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Variables associated with the prevalence of anti-*Leishmania* spp. antibodies in dogs on the tri-border of Foz do Iguaçu, Paraná, Brazil

Variáveis associadas à prevalência de anticorpos anti-*Leishmania* spp. em cães na tríplice fronteira de Foz do Iguaçu, Paraná, Brasil

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Abstract

The aim of this study was to investigate the occurrence of anti-*Leishmania* spp. antibodies in dogs from localities in the city of Foz do Iguaçu, Paraná state, Brazil, on the border with Argentina and Paraguay. Blood samples dogs were collected to perform the following serologic tests: immunochromatographic DPP® rapid test, indirect immunoenzymatic assay (ELISA) and indirect immunofluorescence assay (IFA). In 2012, 285 dogs were analyzed on Argentina border, and in 2013, serum samples from 396 dogs on the border of Paraguay were collected. Using ELISA for screening and IFA for the confirmatory test, the results showed that the antibody prevalence was 1.8% (5/285) on the border of Argentina and 3.0% (12/396) on Paraguay border. When using the DPP® for screening and ELISA as a confirmatory analysis, we observed a seroreagent prevalence in dogs of 2.5% (7/285) on Argentina border and 5.1% (20/396) on Paraguay border. The non-public collection of domestic waste (p= 0.0004) was shown to be associated with leishmaniasis. This study shows the presence of leishmaniasis and suggest the emergence of canine visceral leishmaniasis in state of Paraná due to the confirmed occurrence of seroreactive dogs on Argentina and Paraguay border, which has environmental and geographical characteristics that favor the spread of the parasite.

Keywords: Zoonosis, one health, epidemiology, emergence.

Resumo

O objetivo deste estudo foi investigar a ocorrência de anticorpos anti-*Leishmania* spp. em cães da cidade de Foz do Iguaçu, estado do Paraná, Brasil, fronteira com a Argentina e o Paraguai. Amostras de sangue de cães foram coletadas para realização dos seguintes testes sorológicos: teste rápido imunocromatográfico DPP®, ensaio imunoenzimático indireto (ELISA) e ensaio de imunofluorescência indireta (IFI). Em 2012, 285 cães foram analisados na fronteira com Argentina e, em 2013, amostras de soro de 396 cães na fronteira com o Paraguai. Utilizando ELISA para triagem e IFA para o teste confirmatório, os resultados mostraram uma prevalência de anticorpos de 1,8% (5/285) na fronteira da Argentina e 3,0% (12/396) na fronteira com o Paraguai. Ao usar o DPP® para triagem e ELISA como uma análise confirmatória, observou-se uma prevalência de cães sororreagentes de 2,5% (7/285) na fronteira com a Argentina e 5,1% (20/396) na fronteira com o Paraguai. A não coleta pública de lixo doméstico (p = 0,0004) mostrou-se associada à leishmaniose. Este estudo demonstra a presença de leishmaniose e sugere a emergência da leishmaniose visceral canina no estado do Paraná devido à ocorrência de cães sororreagentes na fronteira Argentina e Paraguai, que possuem características ambientais e geográficas que favorecem a disseminação do parasito.

Palavras-chave: Zoonoses, saúde única, epidemiologia, emergência.

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Introduction

Zoonotic visceral leishmaniasis (VL) is a disease with global distribution. In the Americas, the etiological agent responsible for this disease is the protozoan *Leishmania infantum* (Kinetoplastida, Trypanosomatidae) (NICOLLE, 1908; LAINSON & SHAW, 1987; READY, 2014). In endemic areas, dogs are the reservoirs of greatest epidemiological importance as source of infection for the vectors (ASHFORD, 1996; DANTAS-TORRES, 2007; COSTA, 2011). The incidence of VL in humans is related to the number of canine visceral leishmaniasis (cVL) cases and the presence and density of sand fly species competent to transmit the parasite from dogs to humans (WERNECK, 2014; ORTIZ & ANVERSA, 2015).

In Brazil, female phlebotomine sand flies of the species *Lutzomyia longipalpis* are the main vector responsible for the transmission of *L. infantum* (LAINSON & RANGEL, 2005). This vector was first identified in the southern region of Brazil in São Borja, Rio Grande do Sul (RS), in 2009, but the first canine case was reported in 2008 (SOUZA et al., 2009; FIGUEIREDO et al., 2012). Entomological surveys in Paraná state have been performed for years, and no competent vectors were observed for VL (TEODORO et al., 2003; MEMBRIVE et al., 2004; TOMAZ-SOCCOL et al., 2009; TEODORO et al., 2010). Santos et al. (2012) identified for the first time *Lu. longipalpis* in the state, in the city of Foz do Iguaçu. One of the factors that may have led to the entrance of the vector in Paraná is that on the triple border of Brazil, Argentina and Paraguay are records of VL cases. Reports in Paraguay display an increase in the incidence of human VL (HVL), with the disease appearing in the province of Alto Paraná, a region bordering Foz do Iguaçu (CANESE, 2010; PARAGUAY, 2011). From May 2006 to July 2012, 103 HVL cases were notified in Argentina, and dogs infected with *L. infantum* were reported throughout almost the entire country (SALOMÓN et al., 2008, 2011; GOULD et al., 2013; BARROSO et al., 2015). On the Argentina border of Foz do Iguaçu, in the city of Puerto Iguazú, the presence of the vector *Lu. longipalpis* was verified in a study conducted in 2010 (SALOMÓN et al., 2011), and in 2013, the DNA presence of *L. infantum* was confirmed in samples of dogs from this city (ACOSTA et al., 2015).

Foz do Iguaçu is a vulnerable city to this zoonosis because of the great migratory flow, its border with countries with VL cases and the identification of the vector *Lu. longipalpis* (SANTOS et al., 2012). Therefore, the present study was developed to investigate the occurrence of anti-*Leishmania* spp. antibodies in dogs from localities close to the border of Argentina and Paraguay and to identify the variables associated with VL.

Materials and Methods

Study area

The city of Foz do Iguaçu had an estimated average population of 259,313 inhabitants in 2012 and 2013 (IBGE, 2012, 2013). It is located in the extreme west of Paraná State, on the border of Paraguay and Argentina, at latitude 25º32'45 "S and longitude 54º35'07" W. Foz do Iguaçu shares borders with Itaipulândia (Brazil) in the north, Puerto Iguazú (Argentina) in the south, Santa Terezinha de Itaipu and São Miguel do Iguaçu (Brazil) in the east, and Ciudad del Este (Paraguay) in the west. It experiences a humid subtropical climate, with hot summers, rare frosts and rain during all months of the year. It has nine hydrographic watersheds, seven of which are circumscribed to the municipal perimeter, with the rivers Paraná, Iguaçu, Tamanduá, São João, Almada, M'Boicy and Monjolo (IBGE, 2016; FOZ DO IGUAÇU, 2017).

Sampling site

Due to the emergency appearance of cVL in Foz do Iguaçu, the study was initially performed with authorization from the Ninth Health Region, Paraná State, and was later approved by the Ethics Committee on Animal Use of Londrina State University (CEUA nº. 22530.2013).

The method used to select the residences and to sample the dogs' blood involved selecting locations where sand fly traps were installed in entomological surveys performed by the Ninth Health Region prior to the collections. Houses were randomly chosen out of those in which a dog was present. For the blood collection, the owners signed a term of authorization and awareness and an epidemiological questionnaire that included information about the environment, origin of the animal and its clinical characteristics.

Firstly, from May 4th to 18th of 2012, collections were conducted in localities near the border of Argentina and the rivers Iguaçu and Paraná. In a second sampling stage, from June 17th to July 4th of 2013, blood was collected from dogs in localities close to the border of Paraguay and the Paraná river and from a few other localities far from the borders (Figure 1). The samples were processed at the Ninth Health Region, Paraná State, at the Department of Bioprocess Engineering and Biotechnology laboratory of the Federal University of Paraná (UFPR), and at the laboratory of Protozoology of the Department of Preventive Veterinary Medicine of the Londrina State University.

Serodiagnosis

To determine the presence of anti-*Leishmnia* spp. antibodies and to identify the prevalence of seroreagent animals for leishmaniasis, the serodiagnostics followed two criteria of the screening and confirmatory tests adopted by the Brazilian Ministry of Health: the enzyme-linked immunosorbent assay (ELISA) for screening with the immunofluorescence assay (IFA) as the confirmatory test recommended by the Surveillance and Control Program of Leishmaniasis until 2012, and in order to improve the cVL diagnostic technique this protocol was replaced for the DPP® rapid test (DPP®) for screening with the ELISA as a confirmatory test (BRASIL, 2006, 2011).

The DPP® test was performed using serum samples according to the manufacturer's protocol (Bio-Manguinhos/Fiocruz, Rio de Janeiro, BR). The ELISA test was standardized according to Maziero et al. (MAZIERO et al., 2014). The cut-off point was determined based on the average absorbance of four negative sera

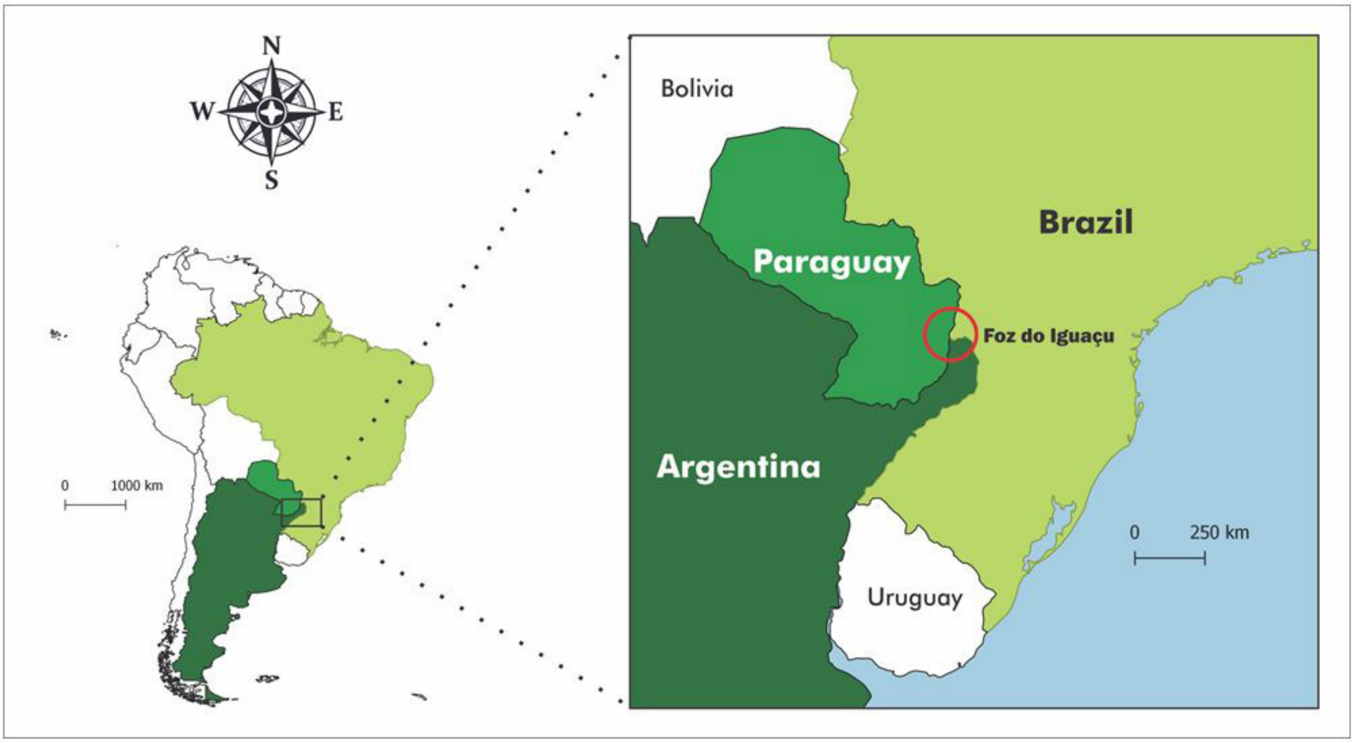

Figure 1. Map showing the location of the tri-border area: Foz do Iguaçu (Brazil) frontier with Puerto Iguaçu (Argentina) and Ciudad del Este (Paraguay).

(dogs born in Curitiba - Paraná) plus three standard deviations, repeated on each plate.

The IFA was standardized according to Marzochi et al. (1980), and the readings were performed under an epifluorescence microscope with a 40x objective. Sera were diluted from 20, and reagents with a titer greater than or equal to 40 were considered (MARZOCHI et al., 1980).

Statistical analysis

The EpiInfo 6 program (DEAN et al., 1994) was used to tabulate and analyze the variables that composed the epidemiological questionnaire along with the serological results found. For the univariate analysis, Pearson's Chi-square test for Independence or Fisher's Exact test were used. The significance level of 5% ($p < 0.05$) was adopted to reject the null hypothesis, and the *odds ratio* (OR) as a measure of association with a 95% confidence interval (CI) was used.

Results

Of the 285 dogs analyzed for anti-*Leishmania* spp. antibodies on the border of Argentina, 3.2% (9/285) were reagent for the DPP® test, 30.5% (87/285) for the ELISA, and 4.6% (13/285) for IFA. Of the 87 reagent animals for ELISA, five (5/87) were also reagent for IFA and seven (7/87) for DPP®. Only one dog was reactive on all three serological tests.

Of the 396 dogs analyzed on the border of Paraguay for anti-*Leishmania* spp. antibodies, 7.6% (30/396) were reagent for the DPP® test, 21.2% (84/396) for the ELISA and 4.3% (17/396) for IFA. Of the 84 reagent animals for ELISA, 12 (12/84) were also reagent for IFA and 20 (20/84) for DPP®. Of the nine dogs that were seroreagent on all three tests, all were autochthonous, two were from the same residence and one dog was from a neighboring residence; all of the dogs were located in the central region of Foz do Iguaçu (Figure 2).

When we used the ELISA as a screening test and the IFA as confirmatory, the prevalence of anti-*Leishmania* spp. antibodies was 1.8% (5/285) on the border of Argentina and 3.0 (12 / 396) on the border of Paraguay. However, when DPP® was used as a screening test and ELISA as confirmatory, a seroreagent animal prevalence of 2.5% (7/285) was observed on the border of Argentina and 5.1% (20/396) on the border of Paraguay (Table 1).

Tables 2 and 3 show the epidemiological data and characteristics of the dogs on the borders of Argentina and Paraguay, respectively, and a significant difference in the non-public collection of domestic waste ($p = 0.0004$; OR = 0.07; 95% CI = 0.02-0.30) was found only on the border of Paraguay (Table 3), demonstrating a variable risk of leishmaniasis in this area.

An analysis of the entire population of dogs (Table 4) showed that most of the animals were female, without an identified breed; were aged between two and eight years old and were from the city of Foz do Iguaçu. The majority lived in urban areas with woods very close to the household. In both collection regions, the presence of other animal species was verified in the peridomiciliary area and mainly included cats (22.1% on the border of Argentina

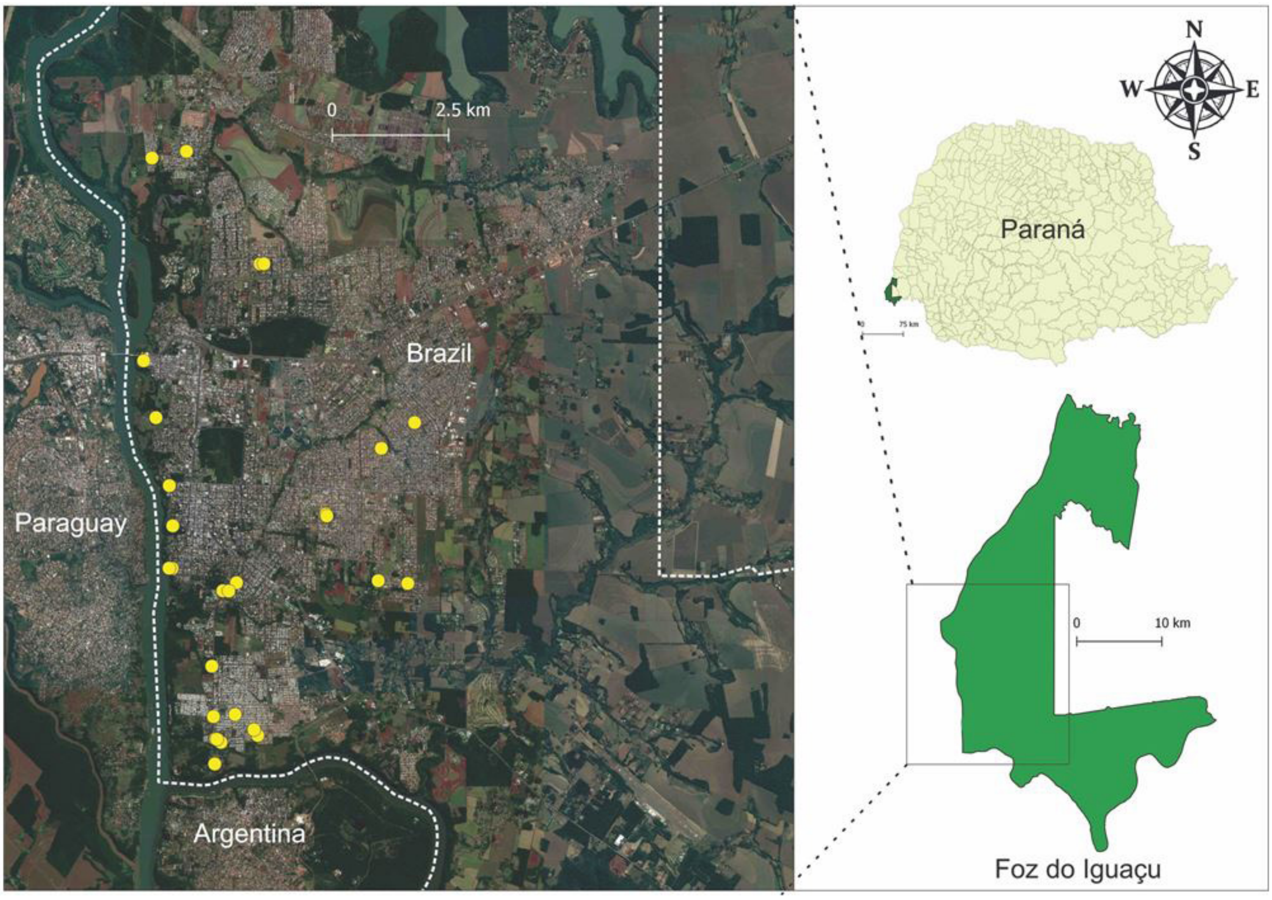

Figure 2. Dispersion of seroreactive dog samples in more than one serological test (immunochromatographic test, enzyme-linked immunosorbent assay and indirect immunofluorescence assay), in Foz do Iguaçu city, Paraná State, Brazil, 2012 - 2013.

Table 1. Prevalence of anti-*L. infantum* antibodies according to the immunochromatographic test (DPP®), the enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescence assay (IFA), in dogs from localities close to the border region of Foz do Iguaçu city, Paraná, in Argentina and Paraguay, 2012 and 2013.

Region	Number of serum samples	DPP® %	ELISA %	RIFI %	ELISA[1] RIFI %	DPP®[2] ELISA %	Prev.[1]	Prev.[2]
Bordering Argentina	285	3.2	30.5	4.6	5	7	1.8%	2.5%
Bordering Paraguay	396	7.6	21.2	4.3	12	20	3.0%	5.1%

Prev. = Prevalence; [1] = Protocol of Brazil Ministry of Health until 2012; [2] = Protocol of Brazil Ministry of Health after 2012.

and 50% on the border of Paraguay), hens (23.9 and 16.7%, respectively) and rats (37.5 and 20.2%); organic matter was also observed due to the presence of gardens (40.4 and 45.7%) and the accumulation of leaves (39.3 and 42.7%).

Most of the domestic sewage was destined for the sewage network (52% on the border of Argentina and 49.1% on the border of Paraguay) or for the septic tank (38.1% on the border of Argentina and 47.8% on the border of Paraguay). Regarding the final destination of domestic waste, most of the households used the public collection system (88% on the border of Argentina and 94.4% on the border of Paraguay) (Table 4).

Of the dogs that tested as reagents based on an ELISA screening test and an IFA confirmatory test in the region bordering Argentina, two (2/5) had clinical signs: one had anorexia, weight loss and prostration; and the other had a skin lesion with a nodular aspect on the limb. With DPP® as a screening test and an ELISA confirmatory test, two (2/7) dogs showed clinical signs: one animal with anorexia, a skin lesion with alopecia, weight loss and prostration; and another with a skin lesion with raised borders on the limb and onychogryphosis.

In the region bordering Paraguay, one dog presented clinical signs of skin lesions and was reactive in an ELISA screening test and IFA confirmatory test (1/12); the dog was also reactive with a DPP® screening test and an ELISA confirmatory test (1/20).

Discussion

This study carried out in Foz do Iguaçu (PR), when based on the diagnosis criteria adopted by the Brazilian Ministry of Health until the year 2012 (ELISA as a screening test and IFA as

Table 2. Epidemiological data and characteristics of the dogs analyzed in Foz do Iguaçu city, Paraná, on the border of Argentina, 2012.

Variables	Prot.[1]			Prot.[2]		
	Seroreactive/ Total (%)	P-value	OR (95%CI)	Seroreactive/ Total (%)	P-value	OR (95%CI)
Place of residence						
Urban area	5/226 (2.2)	0.6553(1)				
Rural area	0/56 (0.0)			2/56 (11.2)		
Presence of woods close to the household						
Yes	4/241 (1.7)	>0.9999(1)	Ind.**	5/241 (2.1)	>0.9999(1)	0.62 (0.07-5.44)
No	0/30 (0.0)			1/30 (3.3)		
Distance of woods from household						
0-300 meters*	0/117 (0.0)			4/117 (3.4)		
300-500 meters	3/50 (6.0)	0.0514(1)	0 (0.0-1.01)	0/50 (0.0)	0.4744(1)	Ind.**
More than 500 meters	1/61 (1.6)	0.6854(1)	0 (0.0-20.3)	1/61 (1.6)	0.8810(1)	2.1(0.20-106.3)
Presence of other animals						
Yes	4/201 (2.0)	>0.9999(1)	1.62 (0.16-80.98)	7/201 (3.5)	0.1813(1)	Ind.**
No	1/81 (1.2)			0/81 (0.0)		
Presence of organic matter in the backyard						
Yes	5/217 (2.3)	0.5533(1)	Ind.**	6/217 (2.8)	>0.9999(1)	1.76 (0.21-82.3)
No	0/63 (0.0)			1/63 (1.6)		
Public collection of domestic waste						
Yes	5/241 (2.08)	>0.9999(1)	Ind.**	5/241 (2.08)	0.4020(1)	0.33 (0.05-3.61)
No	0/33 (0.0)			2/33 (6.1)		
Dog with identified breed						
Yes	1/62 (1.6)	>0.9999(1)	0.85 (0.02-8.79)	0/62 (0.0)	0.3220(1)	0.0 (0.0-2.35)
No	4/211 (1.9)			7/211 (3.3)		
Gender						
Male	2/139 (1.4)	>0.9999(1)	0.67 (0.06-5.97)	5/139 (3.6)	0.4355(1)	2.59 (0.41-27.59)
Female	3/141 (2.1)			2/141 (1.4)		
Age group						
≤ 1 year*	0/54 (0.0)			0/54 (0.0)		
2 ┤ 8 years	4/199 (2.0)	0.7606(1	0 (0.0-5.62)	6/199 (3.0)	0.4659(1)	0 (0.0-2.38)
> 8 years	1/23 (4.4)	0.5974(1)	0 (0.0-16.61)	1/23 (4.4)	0.5974(1)	0 (0.0-16.61)
Dog came from another city						
Yes	0/18 (0.0)	>0.9999(1)	0 (0.0-16.35)	0/18 (0.0)	>0.9999(1)	0.0 (0-10.43)
No	5/257 (2.0)			7/257 (2.7)		

Prot.[1] = Protocol of Brazil Ministry of Health until 2012; Prot.[2] = Protocol of Brazil Ministry of Health after 2012. * = Reference category; ** = Undefined. (1) Fisher's exact test.

confirmatory), showed a anti-*Leishmania* spp. antibodies prevalence of 1.8% (5/285) on the border of Argentina whereas a prevalence of 3.0% (12/396) was found on the border of Paraguay. In a study conducted in São Borja, Rio Grande do Sul (RS), from February 2009 to December 2010, the prevalence was 22.5% in 5,400 dogs serological samples. In the same period, a prevalence of 14% was observed in dogs from Uruguaiana (RS), and a prevalence of 4.1% was observed in dogs from Porto Alegre (RS) (TARTAROTTI et al., 2011). Hirschmann et al. (2015) evaluated 165 dogs from kennels and non-governmental organizations (NGOs) from 12 cities in the state of Rio Grande do Sul, that were disease-free for cVL, and found a prevalence of 3.0% (5/165). In the state of Santa Catarina, which borders the state of Paraná, Figueiredo et al. (2012) described the first autochthonous cVL cases in the city of Florianópolis. Afterwards, an epidemiological survey was conducted on 2,124 dogs from seven districts in the city of Florianópolis, and the prevalence was 1.4% (29/2,124) (STEINDEL et al., 2013). Maziero et al. (2014) showed a rate of 21% in the western part of the Santa Catarina border of Paraná state.

When the criterion adopted by the Ministry of Health was used in this study (DPP® as a screening test and ELISA as a confirmatory test), an anti-*Leishmania* spp. antibodies prevalence of 2.5% (7/285) was found on the border of Argentina and 5.1% (20/396) on the border of Paraguay. In the State of Rio Grande

Table 3. Epidemiological data and characteristics of the dogs analyzed in Foz do Iguaçu city, Paraná, on the border of Paraguay, 2013.

Variables	Prot.[1]			Prot.[2]		
	Seroreactive/ Total (%)	P-value	OR (95%CI)	Seroreactive/ Total (%)	P-value	OR (95%CI)
Place of residence						
Urban area	10/364 (2.8)	0.5034[(2)]	0.43 (0.08-4.17)	16/364 (4.4)	0.1354[(2)]	0.32 (0.10-1.42)
Rural area	2/32 (6.3)			4/32 (12.5)		
Presence of woods close to the household						
Yes	11/345 (3.2)	>0.9999[(2)]	1.61 (0.23-70.86)	17/345 (4.9)	0.9526[(2)]	0.81 (0.22-4.49)
No	1/50 (5.0)					
Distance of woods from household						
0-300 meters*	7/158 (4.4)			11/158 (7.0)		
300-500 meters	2/97 (2.1)	0.5323[(2)]	2.2 (0.41-22.1)	6/97 (6.2)	>0.9999[(2)]	1.13(0.34-3.87)
More than 500 meters	1/80 (1.3)	0.3718[(2)]	3.7 (0.46-30.29)	0/80 (0.0)	0.0195[(2)]	Ind.**
Presence of other animals						
Yes	6/253 (2.4)	0.4469[(2)]	0.54 (0.14-2.08)	10/253 (4.0)	0.2566[(2)]	0.54 (0.19-1.48)
No	6/140 (4.3)			10/140 (7.1)		
Presence of organic matter in the backyard						
Yes	10/328 (3.1)	>0.9999[(2)]	1.02 (0.21-9.81)	17/328 (5.2)	>0.9999[(2)]	1.17 (0.32-6.4)
No	2/67 (3.0)			3/67 (4.5)		
Public collection of domestic waste						
Yes	7/371 (1.9)	0.0004[(1)]	0.07 (0.02-0.30)	14/371 (3.8)	0.0007[(1)]	0.11 (0.03-0.38)
No	5/22 (22.7)			6/22 (27.3)		
Dog with identified breed						
Yes	4/144 (2.8)	>0.9999[(2)]	0.86 (0.19-3.28)	5/144 (3.5)	0.3838[(1)]	0.56 (0.18-1.53)
No	8/249 (3.2)			15/249 (6.0)		
Gender						
Male	6/181 (3.3)	0.9929[(1)]	1.19 (0.36-3.98)	10/181 (5.5)	0.8687[(1)]	1.20 (0.48-3.29)
Female	6/215 (2.8)			10/215 (4.7)		
Age group						
≤ 1 year*	0/69 (0.0)			1/69 (1.5)		
2 ┤8 years	9/277 (3.3)	0.2631[(2)]	0.0 (0.0-2.02)	16/277 (5.8)	0.2263[(2)]	0.24 (0.01-1.6)
> 8 years	3/49 (6.1)	0.1381[(2)]	0.0 (0.0-1.69)	3/49 (6.1)	0.2263[(2)]	0.23 (0.004-2.9)
Dog came from another city						
Yes	0.0/16 (0.0)	>0.9999[(2)]	0.0 (0.0-9.79)	0/16 (0.0)	0.8330[(2)]	0.0 (0.0-4.90)
No	11/367 (3.0)			20/367 (5.5)		

Prot.[1] = Protocol of Brazil Ministry of Health until 2012; Prot.[2] = Protocol of Brazil Ministry of Health after 2012. * = Reference category; ** = Undefined; [(1)] Pearson's chi-squared test; [(2)] Fisher's exact test.

do Sul, Hirschmann et al. (2015) reported a prevalence of 1.8%. In endemic areas, prevalences of 3.1% and 9.2% were found in Campinas, state of São Paulo (VON ZUBEN et al., 2014) and the Federal District (HERENIO et al., 2014), respectively.

DPP® was developed to detect antibodies against rK26/rK39 antigens and shows high specificity (96%) and low sensitivity (47%) to identify dogs without clinical signs of VL. However, in the presence of signs, the sensitivity of the test increases (98%) (GRIMALDI et al., 2012). According to Alves et al. (ALVES et al., 2012), the sensitivity and specificity rates remain high regardless of whether the animals are infected with *Trypanosoma caninum*. The DPP® test as a screening test has an advantage over ELISA because of its easy manipulation and ability to show results in 15 minutes; therefore, it can be used in both the field and the laboratory (SANTIS et al., 2013).

Foz do Iguaçu did not conduct previous studies on leishmaniasis; however, studies conducted in other cities of Paraná demonstrated that the regions analyzed were disease-free for cVL, a fact that allows us to affirm that the area where the study was performed in Foz do Iguaçu was not endemic (TEODORO et al., 2003, 2010; MEMBRIVE et al., 2004; THOMAZ-SOCCOL et al., 2009). The low prevalence showed in the DPP® test can be explained by the

Table 4. Frequency of the characteristics of the environment and dogs analyzed in Foz do Iguaçu city, Paraná, on the border with Argentina and Paraguay, 2012 and 2013.

Variables	Number (%) of residences on the International border	
	Argentina	Paraguay
Place of residence		
Urban area	226 (80.1)	364 (91.9)
Rural area	56 (19.9)	32 (8.1)
Presence of woods close to the household		
Yes	241 (88.9)	345 (87.3)
No	30 (11.1)	50 (12.7)
Distance of woods from household		
0 – 300 meters	117 (51.3)	158 (47.2)
300 – 500 meters	50 (21.9)	97 (28.9)
More than 500 meters	61 (26.8)	80 (23.9)
Presence of other animals		
Yes	201 (71.3)	253 (64.4)
No	81 (28.7)	140 (35.6)
Type of animals		
Wild animal	32 (11.2)	4 (1.0)
Mouse	8 (2.8)	8 (2.0)
Equine	68 (23.9)	66 (16.7)
Hen	55 (19.3)	21 (5.3)
Opossum	63 (22.1)	198 (50.0)
Cat	9 (3.2)	0 (0.0)
Monkey	0 (0.0)	2 (0.5)
Mule	107 (37.5)	80 (20.2)
Presence of organic matter in the backyard		
Yes	217 (77.5)	328 (83.0)
No	63 (22.5)	67 (17.0)
Type of organic matter		
Leaf accumulation	112 (39.3)	169 (42.7)
Vegetable garden	40 (14.0)	59 (14.9)
Garden	115 (40.4)	181 (45.7)
Waste	43 (15.1)	67 (16.9)
Orchard	81 (28.4)	64 (16.2)
Dog with identified breed		
Yes	62 (22.7)	144 (36.6)
No	211 (77.3)	249 (63.4)
Gender		
Male	139 (49.6)	181 (45.7)
Female	141 (50.4)	215 (54.3)
Age group		
≤ 1 year	54 (19.6)	69 (17.5)
2 ┤ 8 years	199 (72.1)	277 (70.1)
> 8 years	23 (8.3)	49 (12.4)
Dog came from another city		
Yes	18 (6.5)	16 (4.2)
No	257 (93.5)	367 (95.8)

fact that Foz do Iguaçu is an area of low incidence of leishmaniasis cases and most canine cases were asymptomatic. Consequently, the DPP® test is likely not efficient for a non-endemic area.

Although some commercial kits are available, most information on the ELISA diagnostic performance comes from in-house tests (PALTRINIERI et al., 2010), which can be performed with crude, synthetic or recombinant antigens (MAIA & CAMPINO, 2008), of which recombinant antigens shows the best results (MIRÓ et al., 2008; MARCONDES et al., 2011). Crude antigens provide a specificity of 87%, whereas the recombinant antigens rK26 and A2 provide specificities of 90% and 96%, respectively (PORROZZI et al., 2007). The advantage in the use of ELISA versus IFA is in its semi-automation, which eliminates the IFA subjectivity; moreover, ELISA is a technique that can be applied to a large number of samples in a short period of time (MAIA & CAMPINO, 2008; LUCIANO et al., 2009).

IFA shows a sensitivity between 83% and 100% and a specificity of approximately 80%. IFA requires specialized, high-cost equipment and trained staff due to the subjective interpretation in the evaluation of the fluorescence intensity by microscopy (EDRISSIAN & DARABIAN, 1979; BARBOSA-DE-DEUS et al., 2002; PALTRINIERI et al., 2010; FARIA & ANDRADE, 2012).

One of the main limitations of ELISA and IFA techniques is the occurrence of cross-reactions with other species of the Trypanosomatidae family, particularly *Trypanosoma* sp., due to the phylogenetic proximity between the species (CAMARGO & REBONATO, 1969; CABALLERO et al., 2007; LUCIANO et al., 2009; PALTRINIERI et al., 2010). To reduce the risks of cross-reaction, the adsorption of sera with *Trypanosoma* sp. antigens could be made, but it is not routinely applicable.

The non-public collection of domestic waste was related as a risk factor for leishmaniasis. The presence of organic matter in the peridomicile represents the possible shelters and vector breeding sites and reflects the importance of the environment to maintain the parasite cycle (GONÇALVES, 2014). When there is regular waste collection, there is a lower leishmaniasis rate, which occurs because of the peridomicile reorganization and cleaning, leading to a reduction in the population of sand flies in these environments and, thus, reducing the risk of transmission of *Leishmania* to humans and domestic animals (MEMBRIVE et al., 2004; LAINSON & RANGEL, 2005).

Most leishmaniasis cases in dogs were autochthonous with the residence located in urban areas and close to the woods. In Brazil, VL was first considered a rural disease; however, since the 1980s, it has been spreading to urban areas (WERNECK, 2014) where dogs are considered the main reservoir of the parasite (MARCONDES & ROSSI, 2013). Environmental degradation, migratory flows and unplanned urban occupation favor the adaptation of vectors in the environment (SILVA et al., 2015). Acosta et al. (2015) reported that VL in Puerto Iguazú, Argentina was established due to changes in the urbanization of the vector and the existence of reservoirs (dogs) and vectors (*Lu. longipalpis*). Moreover, the geographical position of the city (triple border region) with tourist flow throughout the year nearby Iguazu National Park (Iguazu Falls) contributes to the spread of the disease.

The presence of other animals, mainly cats, chickens and rats, was observed in the analyzed residences. In some areas, dogs are not the preferred source of blood of *Lu. longipalpis* (MARCONDES & ROSSI, 2013), and the etiologic agent can remain naturally harbored in wild animals (SCHIMMING & PINTO E SILVA, 2012). Afonso et al. (2012) found evidence of natural infection by *L. infantum* in opossums and found that these animals were feeding sources for the vector. The authors report the possibility that this synanthropic mammal participates in the VL transmission cycle in some regions, particularly those that experienced environmental changes, which facilitates the contact of these animals with human habitation. Controlling the invasion of urban areas by wild animals in search of food are control measures that can be adopted by the community, reducing the link between urban and wild cycles (SCHIMMING & PINTO E SILVA, 2012).

An integrated surveillance system involves human and animal health and wildlife sectors working together to detect unusual disease events that trigger a response to contain and control intervention measures (HÄSLER et al., 2012). This context fits into the One Health concept, with the premise that people, animals and the environment form an interdependent ecosystem that must be considered in a coordinated way (FRANK, 2008; GIBBS, 2014). To achieve this system, a clear leadership and coordination, common goals and objectives, tools for data collection and analysis, integrated contingency plans and a good field communication are required (HÄSLER et al., 2012).

The data obtained in the present study demonstrate the presence of leishmaniasis and suggest the emergence of cVL in Foz do Iguaçu in the tri-border area with Argentina and Paraguay, which presents population characteristics (tourist and commercial region, intense flow of people and animals), environmental (residences with accumulation of organic matter and near to woods), and the presence of autochthonous dogs. All these factors must collaborate in the maintenance of the zoonosis and provide subsidies for a One Health approach.

Since 2013, the technical collaboration of the Pan American Health Organization has assisted in the adoption of strategies in this work, and it has been verified that joint measures with integrated and transdisciplinary eco-socio-systemic approaches must be adopted among the countries to achieve VL control. Thus, prevention and control measures at the local, regional, national and international levels should be conducted in an uniform manner by standardizing collection, processing and analysis of samples, and approaching ecological, epidemiological and socio-cultural variables of interest at these levels and defining control strategies and programs in the three countries.

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