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PARASITOLOGY

Anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in dogs with and without neurological signs

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ABSTRACT: This study investigated the association between neurological signs as well as plausible risk factors and the seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infection in dogs of the Campo Grande region of Mato Grosso do Sul, Brazil. In this study, the dogs were divided into two groups based on the presence and the absence of neurological signs (n=30 in each group). Serological diagnosis was performed using the indirect fluorescent antibody test. In the group with neurological disorders, 23.3% and 30% of the dogs had anti-*T. gondii* and anti-*N. caninum* antibodies, respectively. Moreover, three dogs from this group showed co-infection with both protozoa. In the group without neurological signs, 16.7% and 13.3% of the dogs were seropositive for *T. gondii* and *N. caninum*, respectively. Although presence of neurological signs was not associated with *T. gondii* and *N. caninum* infections (P = 0.747 and P = 0.21, respectively), there was a statistical association between *T. gondii* seropositivity and peripheral neurological alteration (P = 0.016) among dogs with neurological signs. Raw meat ingestion was the only risk factor associated with the presence of anti-*N. caninum* antibodies (P = 0.041). Results revealed evidence of exposure to *N. caninum* and *T. gondii* in dogs irrespective of the presence of neurological signs. Moreover, this study highlighted the need for serological investigation of *T. gondii* in dogs with disturbances in peripheral nervous systems and not offering raw meat to animals to avoid the risk of *N. caninum* infection.
Key words: dogs, *Toxoplasma gondii*, *Neospora caninum*, serology, IFAT, raw meat ingestion, peripheral neurological signs.

Anticorpos anti-*Toxoplasma gondii* e anti-*Neospora caninum* em cães com e sem sinais neurológicos

RESUMO: Este estudo teve como objetivo investigar a associação entre sinais neurológicos, assim como possíveis fatores de risco e soroprevalência de *Toxoplasma gondii* e *Neospora caninum* em cães de Campo Grande, região de Mato Grosso do Sul, Brasil. Nesse estudo, os cães foram divididos em dois grupos, baseado na presença ou ausência de sinais neurológicos (n = 30 em cada grupo). O diagnóstico sorológico foi realizado por meio do teste imunofluorescência indireta (RIFI). No grupo com distúrbios neurológicos, 23.3% e 30% dos cães tinham anticorpos anti-*T. gondii* e anti-*N. caninum*, respectivamente. Além disso, três cães deste grupo estavam coinfectados com ambos os protozoários. No grupo sem sinais neurológicos, 16.7% e 13.3% dos cães foram soropositivos para *T. gondii* e *N. caninum*, respectivamente. Apesar da presença de sinais neurológicos não ter sido associado a infecção por *T. gondii* ou *N. caninum* (P = 0.747 e P = 0.21, respectivamente), houve associação estatística entre soropositividade para *T. gondii* e alteração neurológica periférica (P = 0.016) entre os cães com sinais neurológicos. A ingestão de carne crua foi o único fator de risco que apresentou associação com a presença de anticorpos para *N. caninum* (P = 0.041). Estes resultados mostraram evidência de que a exposição a *N. caninum* e *T. gondii* em cães independe da presença de sinais neurológicos. Além disso, este estudo destaca a necessidade de investigação sorológica para *T. gondii* em cães com distúrbios neurológicos periféricos, e o oferecimento de carne crua aos animais deve ser evitada devido ao risco de infecção por neosporose.
Palavras-chave: cães, *Toxoplasma gondii*, *Neospora caninum*, sorologia, IFI, ingestão de carne crua, sinais neurológicos periféricos.

Dogs are the definitive important hosts of *Neospora caninum* (DUBEY et al., 2012). Moreover, they have been considered sentinels and contact with them is a possible risk factor for *Toxoplasma gondii* infection in humans as they play a role in the mechanical transmission of oocysts (LOPES et al., 2015). Dogs mainly acquire *T. gondii* and *N. caninum* infections through ingestion of food or water contaminated with oocysts, or ingestion of tissue cysts present in raw or under cooked meat; however, vertical transmission may also occur (DUBEY, 2003; DUBEY et al., 2020). *T. gondii* infected dogs are usually asymptomatic. Moreover, dogs with active disease rarely exhibit clinical signs that are primarily

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associated with toxoplasmosis, being observed when they are immunosuppressed, or have co-morbidities (particularly distemper) (GIRALDI et al., 2002). However, neurological signs associated with neosporosis are commonly observed (GIRALDI et al., 2002). There are limited data on the serological prevalence of *T. gondii* and *N. caninum* in the canine population of the Campo Grande, region of Mato Grosso do Sul, Brazil. Furthermore, studies on the prevalence of these protozoa in dogs with and without neurological signs are lacking.

Considering the limited regional data and the importance of *T. gondii* and *N. caninum* infection in dog and other species, this study investigated the association between neurological signs (encephalic, spinal, peripheral, or multifocal) as well as plausible infection risk factors of the occurrence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in the dogs in Campo Grande, Mato Grosso do Sul.

This study included dogs from various regions of Campo Grande, Mato Grosso do Sul, who were treated at the veterinary hospital of the Universidade Federal de Mato Grosso do Sul from April 2019 to December 2019. During this period, 30 dogs presented with neurological signs. After neurological examination, they were categorized according to the anatomical location of the lesion as having in encephalic, spinal, peripheral or multifocal alteration. Additionally, this study included a control group of 30 dogs without neurological signs, who had visited the hospital for problems other than neurological disorders. All dog owners gave their signed consent for participation of the study.

Blood samples were collected for serological diagnosis to verify the presence of anti-*N. caninum* and anti-*T. gondii* immunoglobulin G antibodies using an indirect fluorescent antibody test (IFAT), according to previously described methods (CAMARGO, 1974; CONRAD et al., 1993). Only samples that showed fluorescence of the entire tachyzoites surface were considered to have positive IFAT results. Samples with titers ≥ 1:25 and ≥ 1:16 were considered positive for *N. caninum* and *T. gondii* infection, respectively.

The owners completed a questionnaire regarding the characteristic of their dogs to identify the possible risk factors associated with *T. gondii* and *N. caninum* infections. The questionnaire collected information regarding sex (male/female), age (<5 years/≥5 years), breed (pure breed/mixed breed), place of habitation (urban/rural), presence of other pets (yes/no), contact with wild or wandering animals (yes/no), street access (yes/no), consumption of homemade food (yes/no), ingestion of raw meat (yes/no), contact with carcasses of other animals (yes/no), pre-existing comorbidities (yes/no), and vaccination (yes, if vaccination was updated/no, if not vaccinated or vaccination was delayed). Other diseases involving neurological changes were not excluded.

The association between the serological test results and aforementioned variables from the epidemiological questionnaire was determined by calculating the odds ratio with a 95% confidence interval. Fisher's exact test was performed for the statistical evaluation of neurological signs and seropositivity frequency. All analyses were performed using the Bioestat 5.0.A P-value < 0.05 was considered statistically significant.

In the group with neurological signs, 23.3% and 30% of the dogs were seropositive for *T. gondii* and *N. caninum*, respectively (P = 0.771 for both) (Table 1). In the group without neurological signs, 16.7% and 13.3% of the dogs had antibodies of *T. gondii* and *N. caninum*, respectively. The titres ranged from 1:16 to 1:4096 for toxoplasmosis, and from 1:25 to 1:100 for neosporosis. Three dogs from group with neurological disorders (10%) presented co-infection with both the protozoa.

A significant association was observed only between raw meat ingestion and the presence of anti-*N. caninum* antibodies (P = 0.041). There was no association between other examined variables and the frequency of antibody detection for either of the two protozoa; surprisingly, raw meat ingestion was not associated with *T. gondii* infection (Table 2).

In our study, the proportion of dogs with neurological signs infected by *T. gondii* was 23.3%, which was similar to that reported in Paraná (21.08%) (PLUGGE et al., 2011), but higher than that reported in dogs with clinical suspicion of neosporosis in Germany (13%) (KLEIN & MÜLLER, 2001). The proportion of asymptomatic animals infected with *T. gondii* was 16.7% in our study, which was similar to that reported in other studies conducted in Brazil (12.7% - 20.8%) (LANGONI et al., 2013; LOPES et al., 2014); however, this proportion could reach up to 88.5%, as reported by SANTOS et al. (2009).

Presence of antibodies against *N. caninum* was observed in 13.3% of the dogs without neurological signs, which is similar to what observed in the worldwide canine population (17.14%) (ANVARI et al., 2020), but higher than that in dogs without clinical alterations in Germany (4%) (KLEIN & MÜLLER, 2001). In Brazil, there is a wide variation in the seroprevalence of *N. caninum* in dogs, ranging from 2.6% in Bahia (SICUPIRA et al., 2012)

Table 1 - Total number, frequency (percentage), and statistical association (Fisher's exact test) of dogs seropositive and seronegative for *Toxoplasma gondii* and *Neospora caninum* with and without neurological signs.

Clinical sign	------*Toxoplasma gondii*-----		P-value	-------*Neospora caninum*------		P-value
	Positive	Negative		Positive	Negative	
With neurological sign	7 (23.3%)	23 (76.7%)		9 (30%)	21 (70%)	
Encephalic[1]	2	18	0.026	6	14	0.998
Spinal[2]	1	1	0.419	1	1	1
Peripheral[3]	4	2	0.016	2	4	0.995
Multifocal[4]	0	2	1	0	2	0.566
Without neurological sign	5 (16.7%)	25 (83.3%)		4 (13.3%)	26 (83.7%)	

[1]Encephalic *vs* peripheral, spinal, and multifocal signs.
[2]Spinal *vs* encephalic, peripheral, and multifocal signs.
[3]Peripheral *vs* encephalic, spinal, and multifocal signs.
[4]Multifocal *vs* encephalic, peripheral, and spinal signs.

to 67.6% in Mato Grosso (BENETTI et al., 2009). This could be attributed to several factors, such as sample size, characteristics of the studied population, regional differences, study seasonality, different serological assays, heterogeneous groups, and cut-off titer values (AZEVEDO et al., 2005; ANVARI et al., 2020; GOMES et al., 2020).

Three dogs (10%) from the group with neurological signs showed co-infection with both *T. gondii* and *N. caninum*. This proportion was almost double or even triple of that reported in Campina Grande (4.9%) (AZEVEDO et al., 2005), Paraná (2.72%) (PLUGGE et al., 2011), and in the northeast of the State of São Paulo (5.76%) (VARANDAS et al., 2001).

There are several reports on animals with neosporosis and toxoplasmosis with neurological signs, such as Horner's syndrome (BOYDELL & BROGAN, 2000), necrotizing cerebellitis (CANTILE

Table 2 - Analysis of risk factors for seropositivity for *Toxoplasma gondii* and *Neospora caninum* in 60 dogs from Campo Grande, MS, Brazil.

Risk fator	--------------------*Toxoplasma gondii*----------------			----------------*Neospora caninum*---------------		
	OR	Interval	p-value	OR	Interval	p-value
Neurological signs (Yes/No)	1.52	0.42-5.47	0.747	2.79	0.75-10.33	0.21
Vaccination (Yes/No)	1.15	0.22-6.21	0.802	0.68	0.15-3.06	0.925
Raw meat ingestion (Yes/No)	1.27	0.29-5.57	0.938	4.89	1.26-18.98	0.041
Contact with the carcasses of other animals (Yes/No)	1.72	0.29-10.18	0.920	1.53	0.26-8.96	0.987
Age (<5 years /≥5 years)	1.4	0.39-4.98	0.845	0.77	0.22-2.72	0.933
Pre-existing comorbidities (Yes/No)	1.70	0.46-6.36	0.654	1.23	0.37-4.35	0.958
Breed (Pure/Mixed)	0.34	0.08-1.38	0.218	1.06	0.31-3.64	0.826
Place of habitation (Urban/Rural)	0.33	0.05-2.27	0.559	0.26	0.02-4.48	0.907
Sex (Male/Female)	2.42	0.67-8.84	0.304	1.33	0.37-4.77	0.912
Living with other pets (Yes/No)	1.20	0.35-5.56	0.940	0.60	0.17-2.09	0.634
Contact with wild/ wandering animals (Yes/No)	0.51	0.07-2.48	0.655	0.70	0.08-6.59	0.835
Consumption of homemade food (Yes/No)	1.52	0.43-5.45	0.743	1.88	0.16-22.47	0.829
Street access (Yes/No)	0.47	0.12-1.69	0.397	1.98	0.54-7.34	0.474

& ARISPICI 2002), encephalomyelitis (PATITUCCI et al., 1997; GERHOLD et al., 2014) and behavioral change (PAPINI, 2009).However, there are only few epidemiological studies on the seroprevalence of *N. caninum* and *T. gondii* infections in dogs with neurological signs (PLUGGE et al., 2011; KLEIN & MÜLLER, 2001), since most studies have been conducted on asymptomatic dogs. In this study, the frequency of *N. caninum* and *T. gondii* infections was similar among the dogs with neurological signs (30% vs 23.3%; P = 0.771). This finding is different from that of previous studies, which showed that most dogs with neurological clinical signs were seropositive for *T. gondii* (GIRALDI et al., 2002; PLUGGE et al., 2011; LANGONI et al., 2012). Nevertheless, analyses of the neurological signs by category revealed that peripheral alterations were significantly more often present in *T. gondii* positive dogs relative to encephalic, spinal or multifocal alterations (P = 0.016) (Table 1). There is an interesting explanation for the peripheral nervous system involvement in toxoplasmosis; namely, there exists a molecular similarity between the pathogenic agent and the proteins or glycolipids in peripheral nerve myelin or the axonal Ranvier's membrane in the initial phase of the infection (HOLT et al., 2011). This allows multiplication of the protozoan and delayed immune response, which, in turn, manifests itself as peripheral neurological alterations (HOLT et al., 2011). Although, we reported statistical significance for the encephalic alteration and *T. gondii* infection (Table 1), it is noted that the number of seronegative dogs with this clinical sign is much higher (18) in relation to positive ones (2), so that, in our study, encephalic signs were associated with *T. gondii* seronegative dogs.

Ingestion of raw meat was significantly associated with the presence of anti-*N. caninum* antibodies (P = 0.041), as previously noted by GAO & WANG (2019) in China. The main route of *N. caninum* infection in dogs is by the ingestion of food contaminated with tissue cysts. As there was no association between hunting (i.e., contact with wild animals or with carcasses) and antibodies against *N. caninum*, but there was an association to raw meat ingestion, the infection may probably occur due to the ingestion of beef, as it is the dominant source of meat for dogs in this region of Brazil.

Some studies have shown that the probability of toxoplasmosis and neosporosis of infection increases with age, possibly due to greater exposure to the respective agent (OLIVEIRA et al., 2004). However, there was no association between age and *T. gondii* or *N. caninum* antibodies in our study, similarly to the finding of VARANDAS et al. (2001) and LANGONI et al. (2013).

A limitation of this study is its small sample size, due to the low number of neurological cases during the study period. Future studies with larger sample size are warranted for better understanding the epidemiology of the frequency of antibodies against *N. caninum* and *T. gondii* in dogs with and without neurological signs in this region.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was approved by the Ethics and Use of Animals Committee (CEUA) of the Universidade Federal de Mato Grosso do Sul (UFMS) (protocol 511 1,034/2019).

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