

# Antibody frequency for *Toxoplasma gondii* and *Neospora* spp. in domiciliated and stray cats from Araguaína, Tocantins, Eastern Amazonia

## Frequência de anticorpos para *Toxoplasma gondii* e *Neospora* spp. em gatos domiciliados e errantes de Araguaína, Tocantins, Amazônia Oriental

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### Highlights

First report of antibodies against *T. gondii* and *Neospora* spp. in cats from Araguaína. There was no difference in the risk of infection between domiciliated and stray cats. Cats with defined breed have more risk to *Neospora* spp. infection.

### Abstract

*Toxoplasma gondii* and *Neospora* spp. are protozoa that have a significant impact on animal health due to the diseases they cause in domestic and wild animals. The aim of the present study was to investigate the presence of antibodies against *T. gondii* and *Neospora* spp. in cats from northern Brazil. Serum samples were collected from 180 cats in the municipality of Araguaína, Tocantins and used to evaluate the presence

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of anti-*T. gondii* and anti-*Neospora* spp. antibodies using the indirect fluorescent antibody test, with a cutoff of 1:64 and 1:25, respectively. The association between infection and individual animal characteristics (age, sex, origin, breed, and clinical signs) was tested using univariate analysis, followed by multivariate logistic regression. We found that 48.3% (87/180) of the animals had anti-*T. gondii* (95% CI: 40.8%–55.90%) and 3.9% (7/180) had anti-*Neospora* spp. (95% CI: 1.6%–7.8%) antibodies. There was no association between age, sex, breed origin, clinical signs, and seropositivity for *T. gondii*. Cats of defined breeds were more likely to be infected by *Neospora* spp. (OR = 10.7). Therefore, we found a high rate of seropositivity for *T. gondii* and a high rate of occurrence of *Neospora* infections in cats from the Araguaína region. The exposure of the feline population to the studied coccidia indicates the need to monitor the feline population for these infections and underscores the importance of effective sanitary measures against such pathogens.

**Key words:** Felines. Neosporosis. Public Health. Toxoplasmosis.

## Resumo

*Toxoplasma gondii* e *Neospora* spp. são protozoários com impacto relevante na saúde animal devido às doenças que causam em animais domésticos e silvestres. O objetivo do presente estudo foi investigar a presença de anticorpos contra *T. gondii* e *Neospora* spp. em gatos do norte do Brasil. Amostras de soro foram coletadas de 180 gatos no município de Araguaína, Tocantins utilizadas para avaliar a presença de anticorpos anti-*T. gondii* e anti-*Neospora* spp. pela reação de imunofluorescência indireta, utilizando um ponto de corte de 1:64 e 1:25, respectivamente. Associação da infecção com as características individuais dos animais (idade, sexo, origem, raça e sinais clínicos) foram testadas por meio de análise univariada, seguida de análise multivariada por regressão logística. Como resultado, 48,3% (87/180) dos animais apresentaram anticorpos anti-*T. gondii* (IC 95%: 40,8% -55,90%) e 3,9% (7/180) anti-*Neospora* spp. (IC 95%: 1,6% -7,8%). Não foi encontrada associação entre idade, sexo, raça, origem e presença de sinais clínicos e a soropositividade para *T. gondii*. Gatos com raça definida apresentaram maior chance de infecção por *Neospora* spp. (OR=10,7). Portanto, registra-se elevada soropositividade de gatos para *T. gondii* e a ocorrência de infecção por *Neospora* em gatos da região de Araguaína, Tocantins. A exposição da população felina aos coccídios estudados indica a necessidade de monitoramento da população de felinos quanto a estas infecções e aplicação de medidas sanitárias eficazes contra tais patógenos.

**Palavras-chave:** Felinos. Neosporose. Saúde pública. Toxoplasmose.

## Introduction

Currently, the population of cats in Brazilian households is estimated to be 22.1 million (Instituto Brasileiro de Geografia e Estatística [IBGE], 2015). The domestic cat has retained its predation instinct even though domestication occurred more than 10,000 years ago. However, this instinct is associated with the transmission of diseases

of clinical relevance in feline medicine, such as toxoplasmosis (Karakavuk et al., 2018).

*Toxoplasma gondii* is a cosmopolitan protozoan with an impact on public health, and its definitive hosts are several species of felids. In addition to water intake and ingestion of uncooked meat, these mammals become infected through the predation of rodents and birds (Aguirre et al., 2019). Clinical signs of feline toxoplasmosis include

muscle hyperesthesia, pneumonia, hepatitis, dyspnea, tachypnea, jaundice, diarrhea, fever, and seizures (Hartmann et al., 2013).

Infection of cats with *Neospora caninum* and *Neospora hughesi* has also been demonstrated experimentally (Cerqueira-Cézar, Calero-Bernal, Dubey, & Gennari, 2017). These infections result in musculoskeletal necrosis, necrotizing hepatitis, pneumonia, tubular renal necrosis, and transplacental transmission of *N. caninum* (Dubey & Lindsay, 1989; Dubey, Lindsay, & Lipscomb, 1990).

There have been few investigations on the circulation of *T. gondii* and *Neospora* spp. in cats in the northern region of Brazil. This area covers the Amazon ecosystem, where cats are present in 22.7% of households (IBGE, 2015; Calero-Bernal & Gennari, 2019). It is noteworthy that these animals can become a source of environmental dissemination and infection in humans because of the fecal elimination of *T. gondii* oocysts in felines (Teixeira et al., 2019).

Because of the relevance of these protozoa in veterinary medicine and the diseases that they cause in felines, this study aimed to investigate the presence of antibodies against *T. gondii* and *Neospora* spp. in cats in the municipality of Araguaína (Tocantins) and to verify their association with epidemiological variables.

## Materials and Methods

### Study area

The municipality of Araguaína (7°11'28"S and 48°12'26"W) is located in the northern region of Tocantins in the Brazilian

Eastern Amazon Region. The Aw (hot and humid with a winter dry season) type climate predominates, with an average annual temperature of 26°C and average rainfall of 1,869 mm per year, and a rainy period from October to April (Penereiro, Martins, & Beretta, 2016).

### Sample and data collection

The convenience sampling method was used in this study. The study sample consisted of 180 blood samples collected from domestic cats from veterinary care facilities and cats caught by the Araguaína Zoonoses Control Center from 2015 to 2018.

After the study was explained to them, the owners authorized the collection of biological samples from the animals on their property by signing an informed consent form. At the time of blood collection, the animals were physically restrained and underwent a clinical examination (including medical history, measurement of temperature and capillary filling time, and abdominal palpation), and data of the individual characteristics were collected.

After the initial examination, approximately 1–4 mL of blood from the cephalic or jugular vein was collected using a vacuum suction system in tubes with tripotassium ethylenediaminetetraacetic acid (K3 EDTA) and tubes with a coagulation activator. The serum samples obtained after centrifugation were stored at -20°C in sterile polystyrene microtubes.

The individual epidemiological characteristics evaluated were age ( $\geq 6$  months or  $< 6$  months), sex (male or female),

clinical signs (presence or absence of any signs of a clinical abnormality after complete medical history was obtained and physical examination performed), breed (defined breed or no defined breed), and origin (stray or domiciliated).

### *Detection of anti-*T. gondii* and anti-*Neospora* spp. IgG antibodies*

IgG antibodies against *T. gondii* and *Neospora* spp. were detected using the indirect fluorescent antibody test (IFAT), based on tachyzoite antigens from the *T. gondii* RH strain and tachyzoite antigens from *N. caninum* isolate NC-1.

The serum was diluted with 1x phosphate-buffered saline, and 1:64 and 1:25 dilutions were used as cutoffs for positivity for *T. gondii* and *Neospora* spp., respectively (Camargo, 1964; Braga et al., 2012). The samples considered positive ( $\geq 1:64$  for *T. gondii* and  $\geq 1:25$  for *Neospora* spp.) were titrated in dilutions with a base dilution factor of 2 until fluorescence occurred. Goat anti-cat IgG conjugate labeled with fluorescein isothiocyanate (F4262; Sigma-Aldrich, St. Louis, MO, USA) was diluted to 1:64 to perform the test.

The reading was performed in a dark room with a microscope equipped for fluorescence (Model Eclipse E200; Nikon, Tokyo, Japan) at 40x magnification. Samples that showed complete fluorescence of the tachyzoite surface used as antigens were considered positive.

### *Statistical analysis*

The analysis of the association between the presence or absence of anti-*T. gondii* and anti-*Neospora* spp. antibodies in the feline population and epidemiological variables (age, sex, clinical signs, and origin) were performed using univariate analysis, followed by unconditional logistic regression using EpiInfo 7.2.® Variables with a P-value  $< 0.25$  were included in the logistic regression model.

A two-tailed chi-square ( $\chi^2$ ) statistical method with Yates correction and Fisher's exact test was used. The threshold for statistical significance was set at  $P \leq 0.05$ .

The study was approved by the Animal Use Ethics Committee of the Federal University of Tocantins (protocol No. 23101.000.988/2018-10).

## **Results and Discussion**

The frequency of antibodies against *T. gondii* was 48.3% (87/180; 95% CI: 40.8%–55.9%), and the titers were 1:64 (n = 53), 1:128 (n = 20), 1:256 (n = 11), 1:2048 (n = 2), and 1:4096 (n = 1).

The frequency of antibodies against *T. gondii* found in the present study was lower than that found in the studies conducted by Magalhães et al. (2017) on the island of Fernando de Noronha-PE (71.26%; 248/348), which can be attributed to the fact that these researchers used a lower cutoff for IFAT (IFAT cut-off value of  $\geq 1:16$ ) than in the present study.

Environmental and cultural differences may also have influenced the frequency of *T. gondii* infection. No restriction of movement, predation of birds and rodents, and consumption of uncooked meat products by stray and domestic cats, may increase the frequency of *T. gondii* infection.

The frequency of antibodies against *T. gondii* was similar to that reported by Braga et al. (2012) in São Luís-MA (50.5%; 101/200) and Feitosa et al. (2014) in Patos-PA (43.8%; 88/201). Such results may be attributed to the environmental similarities between Araguaína and São Luís. Both localities are in the Amazon Biome, where sporulation of oocysts is favored due to the large area of preserved forests close to the urban area and the hot and humid climate. The results found in Patos, in the state of Paraíba, can be attributed to the lowest established cutoff (1:16), which favors greater sensitivity of the technique. However, such samples were obtained from regions with unfavorable environmental conditions (semi-arid) (Feitosa et al., 2014).

Lower *T. gondii* frequency values than those in the present study have been reported by other authors in Brazil using IFAT with different cut-offs: for instance, Sousa et al. (2014) in Campo Grande-MS (32.5%; 49/151; 1:40), Souza et al. (2015) in Rio Branco-AC (24.7%; 22/89; 1:64), Caldart et al. (2015) in Londrina-PR (20.9%; 87/415; 1:16) and Koch et al. (2019) in Curitiba-PR (21%; 21/100; 1:50).

The difference in frequency reported in these previous studies may be related to the fact that cats in the countryside and peripheral regions are widely used to control rodent and synanthropic bird populations, have free outdoor access, and are frequently fed uncooked meat. In addition, these cats are not often seen by veterinarians, thus increasing the risk of exposure to *T. gondii* (Mendes-de-Almeida et al., 2007).

The frequency of seropositivity suggests that there is exposure to *T. gondii* in the feline population in the municipality. Furthermore, some animals could have an active infection, at least those with titers above 1:1024 (Bresciani et al., 2007). The three animals that had the highest titers (1:4096 and 1:2048) were less than 6 months old and showed clinical signs such as ataxia of the pelvic limbs, splenomegaly, and uveitis. Despite being non-specific, these clinical signs are compatible with cases of feline toxoplasmosis (Calero-Bernal & Gennari, 2019). Although morbidity rates are low for felines, the high frequency of *T. gondii* infection in the municipality may indicate that detectable titers in sick animals are possible.

No significant association was found between age, sex, breed, presence of any clinical alteration, or animal origin, and seropositivity for *T. gondii* (Table 1).

**Table 1**  
**Results of univariate and multivariate analysis for individual characteristics and infection for *Toxoplasma gondii* in cats from the municipality of Araguaína, Tocantins, Brazil**

Variable	Total (180)	Positives N(%)	Univariate			Multivariate	
			Crude odds ratio	CI 95%	P-value	Adjusted odds ratio (IC 95%)	P-value
Age							
Young	30	16 (53.3)	1.27	0.58- 2.79	0.69	-	-
Adult	150	71 (47.3)					
Sex							
Male	81	33 (40.7)	1.74	0.94- 3.17	0.09	0.55(0.30-1.01)	0.054
Female	99	54 (54.5)					
Clinical signs							
Present	51	31 (60.8)	2.02	1.04- 3.91	0.05	1.77 (0.83-3.76)	0.139
Absent	129	56 (43.4)					
Origin							
Stray	73	40 (54.8)	1.55	0.85- 2.83	0.20	0.80(0.41-1.59)	0.525
Domiciled	107	47 (43.9)					
Breed							
Defined breed	8	07 (87.5)	7.98	1.198-184.6	0.02*	6.91(0.80-60.02)	0.080
No defined breed	172	80 (46.5)					

\*Fisher exact test.

In previous studies, a relationship with older age was found because older animals have been exposed to parasites for longer than younger animals have and can re-eliminate oocysts when infected with heterologous strains (Bresciani et al., 2007; Cardia, Camossi, Silveira, Langoni, & Bresciani, 2013; Feitosa et al., 2014; Zulpo et al., 2018); however, the results of our study indicate that young and adult animals are exposed to a similar risk of infection.

The lack of association observed for the sex variable corroborates the findings of Bresciani et al. (2007) and Cruz et al. (2011), although there is an indication that females may be more resistant to parasitic infections

than males because of a more effective immune response (Morales-Montor et al., 2004). Although in the univariate analysis, the breed was associated with seropositivity for *T. gondii* (OR =7.98), this was not seen in the logistic regression analysis (P = 0.08). A similar result was reported by Souza et al. (2015).

Although domiciliated cats were expected to have a lower risk of infection because they had more preventive care, this was not observed. Environmental or social characteristics, such as cats having unrestricted access to outdoor areas and being fed uncooked food (meat products and by-products), lead to a different epidemiological scenario, and such characteristics are more

relevant than the origin of the animal (Feitosa et al., 2014).

Regarding *Neospora* spp., the frequency of antibodies was verified in 3.9% (95% CI: 1.6%–7.8%) of the sampled animals, and titers were 1:25 (n = 5), 1:50 (n = 1), and 1:100 (n = 1).

By assessing the frequency of antibodies against *Neospora* spp. (3.9%), it can be inferred that there is a low rate of infection by the pathogen in the feline population. Only two animals had titers above the 1:25 cutoff, demonstrating, in addition to the small number of infected animals, a low rate of seroconversion, corroborating the results of another study (Sedlák, Bartova, & Machacova, 2014). According to Meneses et al. (2014) and Feitosa et al. (2014), natural infections in cats by *Neospora* spp. have never been reported, which could suggest low seroconversion or resistance to the pathogen, despite reports of experimental infection in immunosuppressed animals.

The frequency of antibodies against *Neospora* spp. found in felines from Araguaína was lower than that reported by Bresciani et al. (2007) in São Paulo (24.5%; 100/400; 1:16), Braga et al. (2012) in Maranhão (27%; 54/200; 1:25), and Koch et al. (2019) in Paraná (42%; 42/100; 1:50). However, the frequency of antibodies against *Neospora* spp. was

similar to that reported by Sousa et al. (2014) in Mato Grosso do Sul (6.6%; 10/151), by Lima et al. (2018) on the Island of Fernando de Noronha (3.11%; 8/257), and by Meneses et al. (2014) in Bahia (2.9%; 8/272). Therefore, the percentage of frequency of *Neospora* spp. observed nationally were lower than those observed for *T. gondii*. However, such an occurrence is expected, as *Neospora* spp. appear to be less widespread than *T. gondii*. With the exception of Lima et al. (2018), who used the agglutination technique and a 1:20 cutoff, the other authors mentioned above used IFAT with a 1:50 cutoff.

Except for breed, no other epidemiological variables evaluated were associated with seroreactivity to *Neospora* spp. (Table 2). Bresciani et al. (2007) also observed a higher frequency of seropositivity for *Neospora* in animals of a defined breed. However, they found no statistically significant differences between the two groups. The breed was identified as an associated factor because a higher frequency was found in animals of a defined breed ( $P < 0.05$ ), which may suggest a greater exposure of these animals to sources of infection. However, it is worth noting that the confidence interval was quite large due to the small number of animals of a defined breed, which could have influenced the results.

**Table 2**  
**Results of univariate and multivariate analysis for individual characteristics and infection for *Neospora* spp. in cats from the municipality of Araguaína, Tocantins, Brazil**

Variable	Total (180)	Positives N(%)	Univariate			Multivariate	
			Crude odds ratio	CI 95%	P-value	Adjusted odds ratio (IC 95%)	P-value
Age							
Young	30	1 (3.3)	0.82	0.09-7.14	0.73	-	-
Adult	150	6 (4.0)					
Sex							
Male	81	4 (4.9)	1.66	0.36-7.65	0.79	-	-
Female	99	3 (3.0)					
Clinical signs							
Present	51	3 (5.9)	1.95	0.42-9.05	0.66	-	-
Absent	129	4 (3.1)					
Origin							
Stray	73	5 (6.8)	3.86	0.73-20.46	0.19	0.27(0.05-1.47)	0.130
Domiciled	107	2 (1.9)					
Breed							
Defined breed	8	2 (25)	10.75	1.23-68.31	0.03*	10.70(1.62-70.6)	0.014
No defined breed	172	5 (2.9)					

\*Fisher exact test.

There was no significant difference in sex, corroborating the findings of Bresciani et al. (2007). In the present study, age was not associated with seroreactivity to *Neospora* spp., which differs from the findings of Bresciani et al. (2007).

It is important to highlight that frequency studies are fundamental as they act as a direct indicator of the degree of spread of infectious and parasitic agents and are the basis for preventive measures for sanitary control in animals; consequently, they directly reflect on human health when dealing with zoonoses (Calero-Bernal & Gennari, 2019).

## Conclusions

This study reported the presence of antibodies against *T. gondii* and *Neospora* spp. in cats of Araguaína, highlighting the high proportion of cats that are seropositive for *T. gondii*. Cats of defined breeds were more likely to be infected by *Neospora* spp. Further studies are needed to assess other epidemiological variables that allow for a better understanding of the epidemiological scenario for the two coccidians in the feline population in the region.



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