



Revista Brasileira de Parasitologia
Veterinária

ISSN: 0103-846X

zacariascbpv@fcav.unesp.br

Colégio Brasileiro de Parasitologia
Veterinária
Brasil

Constantino, Caroline; Pellizzaro, Maysa; Ferraz Evaristo de Paula, Edson; Wischral
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Revista Brasileira de Parasitologia Veterinária, vol. 25, núm. 4, octubre-diciembre, 2016,
pp. 504-510
Colégio Brasileiro de Parasitologia Veterinária
Jaboticabal, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=397848910020>

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Serosurvey for *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum* in neighborhood dogs in Curitiba-Paraná, Brazil

Inquérito sorológico para *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* e *Neospora caninum* em cães comunitários em Curitiba-Paraná, Brasil

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Received April 13, 2016

Accepted June 27, 2016

Abstract

Neighborhood dogs may act as reservoirs for several zoonotic protozoan infections, particularly in urban areas, thus constituting a potential public health threat. Accordingly, the aim of the present study was to evaluate the exposure of neighborhood dogs to four protozoan pathogens in public areas with high levels of human movement in Curitiba, southern Brazil. Blood samples from 26 neighborhood dogs were screened by means of the indirect immunofluorescent antibody test (IFAT) for *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum*, and a questionnaire was answered by the respective keeper. A total of 8/26 dogs (30.7%) seroreactive to *T. gondii*, 3/26 (11.5%) to *N. caninum* and 2/26 (7.7%) to both were identified. All the samples were seronegative for *T. cruzi* and *Leishmania* spp. Pathogen seroreactivity was not associated with the daily human movements or other epidemiological variables investigated ($p > 0.05$). In conclusion, the low seroprevalence for *T. gondii* and *N. caninum* indicated low environmental and food risk for animal infection and the seronegativity for *Leishmania* spp. and *T. cruzi* may reflect the absence of these pathogens in urban areas of Curitiba. Moreover, neighborhood dogs may be used as environmental sentinels for the presence of protozoan pathogens and their vectors.

Keywords: Community dogs, urban area, sentinel animals, toxoplasmosis, neosporosis, IFAT.

Resumo

Cães comunitários podem atuar como reservatórios para algumas zoonoses causadas por protozoários, principalmente em áreas urbanas, constituindo potencial ameaça à saúde pública. Portanto, o objetivo do presente estudo foi avaliar a exposição de cães comunitários a quatro protozoários em áreas públicas com alta circulação de pessoas, em Curitiba, Sul do Brasil. Amostras de sangue de 26 cães comunitários foram testadas pela reação de imunofluorescência indireta (RIFI) para *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* e *Neospora caninum*, e um questionário foi respondido pelo respectivo mantenedor. Um total de 8/26 (30,7%) foram sororreagentes para *T. gondii*, 3/26 (11,5%) para *N. caninum* e 2/26 (7,7%) para ambos. Todas as amostras foram soronegativas para *T. cruzi* e *Leishmania* spp. Não houve associação entre sororeatividade para os patógenos pesquisados e o tráfego diário de pessoas e outras variáveis epidemiológicas analisadas ($p > 0.05$). Conclui-se a baixa soroprevalência para *T. gondii* e *T. cruzi* indica baixo risco ambiental e alimentar para a infecção dos animais, e a soronegatividade para *Leishmania* spp. e *T. cruzi* pode refletir a ausência desses patógenos em áreas urbanas de Curitiba. Além disso, os cães comunitários podem atuar como sentinelas ambientais quanto à presença de protozoários e seus vetores.

Palavras-chave: Cães comunitários, área urbana, animais sentinela, toxoplasmose, neosporose, RIFI.

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Neighborhood dogs, defined as semi-restricted or free roaming animals with semi-dependence on one or more individuals for food and shelter (WHO & WSPA, 1990), may act as reservoirs or sentinels for several zoonotic and protozoan infections, particularly in urban areas, thus constituting a potential threat to animal and public health (CABEZÓN et al., 2010; CASTILLO-NEYRA et al., 2015; GÜRTLER & CARDINAL, 2015; MEIRELES et al., 2004; ROBERTSON et al., 2000; SALB et al., 2008). Although protozoan infection has previously been assessed in different canine populations (AZZAG et al., 2015; COLLANTES-FERNÁNDEZ et al., 2008; LANGONI et al., 2013; TENNEY et al., 2014), the health status of neighborhood dogs remains to be fully established.

In the city of Curitiba, state of Paraná, southern Brazil, neighborhood dogs have spent mostly of their free-roaming lives in densely occupied public areas, such as bus stations and public parks, thus sharing their environment with around 1.1 million daily bus users. Because of the outdoor and free-roaming habits of neighborhood dogs, these dogs may present greater exposure to environmental and vector-borne diseases such as *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum* (CABEZÓN et al., 2010; SEABRA et al., 2015; TENNEY et al., 2014). For dogs living in stray situation in Brazil, as neighborhood dogs, the seroprevalence for *Leishmania* spp. have been ranging from 0.0027% to 32.5% (FREHSE et al., 2010; SEABRA et al., 2015; TRONCARELLI et al., 2009; VALADAS et al., 2010), for *T. gondii* from 31.6% to 68.4% (MEIRELES et al., 2004; SEABRA et al., 2015; SOUZA et al., 2003; VALADAS et al., 2010), for *T. cruzi* from 2.0% to 4.0% (MORAIS et al., 2013; TRONCARELLI et al., 2009) and for *N. caninum* from 3.2% to 22.1% (FRIDLUND-PLUGGE et al., 2008; SEABRA et al., 2015; SOUSA et al., 2012; VALADAS et al., 2010).

Accordingly, the aim of the present study was to evaluate the exposure of neighborhood dogs to four protozoan pathogens in public areas with highly levels of human movement in the city of Curitiba (bus stations and public parks), the eighth biggest city in Brazil.

This study was approved by the Ethics Committee for Animal Experimentation and Animal Welfare of the Federal University of Paraná, state of Paraná, Brazil (protocol number 027/2015).

Bus stations and public parks were included in this study because they are public areas with high and daily human concentration and movement, besides to their good conditions for neighborhood dogs housing. Dog inclusion criteria was applied according to the WHO definition (WHO & WSPA, 1990) and dogs only included if their correspondent keeper were identified. All 22 bus stations of the city of Curitiba were visited in attempt to identified neighborhood dogs and their respective keeper. In 26 public parks, the neighborhood dogs and their corresponding keeper were registered by population demand by means of city Hall Curitiba phone.

A total of 26 neighborhood dogs were identified in 10/22 bus stations (45.5%) and 2/26 (7.7%) public parks in the city of Curitiba (25°25'40" S; 49°16'23" W), state of Paraná, southern Brazil, between February and August 2014. These dogs were all of mixed breed, aged ≥ 1 year, and comprised 9/26 females (34.1%) and 17/26 males (65.4%). An epidemiological questionnaire was applied to corresponding keeper about the dog's environmental

conditions and habits such as organic material accumulation, raw meat intake and hunting practices.

Whole blood samples (5 mL) were collected by means of venipuncture of the jugular vein using sterile vacuum tubes containing serum separator gel (BD Vacutainer, Franklin Lakes, NJ, USA) and were stored at room temperature (25 °C) until visible clot retraction. The samples were centrifuged at 1500 g for 5 minutes, serum separated and stored at -20 °C until serological analysis. The serum samples were tested for specific IgG antibodies against *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum* by means of the indirect immunofluorescent antibody test (IFAT), as previously described (CAMARGO, 1974). Both, positive and negative, control sera were provided by the Núcleo de Pesquisa em Zoonoses (NUPEZO), Universidade Estadual Paulista (UNESP), Botucatu – SP for all the four diseases. Samples were considered reactive when antibody titers ≥ 40 for *Leishmania* spp., ≥ 16 for *T. gondii*, ≥ 20 for *T. cruzi* and ≥ 25 for *N. caninum* were found. The titers were determined to the largest dilution at which fluorescence was viewed around the protozoa (endpoint titers). Fisher's exact test was used to determine differences relating to whether individual factors were associated with seroreactivity through IFAT. Moreover, the Spearman correlation coefficient was calculated to verify the correlation between daily human movement and seroreactivity. Results were considered to be statistically significant when $p < 0.05$. Data were stored and analyzed using commercially available software (Epi Info, version 7.1.5.2, CDC, Atlanta, USA).

In total, 3/26 neighborhood dogs (11.5%; 95% CI: 2.35-29.16%) were seroreactive to *N. caninum*, with antibody titers ranging from 25 to 200; 8/26 (30.7%; 95% CI: 14.33-51.79%) were seroreactive to *T. gondii*, with antibodies titers ranging from 16 to 64; and only 2/26 (7.7%) were seroreactive to both (Figure 1). All the dogs were seronegative to *Leishmania* spp. and *T. cruzi*. No significant associations were found between gender, diet, raw meat intake or hunting practices and seroreactivity to *N. caninum* and/or *T. gondii*. The seroprevalence results for *N. caninum* and *T. gondii* among the neighborhood dogs and the respective variables are presented in Table 1 and Table 2. Likewise, no correlation was found between daily high human movement and seroreactivity to *N. caninum* (Spearman correlation coefficient = - 0.174; $p = 0.631$) and/or *T. gondii* (Spearman correlation coefficient = 0.302; $p = 0.397$) (Figure 1). However, the neighborhood dogs appear at bus stations where there are a higher population density, and also that the dogs seronegative for all surveyed pathogens represent the most these locations (Figure 2).

Surprisingly, to the best of our knowledge, this study was the first serosurvey on this dog population worldwide. Despite the outdoor and free-roaming habits of neighborhood dogs, which may increase their exposure to protozoan infections (AZEVEDO et al., 2005; COLLANTES-FERNÁNDEZ et al., 2008; MOURA et al., 2009; NAZIR et al., 2014), they presented in this study a low seroprevalence for *T. gondii* and *N. caninum* compared with literature results (MEIRELES et al., 2004; SEABRA et al., 2015; VALADAS et al., 2010), which may indicate that there was a situation of low environmental oocyst contamination (MEIRELES et al., 2004).

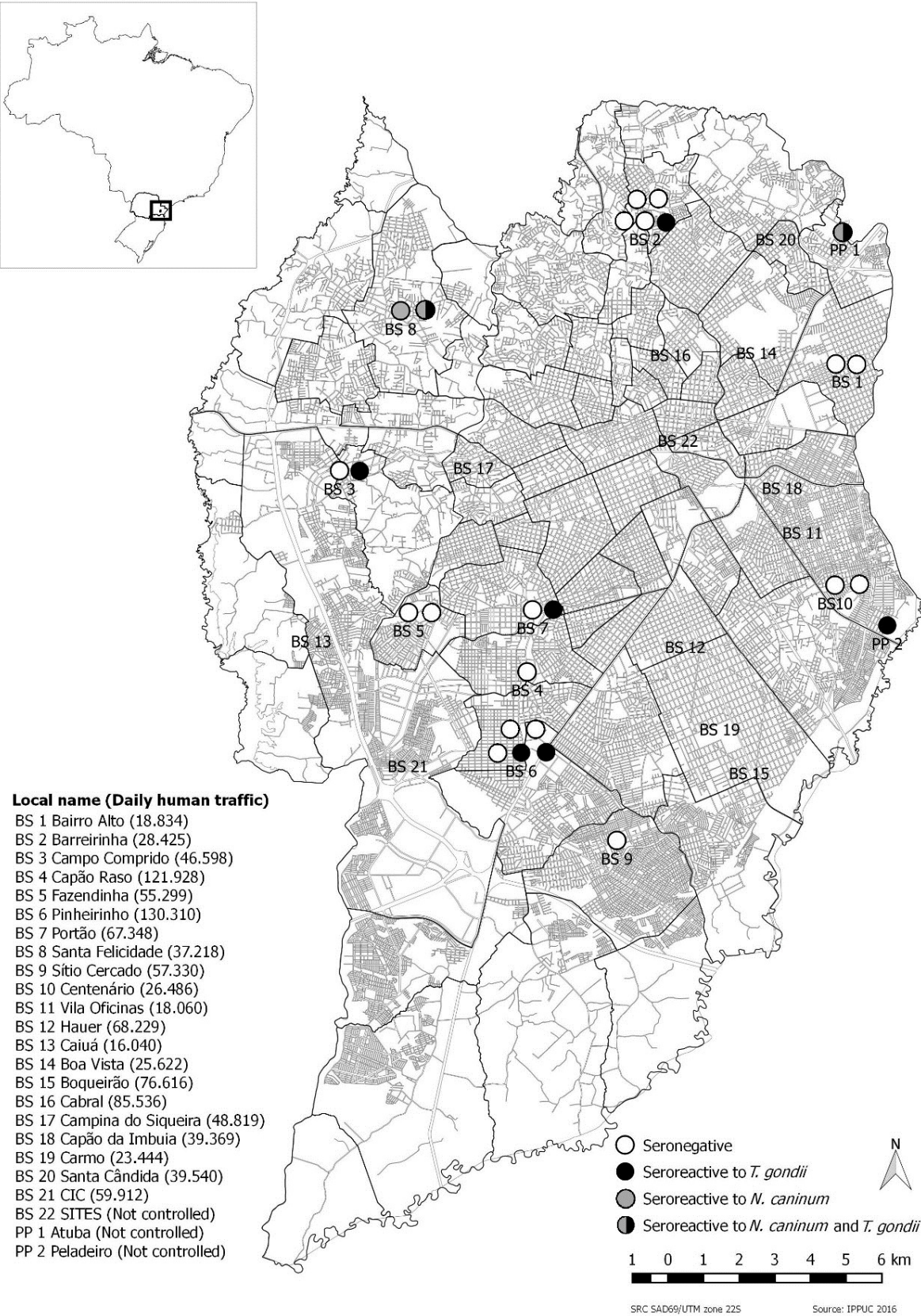


Figure 1. Map of Brazil showing the location of the state of Paraná and city of Curitiba. Enlarged map shows the spatial distribution of neighborhood dogs according to blood test results, in relation to *Leishmania* spp., *Toxoplasma gondii*, *Trypanosoma cruzi* and *Neospora caninum*, and daily human movements in bus stations (BS) and public parks (PP) included in the study, city of Curitiba, state of Paraná, southern Brazil, 2016.

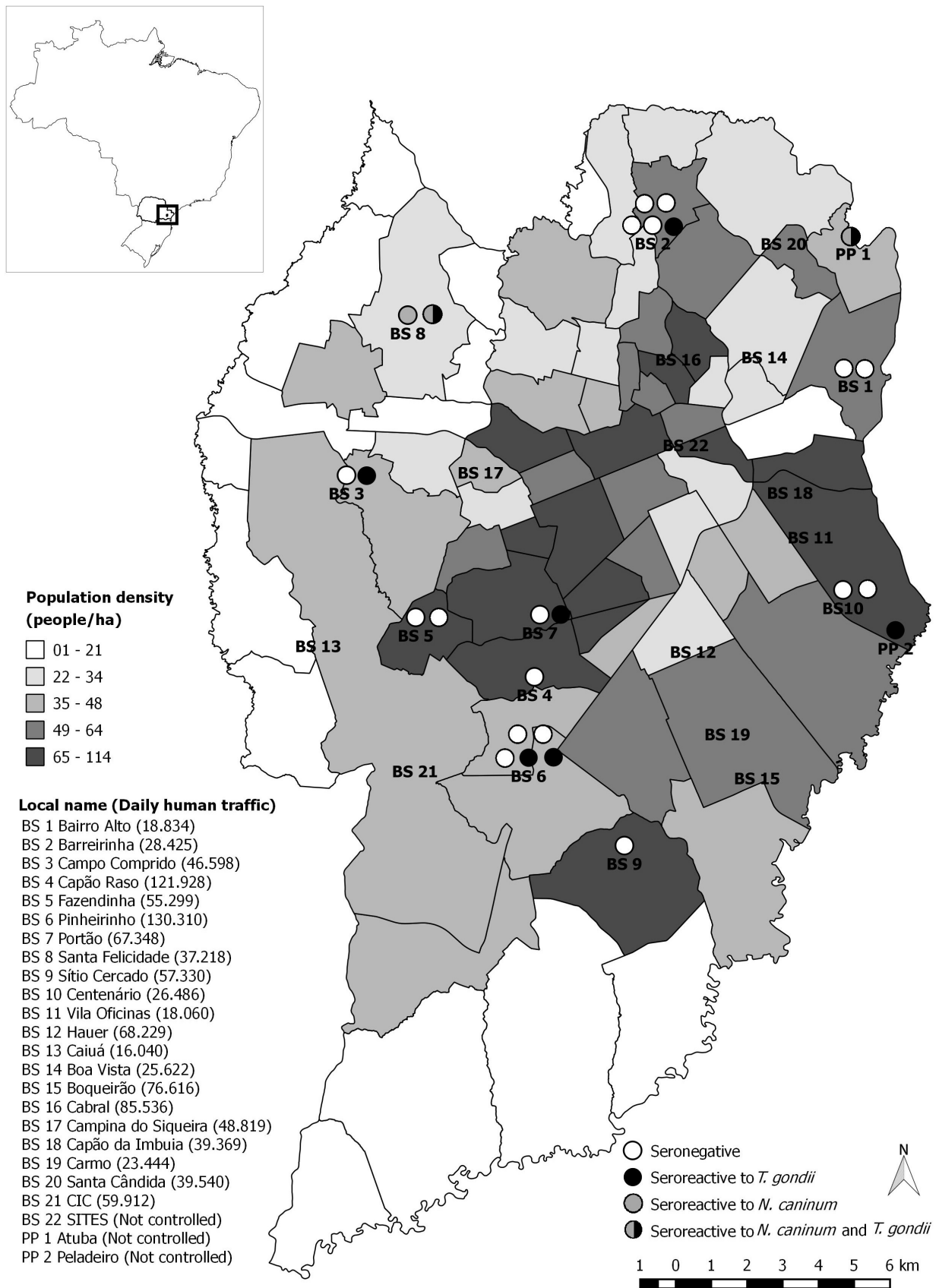


Figure 2. Map of Brazil showing the location of the state of Paraná and city of Curitiba. Enlarged map shows the spatial distribution of neighborhood dogs according to blood test results in relation to population density of city of Curitiba, state of Paraná, southern Brazil, 2016.

Table 1. Seroprevalence for *Neospora caninum* and *Toxoplasma gondii* among neighborhood dogs according to IFAT, in relation to each variable studied, city of Curitiba, state of Paraná, southern Brazil, 2016.

Variable	<i>Neospora caninum</i>				<i>Toxoplasma gondii</i>			
	+/N (%)	OR	95% CI	p-value	+/N (%)	OR	95% CI	p-value
Gender								
Male	1/17 (5.9%)	0.50	0.01-44.10	1.0000	3/17 (17.6%)	0.17	0.02-1.42	0.0781
Female	2/9 (22.2%)	Ref.			5/9 (55.6%)	Ref.		
Hunting practice								
Yes	1/8 (12.5%)	1.14	0.02-25.42	1.0000	2/8 (25.0%)	0.66	0.05-5.56	1.0000
No	2/18 (11.1%)	Ref.			6/18 (33.3%)	Ref.		
Raw meat intake								
Yes	1/5 (20.0%)	2.4	0.03-55.07	0.9769	1/5 (20.0%)	0.50	0.09-6.63	1.0000
No	2/21 (9.5%)	Ref.			7/21 (33.3%)	Ref.		

+: number of positive animals; N: number of samples per variable; OR: odds ratio; 95% CI: 95% confidence interval; Ref.: variable used as a reference value; -: undetermined.

Table 2. Seroprevalence for *Toxoplasma gondii* and *Neospora caninum* among neighborhood dogs according to IFAT, in relation to each gender and housing location of neighborhood dogs, city of Curitiba, state of Paraná, southern Brazil, 2016.

HOUSING LOCATION	N	SEROREACTIVES FOR <i>T. gondii</i>		SEROREACTIVES FOR <i>N. caninum</i>	
		M/n (%)	F/n (%)	M/n (%)	F/n (%)
BS Bairro Alto	2	0/2 (0)	0/0 (0)	0/2 (0)	0/0 (0)
BS Barreirinha	5	0/3 (0)	1/2 (50)	0/3 (0)	0/2 (0)
BS Campo Comprido	2	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
BS Capão Raso	1	0/0 (0)	0/1 (0)	0/0 (0)	0/1 (0)
BS Centenário	2	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
BS Pinheirinho	5	2/4 (50)	0/1 (0)	0/4 (0)	0/1 (0)
BS Portão	2	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
BS Santa Felicidade	2	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)
BS Sítio Cercado	1	0/1 (0)	0/0 (0)	0/1 (0)	0/0 (0)
BS Fazendinha	2	0/2 (0)	0/0 (0)	0/2 (0)	0/0 (0)
PP Atuba	1	0/0 (0)	1/1 (100)	0/0 (0)	1/1 (100)
PP Peladeiro	1	1/1 (100)	0/0 (0)	0/1 (0)	0/0 (0)
TOTAL	26	3/17 (17.6)	5/9 (55.6)	1/17 (5.9)	2/9 (22.2)

BS: bus station; PP: public park; N: number of samples tested; M: male; F: female; n: number of samples per gender.

Since dogs play a secondary role in the toxoplasmosis cycle, neighborhood dogs may be considered to be true environmental sentinels for *T. gondii*, particularly when sharing a contaminated urban environment and contaminated food and water (MEIRELES et al., 2004). In contrast, as the definitive hosts of *N. caninum*, dogs may play an important role in spreading this disease (NAZIR et al., 2014).

As expected, all the neighborhood dogs tested negative for *Leishmania* spp. and *T. cruzi* in the present study. In fact, the city of Curitiba has been considered to be a non-endemic area for both of these diseases and, thus, these results may reflect the historical absence of vectors and pathogens (BRASIL, 2014a, b). However, canine visceral leishmaniasis was previously detected in 1/364 stray dogs (0.0027%) from a city situated close to Curitiba (FREHSE et al., 2010), and triatomine (*T. cruzi* vector) infestation was detected in 7/9 cities (77.8%) in the northern part of the state of Paraná (FALAVIGNA-GUILHERME et al., 2004). Moreover, the first autochthonous case of human visceral

leishmaniasis in the state of Paraná was registered in 2015 in city of Foz do Iguaçu, and the first death due to this disease was reported in January 2016 (AEN, 2015). Since infection of dogs by *Leishmania* spp. may precede human cases (OLIVEIRA et al., 2001), periodic serosurveys among neighborhood dogs may be used as a means of active surveillance in non-endemic areas such as the city of Curitiba.

In conclusion, despite daily exposure of these neighborhood dogs to risk of infection, in public areas of Curitiba, the protozoan seroprevalence for *N. caninum* and *T. gondii* was within the general population range and may indicate that there was a situation of low environmental and food risk with regard to animal infection. Furthermore, the absence of antibodies anti-*Leishmania* spp. and *T. cruzi* may reflect the absence of these pathogens in urban areas of Curitiba. Moreover, neighborhood dogs may be used as environmental sentinels for the presence of these protozoan pathogens and their transmission vectors in non-endemic areas.

Acknowledgements

We thank the staff of the Zoonosis Research Center, UNESP, city of Botucatu and Curitiba Animal Service for their in-field assistance. We thank the Araucaria Support Foundation for Scientific and Technological Development of the State of Paraná for the full financial support for this research and the Coordination Office for Improvement of Higher-Education Personnel for the master's fellowship.

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