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## Serological and molecular detection of *Toxoplasma gondii* in dogs of urban and rural areas of Cuiabá, Mato Grosso

### Detecção sorológica e molecular de *Toxoplasma gondii* em cães de áreas urbanas e rurais de Cuiabá, Mato Grosso

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#### Abstract

This study aimed to determine the prevalence of *Toxoplasma gondii* infection using serological and molecular analyses in dogs from Cuiabá, a municipality of the Brazilian Cerrado, and the associated factors involved in the transmission cycle. A cross-sectional study was conducted with dogs from the rural and urban areas of Cuiabá (Mato Grosso) from February 2010 to January 2011, and antibodies and the DNA of *Toxoplasma gondii* were evaluated using indirect immunofluorescence (IFA) and polymerase chain reaction (PCR). In the study, a total of 269 dogs were evaluated using IFA considering positive titer threshold of  $\geq 16$  and buffy coat PCR. Of the 269 dogs surveyed, 48.7% had anti-*Toxoplasma gondii* antibodies according to IFA, and 15.6% had the parasite's DNA. The seroprevalence was 62.4% in the rural districts and 40.4% in the urban areas, and the difference between these areas was significant ( $p = 0.0007$ ). The most common levels of antibody titers were 256 in 51 (39%) dogs, followed by 1024 in 37 (28.2%) dogs. The infectious agent was associated with breed, age, access to the street and the environment in which the animal lived ( $p < 0.05$ ). The serological and molecular results showed that *T. gondii* infection is active in the canine population in the rural and urban areas of Cuiabá, with a higher risk in dogs residing in rural areas.

**Key words:** Dog, associated factors, PCR, serology, toxoplasmosis

#### Resumo

Este artigo teve por objetivo determinar a soroprevalência da infecção por *Toxoplasma gondii* através de análise sorológica e molecular em cães de Cuiabá, município do cerrado brasileiro, associando com os fatores de risco envolvidos na cadeia de transmissão. Estudo transversal foi conduzido com cães de áreas rurais e urbanas de Cuiabá (Mato Grosso), entre fevereiro de 2010 a janeiro de 2011, através da pesquisa de anticorpos por imunofluorescência indireta e DNA de *Toxoplasma gondii*. Foram avaliados no estudo um total de 269 cães por IFI, considerando reagente cães com título  $\geq 16$  e por PCR de capa

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leucocitária. Dos 269 cães pesquisados, 48,7% apresentaram anticorpos anti-*Toxoplasma gondii* pela IFI e 15,6% DNA do parasito. Os bairros localizados em ambiente rural apresentaram uma soroprevalência de 62,4% e os urbanos de 40,4%, dados estes estatisticamente significativos ( $p=0,0007$ ). Os títulos mais frequentemente observados foram de 256 em 51 (39%) cães, seguido por 1024 em 37 (28,2%) cães avaliados. A infecção pelo agente esteve associada a raça, idade, acesso à rua e ao ambiente em que o animal vivia ( $p<0,05$ ). Os resultados sorológicos e moleculares permitiram inferir que a infecção por *T. gondii* encontra-se ativa na população canina nas regiões rurais e urbanas do município de Cuiabá, com risco maior em cães residentes em áreas rurais.

**Palavras-chave:** Cão, fatores de risco, PCR, sorologia, toxoplasmose

## Introduction

*Toxoplasma gondii* is an obligate intracellular parasite that belongs to the Phylum Apicomplexa and infects approximately one-third of the human population and various animal species, including dogs (REID et al., 2012). In humans, toxoplasmosis is a clinical disease that is rare; however, in immunosuppressed patients with cancer and AIDS, this disease is recognized as a major cause of death (MARTINS; VIANA, 1998). Similarly, in dogs, the disease is often associated with immunosuppressive diseases such as canine distemper (MORETTI et al., 2002).

In Brazil, the prevalence of infection in humans is more than 68% (SROKA et al., 2010; SANTOS et al., 2009) depending on the study area, whereas studies in dogs have shown infection rates ranging from 20.8% (ROMANELLI et al., 2007) to 88% (SANTOS et al., 2009). A high prevalence of toxoplasmosis in dogs is correlated with a highly contaminated environment, as it facilitates infection in these species because of eating habits and ground contact (GARCIA et al., 1999).

To confirm the risk factors of *T. gondii* infection in dogs, this study aimed to determine the presence of anti-*T. gondii* and the parasite's DNA in pet dogs in the urban and rural areas of Cuiabá, a Brazilian cerrado municipality.

## Materials and Methods

A cross-sectional study was conducted from February 2010 to January 2011 in rural (Barreiro

Branco and Coxipó do Ouro) and urban (Osmar Cabral and Jardim União) areas in Cuiabá (15°35'56" S and 56°06'01" W), a city located in the south-central state of Mato Grosso in Brazil's cerrado. The city covers 3362.755 square kilometres, is at an altitude of 165 metres above the sea level, has a warm tropical and sub-humid climate, has an average annual rainfall of 1750 mm and temperature of 43 ° C to 14 ° C and has a population of 551,098 inhabitants (IBGE, 2011) and 96502 dogs (CCZ, 2007).

Based on a sample calculation considering a disease prevalence of 22.3% (MOURA et al., 2009), 95% confidence intervals and an error rate of 5%, 269 dogs of various breeds and ages were examined. The samples were taken from each street block from every 5 homes, and home visits were conducted. After an explanation of the study was provided and consent of the owners was obtained, a questionnaire was administered to obtain data on the following: sex, breed, age, eating habits, presence of cats, street access and features of the floor where the animal lived. From each dog, blood samples with and without anticoagulant were collected by puncturing the cephalic or jugular vein and stored at - 20 ° C.

To perform the indirect immunofluorescence (IFA), we used antigen tachyzoites of *T. gondii* (RH strain) at a concentration of  $1 \times 10^7$  taq / mL and conjugated anti-IgG dog (Sigma Aldrich®) (CAMARGO, 1964). Reagents were used in the dog samples with a titration threshold of  $\geq 1:16$  (MOURA et al., 2009), and the samples were compared with positive and negative controls.

To extract DNA from the blood samples, we used the phenol-chloroform-isoamyl method (SAMBROOK et al., 1989). The polymerase chain reaction (PCR) was performed using TOXO 1 oligonucleotides: 5'-GGAAGTGCATCCGTTTCATGAG-3' and TOXO 2: 5'-TCTTTAAAGCGTTCGTGGTC-3', which amplify a DNA fragment of 194 bp (BURG et al., 1989).

The epidemiological variables were analysed using the chi-square test and Fisher's exact test ( $p < 0.05$ ) based on the IFA as the standard technique. The Epi Info version 6 (CDC) program was used to perform the analyses using a statistical significance level of 5%.

This research followed the Animal Experimentation Ethical Principles adopted

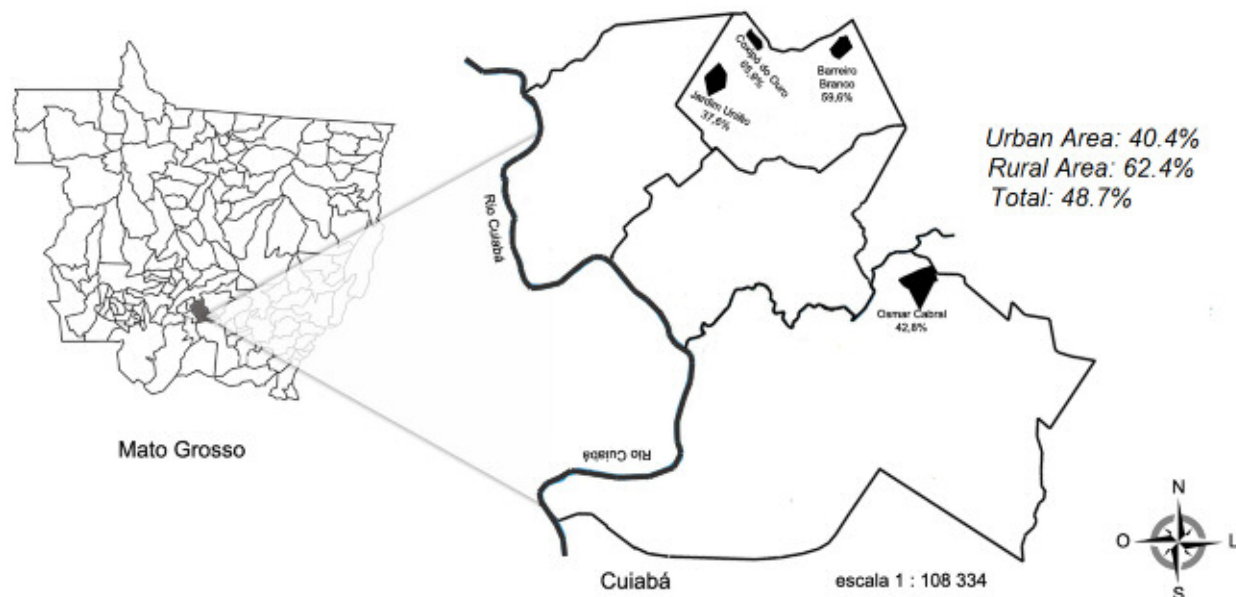
by the Brazilian Society of Laboratory Animal Science (SBCAL) and was approved by the Ethics Committee for Animal Research (CEPA) -UFMT under the Protocol N ° 23108.014445 / 10-7.

## Results

Of the 269 samples tested, we found a prevalence of 131 (48.7%) cases of infection that were reactive according to IFA and 42 (15.6%) that were positive according to PCR, which resulted in a total of 153 (56.9%) dogs with the infection. Of the 42 positive PCR results, 20 cases also had titers of antibodies against *T. gondii*.

The seroprevalence was 62.4% in the rural neighbourhoods and 40.4% in the urban areas, and the difference between these areas was significant ( $p = 0.0007$ ) (Figure 1).

**Figure 1.** Distribution of dogs with *Toxoplasma gondii* in city of Cuiabá, Mato Grosso, Brazil.



The antibody titers in seropositive dogs were as follows: 16 in 7 dogs (5.3%); 64 in 26 dogs (19.8%); 256 in 51 dogs (39%); 1024 in 37 dogs (28.2%); and 4096 in 10 dogs (7.7%).

On physical examination, 58 (44.3%) dogs infected with *T. gondii* showed clinical signs

during the home visit, including weight loss (20.6%), generalized lymphadenopathy (16%), splenomegaly (15.2%), uveitis and conjunctivitis (11.4%). However, these signs were observed in 13.8%, 22.5%, 8%, and 13.8%, of non-reactive dogs, respectively.

There was no sex predisposition to infection with *T. gondii* ( $p = 0.95$ ), despite the greater percentage of male dogs with antibodies against the agent. However, a significant association was observed between infection and dogs older than six years ( $p = 0.03$ ) regardless of breed ( $p = 0.04$ ) (Table 1).

Of the dogs that were seropositive, 27 (20.6%) ate raw meat mixed with feed and / or human food leftovers, and 48 dogs (36.6%) shared the same environment with cats. Free access to the street was significantly associated with infection ( $p = 0.03$ ) (Table 1).

**Table 1.** Association between the variables and the presence of anti-*Toxoplasma gondii* antibodies in dogs in the city of Cuiaba - Mato Grosso.

Variables	Total		P	Odds Ratio	CI
	Positive	%			
<b>Breed</b>					
SRD	115	87.8	0.04 <sup>a</sup>	2.08	1.03 - 4.24
CRD	16	12.2			
<b>Sex</b>					
Male	65	49.6	0.95	-	-
Female	66	50.4			
<b>Age groups</b>					
< 3 years	63	42	0.03 <sup>a</sup>	2.14*	1.03 - 4.24
3-6 years	37	54.4			
> 6 years	31	60.8			
<b>Diet</b>					
Commercial	22	16.8	0.46	-	-
Home-cooked	30	22.9			
Raw meat	27	20.6			
Mixed	52	39.7			
<b>Contact with cats</b>					
Yes	48	36.6	0.10	-	-
No	83	63.4			
<b>Access to the street</b>					
Yes	105	80.2	0.03 <sup>a</sup>	1.89	1.04 - 3.44
No	26	19.8			
<b>Area</b>					
Urban	68	51.9	0.00 <sup>a</sup>	2.44	1.42 - 4.18
Rural	63	48.1			

<sup>a</sup> Statistical significance 5%; SRD - Without defined Breed; CRD - With defined Breed.

In urban areas, there was a significant difference in the number of seropositive dogs between the types of ground floor (mixed (cemented and ground) and cement only) ( $p = 0.01$ ). However, in the countryside, no seropositive dogs lived in a cemented or mixed environment but instead lived on predominantly dirt floors.

## Discussion

The prevalence of 56.9% of dogs with a *T. gondii* infection is greater than the 35% prevalence (GRÖSZ et al., 2002) found between 1997 and 1998 in the sample municipality. This difference may be related to the sample in this study, which included urban and rural areas. In addition to the technique employed, we used two diagnostic techniques with

different principles. The use of PCR for canine surveys of toxoplasmosis has not been described; however, this methodology (KOMPALIC-CHRIST et al., 2005) using blood samples is able to distinguish acute infections that result in parasitemia, in addition to confirming the transmission cycle of the disease in these environments.

In the serological analysis, high titers of antibodies were detected, in which titers of 256 and 1024 were the most common, and these values suggest chronic infection in most animals (CAMARGO, 1975). This finding differs from the results of other authors (DREER et al., 2013) who found that most animals had low titers. High titers can be justified if highly immunogenic and minimally cystogenic strains lead to the emergence of high antibody levels without clinical signs (DUBEY et al., 1995), an aspect that could explain the high incidence of clinically healthy seropositive dogs found in this study. The clinical signs presented by the dogs were weight loss, lymphadenopathy, splenomegaly and ophthalmic changes, which have also been described by other authors (BRESCHIANI et al., 1999; ABREU et al., 2001; MORETTI et al., 2002).

The prevalence depends on the presence of predisposing factors (BARBOSA et al., 2003), which explain the varying prevalence in the literature (SOUZA et al., 2003; CAÑÓN-FRANCO et al., 2004; AZEVEDO et al., 2005; ROMANELLI et al., 2007; MOURA et al., 2009) and suggests the risk to the human population. The factors that were significantly associated with *T. gondii* infection were the following: free access to the street (two-fold increased chance of infection) and age older than six years compared to dogs younger than three years (regardless of breed). These factors may result from the management of these animals, as they are probably exposed more to the source of infection, such as rodents and oocysts eliminated by cats, and older animals have a longer exposure to the agent compared to younger dogs (MINEO et al., 2004; LANGONI et al., 2006; SILVA et al., 2009).

The presence and close contact with cats is an important aspect in the epidemiology of canine toxoplasmosis due to environmental contamination, as cats prefer defecating in an environment that contains soil (SANTOS et al., 2009). This factor may explain why dirt only and mixed environments were risk factors of infection in the dogs in this study ( $p = 0.01$ ). However, the presence of cats was not considered a potential risk factor of canine infection. Similarly, diet was not related to risk of infection, although this variable is considered an important risk factor in the epidemiology of toxoplasmosis (ARAÚJO et al., 2001).

Climate change, sanitation, and, most importantly, cultural population can affect the prevalence of *T. gondii* infection and contribute to the development and transmission of the parasite in the environment (FERREIRA et al., 2014). The districts in this study did not have sewage systems, and public garbage collection was irregular. These characteristics may have enabled the high prevalence of infected dogs in these places due to the exposure of the dogs to waste and environmental contamination with organic residue buildup.

## Conclusion

The serological and molecular results revealed that infection with *T. gondii* is active in the canine population in the rural and urban areas of Cuiabá, with a higher risk of infection in rural areas, in adult dogs and in those dogs with free access to the street.

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