside communities of the Cuiabá River are characterized by subsistence farming, and some, like Bom Sucesso and Passagem da Conceição, in the municipality of Várzea Grande and São Gonçalo Beira Rio in Cuiabá, are considered tourist sites, presenting the best infrastructure conditions of the communities located in Santo Antônio de Leverger. However, this feature did not reduce the occurrence of infection of dogs by *T. gondii*. In a study conducted in the urban and rural areas of the city of Cuiabá, the prevalence (BOA SORTE et al., 2015) was similar to the average of the coastal communities; however, the prevalence was lower compared to the community of São Gonçalo Beira Rio in the same municipality.

Table 1. Analysis of the variables investigated in accordance with the seroprevalence of *Toxoplasma gondii* in the riverside communities of Mato Grosso, Central-Western Brazil.

Variables	Dogs		Univariate		Multiple	
	Total	Positive (%)	P-value	OR (CI 95%)	P-value	OR (CI 95%)
Breed						
Mixed	220	98 (44.54)	0.20	0.69	---	---
Pure	28	09 (32.14)		(0.21-1.36)		
Gender						
Male	159	69 (43.39)	0.91	0.97	---	---
Female	89	38 (42.69)		(0.56-1.64)		
Age groups						
Indefinite age	14	07 (50.00)	0.15	---	---	---
≤ 1 year old	46	15 (32.60)				
> 1-3 years old	85	40 (47.05)				
> 3-6 years old	74	28 (37.83)				
> 6 years old	29	17 (58.62)				
Diet						
Commercial	32	11 (34.37)	0.08	---	---	---
Home-cooked	64	35 (54.68)				
Mixed	152	61 (40.13)				
Contact with cats						
Yes	61	34 (55.73)	0.02	1.96	0.02	1.96
No	187	73 (39.03)		(1.08-3.46)		(1.09-3.52)
Access to the street						
Yes	189	87 (46.03)	0.09	1.63	---	---
No	59	20 (33.89)		(0.91-3.26)		
Livestock						
Yes	191	81 (42.40)	0.66	0.87	---	---
No	57	26 (45.61)		(0.48-1.65)		
Types of floor						
Ground	213	95 (44.60)	0.34	---	---	---
Cemented	09	02 (22.22)				
Mixed	26	10 (38.46)				

Similar to Dreer et al. (2013), 88.8% of seropositive dogs had antibody titres between 1:16 to 1:256, which is considered low, diverging from the study in Cuiabá, Mato Grosso (BOA SORTE et al., 2015) where high titres of antibodies were prevalent. Differing rates of infection in clinical toxoplasmosis can be related to distribution and parasite efficiency. Low titres have been mentioned as resulting from a chronic stage or an early stage of infection (BARBOSA et al., 2003).

As in humans, *T. gondii* infection in dogs tends to be asymptomatic (HOSSEININEJAD et al., 2011). In this research, the frequency of healthy dogs and those presenting any clinical changes were similar; however, it is not possible to attribute such changes to infection by *T. gondii*, given the endemic nature of other diseases such as canine ehrlichiosis and canine visceral leishmaniasis (SILVA et al., 2010; ALMEIDA et al., 2012).

There was no age, gender, or racial predisposition, despite the prevalence being higher in adult dogs. This observation relates to the longer exposure time to the agent (AHMAD et al., 2014).

In relation to the environmental and management factors, such as the type of diet, free access to the street, a chicken coop and type of room floor, there was no significant association with infection by *T. gondii*. Although the diet was not associated with infection in this study, the supply of undercooked meat and fruits and vegetables unwashed increases the likelihood of infection by the possibility of ingestion of bradyzoites or oocysts (BRESCIANI et al., 2007; CARLOS et al., 2010). In addition, a study conducted in Brazil (BRESCIANI et al., 2007) found that dogs that living in an environment with a dirt yard were 11.5 times more likely to acquire the infection, reinforcing the possible soil contamination by staff members in areas surveyed. However, this aspect was not observed in this study, although 88.8% of positive dogs reside in an environment with a dirt yard.

Dogs that live with a domestic cat are approximately two times more likely to acquire infection. Living with cats has been associated with canine and human infection (FERREIRA et al., 2014), which is related to the ingestion of oocysts released in the cat faeces or environmental contamination by increasing exposure. However, a study of dogs in Mexico found no association between living with cats and *T. gondii* infection (ALVARADO-ESQUIVEL et al., 2014).

In conclusion, seroprevalence in the dogs of the riverside communities in the Baixada Cuiabana demonstrated high rates of infection, and contact with domestic cats was the main factor associated with infection.

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