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ScienceDirect
Experimental Parasitology 117 (2007) 13–21
Experimental Parasitology
www.elsevier.com/locate/yexpr

Leishmania (*Viannia*) *braziliensis*: Epidemiology of canine cutaneous leishmaniasis in the State of Paraná (Brazil)

Edilene A. Castro [a], Vanete Thomaz-Soccol [a,*], Christopher Augur [b], Ennio Luz [a]

[a] *Laboratório de Parasitologia Molecular, Departamento de Patologia Básica, Universidade Federal do Paraná, Centro Politécnico, Jardim das Américas, CEP81531-990, Curitiba, Paraná, Brazil*

[b] *IRD, Laboratoire BioTrans, UR 185, Boite-441, IMEP, Université Paul Cézanne, Faculté des Sciences de St. Jérome, Avenue Escadrille Normandie-Niemen, F-13397, Marseille Cedex 20, France*

Received 26 July 2006; received in revised form 2 March 2007; accepted 9 March 2007
Available online 16 March 2007

Abstract

The present study examines the role that dogs play in the maintenance of the *Leishmania* cycle in the State of Paraná, Southern Brazil. Dogs were examined in three regions where cutaneous leishmaniasis is endemic or epidemic (**R1**—Vale da Ribeira; **R2**—Central region of Paraná State and **R3**—Northern region). To determine serum prevalence rates ELISA was used. In regions endemic for *Trypanosoma cruzi* (**R1** and **R3**), serum from dogs seroreactive towards *Leishmania* antigen was subjected to *T. cruzi* adsorption in order to eliminate cross-reaction with common antigen epitopes. Concomitantly, dogs with cutaneous lesions were biopsied to isolate and identify parasites using RAPD. *Leishmania* were classified by the phenetic method using the Jaccard coefficient of similarity, and grouped by Unweighted Pair-Group Method using an Arithmetic Average (UPGMA). A total of 410 dogs were studied. In **R1** (Vale da Ribeira) 159 dogs were evaluated of which 10 had anti-*Leishmania* antibody. In **R2** (Central Paraná), 39 animals were examined of which 8 were seropositive. In **R3** (the North) 212 dogs were evaluated of which 39 animals were seropositive. Thirteen dogs had cutaneous lesions and the parasites were isolated from a dog with mucocutaneous lesion in **R1**, two animals with simple skin lesions in **R2** and 10 dogs with multiple lesions in **R3**. The identification of the parasite by molecular methods showed it to be *L.* (*Viannia*) *braziliensis.* Based on this information, the role of domestic dogs in *Leishmania* infection of cutaneous leishmaniasis in Paraná is discussed.
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Index Descriptors and Abbreviations: *Leishmania* (*Viannia*) *braziliensis*; Cutaneous leishmaniasis; Dogs; Enzyme linked immunosorbent assay (ELISA); Random amplified polymorphic DNA (RAPD); Brazil

1. Introduction

The genus *Leishmania* Ross, 1903 is currently found in 22 countries of the New World, from the Yucatan Peninsula in Mexico to Northern Argentina and Southern Brazil. Within this same region there are at least 20 species of *Leishmania* causing a wide variety of clinical syndromes (Desjeux, 1996). While the epidemiology, with respect to vectors and reservoirs, of many species is well known, that of *Leishmania* (*Viannia*) *braziliensis* is poorly known, yet it is found throughout the neotropics, and infections have been found in a variety of animals (Lainson and Shaw, 1987; WHO, 1990; Shaw, 2002). Recently rodents have been implicated as potentially important reservoirs (Brandão-Filho et al., 2003).

In Brazil, cases of cutaneous leishmaniasis have been increasing since the 1980s with, on average, 28 thousand new cases per year. In the State of Paraná, Southern Brazil, 3906 human cases were recorded between 2000 and 2005. Autochthonous transmission is found in three regions: in Vale da Ribeira where the disease has been recorded for more than a century, with 49 human cases from 2000 to

* Corresponding author. Fax: +1955413266042.
E-mail address: vanete.soccol@pesquisador.cnpq.br (V. Thomaz-Soccol).

0014-4894/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2007.03.003

2005; in the Central region, where leishmaniasis has only been reported since 2002 with 116 cases, and in the Northern region of the State, where cutaneous leishmaniasis cases have been colonized with an outbreak in 1994 (Brazil, 2006). The parasite isolated from humans in the different regions is *L.* (*V.*) *braziliensis* (Silveira et al., 1996; Thomaz-Soccol et al., 2003; Castro et al., 2005). The vector, *Lutzomyia whitmani,* has been found infected with the same species (Luz et al., 2000). Only one wild animal (*Nectomys* sp.) has been found infected with *L.* (*V.*) *braziliensis* (Thomaz-Soccol et al., 2003). However, domestic dogs have been found with *Leishmania* infection (Silveira et al., 1996; Castro et al., 2005). Could dogs be replacing wild animals as a reservoir?

To incriminate a host as a reservoir, extensive ecological studies are necessary to demonstrate that the parasite is indistinguishable from those in man and vector. In addition, the animal population must be abundant, live long enough to be associated with sand flies, have intense contact with the vectors, and have a high proportion of animals infected. The infection should also be relatively nonpathogenic for the reservoirs (WHO, 1990).

In the absence of wild reservoir species with the same geographic distribution as *L.* (*V.*) *braziliensis*, many researchers have turned their attention to domestic dogs living in the same area where cutaneous leishmaniasis occurs. The first *Leishmania* infection in dogs in Brazil was reported in the State of São Paulo (Pedroso, 1913). The parasite was later found in dogs in Argentina (Mazza, 1926) and Peru (Herrer, 1949/51).

Since the 1970s, dogs have been frequently implicated in leishmaniasis transmission in Latin America and their importance as a reservoir is still being discussed (Dias et al., 1977; Falqueto et al., 1986; Le Pont et al., 1989; Vasconcelos et al., 1994; Madeira et al., 2005).

The aim of this work was to examine the role of the domestic dog (*Canis familiaris*) in the maintenance of the *Leishmania* cycle in three zones in the State of Paraná, Southern Brazil where cutaneous leishmaniasis transmission is endemic or epidemic.

2. Materials and methods

2.1. Study area

The State of Paraná is situated in Southern Brazil between 22°29′23″ and 26°42′59″S, and 48°02′24″ and 54°37′38″W. In this State cutaneous leishmaniasis is endemic or epidemic and the present study was carried out in three main zones transmission.

The first (**R1**) is located in the Vale da Ribeira region, from 48°59′00″W (Adrianópolis) to 49°10′22″W (Rio Branco do Sul) and 24°34′00″S (Doutor Ulysses) to 25°10′22″S (Rio Branco do Sul) with a very humid tropical climate without a dry season (18 °C average temperature, 115 mm/month rainfall, 85% relative humidity, and 100–300 m elevation). Dogs from six places in different valleys in the Adrianopólis county: Barra Grande (1), Laranjal (2), Perau (3), Capela (4), Colônia (5), Ribeirão do Rocha (6) were studied (Fig. 1).

The second zone (**R2**) is located in the Central area (22 °C average temperature, 107 mm/month rainfall, 70–75% relative humidity, and 600 m elevation). The study included the counties of Manoel Ribas (1), Cândido de Abreu (2) and Prudentópolis (3) from 50°58′50″W (Prudentópolis) to 51°43′00″W (Manoel Ribas), and 24°32′00″S (Manoel Ribas) to 25°12′40″S (Prudentópolis) (Fig. 1).

The third zone (**R3**), in Northern Paraná (600–700 m altitude), has a subtropical climate, average temperature <18 °C in the coldest month and average temperature >22 °C in the hottest month, with hot summers and infrequent frosts. Rainfall is somewhat greater during the summer, without a definite dry season. It is separated from **R2** by a range of mountains. The study was carried out in the counties of Florestópolis (1), Londrina (2), Arapongas (3), Apucarana (4), Sabáudia (5), Cambira (6), Borrazópolis (7), Jardim Alegre (8), Kaloré (9) and Mariluz (10), from 51°11′30″W (Londrina) to 51°50′10″W (Jardim Alegre), and 22°53′00″S (Florestópolis) to 24°10′00″S (Jardim Alegre) (Fig. 1).

2.2. Sample collection

Dogs clearly established in the region (born there, or in the area for more than one year) were used in this study. Those without an owner, or younger than six months, or pregnant were excluded. Before sample collection, dogs were tranquilized by intramuscular administration of ketamine (10 mg/kg) and immobilized mechanically.

Blood was collected without anticoagulant for serological tests. Peripheral lymph node contents and blood (with anticoagulant) were collected for parasite cultures. Dogs with cutaneous lesions were biopsied under local anesthesia (2% xylocaine).

In dogs without lesions (intact skin) were biopsied on the snout, to determine whether animals within the endemic zone of the disease were carriers. Biopsies were placed in 0.85% sodium chloride solution with penicillin (25,000 UI) and streptomycin (100 μg/mL) and transported to the laboratory, where they were macerated and homogenized before inoculation in culture medium (NNN). For each biopsy, eight tubes with culture media were incubated at 24 °C, examined and subcultured every week. After the 4th week they were discarded if negative. Positive cultures were cryopreserved and prepared for RAPD analysis.

Dogs with positive parasitological exams were sacrificed and necropsied. Organs (liver, kidneys, spleen, bone marrow and heart) were separately placed in 0.85% sodium chloride with penicillin (25,000 UI) and streptomycin (100 μg/mL). They were then washed twice, macerated and inoculated into a NNN medium at 24 °C. This solution was also inoculated (0.3 mL) into the hind feet of hamsters (*Mesocricetus auratus*).

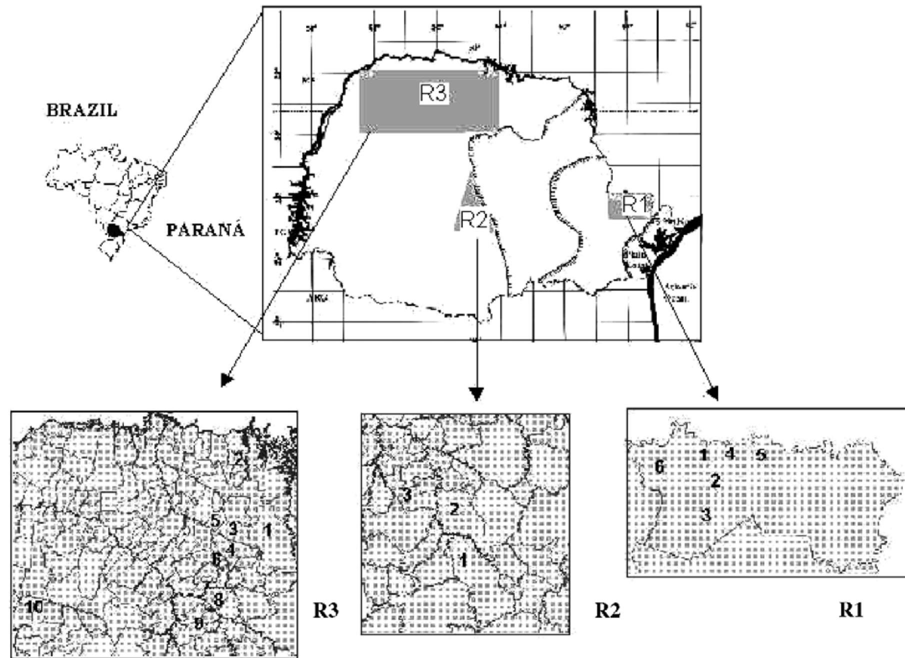

Fig. 1. Geographic location of three cutaneous leishmaniasis regions studied in the State of Paraná, Southern Brazil. Region 1 (**R1**) is located in the Vale da Ribeira (Adrianópolis county) and the dogs' population was studied in six areas: (1) Barra Grande, (2) Laranjal, (3) Perau, (4) Capela, (5) Colônia, (6) Ribeirão do Rocha. Region R2 (**R2**) is located in the Central region and dogs were studied in three counties: (1) Manoel Ribas, (2) Cândido de Abreu, (3) Prudentópolis. Region 3 (**R3**) included 33 rural properties from 10 counties: (1) Florestópolis, (2) Londrina, (3) Arapongas, (4) Apucarana, (5) Cambira, (6) Borrazópolis, (7) Kaloré, (8) Jardim, (9) Alegre and (10) Mariluz.

2.3. Serological analysis

For the Enzyme Linked Immunosorbent Assay (ELISA), a soluble antigen was produced with promastigotes following the method of Engwall and Perlamann (1972), modified for microplating on polystyrene. Reactivity was defined as readings higher than cut-off ($\geqslant 0.178$) which was calculated by the mean values of sera from non-endemic areas plus three times standard deviation (Castro et al., 2003).

ELISA sensitivity was tested on dogs by positive parasitological examination. To test for potential cross-reactions with other kinetoplastid protozoa, serum was tested for reactions to recombinant antigen of *T. cruzi* (following Krieger et al., 1992) using the Kit developed by Biomanguinhos Instituto Oswaldo Cruz, Rio de Janeiro.

Cross-reactive sera for *T. cruzi* and *Leishmania* were adsorbed to metacyclic forms of *T. cruzi* (MDID/BR/97/CUR5 strain; see Thomaz-Soccol et al., 2002). Adsorption was carried out as described by Camargo and Rebonato (1969), after which the sera were retested by ELISA for *T. cruzi* and *Leishmania*. Those animals which reacted positively only to *T. cruzi* antigen were excluded from epidemiological analysis of leishmaniasis.

2.4. Protozoan identification

The isolated strains were identified by RAPD approach using primers OPA2 (TGCCGAGCTG), OPA3 (AGT CAGCCAC), OPA9 (GGGTAACGCC) and OPA10 (GTGATCGCAG) (Bañuls et al., 2002). Initially DNA was extracted from promastigotes obtained from mass cultivation on RPMI medium supplemented with 10% fetal bovine serum. The culture was washed twice with PBS. In order to gather 50–100 mg of cells, 400 μL of Tris–HCl, pH 8, 10 mM and EDTA 1 mM buffer was added and incubated for 2 h at 37 °C with 200 μg/mL RNAse. Subsequently, proteinase K (20 mg/mL) was added and incubated for 2 h at 55 ± 1 °C. Deproteination was done by phenol/chloroform/isoamyl alcohol (24:25:1) and centrifuged at 10,000*g*/5 min. The supernatant was transferred to another tube and DNA was precipitated by addition of pure ethanol (600 μL) and 10% sodium acetate 3 M (30 μL) and subsequently centrifuged at 12,000*g* for 30 min. The DNA was washed twice with 600 μL of 70% ethanol followed by centrifugation (12,000*g*/15 min). The DNA pellet was resuspended in 200 μL of ultra pure water and stored at 4 °C until use.

Amplification reactions were carried out on 25 μL aliquots with 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP (deoxinucleotide triphosphate), 10 pmol primer, dilute genomic DNA 1:40 and 2.5 U of Taq DNA polymerase (Invitrogen). Amplification cycles included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. Amplified products were stained with ethidium bromide and observed under UV after electrophoresis in agarose gel (1.6%) (Sambrook et al.,1989). Negative and positive controls were used.

Reference strains in these analyses were *L.* (*V.*) *braziliensis* (MHOM/BR/75/M2904), *Leishmania* (*Leishmania*) *amazonensis* (MHOM/BR/73/M2269) and *Leishmania* (*L.*) *infantum* (MHOM/TN/80/IPT1).

2.5. Statistical analysis

To determine the molecular weight of DNA fragments generated by RAPD analysis, the bands were analyzed by Gel Proanalyzer computer program and the data were transformed in a disjunctive matrix of shared bands using NTSYS program. The matrix was constructed based on all bands generated by four primers (OPA2, OPA3, OPA9 and OPA10). *Leishmania* were classified by the phenetic method using the Jaccard coefficient of similarity, and grouped by Unweighted Pair-Group Method using an Arithmetic Average (UPGMA) genering a dendrogram. Clusters were validated by bootstrap analysis and considered consistent with values more than 85.

3. Results

3.1. Clinical observation and parasitological study

A total of 410 dogs were studied: 159 from Vale da Ribeira (**R1**), 39 from Central Paraná (**R2**) and 212 from Northern Paraná (**R3**). Out of 410 animals, 28 had lesions that were compatible with cutaneous leishmaniasis of which 61.5% had simple skin lesions, 34.9% multiple skin lesions and 3.6% had mucosae lesions. In **R1** seven dogs had lesions compatible with leishmaniasis and one had a nasal mucosal lesion. Two dogs in **R2** had simple skin lesions on the scrotum compatible with leishmaniasis; and in **R3**, 19 animals had ulcerated lesions in different areas (scrotum, nose, ear) or scabs on the snout that, when removed, had granulomatous reactions (Table 1).

Fourteen strains of *Leishmania* were isolated from cutaneous and mucosal lesions in 13 dogs: one animal from **R1** (Adrianópolis county); two from **R2** in Manoel Ribas (1) and Prudentópolis (1); and 10 from **R3**: in Florestópolis ($n = 3$), Arapongas (3), Jardim Alegre (1), Londrina (1) and Mariluz (2). Lesions were found on the inner surface of the ear, snout, scrotum and mouth (Table 1).

The thirteen dogs showing cutaneous and mucosal lesions and presence of promastigotes isolated in culture were euthanized. Parasites were not found in deep organs. Parasites were not detected in the seropositive animals ($n = 57$) in which intact skin and lymph nodes were examined.

3.2. Isolates identification

By RAPD, DNA amplification of all stocks resulted in multiple reproducible fragments varying from 500 to 3000 bp. Each primer gave a characteristic profile of bands (see example in Fig. 2a for OPA10 primer). To compare the strains isolated from dogs with human isolates, the strains Cur 263, from North of Paraná, and Cur 266, Cur 267, Cur 264, Cur 218 from Central Paraná were included. They showed a similar profile to those isolated from dogs (Fig. 2b).

The phenogram produced with RAPD data (primers OPA2, OPA3, OPA9 and OPA10) clustered the isolates together, including *L.* (*V.*) *braziliensis* reference strain and strains isolated from humans. A great genetic diversity among stocks isolated from dogs was observed. The RAPD results analyzed by UPGMA showed distinct branches. One branch with 83% similarity grouped 16 strains from 12 samples isolated from dogs, 3 strains isolated from

Table 1
Dogs with positive culture for *Leishmania* from three leishmaniasis foci in the State of Paraná, Southern Brazil

Animal origin	Number of infected dogs	Clinical lesions	Number of lesions	Strain code
Region 1				
Adrianópolis	1	Muco-cutaneous	2	MCAN/BR/04/CUR 292
Region 2				
Manoel Ribas	1	Cutaneous in Ear	1	MCAN/BR/03/CUR 247
Prudentópolis	1	Cutaneous in Snout	1	MCAN/BR/04/CUR 266
Region 3				
Arapongas	3	Cutaneous in Ear	2	MCAN/BR/99/CUR 127
		Cutaneous in Snout	2	MCAN/BR/99/CUR 128
		Cutaneous in Ear, Snout	2	MCAN/BR/99/CUR 200
Florestópolis	3	Cutaneous in Scrotum, Ear	2	MCAN/BR/99/CUR 111
		Cutaneous in Snout, Scrotum	2	MCAN/BR/99/CUR 110
		Cutaneous in Snout, Scrotum	2	MCAN/BR/99/CUR 112
Jardim Alegre*	1	Cutaneous in Mouth	1	MCAN/BR/99/CUR 136
		Cutaneous in Scrotum	1	MCAN/BR/99/CUR 137
Londrina	1	Cutaneous in Ear	1	MCAN/BR/03/CUR 261
Mariluz	2	Cutaneous in Snout	1	MCAN/BR/03/CUR 237
		Cutaneous in Scrotum	1	MCAN/BR/03/CUR 238

Fourteen strains were isolated from skin lesions of 13 dogs.
* Two strains were isolated from one dog (mouth and scrotum).

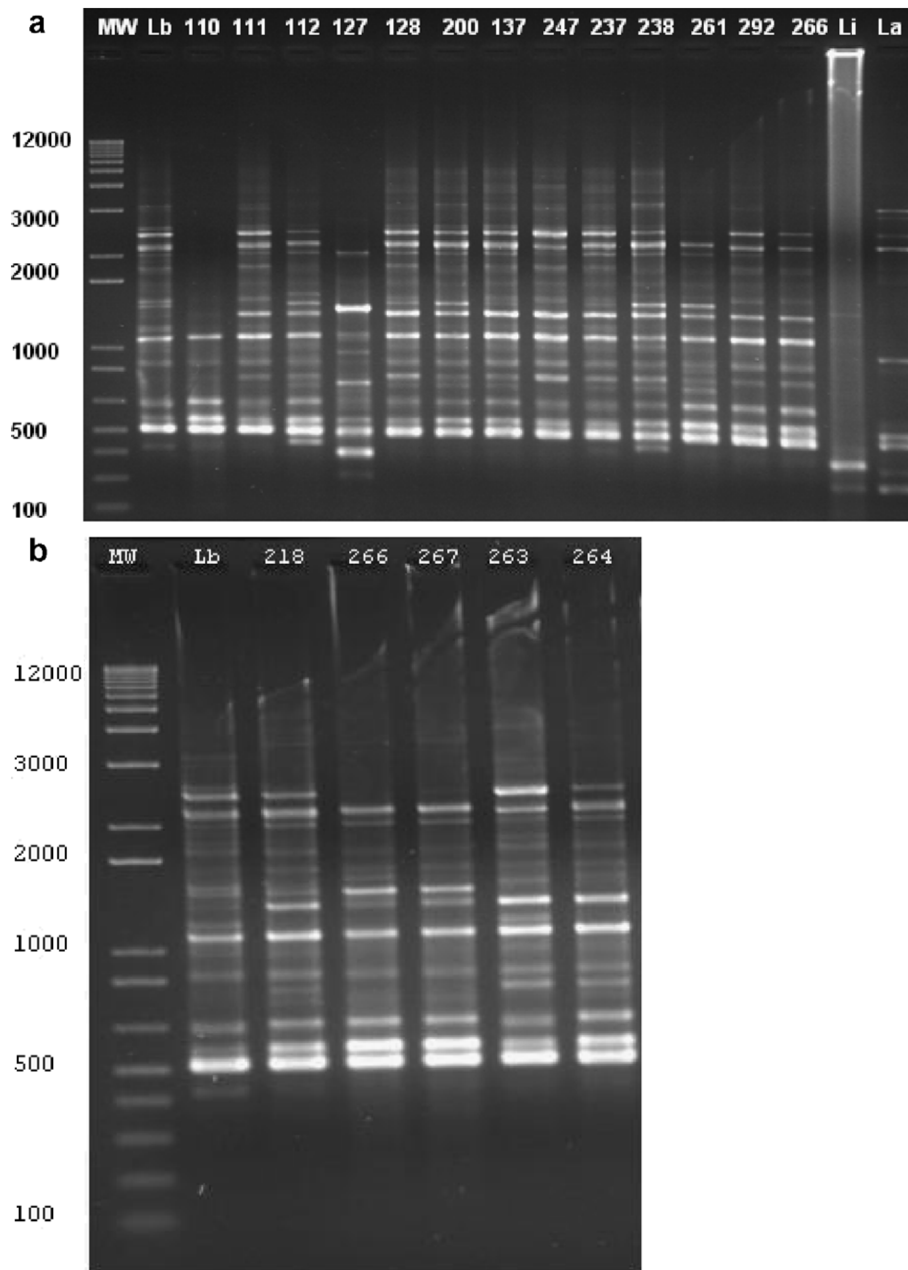

Fig. 2. (a) Profile of the RAPD amplification of the *Leishmania* isolates from dogs amplified with OPA10 primer. Line 1: Ladder 1 Kb; Line 2: Reference strain *L. braziliensis*; Line 3: Cur 110; Line 4: Cur 111; Line 5: Cur 112; Line 6: Cur 127; Line 7: Cur 128; Line 8: Cur 200; Line 9: Cur 137; Line 10: Cur 247; Line 11: Cur 237; Line 12: Cur 238; Line 13: Cur 261; Line 14: Cur 292; Line 15: Cur 266; Line 16: *L.* (*L.*) *infantum*, Line 17: *L.* (*L.*) *amazonensis*. (b) Profile of the RAPD amplification of *Leishmania* isolates from humans and dogs amplified with OPA10 primer. Line 1: Ladder 1 Kb; Line 2: Reference strain *L.braziliensis*; Line 3–7: Cur 218, Cur 266, Cur 267, Cur 263, Cur 264.

cutaneous leishmaniasis in humans and the *L.* (*V.*) *braziliensis* reference strain (Fig. 3). A second branch with 60% similarity grouped two strains, one isolated from dogs (CUR266) and another from human (CUR264).

3.3. Serology

From the 410 sera examined, 353 were non reactive and 57 (13.9%) reacted with the *Leishmania braziliensis* antigen. In Vale da Ribeira (**R1**), 10 (6.3%, $n = 159$) animals had anti-*Leishmania* antibodies as shown by ELISA. In the Central region (**R2**), eight (20.5%, $n = 39$) animals reacted to the *L.* (*V.*) *braziliensis* antigen and in Northern Paraná (**R3**), 39 (18.4%, $n = 212$) dogs were positive (Table 2).

Sensitivity of ELISA was calculated using positive animals confirmed by parasitology. From 13 dogs that were parasite positive, 12 reacted with the *L. braziliensis* antigen giving a sensitivity of 92.3%.

Cross-reactivity was estimated by recombinant-ELISA for *T. cruzi*. In the Vale da Ribeira region (**R1**) a total of 36 dogs reacted with recombinant ELISA-EIE for *T. cruzi.*

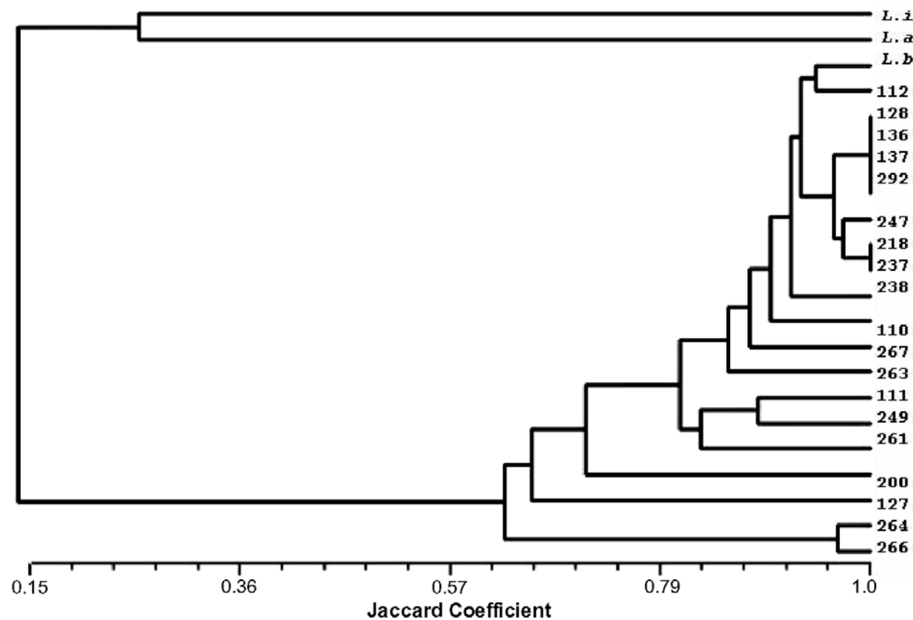

Fig. 3. UPGMA dendrogram built with the Jaccard similarity index and RAPD data using four primers (OPA2, OPA3, OPA9 and OPA10) and strains isolated from dogs and human from three leishmaniasis zones in the State of Paraná, Southern Brazil.

Table 2
Enzyme Linked Immunosorbent Assay (ELISA) for the detection of antibodies for *Leishmania braziliensis* in dogs from Vale da Ribeira region (**R1**), central region (**R2**) and Northern area (**R3**) of the State of Paraná, Southern Brazil

Counties	Localities number	Dogs number	Not reactive N (%)	ELISA O.D. > 0.178 N (%)
Region 1 - **R1**				
Adrianópolis	6	159	149 (93.7%)	10 (6.3%)
Region 2 - **R2**				
Manoel Ribas	1	1	0	1
Cândido de Abreu	2	18	14	4
Prudentópolis	6	20	17	3
Sub-total	9	39	31 (79.5%)	8 (20.5%)
Region 3 -**R3**				
Florestópolis	1	6	2	4
Londrina	1	10	10	0
Arapongas	20	65	58	7
Apucarana	5	16	10	6
Sabáudia	1	5	3	2
Cambira	1	4	3	1
Borrazópolis	1	3	1	2
Kaloré	1	2	2	0
J. Alegre	1	99	82	17
Mariluz	1	2	2	0
Sub-total	33	212	173 (81.6%)	39 (18.4%)

After serum adsorption with *T. cruzi* antigen, a new ELISA was performed and only seven reacted with the *Leishmania* antigen. In the Northern region (**R3**), 53 dogs reacted with recombinant ELISA-EIE for *T. cruzi*, and after specific antigen adsorption, only 16 of them reacted with the *Leishmania* antigen (Table 3). In Central Paraná, antibodies for *T. cruzi* were not tested since it is not endemic in that region. Sixty six seropositive dogs for *T. cruzi* were excluded from epidemiological analysis.

4. Discussion

RAPD method was used to identify parasites and phenetic analysis to evaluate the chemotaxonomy of *Leishmania* isolates from dogs. Dendrograms were generated grouping the isolates with *L.* (*V.*) *braziliensis* including reference strains and strains isolated from humans with cutaneous lesions. The clusters formed among *Leishmania* isolates from dogs could not be attributed to geographical

Table 3
Comparison of ELISA methods for the detection of antibodies anti-*Leishmania* and anti-*Trypanosoma cruzi* in dogs in the State of Paraná, Southern Brazil

		ELISA anti-Leishmania					
		Reactive		Not reactive		Total	
		R1	**R3**	**R1**	**R3**	**R1**	**R3**
ELISA	Reactive	7	16	29	37	36	53
anti-T. cruzi	Not reactive	17	44	106	115	123	159
	Total	24	60	135	152	159	212

distribution of the animals. RAPD revealed intra-specific polymorphism. *L.* (*V.*) *braziliensis* seems to show greater genetic variation among the species of *Leishmania* independently of the method employed. The studies of Thomaz-Soccol et al. (1993), Bañuls et al. (2002) and Ishikawa et al. (2002) and the present study are, at least in part, in agreement in demonstrating this variation.

The parasitological examination revealed that 3.17% of dogs tested had positive cultures of *Leishmania.* In many regions of Latin America, studies have shown leishmaniasis prevalence in dogs ranging from 3% to 36% (Herrer and Christensen, 1976; Aguilar et al., 1989; Le Pont et al., 1989; Reithinger and Davies, 1999). In Brazil, based on parasite isolation or on the presence of lesions, leishmaniasis prevalence in dogs varies from 2% to 32% (Dias et al., 1977; Falqueto et al., 1986). However, more than one species of *Leishmania* has been detected circulating sympatrically in different regions of Latin America (Quinnell et al., 1997; Reithinger et al., 2003) which makes the role of domestic dogs in the epidemiology of leishmaniasis difficult to interpret. It is worth noting that in Paraná State, *L.* (*V.*) *braziliensis* is the only species present (Thomaz-Soccol et al., 2003; Castro et al., 2005). This facilitates interpretation of the role of dogs as primary or secondary reservoirs or as accidental hosts.

In order to propose animals as reservoirs in the cycle of *Leishmania*, parasitological methods are required to demonstrate that parasites circulating in different hosts (human and animals) are the same (WHO, 1990). Without parasite isolation it is not possible to evaluate the role of the host as reservoir or to say whether the infection is anthroponotic or zoonotic. In addition to parasitology, other methods such as PCR have been used to detect leishmanial kDNA and rDNA in cryopreserved tissue samples from dogs in other endemic *L.* (*V.*) *braziliensis* areas (Brandão-Filho and Shaw, 2006). Reithinger et al. (2003) and Velasquez et al. (2006) used blood samples to detect *Leishmania* DNA from dogs using PCR. However, it should be noted that molecular tools do not allow proper evaluation of intra-specific genetic variability nor do they describe new species.

Because of the difficulties imposed by conventional parasitological methods, serological techniques have been chosen as a reliable alternative for epidemiological analysis. They have been employed for prevalence studies of leishmaniases (Padilla et al., 2002; Ryan et al., 2003; Reithinger et al., 2003; Castro et al., 2002, 2003, 2005). In the present research carried out in the State of Paraná South of Brazil, serological analysis of material collected from 410 dogs showed that seroprevalence of leishmaniasis should substantial differences from region to region. In Vale da Ribeira, where leishmaniasis has been reported ever since colonization in the 1920s, the seroprevalence rate in dogs was 6.3%. The role of the dog is apparently that of a link between the wild and domestic cycles, being an amplifier to the cutaneous leishmaniasis ecosystem. The data obtained by Gomes et al. (1990) in São Paulo State, corroborates this finding. In the Central region leishmaniasis is more recent, being reported only since 2002 (Brazil, 2006) and the seroprevalence in the dog was 20.5%. Dogs and human show similarities with regards to infection by *Leishmania*. In the North, leishmaniasis was first detected in the 1940s when colonization took place and again in late 1990s when an outbreak was reported (Brazil, 2006) and canine seroprevalence was 18.4%. Similar rate was observed by Silveira et al. (1996) in a study performed in North West of Paraná. Therefore, dog and man appear to be accidental hosts in that region and transmission to humans appears to originate in wooded areas. This is suggested by the finding of one rodent and phlebotomine flies (*Lutzomyia whitmani*) that were captured on the forest edge with *L.* (*V.*) *braziliensis* (Luz et al., 2000; Thomaz-Soccol et al., 2003).

In the three foci studied, the proportion of infected animals was low (3.17% using only parasitological results). However, the seroprevalence rate was greater (13.9%). As mentioned earlier, rates given by serological surveys of canine leishmaniasis has ranged from 3% to 36% in the New World. However, antibodies in animals do not necessarily imply active infection, rather show only contact with the parasite and elevated serum rates may mean cross reaction (due to other Trypanosomatidae) as observed in the present study or may be due to other species of *Leishmania* such as *L.* (*L.*) *infantum*, *Leishmania* (*V.*) *guyanensis* and *Leishmania* (*V.*) *peruviana* circulating sympatrically.

In the present work, parasites were not detected in cultures of intact skin, lymph nodes or deep organs. In recent studies by Madeira et al. (2005, 2006) parasites were only isolated from skin lesions which corroborate data observed here. In addition, Reithinger et al. (2003) observed that seropositive animals had become seronegative and PCR+ had turned into PCR− after a year. A possible cure is the explanation given by the authors. They also propose that dogs develop a cell-mediated Th1 immune response

that controls *L.* (*V.*) *braziliensis* infections decreasing the number of parasites in the blood. Nevertheless, if dogs became negative in immunological and PCR tests, this would indicate that the parasite was temporarily in the blood circulation at the time of the original testing. For an animal to be a good reservoir, parasite abundance and duration are crucial for sand fly infection. This would suggest that dogs are not good reservoirs of *L.* (*V.*) *braziliensis*.

Acknowledgments

This work was supported by UGF/SETI - process number: 86. The authors thank Biomanguinhos - Instituto Oswaldo Cruz for their diagnostic "Kit" for Chagas Diseases.

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