

## Serological prevalence of *Toxoplasma gondii* infection in cats (Belém, Pará, Brazil)

### Prevalência sorológica de infecção por *Toxoplasma gondii* em gatos (Belém, Pará, Brasil)

Katarine de Souza Rocha<sup>1</sup>; Michele de Souza Lima<sup>2</sup>; Thamillys Rayssa Marques Monteiro<sup>3</sup>; Betsy Emely Tavares Honorio<sup>1</sup>; Ana Paula Vilhena Beckman Pinho<sup>4</sup>; Giselle Souza da Paz<sup>5</sup>; Alessandra Scofield<sup>6</sup>; Gustavo Góes Cavalcante<sup>6</sup>; Paulo Cesar Magalhães-Matos<sup>7</sup>; Francisco Dantas Sampaio Junior<sup>6</sup>; Isis Abel<sup>8</sup>; Helio Langoni<sup>5</sup>; Carla Cristina Guimarães de Moraes<sup>1\*</sup> 

<sup>1</sup> Laboratório de Zoonoses e Saúde Pública, Faculdade de Medicina Veterinária, Instituto de Medicina Veterinária, Universidade Federal do Pará – UFPA, Castanhal, PA, Brasil

<sup>2</sup> Médica Veterinária Autônoma, Belém, PA, Brasil

<sup>3</sup> Laboratório de Biologia Molecular, Instituto de Saúde e Produção Animal – ISPA, Universidade Federal Rural da Amazônia – UFRA, Belém, PA, Brasil

<sup>4</sup> Agência de Defesa Agropecuária do Pará – ADEPARÁ, Belém, PA, Brasil

<sup>5</sup> Departamento de Higiene Veterinária e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista “Júlio de Mesquita Filho” – UNESP, Botucatu, SP, Brasil

<sup>6</sup> Laboratório de Parasitologia Veterinária, Faculdade de Medicina Veterinária, Instituto de Medicina Veterinária, Universidade Federal do Pará – UFPA, Castanhal, PA, Brasil

<sup>7</sup> Curso de Medicina Veterinária, Centro de Ciências Biológicas e da Saúde, Universidade da Amazônia – UNAMA, Belém, PA, Brasil

<sup>8</sup> Laboratório de Epidemiologia e Geoprocessamento, Faculdade de Medicina Veterinária, Instituto de Medicina Veterinária, Universidade Federal do Pará – UFPA, Castanhal, PA, Brasil

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#### Abstract

We evaluated the prevalence of anti-*Toxoplasma gondii* antibodies in the serum samples collected from domestic cats in Belém, Pará, Brazil. We also correlated the presence of *T. gondii* antibodies with environmental variables and cat-owner habits. Four-hundred and forty-seven serum samples from domestic cats were analyzed. The sera were tested using an indirect immunofluorescence assay. Among the animals analyzed, 21.92% (98/447) were seropositive. A statistically significant association was found in relation to age and serology among the animals over 1 year old ( $p < 0.01$ ): in the group up to 1 year old, 12.82% (20/156) of the animals were positive, and in the group over 1 year old, 26.80% (78/291) were positive. Our results show that the cats in Belém, Pará region have anti-*T. gondii* antibodies, and their owners are not aware of toxoplasmosis or how to prevent its transmission.

**Keywords:** Toxoplasmosis, feline, IFA.

#### Resumo

Foi avaliada a prevalência de anticorpos anti-*Toxoplasma gondii* em amostras de soro, coletadas de gatos domésticos, em Belém, Pará, Brasil. Também correlacionou-se a presença de anticorpos contra *T. gondii* com variáveis ambientais e hábitos de proprietários. Quatrocentas e quarenta e sete amostras de soro de gatos domésticos foram analisadas. Os soros foram testados por ensaio de imunofluorescência indireta, e as amostras de fezes foram analisadas por meio de reação em cadeia da polimerase. Entre os animais analisados, 21,92% (98/447) foram soropositivos. Foi encontrada associação estatística significativa em relação à idade e à sorologia entre os animais acima de 1 ano ( $p < 0,01$ ): no grupo de até 1 ano, 12,82% (20/156) dos animais positivos e, no grupo acima de 1 ano, 26,80% (78/291) positivos. Os resultados mostram que os gatos daquela região possuem anticorpos anti-*T. gondii*, e seus donos não estão cientes da toxoplasmose ou de como evitar sua transmissão.

**Palavras-chave:** Toxoplasmose, felinos, IFA.

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\*Corresponding author: Carla Cristina Guimarães de Moraes. E-mail: ccmoraes@ufpa.br



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## Introduction

*Toxoplasma gondii* (phylum Apicomplexa, family Sarcocystidae) is an obligate intracellular enteric coccidian parasite that infects domestic cats and other felids (Bowman, 2010; Frenkel & Bermudez, 2006). This agent has a wide geographic distribution and infects several warm-blooded animals, including aquatic mammals and humans. Therefore, the disease caused by this pathogen, toxoplasmosis, is an anthroozoonosis (Kawazoe, 2005). Cats are the definitive hosts of *T. gondii*, while the other susceptible species are intermediate hosts. Infection usually occurs by ingesting tissue cysts or pseudocysts present in different animal tissues or by ingesting food or water contaminated with sporulated oocysts (Dabritz et al., 2007; Elmore et al., 2010).

In Belém, the state capital of Pará (Brazil), the household cat population was estimated at 48,838, per the Center for Zoonosis Control of Belém (personal contact, 2010). The state capital, despite having a high rate of congenital toxoplasmosis in humans, which is characterized by primary infection in pregnant women that is consequently transferred to the fetus through the placenta in the first trimester of pregnancy and can lead to serious problems such as abortion, fetal death, premature births, or late side effects such as visual problems (Bichara et al., 2012), does not have a program for controlling population of stray cats, and there is no government incentive to raise awareness of responsible pet ownership.

Several studies have provided data on the seroprevalence of *T. gondii* in cats domiciled in different regions of the country (Lucas et al., 1999; Langoni et al., 2001; Netto et al., 2003; Cavalcante et al., 2006; Dalla Rosa et al., 2010), however, data on cats in Belém are lacking. Therefore, the present study aimed to evaluate the prevalence of anti-*T. gondii* IgG antibodies in the sera of domestic cats in Belém. We also examined the possible risk factors and cat-owner's knowledge of toxoplasmosis.

## Materials and Methods

### Sampling and collection location

Biological samples were collected from one cat per household in Belém, Pará (01° 27'21" S, 48° 30'16" W) totaling 447 blood samples, regardless of gender, age, or breed. From the estimate of the number of cats living in the city provided by the Zoonosis Control Center on the basis of the vaccine dose given to dogs and cats during the 2009 rabies vaccination campaign, sample size calculation was performed, with an expected frequency prevalence of 50% and 95% confidence interval using the simple random sample formula proposed by Thrusfield (2007).

Blood collection was performed aseptically by venipuncture of the cephalic or jugular vein. Approximately 2-5 mL of blood was collected using 5 mL syringes and tubes without anticoagulants. The samples were transported to the Zoonoses and Public Health Laboratory of the Institute of Veterinary Medicine of the Federal University of Pará (IMV/UFGPA), where they were centrifuged at 1200 rpm for 15 minutes to separate coagulated blood from serum. The serum was placed in 1.5 mL Eppendorf® tubes, labeled, and stored at -20 °C until analysis.

The animals were divided into two groups according to age. Group 1 consisted of animals less than or equal to 1 year old, and group 2 consisted of cats older than 1 year in age. This study was approved by the Ethics Committee on Animal Use at the State University of Pará under protocol n° 23/2012. In addition, informed consent forms (ICFs) were signed by the animal owners, who also completed epidemiological surveys.

### Data analysis

For analysis of the responses to the questionnaires, a database using the SPSS v.20.0 software (© 2011 IBM corporation, United States) was created. The verification of associations between variables was conducted using the chi-square test. When associations were present, the odds ratio was calculated. The 95% CI was used to determine the significance of differences.

### Sample analysis

Analysis of the anti-*T. gondii* antibody was performed by detecting specific IgG using an indirect immunofluorescence assay (IFA), using a 1:16 dilution as the cut-off (Camargo, 1974). The sera were diluted up to 1:1024 in phosphate-buffered saline and placed on glass slides coated with the *T. gondii* tachyzoites antigen (RH strain). Then, we used fluorescein-conjugated anti-cat IgG (SAB700065-2MG anti-cat IgG FC specific + fluorescein isothiocyanate (©SIGMA-Aldrich, San Luis, Missouri, EUA). As positive control, we used a positive cat serum sample for *T. gondii* treated at the veterinary hospital at UNESP-Botucatu. Samples were considered positive when at least 50% of the tachyzoites per field exhibited fluorescence. The positivity titer was related to the last dilution that still showed fluorescence.

## Results

Among the 447 samples analyzed using IFA, 21.92% (98/447) of the animals were found to be seropositive. The values for the antibody titers ranged from 16 to 1024. Most of the animals, 39.80% (39/98), had a titer of 64, and the fewest proportion of animals, 6.12% (6/98), had a titer of 1024. The variables, gender, race, diet, and behavioral habits, were not found to be associated with serological results. With respect to age, being over 1 year old was found to be a risk factor for *T. gondii* seropositivity; 12.82% (20/156) of the animals in group 1 were positive, with animals in that group 2.49 (odds) chances of presenting positive results, while 26.80% (78/291) in group 2 were positive, indicating an association between age and serological findings (Table 1). It was observed that 74% (331/447) and 93.50% (418/447) of cat breeders were unaware of the disease and its modes of transmission, respectively (Table 2).

**Table 1.** Factors associated with the seroprevalence of *Toxoplasma gondii* in domestic cats (Belém, Pará, Brazil).

Variable	RIFI and Univariate analysis				Logistic regression			
	Positive (n=98)		Negative (n=349)		P-value <sup>(2)</sup>	OR <sup>(3)</sup>	P-value <sup>(4)</sup>	95% CI <sup>(5)</sup>
	n	%	n	%				
<b>Sex</b>					0.57	1.140	0.57	0.717-1.812
<i>Male</i> <sup>(1)</sup>	36	36.73	139	39.82				
<i>Female</i>	62	63.26	210	60.17				
<b>Breed</b>					0.217	0.761	0.274	0.467-1.242
<i>Breed</i> <sup>(1)</sup>	27	27.55	73	21.19				
<i>Mixed-breed</i>	71	72.44	275	78.79				
<b>Age</b>					0.01	2.490	0.001	0.264-0.725
<i>Up to 1 yr. old</i> <sup>(1)</sup>	20	20.41	136	38.97				
<i>&gt;1 yr. old</i>	78	79.59	213	61.03				
<b>Outdoor access</b>					0.56	1.489	0.318	0.678-3.310
<i>Yes</i> <sup>(1)</sup>	85	86.73	310	88.82				
<i>No</i>	13	13.26	39	11.17				
<b>Eating raw meat</b>					0.092	0.947	0.274	0.863-1.038
<i>Yes</i> <sup>(1)</sup>	41	41.83	114	32.66				
<i>No</i>	57	58.16	235	67.33				
<b>Hunting</b>					0.63	1.153	0.634	0.642-2.072
<i>Yes</i> <sup>(1)</sup>	81	82.65	281	80.51				
<i>No</i>	17	17.35	68	19.48				

n: number; <sup>(1)</sup>Reference categories; <sup>(2)</sup>P-value (Chi-square test); <sup>(3)</sup>Odds ratio; <sup>(4)</sup>P-value (Logistic regression); <sup>(5)</sup>CI: confidence interval.

**Table 2.** Association between owner awareness and seroprevalence of *Toxoplasma gondii* in domestic cats (Belém, Pará, Brazil).

Variable	RIFI and Univariate analysis				Logistic regression			
	Positive (n=98)		Negative (n=349)		P-value <sup>(2)</sup>	OR <sup>(3)</sup>	P-value <sup>(4)</sup>	95% CI <sup>(5)</sup>
	n	%	n	%				
<b>Origin</b>					0.21	0.723	0.195	0.443-1.181
<i>Born at the house</i> <sup>(1)</sup>	68	20.48	264	79.51				
<i>Adopted</i>	30	26.08	85	73.91				
<b>Disease awareness</b>					0.52	0.844	0.526	0.500-1.426
<i>Yes</i> <sup>(1)</sup>	23	19.82	93	80.17				
<i>No</i>	75	22.65	256	77.34				
<b>Awareness of the modes of toxoplasmosis transmission</b>					0.27	0.551	0.280	0.187-1.624
<i>Yes</i> <sup>(1)</sup>	4	13.79	25	86.20				
<i>No</i>	94	22.48	324	72.48				

n: number; <sup>(1)</sup>Reference categories; <sup>(2)</sup>P-value (Chi-square test); <sup>(3)</sup>Odds ratio; <sup>(4)</sup>P-value (Logistic regression); <sup>(5)</sup>CI: confidence interval.

## Discussion

Our results indicate that cats are exposed to *T. gondii*, thus suggesting a high degree of environmental contamination by infective oocysts, similar to the findings of Cavalcante et al. (2006) in Monte Alegre-Rondônia.

In the present study, no statistical association was found between animal sex and serological results ( $p > 0.05$ ). This is in line with the results of previous studies showing that men and women are equally at risk of protozoan infection (Dalla Rosa et al., 2010; Pereira et al., 2018).

Hunting and the consumption of raw meat were also not associated with serological results, an observation similar to that reported by Dalla Rosa et al. (2010) and López et al. (2011). Although our study revealed no correction between eating and behavioral habits and the risk of infection in cats, Langoni et al. (2001) and Netto et al. (2003) observed that the prevalence of seropositive animals is directly related to cat behavior. Animals that have unrestricted access to the street are more likely to catch prey, which facilitates infection via the consumption of intermediate parasite hosts (Sroka et al., 2018; Mohammed et al., 2019).

Lucas et al. (1999), who demonstrated that the infection rates in cats that consumed raw meat were significantly different from those of animals that consumed homemade or commercial food ( $p < 0.05$ ). However, Mohammed et al. (2019) report that infection via the ingestion of the parasite can affect both free-ranging cats that have a habit of hunting, and those that live indoors and are fed only canned food, provided the food is not cooked properly, similar to the results of the present study.

With respect to age, age greater than 1 year constitutes a risk factor for *T. gondii* seropositivity for animals in group 2 (Table 1), revealing an association between age and the serological findings. It has been suggested that a gradual increase in age is directly related to *T. gondii* exposure (López et al., 2011; Mohammed et al., 2019).

For a better understanding of our study group, we provided epidemiological findings with questions designed to assess owner awareness, regardless of educational level, regarding toxoplasmosis and modes of transmission to humans. Most were unaware of the disease and its modes of transmission.

These data indicate that the lack of awareness of toxoplasmosis in many owners is probably because the infected cat is asymptomatic. Hence, the agent is eliminated to the environment in imperceptible ways and may contaminate food and water, thereby promoting the transmission of *T. gondii* to humans. Similar assumptions were corroborated by Elmore et al. (2010), Dabritz & Conrad (2010), and VanWormer et al. (2013). We also observed no significant association between owner awareness and the seroprevalence of their animals ( $p > 0.05$ ).

Although only the age variable correlates with the result of the serological test, research using indirect diagnostic techniques has been shown to be useful in epidemiological studies (Sroka et al., 2018), allowing us to gain insight into the current health status with respect to *T. gondii* infection.

## Conclusions

Many domestic cats in Belém had anti-*T. gondii* antibodies. Owners also tended to have poor knowledge regarding the modes of transmission of the infectious agent to humans and animals.

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