

Prevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in dogs in the municipality of Poconé, state of Mato Grosso, Brazil

Prevalência de anticorpos contra *Neospora caninum* e *Toxoplasma gondii* no município de Poconé, estado de Mato Grosso, Brasil

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ABSTRACT

This study evaluated the presence of *Neospora caninum* and *Toxoplasma gondii* antibodies in 320 domestic dogs living in urban and rural areas of the municipality of Poconé, located in the Pantanal wetlands of the state of Mato Grosso, Brazil. Anti-*N. caninum* (cut-off point 50) and anti-*T. gondii* (cut-off point 16) antibodies were detected by means of immunofluorescence assays (IFA), using NC-Bahia and RH strains as antigens, respectively. Anti-*N. caninum* antibodies were detected in 69 (21.56%; 95% CI: 17.27%-26.56%) dogs, 31 (44.93%) of which lived in urban areas and 38 (55.07%) in rural areas, and endpoint titers ranged from 50 to 3200. Anti-*T. gondii* antibodies were found in 132 (41.25%; 95% CI: 35.84%-46.87%) dogs, 58 (43.94%) from urban areas and 74 (56.06%) from rural areas, and endpoint titers ranged from 16 to 8192. A total of 33 dogs (10.3%) (12 urban and 21 rural animals) reacted to both agents ($P < 0.05$). This study showed a higher prevalence of anti-*T. gondii* compared to anti-*N. caninum* antibodies in the dogs living in the Pantanal region, suggesting that both protozoans circulate in the studied region. This information is relevant, in view of its implications for animal and public health.

Keywords: Protozoans. Seroprevalence. IFA. Canids. Pantanal.

RESUMO

Este estudo avaliou a presença de anticorpos contra *N. caninum* e *T. gondii* em 320 cães domésticos das áreas urbana e rural do município de Poconé, no estado do Mato Grosso, região pantaneira do Pantanal. A Reação de Imunofluorescência Indireta (RIFI) foi utilizada para detectar anticorpos anti-*N. caninum* (ponto de corte igual a 50) e anticorpos anti-*T. gondii* (ponto de corte igual a 16) utilizando as cepas NC-Bahia e RH como antígenos, respectivamente. Anticorpos anti-*N. caninum* foram detectados em 69 (21,56%; IC 95%: 17,27%-26,56%) cães, dos quais 31 (44,93%) eram da área urbana e 38 (55,07%) eram da área rural e os títulos variaram entre 50 a 3200. Em relação aos anticorpos contra *T. gondii* 132 (41,25%; IC 95%: 35,84%-46,87%) cães foram sororeagentes sendo que 58 (43,94%) eram da área urbana e 74 (56,06%) da área rural e os títulos variaram entre 16 a 8192. Um total de 33 (10,31%) (12 cães urbanos e 21 cães rurais) cães reagiram para ambos os agentes testados pela RIFI ($P < 0,05$). Este estudo mostrou uma maior ocorrência de anticorpos contra *T. gondii* em relação a *N. caninum* nos cães avaliados, mostrando que na região do Pantanal há circulação desses importantes protozoários. Essa informação é relevante dada sua importância para a saúde animal e pública.

Palavras-chave: Protozoários. Seroprevalence. RIFI. Canídeos. Pantanal.

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Introduction

Neospora caninum and *Toxoplasma gondii* are important apicomplexan pathogens characterized as obligatory intracellular parasites that can infect dogs (Reid et al., 2012). The definitive hosts of the protozoan *N. caninum* are canids, which eliminate oocysts in their feces. Dogs, together with other warm-blooded animals, are also considered intermediate hosts (Gondim et al., 2004; King et al., 2010; McAllister et al., 1998). *Neospora caninum* is associated with cases of abortion in cattle and neuromuscular disease in newborn dogs (Dubey et al., 2007).

Toxoplasma gondii, on the other hand, is one of the most common coccidian parasites that can infect animals and humans, and is considered a major public health problem in several parts of the world. The life cycle of this protozoan includes felids, which are its definitive hosts, since they eliminate the oocysts through their feces. *T. gondii* has a wide variety of intermediate hosts, including mammals and birds (Dubey & Jones, 2008). *T. gondii* infections may be congenital or postnatal. Most infections in humans are asymptomatic, but in some cases they may be severe, particularly in immunosuppressed people (Hill & Dubey, 2016).

Information about the epidemiology of neosporosis and toxoplasmosis has been reported in several places in Brazil, proving the widespread circulation of these protozoans (Acosta et al., 2016; Brasil et al., 2018; Cerqueira-Cézar et al., 2017; Langoni et al., 2014). The presence of anti-*N. caninum* antibodies has also been reported in different animals, including cattle and dogs, in the state of Mato Grosso (Benetti et al., 2008; Benetti et al., 2009; Boa Sorte et al., 2015; Melo et al., 2012; Rodrigues et al., 2016). In the Pantanal wetlands, equids have been found seropositive for both

N. caninum and *T. gondii* (Borges et al., 2017). In addition, a serological survey of free living jaguars (*Panthera onca*) in this region revealed high prevalence rates of antibodies to both parasites (Onuma et al., 2014). Given that dogs can act as sentinels for these diseases (Ullmann et al., 2008) and are usually in close contact with humans, the purpose of this study was to determine the prevalence of anti-*N. caninum* and anti-*T. gondii* antibodies in domestic dogs in the municipality of Poconé, located in the northern region of the Pantanal in the state of Mato Grosso, Brazil.

Materials and Methods

A total of 320 dogs in the municipality of Poconé (56°37'22" W; 16°15'24" S) were examined for rickettsial diseases between July and September 2009 (Melo et al., 2011), and for comparison, 160 of these dogs came from urban areas and 160 dogs from rural areas. Blood samples were collected by jugular venipuncture to obtain serum. Immunofluorescence assays (IFA) were performed to detect antibodies to these pathogens, using as antigens tachyzoites of the NC-Bahia strain of *N. caninum* (1:50 cut-off) (Aguiar et al., 2006) and RH strain of *T. gondii* (1:16 cut-off) (Camargo, 1964). Each IFA slide contained sera previously known to be non-reactive (negative control) and reactive (positive control).

The owners of the dogs selected for blood collection were asked to fill out a comprehensive questionnaire that included information about the animal, as described in Table 1. Associations between the serology results and the variables obtained from the questionnaire were then inferred based on the chi-square test (χ^2) or Fisher's exact test, using the Epi Info 7.1 software package. Our procedures were approved by the Bioethics Committee on Animal Research of the Federal University of Mato Grosso, under protocol no. 23108.019742/09-9.

Results and Discussion

The dogs, ranging in age from 1 to 72 months, were of different breeds and appeared to be healthy. Anti-*N. caninum* antibodies were detected in 69 (21.56%; 95% CI: 17.27%- 26.56%) dogs, 31 (44.93%) of them from urban and 38 (55.07%) from rural areas. Endpoint titers were 50 (n=6), 100 (n=13), 200 (n=12), 400 (n=11), 800 (n=11), 1600 (n=9) and 3200 (n=7). Anti-*T. gondii* antibodies were detected in 132 (41.25%; 95% CI: 35.84%-46.87%) dogs, 58 (43.94%) from urban and 74 (56.06%) from rural areas. Endpoint titers were 16 (n=5), 32 (n=16), 64 (n=15), 128 (n=30), 256 (n=29), 512 (n=17), 1024 (n=13), 2048 (n=2), 4096 (n=4) and 8192 (n=1). A total of 33 dogs (10.3%) (12 urban and 21 rural dogs) reacted to both agents tested by IFA.

Table 1 – Analysis of variables and IFA results for anti-*Neospora caninum* and anti-*Toxoplasma gondii* antibodies from dogs in the municipality of Poconé, Mato Grosso, Brazil – 2009

Analyzed variables	No. of dogs				
	Analyzed	Seropositive for <i>N. caninum</i>		Seropositive for <i>T. gondii</i>	
		Number (%)	P-value	Number (%)	P-value
Living habitat					
Urban	160	31 (44.93)	0.41	58 (43.94)	0.08
Rural	160	38 (55.07)		74 (56.06)	
Sex					
Male	180	41 (59.42)	0.64	79 (59.85)	0.33
Female	140	28 (40.58)		53 (40.15)	
Access to the street					
Yes	127	30 (43.48)	0.55	54 (40.91)	0.79
No	193	39 (56.52)		78 (59.09)	
Access to rural areas					
Yes	196	46 (66.67)	0.36	94 (71.21)	0.00
No	124	23 (33.33)		38 (28.79)	
Type of diet*					
Home-cooked food	174	42 (60.87)	0.30	81 (61.83)	0.04
Mixed**	144	27 (39.13)		50 (38.17)	
Contact with dogs					
Yes	260	57 (82.61)	0.87	103 (78.03)	0.27
No	60	12 (17.39)		29 (21.97)	
Contact with felids					
Yes	145	37 (53.62)	0.15	62 (46.97)	0.70
No	175	32 (46.38)		70 (53.03)	
Contact with animal food products					
Yes	185	44 (63.77)	0.32	87 (65.91)	0.01
No	135	25 (36.23)		45 (34.09)	
Contact with wild animals					
Yes	188	45 (65.22)	0.27	89 (67.42)	0.01
No	132	24 (34.78)		43 (32.58)	
Hunting behavior					
Yes	100	30 (43.48)	0.01	46 (34.85)	0.29
No	220	39 (56.52)		86 (65.15)	
Age					
0-12 months	99	14 (20.29)	0.04	21 (15.91)	0.00
12-24 months	67	20 (28.99)	0.09	25 (18.94)	0.55
24-48 months	72	14 (20.29)	0.73	38 (28.79)	0.03
>48 months	78	21 (30.43)	0.24	46 (34.85)	0.00
Indeterminate	4	0 (0.0)	0.58	2 (1.52)	1.00

*The diet of 2 dogs could not be determined; **Mixed: a combination of home-cooked and commercial dog food.

The prevalence of anti-*N. caninum* antibodies was associated with age and hunting behavior ($P < 0.05$). On the other hand, anti-*T. gondii* antibody prevalence was associated with age, access to rural areas, type of diet and contact with farm and wild animals ($P < 0.05$; Table 1).

Our findings indicated that urban and rural dogs in the northern region of Pantanal were equally exposed to *N. caninum* and *T. gondii*. Although no association was found between the variables of urban/rural environment and the prevalence of the two agents, associations were found between several other variables. The association between young aged animals and seropositivity for *N. caninum* in dogs appears to suggest possible transplacental transmission

(Cavalcante et al., 2012), which is also supported by the non-hunting behavior of many of these dogs. In contrast, an association was found between older dogs and the prevalence of *T. gondii*, indicating that the dogs in this region were exposed to this coccidian parasite for longer periods of time during their life, which suggests that the main route of infection in dogs is horizontal transmission (Cañón-Franco et al., 2003; Cano-Terriza et al., 2016).

The findings about *T. gondii* are supported by other associations, including access to rural areas, home cooked diet, contact with animal food products and wild animals. All these variables may be related to contaminated environments or food to which these dogs may have been exposed during

their life. The association between home-cooked diets and the seropositivity to the protozoan *T. gondii* is relevant, given that infections by this agent may also be acquired by ingesting tissue-inhabiting stages of the parasite and that any animal tissues, especially undercooked meat containing cysts with bradyzoites, may be a source of infection for dogs (Hill et al., 2005). Borges et al. (2017), who evaluated equids in this region, found a large number of seropositive animals. In this context, the possible contribution of domestic or wild felids should be considered, since the presence of cats in urban areas and on farms is common; in fact, 50% of the dogs in our study have contact with cats. Moreover, the presence of wild felids in this region is frequent, and high titers of anti-*T. gondii* antibodies have been detected in local free-living jaguars (Onuma et al., 2014).

Conclusions

The prevalence of anti-*T. gondii* and anti-*N. caninum* antibodies among these dogs indicates that these coccidia are circulating in the Pantanal. This information is relevant,

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- Conflict of Interest**
- The authors declare that there is no conflict of interest.
- Ethics Statement**
- This study was evaluated by the Animal Research Ethics Committee of the Federal University of Mato Grosso and was approved under Protocol nº 23108.019742/09-9.
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