AMERICAN VISCERAL LEISHMANIASIS: DISEASE CONTROL STRATEGIES IN DRACENA MICROREGION IN ALTA PAULISTA, SP, BRAZIL

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ABSTRACT: Despite measures adopted to control American visceral leishmaniasis (AVL), the disease is spreading in a fast and worrying way throughout western São Paulo state. The aim of this work was to study the variables involved in the disease cycle as well as the effectiveness of controlling measures. The study was carried out in the microregion of Dracena, which is composed of twelve cities and belongs to Alta Paulista, a region of western São Paulo. The necessary data were provided by the Superintendence for Endemic Disease Control and Adolfo Lutz Institute, Regional Laboratory of Presidente Prudente. From August 2005 to January 2008, the following factors were observed: detection of phlebotomine sandflies in the cities and periods in which dogs or humans were diagnosed; number of human deaths; prevalence of suspected dogs tested by serology; percentage of euthanasia in suspected dogs; a possible correlation between positive dogs and cases of the disease in humans; and the disease prevalence among municipalities from the studied region. It was verified that, despite the strategies adopted in Dracena microregion to control AVL, the disease continues to rise. Thus, some procedures of the AVL Monitoring and Control Program should be reviewed, to grant the initiative more credibility and effectiveness.

CONFLICTS OF INTEREST: There is no conflict.

KEY WORDS: visceral leishmaniasis, seroprevalence, control strategies, São Paulo, Brazil.

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INTRODUCTION

Leishmaniasis is considered a serious zoonosis, provoked by a protozoan of the genus *Leishmania*, that may affect humans who get into contact with the parasite transmission cycle, thus becoming an antropozoonosis (1). The American visceral leishmaniasis (AVL) is caused by a species of the subgenus *Leishmania*, *Leishmania chagasi* (2). Clinical manifestations of the disease and its severity are associated with malnutrition and to host immune response (3, 4). There is a connection between AVL and immunosuppression caused by acquired immunodeficiency syndrome (AIDS) and other diseases including microbacteriosis, cytomegalovirus infection and Kaposi's sarcoma; hence, it constitutes an opportunistic pathology among immunosuppressed patients who lives in endemic areas (5-7).

Different types of phlebotomine sandflies can transmit AVL. In the Americas, *Lutzomyia (Lutzomyia) longipalpis* is the main species, present in virtually all areas of occurrence of leishmaniasis (8). However, there are reports of transmission by *L. cruzi* in cities like Corumbá and Ladário (Mato Grosso do Sul state, Brazil) (9, 10). Additionally, Marzochi and Marzochi (2) suggested an association between the disease and *L. intermedia* in coastal areas of Rio de Janeiro city. *L. longipalpis* life cycle takes place in soil, in wet and shaded places that are also rich in organic matter. Only females are bloodsuckers, feeding on numerous warm-blooded vertebrates (11). According to a recent study carried out in São Paulo state, it appears that *L. longipalpis* prefer to feed on dogs (12). Although this species can be found throughout the year, there are seasonal variations of population density, depending on geographic region and some factors like temperature, humidity and wind speed (13).

Despite its wide worldwide distribution, ALV is predominant in tropical and subtropical regions, occurring in Asia, Europe, Middle East, Africa and the Americas. In Latin America, the disease has already been described in at least 12 countries, with 90% of cases in Brazil. Nowadays, leishmaniasis is present in four out of five Brazilian geographic regions, mainly in the Northeast. However, over the years, it has spread to the north, southeast and central west, with different geographical, climatic and social aspects, due to its wide distribution (14).

The first record of *L. longipalpis* in the State of São Paulo (SSP) occurred in the 1970s, in a specific area on the Mantiqueira mountain range, in the east of the state (15). In 1997, this vector was identified in western SSP, in Araçatuba city, followed by

the discovery of *L. chagasi* infection in dogs, in 1998, and in humans, in 1999. Since then, the disease has been reported in several regions, with exclusively urban transmission (16-18). In eastern SSP, the spreading occurred from west to east and, more recently, from north to south of Minas Gerais state (12). Therefore, control measures are being implemented in order to reduce human morbidity and mortality by AVL in SSP (14, 19).

There have been important changes in the disease transmission pattern. Initially, it was predominant in rural and periurban areas and, later, in urban centers including the cities of Rio de Janeiro (RJ), Corumbá (MS), Belo Horizonte (MG), Araçatuba (SP), Palmas (TO), Três Lagoas (MS) and Campo Grande (MS).

The adaptation of the vector to urban environment happened slowly. It was first recorded in municipalities neighboring Araçatuba and subsequently in cities that had migratory flow of both population and goods. Then, it was detected in other administrative regions – such as Bauru (SP), Marília (SP) and Presidente Prudente (SP) – crossed by major roads and railways axes. Recently, it was found in the region of São João da Boa Vista (SP), which presents different climatic and topographic characteristics from those observed in western Planalto Paulista; thus, proving *L. longipalpis* ability to adapt to anthropic environments (12).

The São Paulo State Secretary of Health created the AVL Monitoring and Control Program (AVLMCP) in order to reduce both the mortality and lethality of the disease. The program actions are based on three strategic pillars, similar to the Ministry of Health Program: early diagnosis and treatment of human cases, monitoring and reduction of sandfly population density, and control of *L. (L.) chagasi* domestic reservoirs represented by seropositive dogs. The antivectorial activities include epidemiological surveillance plans such as: entomological survey; entomological research of infestation focus and fixed units; notification of insects by population; prevention and control initiatives that include environmental management, chemical control and educational projects. Another key point of AVLMCP is the control of canine reservoirs through serological surveys and sample census in affected cities (14).

Canine infection has usually preceded the occurrence of human cases and has been more prevalent than the human disease (20). Therefore, it is very important to know the prevalence of infected dogs in a given region.

This study aimed carry out a time-space analysis of the prevalence of circulating antibodies against *Leishmania* sp. in blood samples of dogs and its correlation with the incidence of human cases. Additionally, we assessed the effectiveness of removing canine reservoirs as a controll strategy in cities from the microregion of Dracena, Alta Paulista (SP), from August 2005 to January 2008.

MATERIAL AND METHODS

Description of the Studied Area

The study was conducted in Alta Paulista, a region that comprises the Dracena microregion, between the rivers Feio (or Aguapeí) and Peixe, which is composed by twelve cities: Dracena, Junqueirópolis, Monte Castelo, Nova Guataporanga, Ouro Verde, Panorama, Paulicéia, Santa Mercedes, São João do Pau d'Alho, Tupi Paulista, Flora Rica and Irapuru. The total human population estimated in this region is 121,976 inhabitants while dogs are 30,022 (Table 1) (21, 22). Alta Paulista is located at 21°28'57S latitude and 51º31'58"W longitude, being a hot and humid tropical region, with one or two months of drought, whose average temperature is above 18°C throughout the year (23). It is part of the 10th administrative region of São Paulo state, 55 km away from Adamantina, 110 km from Presidente Prudente, 200 km from Marília, 647 km from São Paulo city and 40 km from the state border with Mato Grosso do Sul, near Paraná River's new lake that turned the region into a tourist attraction of the state. It is one of the poorest regions in São Paulo state, along with the Vale do Rio Ribeira de Iguape, with a literacy rate of 83.93% (24).

Table 1. Estimated distribution of human and canine population in Dracena microregion, Alta Paulista (SP), 2007

Cities	Human population*	Canine population**
Dracena	42,107	10,052
Flora Rica	2,019	505
Irapuru	7,556	1,890
Junqueirópolis	18,628	4,660
Monte Castelo	4,014	1,003
Nova Guataporanga	2,101	525
Ouro Verde	7,668	1,917
Panorama	13,944	3,486
Paulicéia	5,506	1,376
Santa Mercedes	2, 589	647
São João do Pau d'Alho	2,132	533
Tupi Paulista	13 712	3,428
Total	**121,976**	**30,022**

*IBGE (21); **Alves *et al.* (22).

Sampling

Data herein presented are from serological surveys officially accomplished by SUCEN in accordance with AVLMCP. The tests were performed in all cities belonging to Dracena microregion, from August 2005 to January 2008.

AVL Diagnosis

Human and canine AVL diagnoses were carried out by the Adolfo Lutz Institute, Regional Laboratory of Presidente Prudente (SP), which is the regional reference for this disorder. Samples were processed in the Medical Biology section, Serology and Parasitology sector, after being sent by Superintendence for Endemic Disease Control, SUCEN, of Presidente Prudente, Brazil.

Direct Parasitological Diagnosis

The direct technique for leishmaniasis diagnosis was utilized to detect the amastigote form of the parasite, both in human and animal hosts. Several procedures were employed, including bone marrow puncture, leucocitary cream, aspiration of wounds and lymph nodes, biopsy of injuries and guts. The samples were prepared on stained laminas with Giemsa or Leishman and examined on an optical microscope (100x).

Serological Diagnosis

Samples of whole blood tested for dog AVL were obtained by lancet puncture of the marginal ear vein and immediately transferred to a standardized filter paper, such as Watmann number 1 or Klabin (Klabin Co., Brazil), as recommended by the Manual of Surveillance and Control of American Visceral Leishmaniasis of São Paulo state. The blood drenched area covered a diameter of 3 cm (14). This material was subsequently fixed in phosphate buffered saline solution (PBS), pH 7.2, originating the eluate. All samples were labeled with the animal name, registration number, date, city, sector and the collection responsible. The samples were kept at room temperature (RT) or at 4°C for a period of 30 days after collection or up to 60 days at −20°C, protected in moisture-free plastic bags.

The EIE Bio-Manguinhos/Fundação Oswaldo Cruz kit was employed for the canine leishmaniasis diagnosis, according to their recommendations. Filter paper was used in this immunoenzymatic assay, as described by Evans *et al*. (25), with 79.45% sensitivity and 90.24% specificity. The indirect immunofluorescence Bio-Manguinhos/Fundação Oswaldo Cruz kit for canine leishmaniasis diagnosis was also used, according to the manufacturer recommendations. The reaction of indirect fluorescent antibody test (IFAT) was carried out on samples collected on filter paper, to detect class IgG antibodies, according to the methodology described by Camargo and Rebonato (26) and Costa *et al*. (27) with 90% sensitivity and 80% specificity. Slides were prepared with a 10-µL suspension of *L. like major* promastigotes, fixed in air for 12 hours or for two hours at 37°C and incubated with 1:40 diluted eluate. Connected antibodies were detected with the total conjugated anti-immunoglobulin of dog stained with fluorescein, diluted in PBS with Evans Blue at 0.004%.

Statistical Analysis

The average prevalence of *Leishmania* sp. antibodies was calculated taking into account the total number of animals tested and seroreactives. The chi-square (χ^2) test was employed to compare the prevalence among the cities. The simple linear coefficient was used to verify a possible association between positive dogs and AVL human cases (28).

RESULTS AND DISCUSSION

The cities of Dracena and Nova Guataporanga were the first where the presence of *L. longipalpis* was detected, in 2003. Subsequently, in 2004, the sandfly was also observed in Tupi Paulista, Junqueirópolis, Monte Castelo, Flora Rica, Santa Mercedes and São João do Pau D'Alho. In 2005, all the cities comprising Dracena microregion had already registered the presence of this vector. Both canine and human infections were identified from one to three years after *L. longipalpis* detection. Its spread reached even adjacent cities, such as President Venceslau, where the vector was found in January 2008 (Table 2).

Table 2. Years of detection of *L. longipalpis* and canine and human AVL transmission in 12 cities belonging to Dracena microregion, Alta Paulista (SP), as well as neighboring cities

	Year of detection		
Cities	***L. longipalpis***	**Canine transmission**	**Human transmission**
Dracena	Mar. 2003	2005	2005
Ouro Verde	Feb. 2004	2006	2006
Tupi Paulista	Apr. 2004	2006	2006
Nova Guataporanga	Apr. 2003	2007	2007
Junqueirópolis	Apr. 2004	2006	2006
Monte Castelo	Feb. 2004	2008	–
Flora Rica	Dec. 2004	–	–
Santa Mercedes	Dec. 2004	2007	2007
S. J. P. Alho	Dec. 2004	–	–
Paulicéia	Feb. 2005	2007	2007
Panorama	Feb. 2005	2007	2007
Irapuru	Oct. 2005	2007	2007
Presidente Venceslau	Feb. 2008	–	–

Source: SUCEN/IAL – Presidente Prudente.

The parasite detection and both clinical and epidemiological criteria were employed to confirm human AVL. A total of 155 AVL human cases were recorded in the microregion of Dracena, Alta Paulista (SP), from August 2005 to January 2008, with seven deaths (Table 3).

Table 3. AVL cases and human deaths in Dracena microregion, Alta Paulista (SP), from August 2005 to January 2008

Cities	2005		2006		2007		2008	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Dracena	8	–	41	1	38	–	2	
Junqueirópolis	–	–	3	–	10	1	2	
Nova Guataporanga	–	–	–	–	2	2	–	
Ouro Verde	1	–	4	1	5	–	1	
Panorama	–	–	–	–	10	1	2	
Paulicéia	–	–	–	–	2	–	2	
Tupi Paulista	–	–	2	–	7	1	1	
Irapuru	–	–	–	–	1	–	–	
Santa Mercedes	–	–	–	–	3	–	1	
Total	**9**	**0**	**50**	**2**	**78**	**5**	**11**	

Source: www.cve.saude.sp.gov.br

In this microregion, canine exams were carried out as recommended by AVLMCP (14), with an overall average prevalence of *Leishmania* sp. antibodies of 23.78%, ranging between 0 and 30.03% (Table 4). Of 29,995 tested dogs, 7,133 were seroreactive to *Leishmania* sp., among which only 62.44% (4,454) were killed in the studied region (Table 5).

Table 4. Mean antibody prevalence of *Leishmania* sp. among dogs from Dracena microregion, Alta Paulista (SP), from August 2005 to January 2008

Cities	Inquiries (n)	Total analyzed	Positive results (n)	Inconclusive results	Mean prevalence (%)
Dracena	3	11,675	3,415	2,263	29.25%
Junqueirópolis	3	5,268	705	1,172	13.38%
Tupi Paulista	2	4,200	761	1,257	18.12%
Ouro Verde	3	2,693	746	776	27.70%
Paulicéia	2	1,150	343	217	29.81%
Santa Mercedes	2	726	218	111	30.03%
Panorama	2	3,190	889	18	27.87%
Flora Rica	1	195	5	0	2.50%
Irapuru	1	348	40	192	11.49%
Monte Castelo	1	224	11	0	4.91%
Nova Guataporanga	1	162	0	0	0.00%
S. J. P. Alho	1	164	0	0	0.00%
Total	**22**	**29,995**	**7,133**	**6,006**	**23.78%**

Source: SUCEN/IAL – Presidente Prudente.

Table 5. Porcentage of euthanasia in AVL seroreactive dogs in the cities of Dracena microregion, Alta Paulista (SP), from August 2005 to January 2008

Cities	Positives	Euthanasia	
		Number	(%)
Dracena	3,415	2,299	67.32
Junqueirópolis	705	644	91.34
Tupi Paulista	761	628	82.52
Ouro Verde	746	379	50.80
Paulicéia	343	169	49.27
Santa Mercedes	218	186	85.32
Panorama	889	528	59.39
Flora Rica	5	–	–
Irapuru	40	–	–
Monte Castelo	11	–	–
Nova Guataporanga	0	–	–
S. J. P. Alho	0	–	–
Total	**7,133**	**4,454**	**62.44**

Source: SUCEN/IAL – Presidente Prudente.

The correlation coefficient was 0.98 (significant level of 1%), revealing an association between the total number of human cases of leishmaniasis and the number of dogs that reacted to *Leishmania* sp. (Table 6).

Table 6. Total number of human leishmaniasis cases (Y) and positive results among dogs (X) in Dracena microregion, Alta Paulista (SP), from August 2005 to January 2008

Cities	Positive dogs (X)	Human leishmaniasis cases (Y)
Dracena	3,415	90
Junqueirópolis	705	16
Tupi Paulista	761	11
Ouro Verde	746	12
Paulicéia	343	4
Santa Mercedes	218	4
Panorama	889	13
Flora Rica	5	0
Irapuru	40	1
Monte Castelo	11	0
Nova Guataporanga	0	4
S. J. P. Alho	0	0
Total	**7,133**	**155**

Correlation coefficient (r = 0.98) significant in the level of 1%, where $\hat{Y} = -3,4224 + 0,0234\ X$

It shows the existence of an association between the number of human leishamaniasis cases (Y) and the number of positive dogs (X).

According Table 7, chi-square test (χ^2), utilized to compare the prevalence among cities of Dracena microregion, revealed that a > b > c > d > e, where different letters indicate significant differences among cities. Cities where *Leishmania* sp. antibodies were more prevalent, like Santa Mercedes (a: 30.03%), Paulicéia (a: 29.83%), Dracena (a: 29.25%), Panorama (a: 27.87%) and Ouro Verde (a: 27.70%), presented no significant difference among them (p > 0.05). On the other hand, Tupi Paulista (b: 18.12%) showed a significant statistical difference when compared to other cities (p < 0.05). There was no difference between Junqueirópolis (c: 13.38%) and Irapuru (c: 11.49 %); however, comparison between Monte Castelo (d: 4.91%) and Flora Rica (d: 2.56%) revealed significant difference. No circulating *Leishmania* sp. antibodies

were found in the cities of Nova Guataporanga (e: 0%) and São João do Pau D'Alho (e: 0%) until January 2008.

Table 7. Comparison of the disease prevalence by chi-squared test of cities from Dracena microregion, Alta Paulista (SP), from August 2005 to January 2008

Cities	Prevalence (%)		Santa Mercedes	Paulicéia	Dracena	Panorama	Ouro Verde	Tupi Paulista	Junqueirópolis	Irapuru	Monte Castelo	Flora Rica	Nova Guataporanga	S. J. P. Alho
Santa Mercedes	30.03	a	–	NS	NS	NS	NS	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.
Paulicéia	29.83	a	–	–	NS	NS	NS	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.
Dracena	29.25	a	–	–	–	NS	NS	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.
Panorama	27.87	a	–	–	–	–	NS	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.
Ouro Verde	27.70	a	–	–	–	–	–	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.
Tupi Paulista	18.12	b	–	–	–	–	–	–	Signif.	Signif.	Signif.	Signif.	Signif.	Signif.
Junqueirópolis	13.38	c	–	–	–	–	–	–	–	NS	Signif.	Signif.	Signif.	Signif.
Irapuru	11.49	c	–	–	–	–	–	–	–	–	Signif.	Signif.	Signif.	Signif.
Monte Castelo	4.91	d	–	–	–	–	–	–	–	–	–	NS	Signif.	Signif.
Flora Rica	2.56	d	–	–	–	–	–	–	–	–	–	–	Signif.	Signif.
Nova Guataporanga	0.00	e	–	–	–	–	–	–	–	–	–	–	–	NS
S. J. P. Alho	0.00	e	–	–	–	–	–	–	–	–	–	–	–	–

NS: results were not significant (p > 0.05); Signif.: results were significant (p < 0.05); a, b, etc.: prevalence followed by the same letter does not differ.

L. longipalpis was first detected in the urban area of Dracena, in March 2003, and the first cases of canine and human AVL were reported in 2005. Since then, the vector has adapted very well in the region, being detected in 12 cities since October 2005, when canine and human AVL cases spread. Initially, up to 2007, *L. longipalpis* was restricted only to municipalities from Dracena microregion. In February 2008, the phlebotomine sandfly was found in President Venceslau, a city contiguous to the affected region. This was an evidence of the great adaptability of *L. longipalpis* in neighboring areas, thus, confirming the spread of the disease. The dissemination of

the disease, in both dogs and humans, may be very rapid once its transmitter is detected, as shown in Table 2. As a result, the monitoring of cities must be on full alert to prevent a possible epidemic (Figure 1).

Dracena microregion.

Presidente Prudente
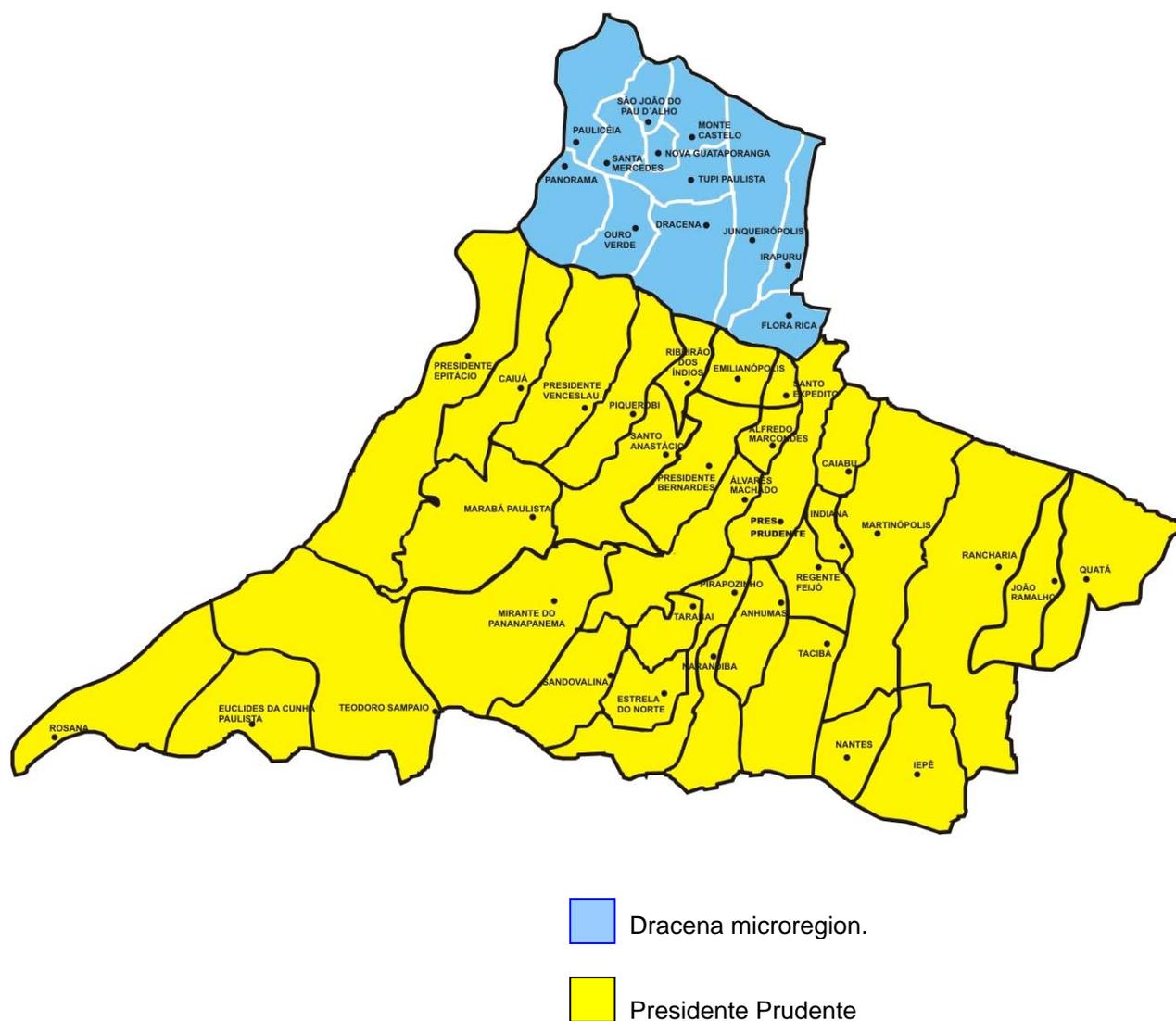
Figure 1. Cities of the 11th Regional Division of Health – Presidente Prudente.

Inadequate environmental supervision and poor socioeconomic conditions constitute a weak point of difficult solution for the program. Regarding environmental management, it is necessary to implement an awareness campaign, both in cities where the disease was not observed and in those where the problem is already

present. The campaign would involve modifications in procedures long employed by the population, such as the use of organic matter as a natural fertilizer, which is the substrate for the vector biological cycle. The expected changes in cultural behavior pattern concerning leishmaniasis will only be achieved over the time through educational campaigns, whose goals are to form eco-literate individuals in all segments of the society, involving children and engaged professionals.

The importance of proper soil management to control the AVL vector and, thus, control the disease, was also described by Lindoso et al (23). Barão *et al.* (29), in a study on the prevalence of AVL in Araçatuba (SP), affirmed that poor socioeconomic and environmental conditions intensify the incidence of the disease. Additionally, Carvalho (30) reported similar conditions that facilitate the proliferation of sandflies, enabling the AVL transmission.

In the cities where AVL has not yet been confirmed, the direct research method should be employed in suspected dogs. Furthermore, it is necessary to identify the species of *Leishmania* sp. in these places, since from this assumption serological surveys are carried out. Two criteria were used for notifying AVL human cases from 2005 to 2008 in the region (Table 3): the laboratorial criterion, based on positivity of direct parasitology, and the clinical/epidemiological one, in those cities where canine and human transmission were confirmed. The clinical/epidemiological criterion has been used due to the shortage of trained and skilled professionals to collect bone marrow puncture. The presumptive diagnosis, as well as the therapeutic one, has helped the program by reducing the number of deaths in suspected patients.

The serological survey census was carried out in all dogs from the area delimited by municipalities with confirmed AVL transmission, vulnerable receptive places and those under investigation (Table 4). The average prevalence of *Leishmania* sp. antibodies was 23.78%, revealing that the disease is installed and spreading through the region. IFAT was employed in investigations of outbreaks and to confirm reagent presence among samples (Table 4).

Despite of this, in cities where the canine AVL had already been confirmed, the São Paulo State Secretary of Health and the Brazilian Ministry of Health required two serological analyses for dogs – anti-*Leishmania* IgG antibody tests – to consider them positive for the disease, ELISA for "sorting" and IFAT to confirm the results. The latter technique is considered the "gold standard" for confirmation of infected animals (31). Following studies could explain which one is the best "gold standard". However,

in the absence of ELISA, IFAT is the technique of choice to define the diagnosis of animals that tested positive for the disease (14). If there is no confirmation of IFAT reactivity, the sample is considered inconclusive; then, the test should be repeated with new sampling, within a minimum period of 15 days and a maximum of 30 days after the first collection. Afterwards, the sample will be subject only to IFAT method (14). According to the suppliers of anti-*Leishmania* antibody tests, there is an inconsistency in use procedures to obtain a final result, regarding sensitivity and specificity, which determines whether or not the dog will be killed, a problem also reported by Braga *et al.* (32). Since IFAT presents greater sensitivity, it would be more useful in the "sorting", while ELISA –with greater specificity – would guarantee the accuracy of the infection suspicion.

During the studied period in Alta Paulista, a total of 6,006 (20.02%) samples presented inconclusive results and were retested in order to verify seroconversion. This fact jeopardizes AVLMCP and contributes to the disease spread, since it overloads the reference laboratory – that has a capacity of 2,000 tests per month – and the cities that are forced to carry out new collections, consequently delaying the program progress and the reservoir elimination. Another problem in serological tests is occurrence of false positive results provoked by *L. (L.) chagasi* infection in cases of ehrlichiosis, babesiosis, cutaneous leishmaniasis, Chagas' disease, among others. Araújo (33) presented an alternative with the rK39 immunochromatographic method (rK39 test), an analysis carried out on immunocromatography paper covered with the recombinant antigen K39. This test is considered rapid, sensitive and specific for confirmation of canine visceral leishmaniasis infections in routine diagnostic and epidemiological studies (33).

The use of such procedures casts doubt on result reliability, because seropositivity alone does not mean that the animal has a latent infection. Therefore, methods such as PCR should be used as "gold standard" to detect causative agents and antibody detection methods used only as tracers.

Taking into account the increased number of AVL human cases and deaths due to the disease in the region (Table 3), as well as the significant correlation between the increase in the canine prevalence and in human cases (Table 6), it is essential to implement more effective measures to control AVL, in order to reduce the infection among animals. Since treatment is not recommended, because treated dogs become inapparent reservoirs, the only option left would be kill of seroreactive dogs. This

initiative, however, is not being carried out in its fullness, since only 62.44% of seroreactive animals were killed (Table 5). This finding corroborates Braga *et al.* (32), who suggest a reduction of the period that an infected animal remains in a community and detection through a more sensitive technique of AVL antibodies.

This study noticed a high presence of antibodies against canine AVL in Dracena microregion. Among the studied cities, Dracena was the first to detect both canine and human transmission and it is among the five places with the higher prevalence, demonstrating the low effectiveness of disease control strategies in the region. It is important to point out that the results shown here date back to January 2008 and that nowadays the transmission of canine AVL can be found in all cities of the region.

CONCLUSIONS

Despite the adopted strategies to control AVL in Dracena microregion, Alta Paulista (SP), the disease continues its spread. Some procedures of the AVL monitoring and control program should be revised, aiming at improving its reliability and effectiveness.

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