


Epidemiological relevance of dogs for the prevention of *Toxoplasma gondii*, *Neospora caninum* and *Leptospira* spp.

Relevância epidemiológica de cães para a prevenção de *Toxoplasma gondii*, *Neospora caninum* e *Leptospira* spp.

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Abstract

This study evaluated the seroprevalence of *Toxoplasma gondii*, *Neospora caninum* and *Leptospira* spp. in dogs from Foz do Iguaçu, Paraná, Brazil. Indirect immunofluorescent antibody test was used to detect antibodies anti-*T. gondii* and anti-*N. caninum*. Immunoenzymatic assay and microscopic serum agglutination were used for screening antibodies anti-*T. gondii* and anti-*Leptospira* spp., respectively. The results were: 67.02% of the samples reactive for *T. gondii* and 1.38% for *N. caninum*, both without statistically significant variables. For *Leptospira* spp. the results indicated seroprevalence of 23.11%. The analysis of the variables without distinction of serovar showed association for intrinsic characteristics as breed, age, nutritional status and dog category. The extrinsic variables as city region and access to the street presented association ($p < 0.05$). The most prevalent serovars were: Canicola 59.47%; Bratislava 13.07% and Butembo 15.68%. Variables that make up the adjusted multiple analysis model using *Leptospira* spp. were: age, breed and nutritional status; serovar Canicola, sex, nutritional status and area ($p < 0.05$); serovar Bratislava, lymphadenomegaly and presence of fleas ($p < 0.05$). Given the results obtained, dogs can be used as sentinels for toxoplasmosis and leptospirosis in Foz do Iguaçu and other cities with similar outcomes. In addition, preventive measures should be taken by health authorities because they are zoonoses and humans are also at risk.

Keywords: Spatial dispersion, toxoplasmosis, neosporosis, leptospirosis, Foz do Iguaçu.

Resumo

Este estudo avaliou a soroprevalência de *Toxoplasma gondii*, *Neospora caninum* e *Leptospira* spp. em cães de Foz do Iguaçu, Paraná, Brasil. O teste de imunofluorescência indireta foi utilizado para detectar anticorpos anti-*T. gondii* e anti-*N. caninum*. Ensaio imunoenzimático e soroprecipitação microscópica foram utilizados para pesquisa de anticorpos anti-*T. gondii* e anti-*Leptospira* spp., respectivamente. Os resultados obtidos foram: 67,02% (435/649) das amostras reativas para *T. gondii* e 1,38% (9/649) para *N. caninum*, ambas com ausência de variáveis significativas estatisticamente. Para *Leptospira* spp. os resultados indicaram soroprevalência de 23,11% (153/649). A análise das variáveis sem distinção de sorovar mostraram associação para características intrínsecas como raça, idade, estado nutricional e categoria de cães. Para as variáveis extrínsecas, a região da cidade e ter acesso à rua mostraram associação estatística ($p < 0,05$). Os sorovares mais prevalentes foram: Canicola com 59,47% (91/153); Bratislava 13,07% (20/153) e Butembo 15,68% (24/153). As variáveis que compõem o modelo de análise multivariada ajustada usando como desfecho *Leptospira* spp. foram: idade, raça, estado nutricional e área. Para o sorovar Canicola, as variáveis significantes ($p < 0,05$) foram sexo, estado nutricional e área; para o sorovar Bratislava, as variáveis significantes ($p < 0,05$) foram linfadenomegalia e presença de pulgas. Dos resultados obtidos, cães podem ser usados como sentinelas para infecção por *T. gondii* e *Leptospira* spp. na cidade de Foz do Iguaçu e em outras cidades com desfechos similares. Além disso, medidas preventivas devem ser tomadas pelas autoridades de saúde, pois são zoonoses e os seres humanos também estão em risco.

Palavras-chave: Distribuição espacial, toxoplasmose, neosporose, leptospirose, Foz do Iguaçu.

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Introduction

Foz do Iguaçu municipality is in the extreme west of the State of Paraná, on the triple border of Brazil, Argentina and Paraguay. Due to its location, it has sizeable migratory flow, and it is an area that is very vulnerable to diseases including the zoonoses. Due to low levels of maritime influence, the area has one of the largest annual thermal amplitudes, with an average temperature difference of approximately 11°C between winter and summer. Its characteristics are very peculiar. There are areas of interposition between rural areas, parks, ecological reserves and urban zones (ZIOBER & ZANIRATO, 2014; IBGE, 2016). Climatic conditions, anthropic intervention in the ecosystems and the socio-cultural and economic characteristics of the city could facilitate the spread of infectious agents such as *Toxoplasma gondii*, *Neospora caninum* and *Leptospira* spp. The close relationship between humans and dogs makes these animals important flags and/or sentinels of microbiological and/or parasitic diseases (FAINE, 1982; GARCIA et al., 1999; MOURA et al., 2009).

Toxoplasma gondii is one of the most studied parasites in the world due to its importance in the medical and veterinary fields. Felids are its final host, and all other endothermic animals (as dogs) are intermediate hosts. In humans this parasite can cause several problems in special in pregnant women because when acquired during pregnancy the toxoplasmosis can produce a severe congenital infection (DUBEY et al., 1970). Dogs have been used as sentinel animals for estimating *T. gondii* infection in the environment due their comportment and proximity with humans (LOPES et al., 2014).

Neospora caninum is a coccidian protozoan that affects mainly production animals and canids, which are the definitive hosts (DUBEY et al., 1988; MCALLISTER et al., 1998). Its zoonotic potential is still uncertain. The pathogenicity and severity of *N. caninum* varies with the host species. Cattle are the most susceptible, with infection resulting in great economic losses in the reproductive sphere (DUBEY et al., 2006).

Leptospira spp. is the bacteria that causes a zoonotic disease endemic in Brazil, has seasonality and occurs at higher rates in times of increased rainfall (FAINE, 1999; LILENBAUM et al., 2005). Dogs are considered reservoirs for some serovars, such as Canicola, and are important for the maintenance of this agent in the environment (GREENE et al., 2006).

Leptospira spp. and *T. gondii* dog infection is epidemiologically important because dogs share habitats with humans, which indicates that the environment represents an ecological niche for these pathogens and, consequently, a potential risk of infection for humans (FAINE, 1982; HEATH & JOHNSON, 1994; GARCIA et al., 1999; MOURA et al., 2009).

Considering the importance of the *T. gondii*, *N. caninum* and *Leptospira* spp. for dogs and other species, the role of dogs as sentinels, as well as the peculiar characteristics of Foz do Iguaçu and the lack of previous studies, the objective of this study was to conduct a cross - sectional study on *Toxoplasma gondii*, *Neospora caninum* and *Leptospira* spp. seroprevalence in dogs in the city of Foz do Iguaçu, Brazil, to identify potential factors related to infection.

Materials and Methods

Study area and data collection

The work was approved by the Ethics Committee on the Use of Animals of the Sector of Agrarian Sciences of the Federal University of Paraná and was registered under number 044/14.

The municipality of Foz do Iguaçu has an estimated population of 263,782 inhabitants and 54,983 dogs. It is located at latitude 25°32'45"S and longitude 54°35'07"W. It has a humid subtropical climate, with hot summers, infrequent frosts, rain in all months of the year, and nine micro watersheds. Seven of the watersheds are circumscribed to the municipal perimeter. The main rivers are Paraná, Iguaçu, Tamanduá, São João, Almada, M'Boicy and Monjolo (IBGE, 2016). According to Instituto Brasileiro de Geografia e Estatística (IBGE, 2016) it has a territorial area of 617,701 km². Of this total area, 191.46 km² are urban areas, 138.17 km² are rural, 138.60 km² are National Parks, 149.10 km² make up Itaipu Lake, and 0.38 km² make up Acaray Island. There are areas of interposition between rural and urban areas and there is great ecological biodiversity in the parks and lakes (Figure 1).

For this study, the city was divided into four areas (A to D) by using a stratified sampling strategy. The stratifications of the urban area were identified using a landscape approach. Thus, by recognizing the patterns of the landscape elements and their spatial distribution, the different landscape areas present in each area were considered to delineate the sampling strata. This method is related to the visual interpretation of the products of remote sensors. In this case, high-resolution images of the study area available from Google Earth were used, and the images were projected on an approximate scale of 1: 50,000. Four strata were identified in the city of Foz do Iguaçu, and each stratum was divided into areas of 400 × 400 m, and then quadrants (between 25 and 40 per area) were randomly selected, and the "best" scenario for the presence of rodents and placement of traps was determined. Five dogs were sampled (irrespective of their clinical status) from each patch according to methodology described in Thomaz-Soccol et al. (2017). When five dogs were not available in this house, dogs from the neighbourhood were included, up to five. Dogs from the houses were blood-sampled by veterinarians.

The city was divided into four areas: A, B, C and D (Figure 2). Area A: corresponds to the commercial region of the city with the highest density of buildings near the Paraná River. This area contains two forest remnants, which occupy a large part of this stratum. Area B: corresponds to one of the residential areas bordering the rural areas. It has medium density housing and is relatively uniform. Area C: located in the northern portion of the city, bordered to the west and north by the Paraná River and rural areas to the east. This area is discontinuous and is interrupted by large spaces of vegetation (e.g., sports fields, small cultivated areas). Area D: has buildings of high and intermediate densities, is at the mouth of the Rio Iguaçu and Rio Paraná, and has patches of vegetation around the coast of the mentioned rivers.

For all collections, the tutors completed the term of science and authorization, and an epidemiological questionnaire which contained information such breed (with defined or non-defined

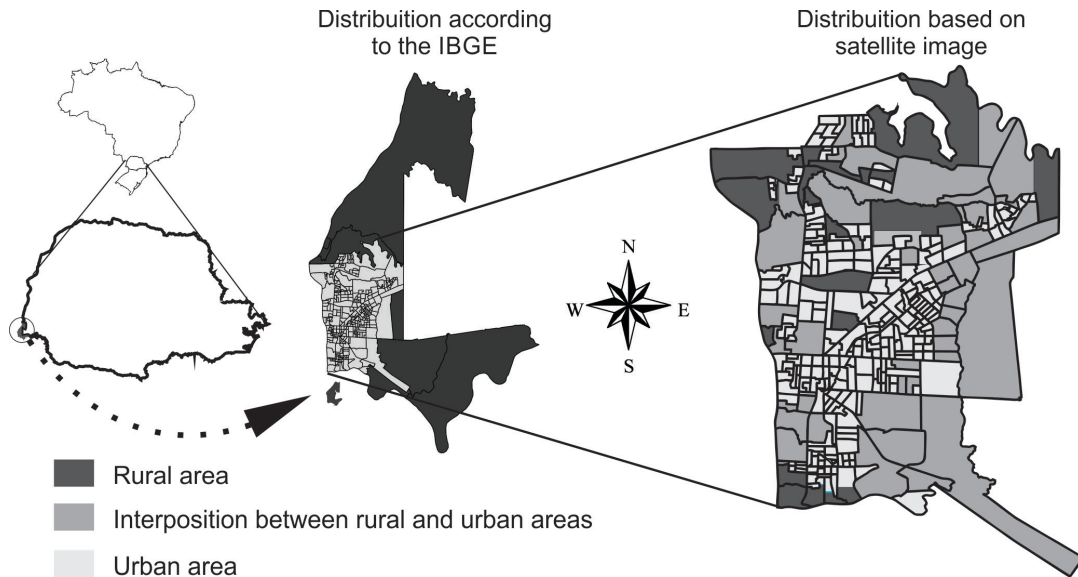


Figure 1. Schematic map with highlights for rural and urban areas, Foz do Iguaçu, Paraná, Brazil, 2014.

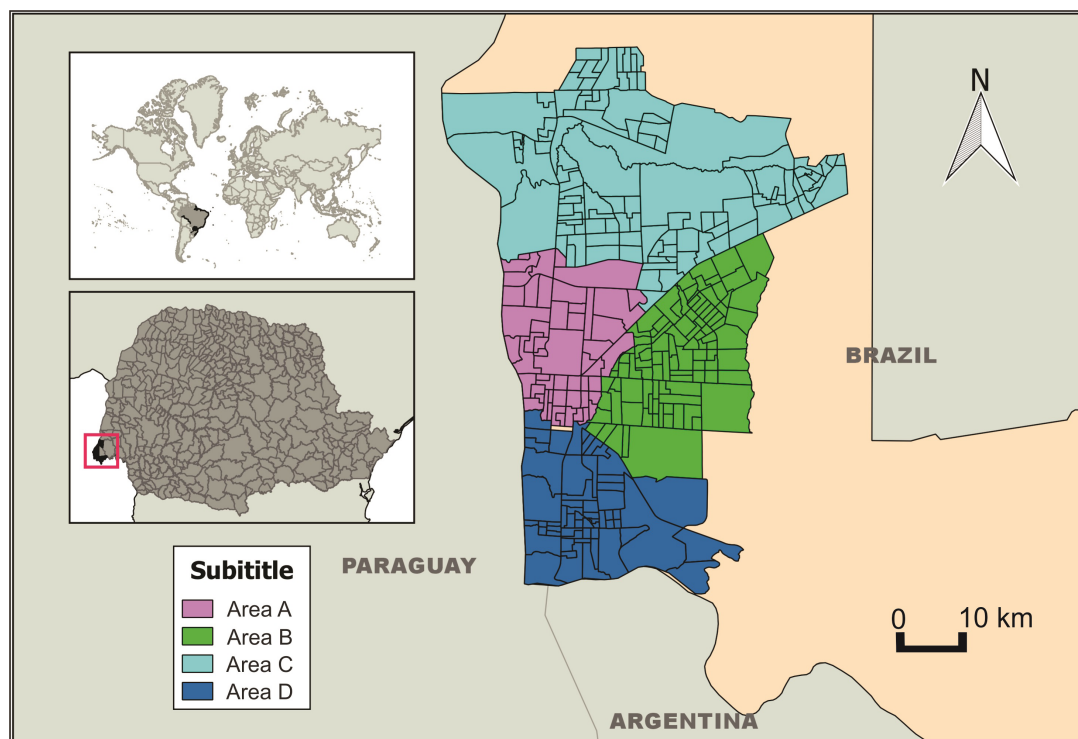


Figure 2. Sampling areas of dog blood, Foz do Iguaçu, Paraná, Brazil, 2014.

breed), sex (male or female), age, sleeping location, access to the street, immunizations (rabies vaccine and polyvalent vaccine), category of dog (company, guard, NGO - Non-governmental Organization or accompanying recyclable waste pickers), and origin (same neighborhood, another neighborhood, another city or other country) was given. Physical examination was carried out by the veterinarians to verify the general state of the animal, observing points such as presence of ectoparasites, body and nutritional status, besides mucosal staining and the presence of cutaneous wounds.

Rodent activity was estimated by 10 traps that were distributed in each quadrant for four consecutive days and were capable of detecting feces, urine and hair, as described by Cavia et al. (2012).

Biological blood samples and serum

Blood collection from 649 dogs was performed in 2014 by puncturing the cephalic or jugular vein using a disposable syringe and needle (25 × 7 mm). The samples were placed in sterilized

tubes without anticoagulants, identified and transported to the laboratory for centrifugation and serum separation. The serum was then aliquoted into polypropylene tubes (1.5 mL) and stored at negative 20 °C until the time of analysis.

Immunoenzymatic assay (ELISA) and Indirect immunofluorescence antibody test for *T. gondii*

Two tests were used for screening for antibodies anti-*T. gondii*, the indirect enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence antibody test (IFAT).

ELISA was performed as described by Garcia et al. (1999). Briefly, concentrations of *T. gondii* antigen, serum and peroxidase-conjugated protein A conjugate (Sigma Aldrich, Missouri, USA) were: 2.5 µg / mL, 1:100, and 1:2,500, respectively. All samples were tested in duplicate. The optimum conditions for the dilutions of antigen, serum and conjugate were established by the greater ratio between the mean optical density of the positive samples and the mean of the negative samples. The cut-off point of each plate was obtained according to Garcia et al. (2006). The general cut-off point was estimated using the ROC curve, which was constructed using the MedCalc Statistical Software program (SCHOONJANS et al., 1995).

To IFAT, the methodology was done according Camargo (1964), where tachyzoites of RH strain were used as antigen, and in each slide positive and negative controls were used. Samples with titers ≥16 were considered positive (FERREIRA et al., 2016) and were diluted at a ratio four until the titer was negative. The results of the two tests were merged to analysis.

Indirect immunofluorescence for anti- *Neospora* spp. antibodies

For the titration of antibodies anti - *N. caninum*, an indirect immunofluorescent antibody test (IFAT) was used (CONRAD et al., 1993), using the tachyzoite forms of the NC-1 strain as antigen. During the analyses, positive and negative controls were included on each slide. Samples with titers ≥ 50 (DUBEY et al., 1988) were considered positive and diluted at a ratio of two until the titer was negative.

Microscopic soroagglutination for *Leptospira* spp.

Serum samples were submitted to microscopic serum agglutination for the detection of antibodies anti- *Leptospira* spp. In general, strains of the different serovars are reported (FAINE, 1999) and for this reason 10 reference serovars were used: Bratislava, Butembo, Castellonis, Canicola, Grippotyphosa, Copenhageni, Icterohaemorrhagiae, Pomona, Pyrogenes and Hardjo. The antigens were maintained at 28 °C for five to ten days in EMJH medium prior to use (FAINE, 1982). Serum samples that had at least 50% of *Leptospira* agglutinated at 1: 100 dilution were considered reactive, and they were then serially diluted 2-fold to determine the maximum positive dilution. The most likely serovar was the one with the highest titer (FAVERO et al., 2002).

Statistical analysis

The EpiInfo program 7.1.5.2 (DEAN et al., 1994) was used to tabulate the epidemiological questionnaire variables, serological results and to determine statistical significance of the variables with a chi-square test or Fisher's exact test, considering a significance level of 5%. Only the variables whose p value was <0.05 in the univariate analysis and which presented some biological meaning were included in the multiple logistic regression models, which were performed with program R 3.4 (TEAM R, 2011). After adjustment, the model residues were checked. Residue analysis verified the presence or absence of systematic models of variation, in addition to the quality of fit. The odds ratio (OR) calculation with a 95% confidence interval (CI) was used as a measure of association.

Geoprocessing

Dog residences were mapped using the Global Positioning System (GPS). Point distribution, cluster analysis (EVERITT, 1974), and kernel density were constructed using the Qgis software (TEAM, 2016). Kernel density is a statistical technique in which the distribution of events is transformed into a continuous surface, allowing the estimation of the density of the event in every area, identifying areas with the highest occurrence of the event (GATRELL et al., 1996). For cluster analysis, the plugin ClusterPoints from Qgis was used and after delimitation of the areas, the chi-square test was applied. Plugin Cluster Points conducts spatial clustering of points based on their mutual distance to each other. The user can select between the K-Means algorithm and (agglomerative) hierarchical clustering with several different link functions (TEAM, 2016).

Results

A total of 649 dogs were included in this study, their geographic distribution were 20.95% (136/649) from area A, 19.72% (128/649) from area B, 36.20% (235/649) from area C, and 23.11% (150/649) from area D (Figure 3). The intrinsic characters

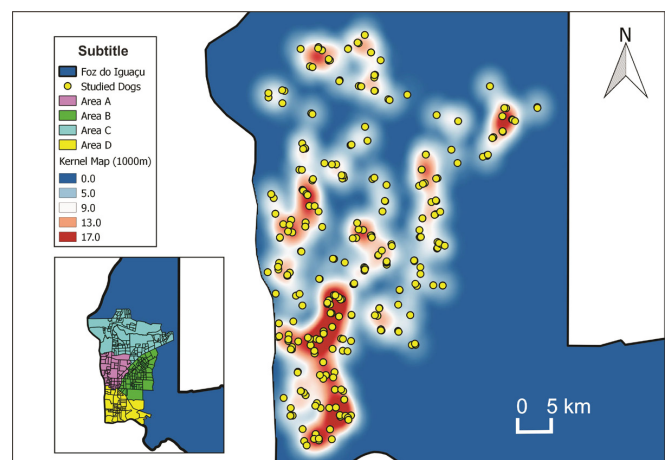


Figure 3. Distribution of dogs sample collected in Foz do Iguacu, Paraná, Brazil, 2014.

as sex were 54.39% female, and 86.44% adults. For the extrinsic characters we observed 73.18% as undefined breed, 92.91% sleep in the outside and 60.86% has street access (Table 1). The numerical differences in age, category and origin are due to the lack of information in the epidemiological questionnaires.

In the search for antibodies anti- *T. gondii*, the positivity varied according to the methodology employed; 51.30% (333/649) by ELISA approach and 40.83% (265/649) by IFAT. When considering both tests, the positivity was 67.02% (435/649) and varied from 60.15% in area B to 77.20% for the area A. We were unable to find any significant variables when using the results from both tests together (Table 2 and Figure 4).

Table 1. Main characteristics of sampled dogs from Foz do Iguaçu, Paraná, Brazil, 2014.

Characteristics	(n/total)	%
Sex		
Female	(353/649)	54.39
Male	(296/649)	45.60
Breed		
Undefined breed	(475/649)	73.18
With defined breed	(174/649)	26.81
Age		
Puppy	(57/642)	8.87
Adult	(555/642)	86.44
Elderly	(30/642)	4.67
Place to sleep		
Inside home	(46/649)	7.87
Out of home	(603/649)	92.91
Street access		
Yes	(395/649)	60.86
No	(254/649)	39.13
Received rabies vaccine		
Yes	(558/649)	85.97
No	(91/649)	14.03
Received polyvalent vaccine		
Yes	(96/649)	14.80
No	(553/649)	85.20
Category of dog		
Company	(350/648)	54.01
Guard	(281/648)	44.36
NGO*	(6/648)	0.92
Accompanies waste picker	(5/648)	0.77
From		
Same neighborhood	(444/644)	68.94
Another city	(34/644)	5.27
Other Neighborhood	(159/644)	24.68
Another country	(7/644)	1.08
Nutritional status		
Good	(605/649)	93.22
Bad	(12/649)	1.84
Regular	(32/649)	4.93
Fleas and ticks		
Yes	(305/649)	46.99
No	(344/649)	53.01

*NGO - non-governmental organization.

For *N. caninum*, only 1.4% (9/649) were seropositive, with absence of significant variables (Table 2). The positivity varied according to the researched area from zero in area A to 2.62% in area C (Figure 4).

For *Leptospira* the microscopic serum agglutination showed 23.57% (153/649) of the dogs presenting antibodies anti-*Leptospira* spp. The most prevalent serovars were Canicola

Table 2. Profile of dogs serologically tested for *T. gondii* and *N. caninum*, Foz do Iguaçu, Paraná, Brazil, 2014.

Characteristics	<i>T. gondii</i>		<i>N. caninum</i>	
	(n/total)	%	(n/total)	%
Unit				
A	(102/136)	77.20	(0/136)	0.00
B	(77/128)	60.15	(2/126)	1.58
C	(156/235)	66.38	(6/229)	2.62
D	(100/150)	66.66	(1/149)	0.06
Sex				
Female	(236/353)	66.85	(6/353)	1.69
Male	(199/296)	67.22	(3/296)	1.01
Breed				
With defined breed	(127/174)	72.98	(2/174)	1.14
Undefined breed	(308/475)	64.84	(7/475)	1.47
Age				
Puppy	(39/57)	68.42	(0/57)	0.00
Adult	(371/555)	66.84	(8/555)	1.44
Elderly	(20/30)	66.66	(1/30)	3.33
Place to sleep				
Inside home	(405/603)	67.16	(8/603)	1.32
Out of home	(30/46)	65.21	(1/46)	2.17
Street access				
Yes	(165/254)	64.96	(2/254)	0.07
No	(270/395)	70.12	(7/395)	1.77
Received vaccine: Rabies				
Yes	(366/558)	65.59	(9/558)	1.61
No	(69/91)	75.82	(0/91)	0.00
Polyvalent				
Yes	(70/96)	72.91	(3/96)	3.12
No	(365/553)	66.00	(6/553)	1.09
Category of dog				
Company	(236/350)	67.42	(5/350)	1.42
Guard	(192/287)	66.89	(4/287)	1.39
NGO	(3/6)	50.00	(0/6)	0.00
Accompanies waste picker	(4/5)	80.00	(0/5)	0.00
From				
Same neighborhood	(289/444)	63.96	(4/444)	0.90
Another city	(25/34)	73.52	(1/34)	2.94
Other Neighborhood	(111/159)	69.81	(4/159)	2.51
Another country	(7/7)	100.00	(0/7)	0.00
Nutritional status				
Good	(406/605)	67.10	(7/605)	1.16
Bad	(8/12)	66.66	(1/12)	8.33
Regular	(21/32)	67.74	(1/32)	3.12
Fleas and ticks				
Yes	(201/305)	65.90	(5/305)	1.64
No	(234/344)	68.02	(4/344)	1.17

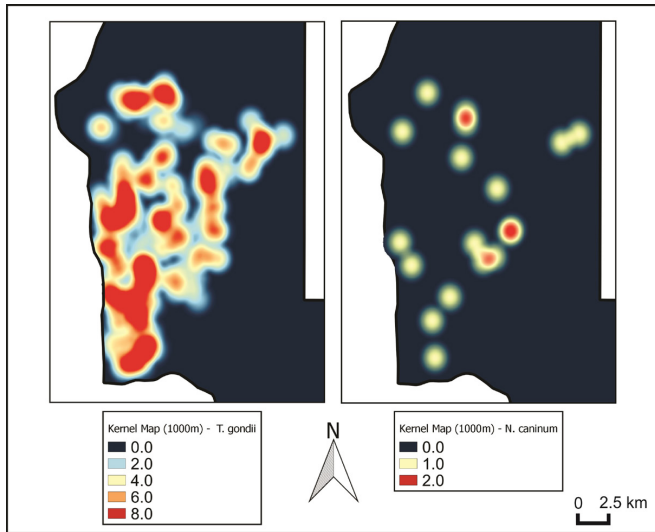


Figure 4. Kernel density analysis of the presence of antibodies anti-*T. gondii* and *N. caninum* in dogs, Foz do Iguaçu, Brazil, 2014.

59.47% (91/153), Bratislava 13.07% (20/153), Butembo 15.68% (24/153), Pyrogenes 2.61% (4/153), Pomona 1.96% (3/153), Copenhageni 1.96% (3/153), Grippotyphosa 2.61% (4/153), and Icterohaemorrhagiae 0.65% (1/153). No samples were seroreactive to the Hardjo and Castellonis serovars. Intrinsic variables as sex, age, breed, nutritional status, mood, presence of fleas and ticks, weight loss, presence of ocular secretion, alopecia and lymphadenomegaly and extrinsic variables as category, street access and origin area were statistically significant for the positivity to *Leptospira* in univariate analysis (Table 3). The adjusted models resulting from the multiple analysis are presented in Table 4. The variables that make up the model whose outcome is *Leptospira* spp. were area, age, breed and nutritional status; for serovar Canicola the adjusted model contained area, sex and nutritional status variables; when the outcome was serovar Bratislava, the variables were: presence of fleas and lymphadenomegaly. The area B presented higher prevalence of animals seropositive to *Leptospira* spp. and serovar Butembo, while area A presented higher prevalence of animals

Table 3. Significant variables in the univariate analysis for antibodies anti-*Leptospira* spp. and serovars analyzed in dogs, Foz do Iguaçu, Paraná, Brazil, 2014.

Variables	Reagents/Total	(%)	p-value	Odds Ratio (CI 95%*)
<i>Leptospira</i> spp.				
Area				
A	24/136	17.64	<0.01	
B	37/128	29.13		
C	23/235	9.78		
D	36/150	24.00		
Breed				
With defined breed	16/174	9.19	<0.01	0.36 (0.20 - 0.62)
Undefined breed	104/474	21.94		
Age				
Puppy	8/56	14.28	0.02	
Adult	99/555	17.83		
Elderly	11/30	36.66		
Nutritional status				
Good	104/604	17.21	<0.01	
Bad	3/9	33.33		
Regular	13/32	40.62		
Category of dog				
Accompanies waste picker	5/5	100.00	<0.01	
Company	46/349	13.18		
NGO	4/6	66.67		
Guard	64/287	22.29		
Weight loss				
Yes	12/36	33.34	0.02	2.33 (1.13 - 4.81)
No	108/612	17.64		
Mood				
Apathetic	6/21	28.57	<0.01	
Active	112/620	18.70		
Lethargic	2/7	28.57		
Street access				

*CI. Confidence interval.

Table 3. Continued...

Variables	Reagents/Total	(%)	p-value	Odds Ratio (CI 95%*)
Yes	57/254	22.44	0.04	1.52 (1.01 - 2.26)
No	63/394	15.98		
Ocular secretion				
Yes	10/23	43.47	<0.01	3.60 (1.54 - 8.42)
No	110/625	17.60		
Alopecia				
Yes	23/80	28.75	0.01	1.96 (1.15 - 3.33)
No	97/568	17.08		
Fleas and ticks				
Yes	24/79	30.37	<0.01	2.15 (1.27 - 3.64)
No	96/569	16.87		
Leptospira spp. Serovar Bratislava				
Area				
A	03/136	2.20	<0.001	
B	13/128	10.15		
C	4/235	1.70		
D	0/150	0		
Lymphadenomegaly				
Yes	05/61	8.19	0.03	3.41 (1.19 - 9.73)
No	15/588	2.56		
Fleas				
Yes	12/234	5.12	0.02	2.75 (1.11 - 6.83)
No	8/415	1.92		
Leptospira spp. Serovar Butembo				
Area				
A	7/136	5.14	<0.01	
B	9/128	7.03		
C	2/235	0.85		
D	6/150	4.00		
Leptospira spp. Serovar Canicola				
Area				
A	28/135	20.74	<0.01	
B	12/128	9.38		
C	22/235	9.36		
D	29/150	19.33		
Sex				
Female	37/352	10.51	<0.01	0.52 (0.34 - 0.83)
Male	54/296	18.24		
Category of dog				
Accompanies waste picker	5/5	100.00	<0.01	
Company	41/350	11.71		
NGO	2/6	33.33		
Guard	42/286	14.68		
Nutritional status				
Good	77/604	12.75	<0.01	
Bad	3/9	33.33		
Regular	11/32	34.37		
Leptospira spp. Serovar Copenhageni				
Fleas and ticks				
Yes	2/79	2.53	0.04	14.78 (1.33 - 165.00)
No	1/570	0.17		

*CI. Confidence interval.

Table 4. Significant variables in the multiple analysis for antibodies anti-*Leptospira* spp. and serovars analysed in dogs, Foz do Iguaçu, Paraná, Brazil, 2014.

	Reagents/total (%)	Logistic Regression	
		p-value	O.R. Gross (IC 95%)
<i>Leptospira</i> spp.			
Area			
A	24/136 (17.64)		1.00
B	37/127 (29.13)	0.022	2.088 (1.121 - 3.980)
C	23/235 (9.78)	0.125	0.596 (0.307 - 1.159)
D	36/150 (24.00)	0.078	1.764 (0.946 - 3.361)
Age			
Puppy	8/56		1.00
Adult	99/456	0.256	1.595 (0.749 - 3.818)
Old	11/19	0.003	5.686 (1.861 - 18.237)
Breed			
With breed defined	16/174 (9.19)		1.00
Undefined breed	104/474 (21.94)	0.003	2.457 (1.401 - 4.562)
Nutritional status			
Good	104/604 (17.21)		1.00
Bad	3/9 (33.33)	0.905	1.087 (0.230 - 3.917)
Regular	13/32 (40.62)	0.006	3.081 (1.368 - 6.807)
<i>Leptospira</i> spp. Serovar Bratislava			
Lymphadenomegaly			
Yes	5/61 (8.19)	0.04	1.00
No	15/588 (2.55)		2.963 (0.925 - 8.084)
Fleas			
Yes	12/234 (5.12)	0.04	2.517 (1.015 - 6.563)
No	8/415 (1.92)		1.00
<i>Leptospira</i> spp. Serovar Canicola			
Area			
A	28/135 (20.74)		1.00
B	12/128 (9.38)	0.031	0.438 (0.201 - 0.911)
C	22/235 (9.36)	0.019	0.464 (0.243 - 0.882)
D	29/150 (19.33)	0.922	0.970 (0.526 - 1.798)
Sex			
Female	37/352 (10.51)		1.00
Male	54/296 (18.24)	0.034	1.651 (1.040 - 2.640)
Nutritional status			
Good	77/604 (12.75)		1.00
Bad	3/9 (33.33)	0.143	2.867 (0.591 - 10.893)
Regular	11/32 (34.27)	0.009	3.062 (1.285 - 7.027)

seropositive to serovar Canicola (Table 4). By spatial analysis, a significant cluster of points was observed for the dogs that were seropositive to Canicola serovar ($p < 0.001$), they were concentrated in a specific region of area D. Area B and C presented a lower occurrence ($p < 0.05$) (Figure 5).

With regard to the percentages of coinfection, 1.38% (9/649) of the animals presented antibodies anti-*T. gondii* and *N. caninum*; 15.56% (101/649), anti-*T. gondii* and *Leptospira* spp.; 0.92% (6/649), anti-*N. caninum* and

Leptospira spp.; 0.77% (5/649) presented antibodies against the three studied agents.

Rodent activity was observed in 70.87% (460/649) of the traps, with no higher concentration in a particular area and no statistical association to the presence of antibodies against the three pathogenic agents studied. It was observed that 70.34% (306/435) of the *T. gondii* seropositive dogs, 77.77% (7/9) of the *N. caninum* seropositive dogs and 73.20% of the *Leptospira* spp. seropositive dogs (112/153) had rodent activity in their homes.

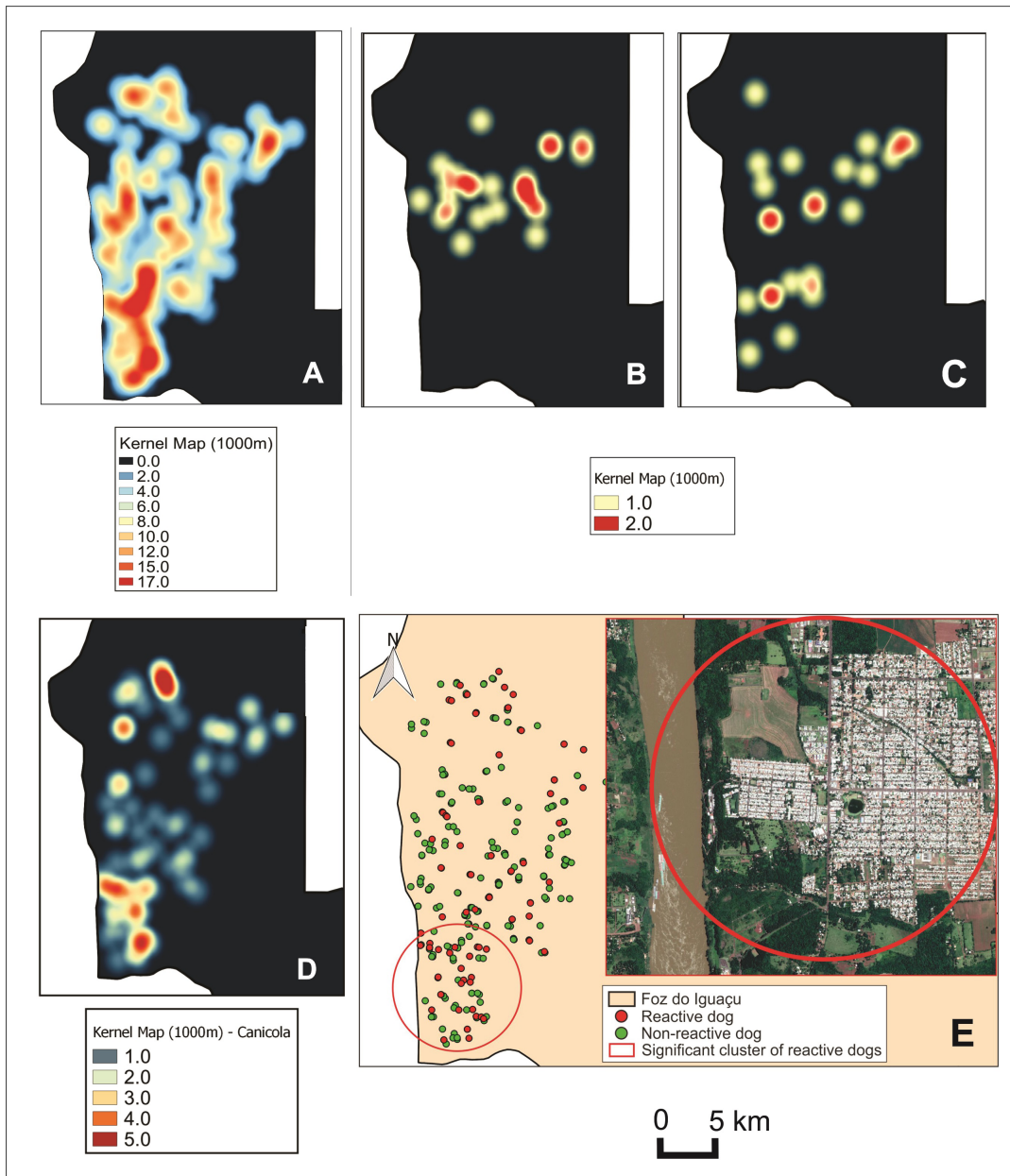


Figure 5. Kernel density analysis (1000m) of the presence of antibodies anti-*Leptospira* spp. (A), anti-serovar Bratislava (B), anti-serovar Butembo (C) and anti-serovar Canicola (D), simple distribution and relative spatial risk for dogs with anti-serovar Canicola antibodies (E), Foz do Iguacu, Paraná, Brazil, 2014.

Discussion

Toxoplasma gondii seroprevalence in dogs from Foz do Iguacu county (67.02%) is close to the upper limit found in Paraná in the last decade: 10.3% and 70.85% (DREER et al., 2013; CALDART et al., 2015; FERREIRA et al., 2016). Even though no epidemiological variables were statistically associated with this high prevalence, dogs that did not have access to the street, slept in the house and has a defined breed showed a higher frequency in the IFAT methodology. These characteristics show a greater affective contact of the tutors and that possibly the shared environment between them may offer conditions for infection by

the pathogenic agent, demonstrating the important role of the dog as a sentinel for toxoplasmosis.

This study showed that dogs from Foz do Iguacu had a low seroprevalence of antibodies anti- *N. caninum* (1.4%), and this was lower than that found in other regions of the country (TEIXEIRA et al., 2006; ROMANELLI et al., 2007; COIRO et al., 2011). The role of the dog in the epidemiology of neosporosis is evident, however, oocyst elimination is intermittent and short-term (LINDSAY et al., 2001) and there may be no seroconversion (DIJKSTRA et al., 2001). On the other hand, the possibility of horizontal transmission among rural dogs is generally greater due to carnivorous and the ease of ingestion of rodents, birds and

wild animals that may contain tissue cysts and serve as a source of parasite infection (WOUDA et al., 1999). Fernandes et al. (2004) and Cunha et al. (2008), in studies carried out in Minas Gerais and Rio Grande do Sul, respectively, described a higher seropositivity to *N. caninum* in dogs from rural areas compared to dogs from urban areas. Rural dogs probably have increased contact with the main intermediate host in the transmission chain, the bovine. It is possible that the low seropositivity for *N. caninum* found in this study is due to the fact that the population studied came from an urban area.

The prevalence of *Leptospira* spp. in dogs, in Brazil, has been shown to vary from 2.66% to 59.25% (BENITEZ et al., 2012; CALDART et al., 2015; SOUZA et al., 2016), the intermediate prevalence observed in this study (23.6%) demonstrates that infection by this agent is important in this region. The area B is a region of medium human population density with areas of interposition between rural and urban land. These environmental characteristics may be associated with the higher prevalence of animals that were seropositive to *Leptospira* spp., serovar Bratislava and serovar Butembo. The studied population has easy access to periurban areas, thus favoring contact between dogs and other possible reservoirs of the bacterium, including production or wild animals (AGUIAR et al., 2007). Dogs are the main reservoirs of the serovar Canicola, the most prevalent in this study (60.00%), they can eliminate the agent in urine for long periods of time, even if they are asymptomatic (FAINE, 1999). Area D, the most prevalent, includes the triple border and relatively uniform residential areas. A statistically significant cluster was found in this area, the specific site of the cluster is a fully residential area, probably with a greater number of dogs which can explain the concentration of seropositivity.

Recyclable garbage collectors have poor working conditions, and the garbage deposits are often in their own yards, which creates ideal conditions for the reproduction of rodents that serves as a source of transmission for various diseases, including leptospirosis. This explains the greater seroprevalence of *Leptospira* spp. ($p < 0.001$) in the dogs that accompanies waste picker and share this environment. Of these animals, 100.00% were reactive to the Canicola serovar. Even the sampling being small the main importance is, that these animals accompany the scavengers, who have a greater contact with street dogs and their contaminated environment. Yasuda et al. (1980) carried out a study in São Paulo city and isolated this serovar from street dogs, showing the important role of these animals as a source of *Leptospira* spp. infection in urban centers for humans and dogs. Sex was another intrinsic variable associated with seropositivity to leptospirosis, which may be due to the propensity of male dogs to travel long distances, following females in heat, and lick the genitalia of the females (BENITEZ et al., 2012). In addition, the territorial behavior of urine marking and/or the urine smell of other dogs are behaviors that favor both direct contact between healthy dogs and asymptomatic carriers and the transmission of leptospires, mainly of the Canicola serovar.

In dogs, leptospirosis is characterized by a variety of clinical signs, ranging from asymptomatic to superacute, acute, subacute or chronic. The presence of lymphadenomegaly (serovar Bratislava; $p = 0.04$), alopecia (*Leptospira* spp.; $p = 0.01$), ocular secretion

(*Leptospira* spp.; < 0.01) and weight loss (*Leptospira* spp.; $p = 0.03$) observed in the seropositive dogs in this study have already been described in case reports of canine leptospirosis, which allows us to suggest that dogs with these clinical signs and positivity to microscopic seroagglutination were infected. The intensity of the clinical signs may vary depending on the vaccination status of the dog, age, serovar virulence, degree of exposure to the disease and host immune response (FREIRE et al., 2007; MAELE et al., 2008; TOCHETTO et al., 2012).

The presence of ectoparasites and nutritional status are variables that may indirectly demonstrate a lack of purchasing power or concern for the dog, important points that reflect on the animal's health and may be associated with a lack of vaccination, inappropriate diet, access to the street, and an inadequate immune response. These factors alone do not lead to *Leptospira* spp. infection but increase the chance of infections in general. In this study, these variables were associated with seropositivity to *Leptospira* spp. and serovar Bratislava in particular.

The city of Foz do Iguaçu underwent a major transformation during the 1970s, the construction of a hydroelectric plant in the north of the municipality with a dam between Paraguay and Brazil (ZIOBER & ZANIRATO, 2014). The migratory process is an important factor related to the construction of a hydroelectric plant, Foz do Iguaçu had a population increase from 28,212 in 1960 to 263,782 to the present day (IBGE, 2016), which may have favored the marginalization of part of the population. The area D, near Argentina, is the poorest and has a set of substandard housing, many dogs wandering and frequent floods, which favors the increase of the population of rodents and the transmission of leptospirosis (SILVA & MOREIRA, 2013). The city has a very different composition and customs, either by the construction of the hydroelectric plant or by the proximity between two other countries or by the ecological tourism and intense shopping that collaborates for the installation and spread of diseases.

Rodent activity was found in 70.87% of the evaluated sites. These sites, even without a significant association, had a higher prevalence of antibodies against the three agents studied. The high occurrence of antibodies anti-*Leptospira* spp. in dogs at these sites is easily explained by the fact that rodents are the main reservoirs of some serovars and disseminators of *Leptospira* spp. (FAINE, 1999). For *T. gondii*, rodents are highly susceptible to infection (INNES, 1997) and are thus considered important prey for felines, the definitive host, and for some intermediate hosts, such as dogs (DUBEY et al., 1970). Romanelli et al. (2007), in Guarapuava, PR, observed the presence of rodents as an important risk factor for canine toxoplasmosis. With respect to *N. caninum*, Dantas et al. (2013) reported that dogs that cohabitated with rodents were 2.34 times more likely to be seropositive to *N. caninum*, the authors also stated that in the wild, naturally infected rodents can function as reservoirs and play an important role in the maintenance and dissemination of the agent and that infection of the dog can occur through hunting. The lack of statistical association between the diseases studied and the presence of rodents in the present study may have occurred due to the wide and homogeneous distribution of rodents throughout the city.

This is the first study that shows the prevalence of three pathogens that cause infectious-parasitic diseases in dogs at Foz do Iguaçu.

We suggest that dogs can be used as sentinels for toxoplasmosis and leptospirosis in Foz do Iguacu city, preventive measures should be taken by health officials because they are zoonoses and humans are also at risk because high prevalence rates have been observed.

Conclusion

The results indicate that *T. gondii* and *Leptospira* spp. are common in the studied canine population. Regarding *N. caninum*, although the parasite is circulating, a low seroprevalence was observed. This article shows that the use of dogs as sentinels for knowledge of the circulation of pathogens responsible for zoonoses is essential, since health is unique and multidisciplinary factors are responsible for installation and maintenance of the diseases. This research generates information that allows health managers to make decisions based on health rather than disease.

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