

# High seroprevalence of *Toxoplasma gondii* and *Neospora* spp. in stray dogs from Rolim de Moura, Rondônia state, Western Brazilian Amazon

## Alta soroprevalência de *Toxoplasma gondii* e *Neospora* spp. em cães errantes de Rolim de Moura, estado de Rondônia, Amazônia Ocidental Brasileira

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### Highlights

*Toxoplasma gondii* and *Neospora* spp. is widespread in dogs of Rolim de Moura, Rondônia.

Seroprevalence of *T. gondii* was 82.20% and of *Neospora* spp. was 73.85%, in stray dog.

First report of seroprevalence of the *T. gondii* and *Neospora* spp. in dogs, Rondônia.

We report the highest seroprevalence of *Neospora* spp. in dogs, in Brazil.

High prevalence of *T. gondii* at Rolim de Moura, Rondônia state, Brazil.

### Abstract

*Toxoplasma gondii* and *Neospora* spp. are important apicomplexan pathogens that can infect dogs and result in a neurological syndrome. The aim of this study was to evaluate the seroprevalence of *T. gondii*

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and *Neospora* spp. in stray dogs in the state of Rondônia, Brazil. A cross-sectional study was conducted from June 2014 to April 2016. A total of 458 dogs blood samples were collected at the Center for Control of Zoonosis (CCZ), and anti-*T. gondii* and anti-*N. spp.* antibody levels were detected and measured using indirect fluorescent antibody test (IFAT). The seroprevalence of *T. gondii* and *Neospora* spp. in dogs it was 82.20% and 73.85%, respectively. The seroprevalence of coinfections was 47.59%. This is the first report of anti-*T. gondii* and anti-*Neospora* spp. antibodies detected in stray dogs in Rolim de Moura, state of Rondônia, Western Brazilian Amazon. Male dogs showed a higher frequency of anti-*T. gondii* antibodies than female dogs (88.0% vs. 75.3%;  $p < 0.001$ ), presenting 2.41 times more chances of having the disease. High seroprevalence of infection (92.4%: *T. gondii* and 89.2%: *Neospora* spp.) was detected in the group  $\geq 1$ -year-old dogs, in all collections, with OR 7.35 and OR 10.27 for the presence of anti-*T. gondii* and anti-*Neospora* spp., respectively. In conclusion, the serological results indicate an important circulation of *T. gondii* and *Neospora* spp. in wandering dogs hailing from Rolim de Moura, state of Rondônia, Western Brazilian Amazonia.

**Key words:** Toxoplasmosis. Neosporosis. Canine. Brazil. Prevalence. IFAT.

## Resumo

*Toxoplasma gondii* e *Neospora* spp. são importantes patógenos apicomplexos que podem infectar cães e ocasionar síndrome neurológica. O objetivo deste estudo foi avaliar a soroprevalência de *T. gondii* e *Neospora* spp. em cães errantes do estado de Rondônia, Brasil. Um estudo transversal foi realizado durante o período de junho de 2014 a abril de 2016. Um total de 458 amostras de sangue de cães foram colhidas no Centro de Controle de Zoonoses (CCZ) e a detecção de anticorpos contra *T. gondii* e *N. spp.* foram realizados pela reação de imunofluorescência indireta (RIFI). A soroprevalência de *T. gondii* e *Neospora* spp. em cães foi 82,20% e 73,85%, respectivamente. A soroprevalência de coinfeções foi 47,59%. Este é o primeiro relato de anti-*T. gondii* e anti-*Neospora* spp. anticorpos detectados em cães errantes em Rolim de Moura, estado de Rondônia, Amazônia Ocidental Brasileira. Os cães machos apresentaram maior frequência de anticorpos anti-*T. gondii* do que as fêmeas (88,0% vs. 75,3%;  $p < 0,001$ ), apresentando 2,41 vezes mais chance de ter a doença. Alta soroprevalência de infecção (92,4%: *T. gondii* e 89,2%: *Neospora* spp.) foi detectada no grupo  $\geq$  cães com 1 ano de idade, em todas as coletas, com OR 7,35 e OR 10,27 para presença do anti-*T. gondii* e anti-*Neospora* spp., respectivamente. Em conclusão, os resultados sorológicos indicam uma importante circulação de *T. gondii* e *Neospora* spp. em cães errantes de Rolim de Moura, estado de Rondônia, Amazônia Ocidental Brasileira.

**Palavras-chave:** Toxoplasmose. Neosporose. Canino. Brasil. Prevalência. RIFI.

## Introduction

*Toxoplasma gondii* and *Neospora* spp. are important apicomplexan pathogens characterized as obligatory intracellular protozoan. Both diseases have widespread

geographical distribution and the capacity to infect homoeothermic animals (Dubey, Lago, Gennari, Su, & Jones, 2012). *Toxoplasma gondii* is responsible for major economic losses in livestock and, it is a public health problem owing to the possibility of its transmission to

humans (Belluco, Simonato, Mancin, Pietrobelli, & Ricci, 2018).

*Toxoplasma gondii* and the related parasite *N. caninum* are cosmopolitan coccidians that cause neurological diseases in domestic dogs (*Canis familiaris*). In addition to behaving as intermediate hosts for *T. gondii* and *Neospora* spp., they can carry oocysts in their fur, thereby favouring human infection in cases of *T. gondii*, due to the close relationship; contaminated the environment and infecting a wide range of intermediate hosts (Langoni, Fornazari, Silva, Monti, & Villa, 2013).

Surveys conducted in Brazil showed that there was variation in the seroprevalence of *T. gondii* and *N. caninum* in dogs from different states. Few studies have been conducted in relation to these parasites in animals in the Amazon region. Therefore, the studies carried out in the state of Rondônia on the occurrence of anti-*T. gondii* antibodies noted 76.4% in dogs, (Canon-Franco et al., 2004) whereas the presence of *N. caninum* antibodies was 8.3% (Canon-Franco et al., 2003) and 12.6% (Aguiar et al., 2006) in dogs.

In this context, the objective of this study was to evaluate the seroprevalence of *T. gondii* and *Neospora* spp. in stray dogs from the state of Rondônia, Brazil and correlate possible variables associated with seropositivity for these protozoans.

In this study, all procedures using animals complied with the Ethical Principles in Animal Research adopted by the College of Animal Experimentation and were approved (protocol number PP010/2014) by the Ethical Committee for Animal Welfare, UNIR, Rolim de Moura, Rondônia, Brazil.

This study was conducted in animals from the municipality of Rolim de Moura

(61°48'13"S, 61°48'12"W), Rondônia state, in the Western Brazilian Amazon. This region presents a climate that, according to Aw of Köppen classification, was characterized as equatorial with changes to the hot and humid tropical climate with a well-defined dry season from March to September, minimum and maximum temperatures of 17° C and 33° C, respectively, with precipitation between high and moderately high (2000 to 2250 mm) and 85% relative air humidity. It has an area of 1,487.35 km<sup>2</sup> with an estimated population of 55,407 inhabitants and an altitude of 261 meters above the sea level.

The number of samples was calculated, assuming that the expected frequency of both *T. gondii* and *N. spp.* are approximately 50% to maximize the sample size, to obtain a minimal confidence level of 97%, and maintain the statistical error under 1%. Calculations were executed using an Epilnfo program (CDC, version 7.1.5.2), thereby resulting in a minimal sample size of 434 dogs (Thrusfield & Christley, 2018). This number was determined based on the human population existing in the municipality and on the calculation of the proportion dog:man, that is, 1:10 (Reichmann, Pinto, & Nunes., 1999), which resulted in a population of 5,541 dogs. A total of 10% of the sample population was added to the sample size; therefore, the final study population size was 478 dogs.

The study was carried out in the municipality of Rolim de Moura in the state of Rondônia, Brazil (Western Brazilian Amazon), and samples were collected from all dog shelters, in Center for Control of Zoonosis (CCZ). The blood samples were collected from 458 mixed breed dogs (*Canis familiaris*) from June 2014 to April 2016, and a cross-sectional study was also performed. The data were

grouped based on the year of collection (1st – 2014, 2nd – 2015, and 3rd – collections of 2016). Amongst these, 152, 167 and 159 samples were collected from the years 2014, 2015 and 2016. Information on age (< one year or ≥ one year) and sex (female or male) were obtained through physical examination. Blood samples from dogs were collected by venepuncture (cephalic or jugular), using identified vacuum tubes, after collection, which were packed in polystyrene with ice and centrifuged at 1000 g for 10 min. Subsequently, the serum was separated in a specific tube. Serum samples were identified and maintained at -20°C until serological tests were performed.

Anti-*T. gondii* and anti-*Neospora* spp. antibody levels were detected and measured by means of indirect fluorescent antibody test (IFAT). The RH strain of the *T. gondii* and *N. caninum* tachyzoites (NC-1) was maintained in Vero cell cultures. We used an anti-dog IgG conjugate label led with fluorescein isothiocyanate (Sigma®, St. Louis, MO), as a secondary antibody, with a cut-off dilutions values of 1:16 (Arraes-Santos et al., 2016) and 1:50 (Gondim, Lindsay, & McAlliester, 2009) for *T. gondii* and *N. caninum*, respectively. Reactions in which tachyzoites presented total fluorescence were deemed positive. Positive and negative control sera were added to each slide.

To compare the infection rates of the two parasites, Pearson's Chi square ( $\chi^2$ ) test or Fisher exact tests were performed to evaluate the differences between variables. For inferential statistics, the seroprevalence of *T. gondii* infection was considered as the dependent variable, and other factors were considered as the explanatory or independent variables. In each year, indexes of the prevalence, Odds ratios (OR), confidence

interval (CI) of 95%,  $\chi^2$ , and P value were separately calculated for each variable. All statistical tests were performed using STATA/SE, Version 16.1, Software (Stata Corp LLC, College Station, Texas, USA), considering a significance level of 5%.

In the present study, the prevalence of toxoplasmosis and neosporosis amongst dogs were 82.20% (392/478; 95% CI: 78.31–85.19) and 73.85% (353/478; 95% CI: 69.73–77.59), respectively.

The seroprevalence of *T. gondii* was 67.76% (95% CI: 59.97–74.68) in 2014, 89.22% (95% CI: 83.61–93.07) in 2015 and 88.05% (95% CI: 82.09–85.19) in 2016. For the presence of anti-*T. gondii* antibodies, a statistical difference was observed in the various collections ( $\chi^2 = 30.728$ ,  $p < 0.0001$ ), where the seroprevalence performed in 2014 was lower and statistically significant that in the other samples in the following years. Regarding *Neospora* spp., referring to the 2014, 2015 and 2016 samples, the percentage of dogs with positive reactions according to IFAT were 79.61% (95% CI: 72.51–85.24), 70.06% (95% CI: 62.73–76.49) and 72.33% (95% CI: 64.91–78.98), respectively. No significant difference was found in the seroprevalence of infection by *Neospora* spp., amongst the 2016 samples ( $\chi^2 = 4,040$ ,  $p = 0.133$ ).

The male dogs had a higher frequency of anti-*T. gondii* antibodies than the female dogs (88.0% vs. 75.3%;  $p < 0.001$ ), showing that the former were 2.41 times more likely to have the antibodies. In the analysis of gender for the presence of anti-*Neospora* spp. antibodies, no statistical difference ( $p > 0.05$ ) was found in all the studied collections. The results related to the comparison between male and female dogs are shown in Table 1.

**Table 1**

**Univariable analysis of risk factors associated with seroprevalence of *T. gondii* and *N. spp.* in dogs from Rolim de Moura, Rondônia state, Western Amazon, Brazil**

Variable	Seroprevalence (IFAT - IgG)									
	<i>Toxoplasma gondii</i>					<i>Neospora</i> spp.				
	Reagent n (%)	Non- Reagent n (%)	Total	OR <sup>a</sup> (95% CI)	p-value <sup>b</sup>	Reagent n (%)	Non- Reagent n (%)	Total	OR <sup>a</sup> (95% CI)	p-value <sup>b</sup>
<b>Sex</b>										
female	171 (75.3)	56 (24.7)	227 (75.3)	1	0.001	168 (74.0)	59 (26.0)	227 (74.0)	1.02 (0.68- 1.53)	0.94
male	221 (88.0)	30 (12.0)	251 (88.0)	2.41 (1.48- 3.92)		185 (73.7)	66 (26.3)	251 (73.7)	1	
<b>Age</b>										
< 1 year	102 (62.2)	62 (37.8)	164 (62.2)	1	< 0.0001	73 (44.5)	91 (55.5)	164 (44.5)	1	
1 year	290 (92.4)	24 (7.6)	314 (92.4)	7.35 (4.36- 12.39)		280 (89.2)	34 (10.8)	314 (89.2)	10.27 (6.41- 16.44)	< 0.0001

<sup>a</sup> OR: odds ratio. Reference group marked as OR = 1;

<sup>b</sup> Pearson's Chi Square

\* Significant association (p < 0.05).

The dogs were further allocated to two groups aged < one year (n = 164) and ≥ one year (n = 314) for statistical analysis (Table 1). Compared with the young dogs (62.2%: *T. gondii* and 45.5%: *N. spp.*), a higher seroprevalence of infection (92.4%: *T. gondii* and 89.2%: *N. spp.*) was detected in the group ≥ one-year-old dogs, in all collections (p < 0.05), with a OR 7.35 (95% CI: 4.36–12.39; p < 0.0001) and OR 10.27 (95% CI: 6.41–16.44; p < 0.0001) for the presence of the anti-*T. gondii* and anti-*Neospora* spp., respectively.

Seroprevalence of *T. gondii* in the order of 82.20% and of *N. spp.* of 73.85% were found in this research. This is the first report of anti-*T. gondii* and anti-*N. spp.* antibodies detected in stray dogs in Rondônia state, Brazil (Western

Brazilian Amazon). This high prevalence may be explained by geographic characteristics such as the hot and humid climate in the Amazon region, which is geographically, socially and ecologically different from the climate in other parts of the world with high levels of precipitation, which may contribute towards higher viability among oocysts in the environment. Figliuolo et al. (2004) showed that differences in altitude and temperature in certain areas may explain serological differences, as hot areas promote higher seroprevalence and sporulation of oocysts than colder regions.

In the present study, the prevalence of co-infections (*T. gondii* and *N. spp.*) was 47.59%. We observed that *T. gondii* and *N. spp.*

are widely spread in the studied region. The *T. gondii* seroprevalence in dogs was higher than the *N. spp.* seroprevalence, which is a frequent finding (Mascolli et al., 2015).

In prevalence studies of the *T. gondii* the rates of positive dogs vary highly. In Brazil, the seroprevalence in dogs can range from 3.1% to 91% in different regions (Dubey et al., 2012). In this survey, the seroprevalence for toxoplasmosis (82.2%) were similar to those reported by Canon-Franco et al. (2004), which showed the presence of anti-*T. gondii* antibodies in 76.4% of domiciled dogs in the city of Monte Negro, Rondônia.

In Brazil, the seroprevalence of *N. caninum* ranges from 0.7% to 67.6% , with a mean of 13.72% (95% CI: 11.32–16.32) (Anvari et al., 2020). In the present study, we report the highest seroprevalence of *N. spp.* (73.85%) in dogs, in Brazil. The results obtained in the present study were similar to those observed by Lasri, De Meerschman, Rettigner, Focant and Losson (2004), with respect to three different dog populations in Belgium, who reported that 76.3% (71/93, ELISA) of canines are positive. Studies carried out in the Rondônia state showed positivity for *N. caninum* (IFAT < 50) in the order of 8.3% (13/157) in domiciled dogs (Canon-Franco et al., 2003) and 12.6% (22/174) in rural dogs (Aguiar et al., 2006). The rates were much lower than those found in this research, and this difference can probably be explained by the wide spread of the agent in the environment.

There was a significant difference between *T. gondii* infection and gender ( $p < 0.001$ ). The male dogs showed 2.41 (1.48–3.92) times the risk of being infected with *T. gondii* than female dogs. Mascolli et al. (2015) have

suggested that sexually active stray male dogs travel long distances and, therefore, they are more exposed to contaminated environments than female dogs, which, in turn, increases the latter's risk of infection. Unlike for neosporosis, the sex of the dogs was not associated ( $p = 0.94$ ) with positive results in serological tests, and these results are in agreement with those cited by Anvari et al. (2020).

High seroprevalence of infection (92.4%: *T. gondii* and 89.2%: *N. spp.*) was detected in the group  $\geq$  one-year-old dogs, in all collections ( $p < 0.0001$ ). This high infection has been related to a greater probability for exposure to *T. gondii* and *Neospora* spp. over time, thereby increasing the susceptibility in older dogs (Watanabe et al., 2020).

High prevalence of *T. gondii* in street dogs than in pets suggests that the habit of eating infected prey is an important source of infection. From an epidemiological point of view, this finding is interesting, especially considering the zoonotic role of *T. gondii*. They do act as important indicators of environmental contamination with *T. gondii* because of their close association with humans (Souza et al., 2019).

In conclusion, these serological results indicate an important circulation of *T. gondii* and *N. spp.* in stray dogs in the Rolim de Moura, Rondônia state, Western Brazilian Amazon. The presence of antibodies against *T. gondii* and *N. spp.* in carnivores is a good indicator of environmental contamination with the parasites. Integrated control strategies are recommended to prevent and control toxoplasmosis and neosporosis, despite the intimate contact between dogs and humans.

## Conflicts of interest/Competing interests

The authors declare that they have no competing interests.

## Ethics approval

In this study, all procedures using animals complied with the Ethical Principles in Animal Research adopted by the College of Animal Experimentation and were approved (protocol number PP010/2014) by the Ethical Committee for Animal Welfare, UNIR, Rolim de Moura, Rondônia, Brazil.

## Competing interests

The authors declare that they have no competing interests.

## Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on request.

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