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Seroprevalence and risk factors for toxoplasmosis and neosporosis in the dog population of Ibiúna, São Paulo, Brazil

Soroprevalência e fatores de risco para toxoplasmose e neosporose na população canina de Ibiúna, São Paulo, Brasil

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Abstract

This study aimed to determine the prevalence of dogs seropositive for *Toxoplasma gondii* and *Neospora caninum* (Apicomplexa) and the risk factors associated with seropositivity in the dog population of Ibiúna, São Paulo, Brazil. A total of 570 animals were examined in the 48 neighborhoods of the city from September 2007 to March 2008. Serological diagnosis of *T. gondii* and *N. caninum* infections was performed using indirect immunofluorescence assay (IFA), with endpoint titers of 1:64 for *T. gondii* and 1:50 for *N. caninum*. Of the 570 animals examined, 314 (55.1%; 95% CI = 50.9−59.2%) were seropositive for *T. gondii* and 40 (7.02%; 95% CI = 5.1−9.4%) for *N. caninum*. The variables presence of rodents (OR = 2.05), ingestion of raw meat (OR= 2.47), and prior sexual activity (OR = 1.79) were identified as risk factors for toxoplasmosis, whereas only prior sexual activity (OR = 3.29) was associated with an increased risk for neosporosis. Both *T. gondii* and *N. caninum* infections were detected by IFA in the dog population of Ibiúna. Rodent control and not feeding raw meat to dogs are important to reduce the risk of infection by *T. gondii* and *N. caninum* in the region.
Key words: Epidemiology, dogs, *Toxoplasma gondii*, *Neospora caninum*, southeastern Brazil

Resumo

O objetivo do trabalho foi determinar a prevalência de animais soropositivos para *Toxoplasma gondii* e *Neospora caninum* (Apicomplexa) e determinar os fatores de risco associados à soropositividade na população canina de Ibiúna, São Paulo, Brasil. Foram examinados 570 animais distribuídos nos 48 bairros do município, no período de setembro de 2007 a março de 2008. O diagnóstico sorológico

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das infecções por *T. gondii* e *N. caninum* foi efetuado com a reação de imunofluorescência indireta (RIFI), adotando-se os pontos de corte de 1:64 para *T. gondii* e 1:50 para *N. caninum*. Dos 570 animais examinados, 314 (55,1%; IC 95% = 50,9% – 59,2%) foram soropositivos para *T. gondii*, e 40 (7,02%; IC 95% = 5,1% – 9,4%) para *N. caninum*. As variáveis presença de ratos (OR = 2,05), ingestão de carne crua (OR= 2,47) e atividade sexual (OR = 1,79) foram identificadas como fatores de risco associados à toxoplasmose, e para neosporose, a variável atividade sexual (OR = 3,29) foi identificada como fator de risco. As infecções por *T. gondii N. caninum*, detectadas pela RIFI, estão presentes na população canina de Ibiúna. O controle de roedores e a não administração de carne crua aos animais são importantes para reduzir o risco de infecção por *T. gondii* e *N. caninum* na região.
Palavras-chave: Epidemiologia, cães, *Toxoplasma gondii*, *Neospora caninum*, sudeste do Brasil

Introduction

The protozoan species *Toxoplasma gondii* and *Neospora caninum* (Apicomplexa) are obligate intracellular parasites that have a worldwide geographic distribution. Their life cycle is heteroxenous and the definitive hosts are mammalian carnivores: felids for *T. gondii* and dogs and some wild canid species for *N. caninum* (DUBEY, 2010).

Dogs play a major role in the life cycle of *N. caninum*, and even though they are not recognized as definitive hosts of *T. gondii*, Lindsay et al. (1997) showed their importance as mechanical disseminators of the latter by acting as sentinels for infection. Thus, dogs are an important link in the epidemiological chain of neosporosis and toxoplasmosis infections, which explains the growing interest in attempting to determine the role of these animals as sources of infection for other parasite species and to help select control measures that affect the natural cycle of the agents (ULLMANN et al., 2008).

The municipality of Ibiúna is located in southern São Paulo (SP) state, Brazil, at 23°39'23" S and 47°13'21" W. Ibiúna is one of the largest cities in the state in land area, covering a total area of 1093 km^2, making it difficult to implement public health and dog population control measures. The average elevation of 996 m determines temperatures ranging from 6 °C to 27 °C and two marked seasons: cold and wet winters with temperatures ranging between 4 °C and 14 °C and summers with average temperatures between 16 °C and 28 °C and maximum temperatures up to 35 °C. Relative humidity ranges from 60% to 90% in most of the territory, and average annual rainfall is 1200 mm with regular rainfall, especially in the mountainous area. To date, no seroepidemiological investigation of *T. gondii* and *N. caninum* antibodies has been done in the dog population of the region. In this study, we aimed to determine the prevalence of dogs seropositive for *T. gondii* and *N. caninum* and the risk factors associated with seropositivity in dogs at Ibiúna, São Paulo, Brazil.

Material and Methods

The study was approved by the Bioethics Committee at University of São Paulo, São Paulo, Brazil, under protocol number 1295/2008.

Blood samples were randomly collected in the 48 neighborhoods of Ibiúna, which were grouped into four regions (1, 2, 3, and 4) according to their land-use characteristics: region 1, consisting of mixed areas of recent urbanization without adequate infrastructure or services and rural areas composed of small farms; region 2, a predominantly rural area, composed mostly of small farms surrounded by forests; region 3, an urban area with adequate infrastructure; and region 4, with a predominance of small agricultural properties and leisure farms surrounded by forests. Sampling was conducted from September 2007 to March 2008, during which time a total of 570 dogs were examined. The four regions had particular characteristics and possible risk factors that could have affected the occurrence of the diseases investigated. Ecosystem aspects and

the breeding conditions of dogs were considered in the analyses. The grouping of a significant number of animals per region was determined according to their proximity. The four regions shared boundaries with other cities surrounding Ibiúna, resulting in a high diversity of animal species, both domestic and wild.

Sample size was calculated based on the dog population of the municipality, which was estimated at 16,065 animals. This estimate was based on a human population of 75,616 people in the city (IBGE, 2006). According to the World Health Organization guidelines for dog population management, most countries report a dog to man ratio between 1:6 and 1:10 (WHO, 1990; REICHMANN et al., 1999); however, based on dog population estimates from the Ibiúna Health Surveillance and Zoonoses Center, we used a 1:4 dog to man ratio (IBIÚNA, 2007). Sample size was calculated with an estimated prevalence of 1% and a 99% significance level. Sampling was weighted in proportion to dog population size using dog population estimates of the 48 neighborhoods.

Blood samples were taken by puncture of the radial cephalic vein and kept under refrigeration until arrival at the laboratory. Serum was obtained from clotted blood after clot retraction, after which samples were centrifuged and serum was stored at –20 °C until required for serological tests.

For the analysis of risk factors for toxoplasmosis and neosporosis in the dog population, dog owners answered a questionnaire that included information about dog gender, age, and prior sexual activity, presence of rodents in the domicile and peridomicile, contact with flooded areas, type of management, type of food, ingestion of raw meat, and whether the owner travel with the dog.

Detection of antibodies to *T. gondii* was conducted using indirect immunofluorescence assay (IFA) (CAMARGO, 1974) with RH-strain tachyzoites recovered from mice. IFA was also used for detection of antibodies to *N. caninum* following the recommendations of Dubey et al. (1988), using NC-1 strain tachyzoites grown in VERO cell culture. Sera to both agents were diluted in phosphate saline buffer (0.1 M, pH 7.2) and endpoint tittered at 1:64 for *T. gondii* and 1:50 for *N. caninum*. The reaction was visualized using commercial conjugated anti-dog IgG antibodies (Sigma, USA) labeled with fluorescein isothiocyanate. Samples with tachyzoites that demonstrated complete peripheral fluorescence were considered positive and subjected to serial dilutions, with the endpoint titer defined as the reciprocal of the last dilution showing a positive result.

Data from the epidemiological questionnaires were analyzed using uni- and multivariate analysis to determine possible risk factors associated with seropositivity for *T. gondii* and *N. caninum*. Animals were grouped into two groups (seropositive and seronegative) in the univariate analysis for comparison of the variables investigated. Variables with $p \leq 0.2$ by the chi-square test or by Fisher's exact test were selected for multivariate analysis, using multiple logistic regression analysis (HOSMER; LEMESHOW, 2000). The significance level was set at $p < 0.05$, and all analyses were performed using SPSS 20.0 for Windows software.

Results and Discussion

Of the 570 animals examined, 314 (55.1%; 95% CI = 50.9−59.2%) were seropositive for *T. gondii* (Table 1), with titers ranging from 64 to 4096.

Table 1. Prevalence of *Toxoplasma gondii* and *Neospora caninum* in the dog population of Ibiúna, São Paulo (SP), Brazil, between September 2007 and March 2008.

Region	Total number of animals	*T. gondii*			*N. caninum*		
		N	Prevalence (%)	95% CI (%)	N	Prevalence (%)	95% CI (%)
1	165	86	52.1	44.1 – 59.9	10	6.1	2.9 – 10.9
2	145	87	60	51.5 – 68.0	8	5.5	2.4 – 10.6
3	149	76	51	42.7 – 59.3	12	8.05	4.2 – 13.6
4	111	65	58.5	48.8 – 67.8	10	9.01	4.4 – 15.9
Total	570	314	55.1	50.9 – 59.2	40	7.02	5.1 – 9.4

Prevalence by region for *T gondii* ranged from 51% to 60%. The seroprevalence of *T. gondii* in dog populations found in several serological surveys conducted in Brazil is high, and similar and even higher rates than the ones observed in this study have been reported. For instance, a prevalence of 60.7% was found by Guimarães et al. (2009) in Lavras, Minas Gerais (MG). However, prevalence rates of 21.3%, 23.1%, 20.8%, 22.3%, and 25.4% were reported, respectively, by Souza et al. (2003) in São Paulo, SP, Bresciani et al. (2007) in Araçatuba, SP, Romanelli et al. (2007) in Guarapuava, Paraná (PR), Moura et al. (2009) in Lages and Balneário Camboriú, Santa Catarina (SC), and Silva et al. (2010) in Ubatuba, SP. Seroprevalence rates of *T. gondii* can vary greatly, because transmission is associated with habits and cultural behaviors as well as socio-economic and health conditions. Habitat is also a factor, because it plays a fundamental role in the viability of the parasite (SILVA et al., 2010).

Of the 314 dogs seropositive for *T. gondii*, 66.9% (210 animals) had titers ranging between 128 and 4096, whereas 33.1% (104 animals) had titers between 16 and 64. Cañón-Franco et al. (2004) reported that most positive dogs (76.4%) in Monte Negro, Rondônia (RO), had titers ranging from 128 to 2048. Conflicting results, in which most positive dogs had titers between 16 and 64, were found by Barbosa et al. (2003) in Salvador, Bahia (BA), Langoni et al. (2006) in Botucatu, SP, and Silva et al. (2010) in Ubatuba, SP. Low titers are indicative of chronic or early phases of infection (CAMARGO, 1975), which may indicate that most examined dogs have been exposed to the parasite and have developed an immunological response by producing specific antibodies.

Forty dogs were seropositive for *N. caninum* (prevalence: 7.02%; 95% CI = 5.1−9.4%), with titers ranging between 50 and 800 (Table 1). The prevalence by region ranged from 5.5% to 9.01%. Similar prevalence rates (< 15%) were observed by Cañón-Franco et al. (2003) in Monte Negro, RO, Fernandes et al. (2004) in Uberlândia, MG, Azevedo et al. (2005) in Campina Grande, Paraíba (PB), Jesus et al. (2006) in Salvador and Lauro de Freitas, BA, and Magalhães et al. (2009) in Ilhéus, BA. Conversely, higher prevalence rates (> 35%) were found by Gennari et al. (2002) in São Paulo, SP, and Moraes et al. (2008) in Botucatu, SP. Prevalence rates of *N. caninum* can vary greatly depending of several factors, including diagnostic technique, sample size, and type of dog population (CAÑÓN-FRANCO et al., 2003).

Of 40 dogs seropositive for neosporosis, 24 (60%) had antibody titers between 50 and 200, while 16 had titer values between 400 and 800. The most common titer value among these dogs was 400 (35%, 14/40). Dubey et al. (1988) reported that antibody titers greater than 400 are indicative of clinical neosporosis. Azevedo et al. (2005) reported that of 24 dogs positive for neosporosis in Campina Grande, PB, 18 (75%) had titer values between 50 and 200, and six dogs had titers between 400 and 12,800. Similar results were reported by Fernandes et al. (2004) in Uberlândia, MG, where

most positive dogs had a titer of 50 (32.7%, 20/63) and the maximum titer value was 3200 (2/63). In the present study, 24 dogs were positive for both *T. gondii* and *N. caninum*, which corresponds to 60% of all dogs positive for neosporosis (24/40), 7.6% of dogs positive for toxoplasmosis (24/314), and 4.2% of all dogs examined (24/570). These results indicate that some dogs in the city may be simultaneously infected by *N. caninum* and *T. gondii*, as a consequence of the dog population of Ibiúna having been exposed to both agents (MINEO et al., 2004).

Tables 2 and 3 show the results of univariate analysis for the risk factors associated with *T. gondii* and *N. caninum* infections. In the final logistic regression models (Table 4), the variables presence of rodents (OR = 2.05), ingestion of raw meat (OR = 2.47), and prior sexual activity (OR = 1.79) were identified as risk factors for *T. gondii* infection, whereas only prior sexual activity (OR = 3.29) was associated with an increased risk for *N. caninum* infection.

Table 2. Univariate analysis for risk factors associated with *Toxoplasma gondii* (Apicomplexa) infection in the dog population of Ibiúna, São Paulo (SP), Brazil, between September 2007 and March 2008.

Variable	Category	No. of dogs	No. of positive dogs (%)	p
gender	male	312	184 (59.0)	
	female	246	127 (51.6)	0.099*
age (years)	≤ 2	116	49 (42.2)	
	2-6	304	172 (56.6)	
	> 6	139	87 (62.6)	0.004*
presence of rodents	no	43	14 (32.6)	
	yes	497	291 (58.6)	0.002*
contact with flooded areas	no	465	258 (55.5)	
	yes	71	44 (62.0)	0.369
management	domiciled	255	114 (44.7)	
	semi-domiciled	110	65 (59.1)	
	stray	191	130 (68.1)	< 0.001*
feeding	commercial food	117	46 (39.3)	
	homemade food	435	262 (60.2)	< 0.001*
ingestion of raw meat	no	222	89 (40.1)	
	yes	329	217 (66.0)	< 0.001*
owner travels with dog	no	518	294 (56.8)	
	yes	3	1 (33.3)	0.582
prior sexual activity	no	201	88 (43.8)	
	yes	339	212 (62.5)	< 0.001*

*Variables selected for multivariate analysis (p ≤ 0.2).

The presence of rodents as a risk factor for toxoplasmosis was expected, because rodents and birds have been described as important intermediate hosts that harbor tissue cysts of *T. gondii* in their bodies (DUBEY, 2010). Because cats and dogs usually hunt and prey upon rodents and birds, some individuals may ingest tissue cysts and become infected. Similar results were observed by Romanelli et al. (2007) in Guarapuava, PR, where the presence of rodents in food storage silos was an important risk factor for canine toxoplasmosis.

Table 3. Univariate analysis for risk factors associated with *Neospora caninum* (Apicomplexa) infection in the dog population of Ibiúna, São Paulo (SP), Brazil, between September 2007 and March 2008.

Variable	Category	No. of dogs	No. of positive dogs (%)	P
gender	male	312	31 (9.9)	
	female	246	14 (5.7)	0.095*
age (years)	≤ 2	116	3 (2.6)	
	2–6	304	25 (8.2)	
	> 6	139	17 (12.2)	0.019*
presence of rodents	no	43	5 (11.6)	
	yes	497	38 (7.6)	0.373
contact with flooded areas	no	465	38 (8.2)	
	yes	71	5 (7.0)	0.927
management	domiciled	255	15 (5.9)	
	semi-domiciled	110	11 (10.0)	
	stray	191	16 (8.4)	0.342
feeding	commercial food	117	10 (8.5)	
	homemade food	435	33 (7.6)	0.881
ingestion of raw meat	no	222	15 (6.8)	
	yes	329	27 (8.2)	0.642
owner travels with dog	no	518	38 (7.3)	
	yes	3	0 (0)	1.000
prior sexual activity	no	201	7 (3.5)	
	yes	339	36 (10.6)	0.005*

*Variables selected for multivariate analysis ($p \leq 0.2$).

Table 4. Risk factors associated with *Toxoplasma gondii* and *Neospora caninum* (Apicomplexa) infections in the dog population of Ibiúna, São Paulo (SP), Brazil, between September 2007 and March 2008, identified by multiple logistic regression analysis.

Variable	Odds ratio	95% CI	p
Toxoplasma gondii			
Presence of rodents	2.05	0.99-4.23	0.051
Ingestion of raw meat	2.47	1.69-3.61	< 0.001
Prior sexual activity	1.79	1.22-2.62	0.003
Neospora caninum			
Prior sexual activity	3.29	1.43-7.54	0.005

The ingestion of oocysts in contaminated water or tissue cysts in raw or undercooked meat is considered the main route of *T. gondii* infection. In our study, dogs that ingested raw meat had 2.47 times the risk of infection than dogs that did not have access to raw meat. Similar results that also support this association were reported by Moura et al. (2009) in Lages and Balneário Camboriú, SC, and by Silva et al. (2010) in Ubatuba, SP. In Brazil, the habit of feeding leftovers to pets is well-established among dog owners, especially in cities where the countryside is close to urban areas, as is the case in Ibiúna. In fact, this type of behavior favors the spread of *T. gondii* infection (SILVA et al., 2010).

Dogs that had previously mated had 1.79 and 3.29 times the risk of being infected with *T. gondii* and *N. caninum*, respectively, than dogs that had never mated. Thus, prior sexual activity can be considered a risk behavior for infection. Moreover, sexually active stray male dogs travel long distances, which may expose these dogs to various contaminated habitats, further increasing the risk for infection.

Conclusions

Toxoplasma gondii and *N. caninum* infections were detected by indirect immunofluorescence assay (IFA) in the dog population of the tourist city of Ibiúna, São Paulo, Brazil. The analysis of risk factors indicates that rodent control and not feeding raw meat to dogs are important to reduce the risk of infection by *T. gondii* and *N. caninum* in the region.

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