

A SURVEY FOR AMERICAN CUTANEOUS AND VISCERAL LEISHMANIASIS AMONG 1,342 DOGS FROM AREAS IN RIO DE JANEIRO (BRAZIL) WHERE THE HUMAN DISEASES OCCUR

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There are areas in the periphery of Rio de Janeiro city where human cases of Visceral and/or Cutaneous Leishmaniasis occur. The parasites have been identified as Leishmania donovani and Leishmania braziliensis braziliensis respectively. A survey for Leishmaniasis was done among 1,342 dogs from those areas using an indirect immunofluorescent test. From the dogs, 616 came from areas where only human cases of Visceral Leishmaniasis occurred, 373 from an area where all human cases were of Cutaneous Leishmaniasis and 353 from a third area (Campo Grande) where both visceral and cutaneous human cases were detected. The prevalence of parasite antibody titers among dogs from areas of Cutaneous Leishmaniasis was significantly higher than that of Visceral Leishmaniasis (8.6% vs. 4.3%, $p < 0.02$). The highest prevalence was observed among dogs from the area where both diseases are present (12.7%).

Since 1977 autochthonous human cases of Visceral Leishmaniasis (Kala-azar) have been diagnosed in the periphery of Rio de Janeiro city (Salazar et al., 1979; Marzochi et al., 1981). They were diagnosed among people living in the continental border of the Pedra Branca mountain chain, where the incriminated vector *Lutzomyia longipalpis* was present (Souza et al., 1981).

Human cases of Cutaneous Leishmaniasis were first detected in Rio de Janeiro city in 1915, by D'Utra e Silva. More recently this disease has been reported by Junqueira (1969), Marzochi et al. (1980), Coutinho et al. (1981), Souza et al. (1982). The majority of human cases of Cutaneous Leishmaniasis in Rio de Janeiro city have been diagnosed among people living in the district of Jacarepaguá, mainly at the Atlantic Ocean border of the Pedra Branca mountain chain. However a few human cases of this disease have also been detected in other peripheral areas of the city, including the district of Campo Grande, localized at the continental border of those mountains where Visceral Leishmaniasis is also transmitted. In Jacarepaguá and others areas of Cutaneous Leishmaniasis within the State of Rio de Janeiro, the sandfly *Lutzomyia intermedia* is considered the main vector. In these areas this sandfly has been captured near or even inside the houses (Aragão, 1922; Araújo Filho, 1978; Lima, Marzochi & Sabroza, 1981).

The parasites isolated from dogs with Cutaneous and Visceral Leishmaniasis in those areas in Rio de Janeiro were respectively *Leishmania braziliensis braziliensis* and *Leishmania donovani*. The methods used for identification of parasites were isoenzyme methods (Momen, Grimaldi & Marzochi, 1983) and kDNA patterns after treatment with restriction endonucleases (Lopes et al., 1984). Monoclonal antibodies were also used (Grimaldi & McMahon-Pratt, 1984).

The present survey was done in order to investigate the prevalence of dog Leishmaniasis in those areas where the human diseases occur.

MATERIAL AND METHODS

The Area: the areas at risk for transmission of human Visceral Leishmaniasis are located at the continental border (North and North East) of the Pedra Branca mountain chain (Fig. 1). They represent peripheral parts of the districts of Realengo, Bangu, Senador Camará and Campo Grande. However, in Campo Grande, a few cases of the cutaneous disease have also been diagnosed.

Another area studied is Pau da Fome (district of Jacarepaguá), at the South border of the Pedra Branca mountain chains, near the Atlantic Ocean (Fig. 1). This is an area at risk for transmission of Cutaneous Leishmaniasis and where human cases of Visceral Leishmaniasis have not been found.

The number of houses as well as the human and canine populations studied from all these areas are shown in Table I.

A total of 1,342 dogs were examined; 616 of them from houses in areas where human cases of Visceral Leishmaniasis occurred; 373 from houses localized in Pau da Fome (Jacarepaguá), with human cases of Cutaneous Leishmaniasis; and 357 from Campo Grande, where both diseases are present.

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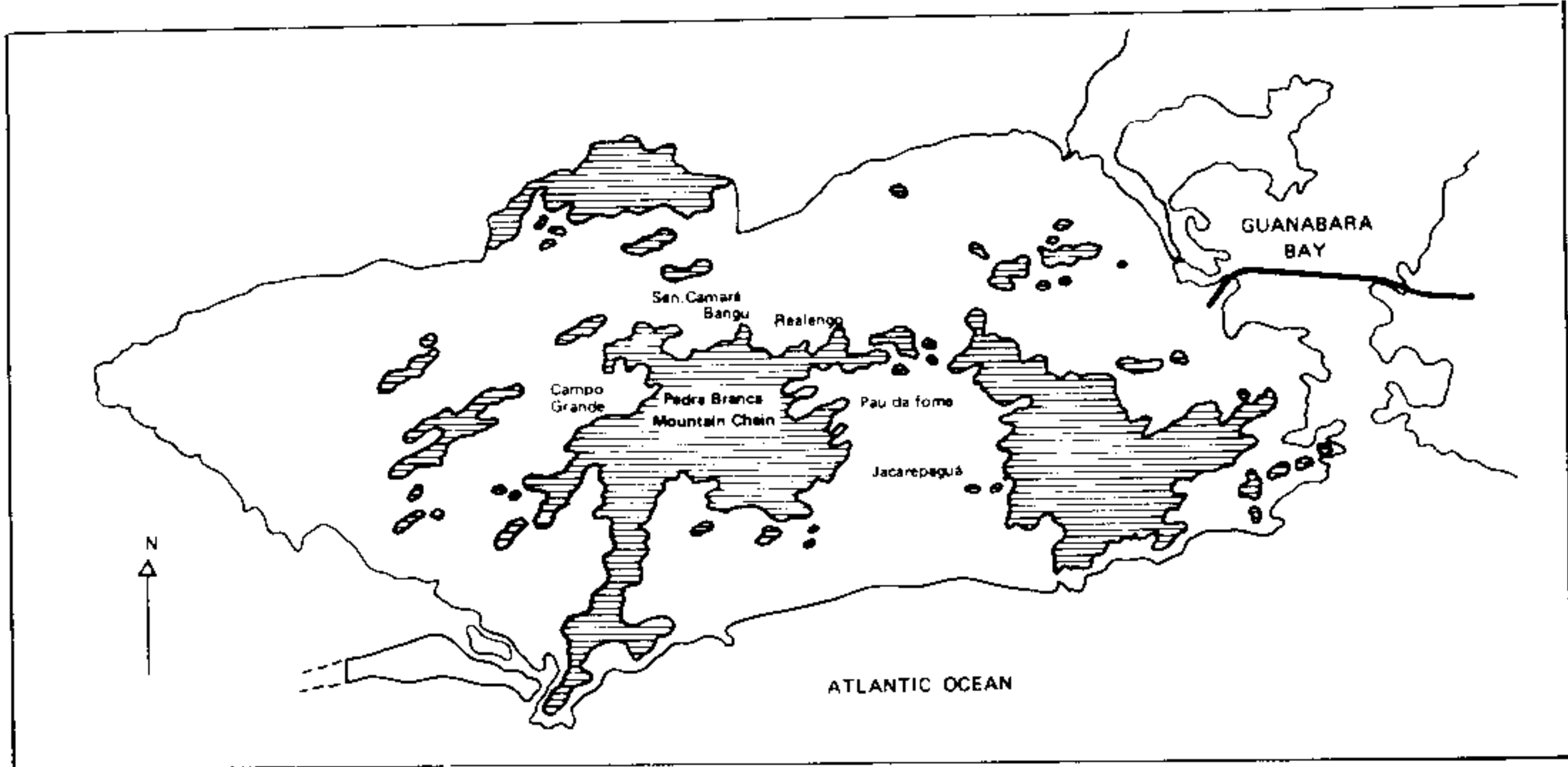


Fig. 1: municipality of Rio de Janeiro, showing sites mentioned in the text.

TABLE I

Number of houses, dogs and human population studied in districts of Rio de Janeiro city, at risk for transmission of Visceral (V) or Cutaneous Leishmaniasis (C)

Areas at risk for transmission	Number of houses	Human population at risk	Number of dogs submitted to the IF test	Number of IF positive tests
Realengo (V)	1,318	4,469	169	6
Bangu (V)	1,066	4,316	147	7
Senador Camará (V)	1,840	7,621	300	14
Campo Grande (VC)	629	2,359	353	45
Jacarepaguá (C) (Pau da Fome)	352	1,504	373	32
Total	5,205	20,269	1,342	104

IF = Indirect Immunofluorescence.

From July to December 1982 the animals were captured by SUCAM (Ministry of Health) in the areas of Realengo, Bangu, Senador Camará and Campo Grande, and from January to April 1983 in Pau da Fome.

Indirect Immunofluorescent Test (IF): this method was used to detect *Leishmania* antibodies among the dogs. Promastigotes of *Leishmania braziliensis* were used as antigen after 7 days culture in NNN medium enriched with RPMI (Flow Laboratories).

Preliminary tests comparing promastigotes of *Leishmania donovani* and *Leishmania braziliensis* as antigen for the IF test showed the latter producing the same or higher titers than the former. This finding was observed even in cases of proved Visceral Leishmaniasis in which *L. donovani* was isolated from the dog spleen. Iverson et al. (1983) had similar results in São Paulo.

The anti-dog Ig fluorescent conjugate was kindly provided by Dr. Mario Camargo, Instituto de Medicina Tropical, University of São Paulo. A few drops of dogs' blood were drawn by ear incision and collected on filter paper (Klabin 125) and a semi-quantitative IF test was performed. An area of the imbibed filter paper was determined experimentally corresponding to a 1:40 serum dilution when eluted in 0.7 ml PBS for 16 hrs at 6°-8°C. Subsequently, a double dilution (1:80) was done.

The SUCAM was in charge of the elimination of all IF positive dogs. Most of these were examined after death for parasites in the skin, spleen, liver and bone marrow. Clinical parasitological and histopathological findings in dogs will be published elsewhere.

Chi square test was used for statistical analysis of data.

RESULTS

A series of 20 parasitized dogs with *Leishmania* isolated from skin or spleen was first submitted to the IF test in order to evaluate accuracy of the test performed on blood samples collected with filter paper. Table II shows that 95% of these dogs had a IF test positive at the 1:40 dilution or higher.

The results of the IF test in these parasitized animals were similar using either the eluate blood or serum samples obtained through venous punctures (data not shown).

TABLE II

Results of the indirect immunofluorescence tests (IF) for Leishmaniasis in 20 proved parasitized dogs from areas where human cases of Visceral Leishmaniasis occurred

Sites of isolation of <i>Leishmania</i> parasites	IF - Titer			Total
	N*	1:40	≥ 1:80	
Spleen	0	1	5	6
Skin lesion	1	2	11	14
Total	1 (5%)	3 (15%)	16 (80%)	20 (100%)

N* = Negative at 1:40 dilution.

A control group of 47 apparently healthy dogs was also submitted to the IF test. These animals were either caught in the street or came from houses not related to areas of Leishmaniasis. All had negative IF tests at 1:40 dilution.

These results showed that IF anti-*Leishmania* antibody titers of 1:40 or higher are indicative of infection.

Among the 1,342 animals examined, 104 (7.7%) had an IF positive test (≥ 1:40). Fig. 2 shows their distribution according to the areas where one or both forms of Leishmaniasis occur. The prevalence of IF positive dogs was similar in the three areas of Visceral Leishmaniasis (Realengo, Bangu and Senador Camará) ($p > 0.10$), the mean rate being 4.3%. Fig. 2 also shows the highest rate of IF positive tests in Campo Grande (12.7%) where both diseases occur. This rate was statistically different from those observed in Realengo, Bangu and Senador Camará ($p < 0.005$), but not from that observed in Pau da Fome (12.7% vs 8.6%; $0.05 < p < 0.10$). The prevalence in Pau da Fome (area of Cutaneous Leishmaniasis) was significantly higher (8.6%) than those observed in Realengo, Bangu and Senador Camará (areas of Visceral Leishmaniasis) ($p < 0.02$).

The *Leishmania* parasite was isolated from most of the IF positive dogs.

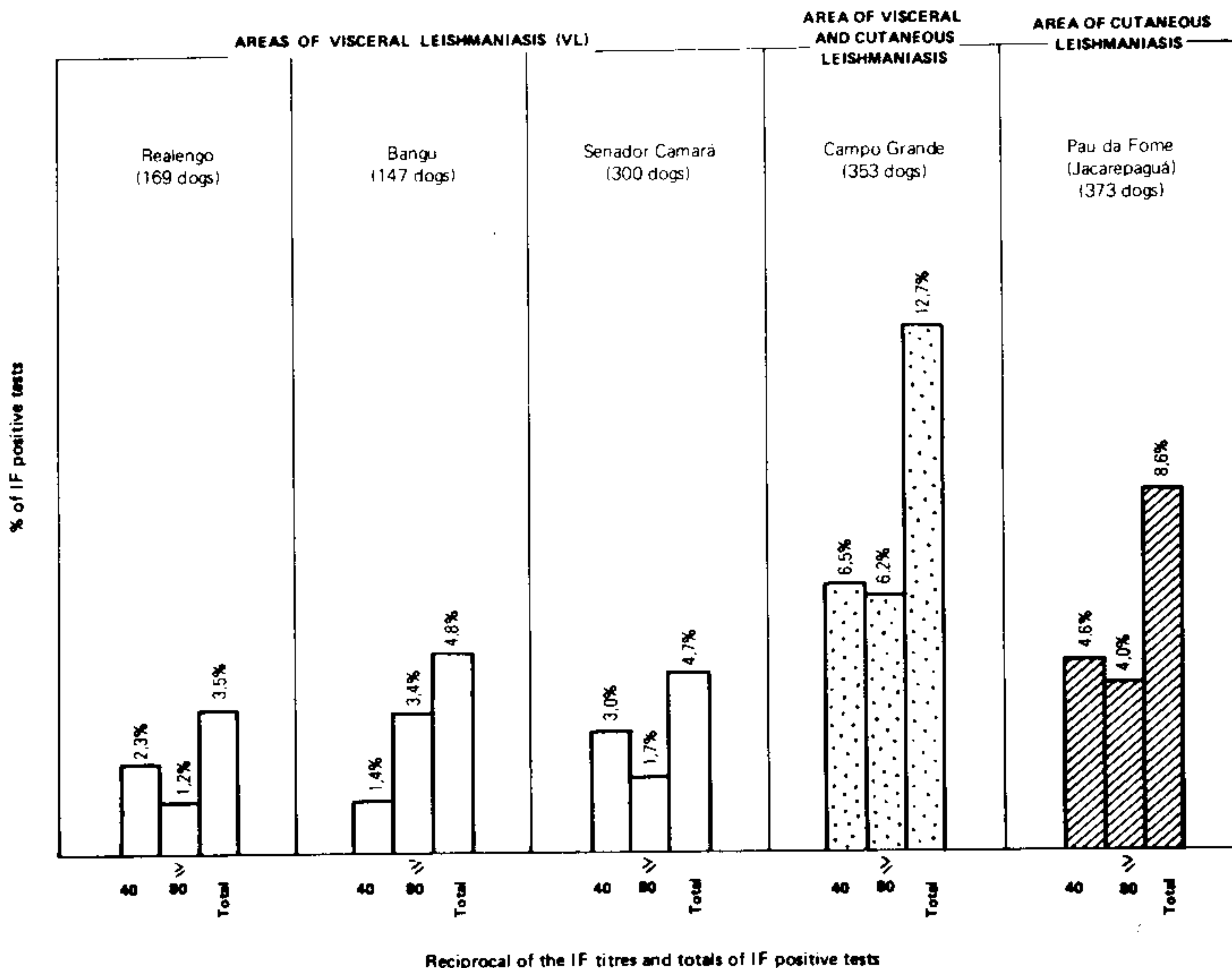


Fig. 2: indirect immunofluorescent test (IF) for visceral and cutaneous leishmaniasis among 1,342 dogs from areas in Rio de Janeiro where the human diseases occur.

DISCUSSION

The majority of human cases of Visceral Leishmaniasis in Brazil has been diagnosed in the North Eastern region of the Country. Alencar (1978) estimated around 6,000 human cases until 1976. According to Marzochi et al. (1981), the endemic area seems to be under expansion to the Southern region including the State of São Paulo (2 cases), Paraná (3 cases) and Rio de Janeiro (17 cases). Recent data have shown a total of 40 cases of acquired Visceral Leishmaniasis in Rio de Janeiro until February 1984, all of them living in an area at risk with about 20,000 inhabitants (Realengo, Bangu, Senador Camará and Campo Grande) (Table II).

A previous survey for canine Leishmaniasis performed in 1980 by SUCAM and the Federal University of Minas Gerais (Brazil) using the complement fixation test, in Bangu, Realengo and Senador Camará detected only 10 positive sera among 526 dogs examined (1.9%). The present results show 4.3% of positive IF tests among dogs from these same areas. This prevalence is in agreement with Alencar (1978) who suggested that in endemic areas dogs infections occur more frequently than human.

Percentage of IF positive dogs in Campo Grande (12.7%) was significantly higher when compared to that observed in the three areas of Visceral Leishmaniasis (Realengo, Bangu, Senador Camará). This result could be explained by the occurrence of both diseases, Cutaneous and Visceral Leishmaniasis in Campo Grande.

The first survey in Brazil for canine Visceral Leishmaniasis in a large number of animals was done by Chagas et al. (1938) in the State of Pará, through liver puncture. Later, with the same method, Deane & Deane (1955a) in Sobral, State of Ceará (North-East Brazil) found infection rates higher than 20% in some places.

Several surveys for canine Visceral Leishmaniasis were done in the State of Ceará using the complement fixation test (CFT). Nussenzweig, Nussenzweig & Alencar (1957) found 23.9% positive sera among 209 dogs. Alencar et al. (1974) found a mean of 3.16% positive tests among 184,243 dogs examined during the period from 1960 to 1964.

In the State of Minas Gerais, Brener (1957) and Magalhães et al. (1980) found high prevalences of positive dogs in some localities.

According to Sherlock & Almeida (1970) prevalence of positive dogs can be higher than 25% in some known focus of human Kala-azar in the State of Bahia. Iverson et al. (1983) in São Paulo found 10 IF positive tests among 389 dogs examined.

The important role played by dogs in the epidemiology of Visceral Leishmaniasis has been emphasized (Deane & Deane, 1955b). Parasites may be found in blood cells and are frequently numerous in the skin, dogs becoming in this way an important source for contamination of sandflies (Deane, 1956; Alencar, 1959).

The presence of both *Lu. longipalpis* (Souza et al., 1981) and infected dogs in this recent focus of Visceral Leishmaniasis in districts of Rio de Janeiro, fits into the usual epidemiological picture of the disease in Brazil.

The work of SUCAM (Ministry of Health) in those areas (spraying DDT in the houses and eliminating the IF positive dogs) has led to a drastic decrease in the number of new human cases of Visceral Leishmaniasis in Rio de Janeiro.

Cutaneous and Mucocutaneous Leishmaniasis in Brazil have been considered diseases acquired mainly in forest regions (Pessoa, 1963). Forest rodents, marsupials and arboreal edentates have been considered the major animal reservoirs in nature (Lainson, 1983). Recent investigations in the States of Rio de Janeiro, Minas Gerais and São Paulo, have demonstrated transmission of Cutaneous and Mucocutaneous Leishmaniasis in rural areas where forests have been destroyed, and even inside houses in urban or peri-urban areas (Menezes, Reis & Vasconcelos, 1974; Araújo Filho, 1978; Gomes, 1979; Mayrink et al., 1979; Sabroza, 1981). Infected dogs found in those areas usually have a low number of parasites in the skin ulcers. The presence of men modifying the original landscape probably has resulted in new ecological conditions for transmission of the disease (Forattini & Santos, 1955; Dias, 1975; Araújo Filho, 1978; Marzochi et al., 1982).

Since the first case of canine Cutaneous Leishmaniasis was described by Pedroso (1913) in Brazil, several other cases have been reported in different places in Latin America (Mazza, 1926; Pifano, 1940; Romaña et al., 1949; Herrer, 1951; Herrer & Christensen, 1976; Bonfante-Garrido, Morillo & Torres, 1981).

Nowadays there is no doubt that dogs are susceptible to contract Cutaneous Leishmaniasis. However their role as a linkhost from parasitized forest reservoirs to humans, or as a real amplifying factor for the transmission of the disease, mainly near human houses (Sabroza, 1981), needs further investigations.

Our results are consistent with the hypothesis that dogs may be important in the epidemiology of Cutaneous Leishmaniasis in Jacarepaguá, since the prevalence of IF positive dogs from that area of transmission of human Cutaneous Leishmaniasis was even higher than that observed in dogs from areas of human Visceral Leishmaniasis. A possible explanation for these different prevalences is that Visceral Leishmaniasis was only recently introduced in Rio de Janeiro. The severe parasitism in dogs with Visceral

Leishmaniasis facilitating the infection of phlebotomines, as opposed to what occurs in Cutaneous Leishmaniasis, can not be underestimated.

Several surveys for Cutaneous canine Leishmaniasis have been done in other Latin American Countries through microscopic skin examination for detection of parasites. Results obtained are similar to the prevalence we observe using the IF test. Pifano (1940) in Venezuela, studying 97 dogs, found 8 positive cases; Romaña et al. (1949) in Argentina found 5.1% positivity among 58 dogs; Herrer (1951) in Peru found 46 positive out of 513 dogs; and Herrer & Christensen (1976) in Panama found 3.3% positivity among 333 dogs. Several surveys have also been done in States of the South Eastern region of Brazil. Guimarães (1955) found one dog probably parasitized among 23 examined. Forattini & Santos (1955) found one positive among 81, Dias (1975) 11 among 355, and Araújo Filho (1978) 10.5% positives among 38 examined.

Canine Cutaneous Leishmaniasis may have a wide range of clinical and epidemiological features. Different parasites may be involved according to each geographical region. All these variables probably play a strong influence on the role of dogs in the epidemiology of the disease according to each specific area. As an example: the dog is regarded by Herrer (1951) as the principal reservoir of *Leishmania braziliensis peruviana* (Uta) in Peru but to Lainson (1983) the role of forest rodents as source for human and canine infections can not be minimized even in the Peruvian form of the disease. It should be noted that in Peru dogs usually have inconspicuous skin ulcers (Herrer, 1951) contrary to what occurs in Cutaneous Leishmaniasis in Brazil, Argentina and Venezuela.

RESUMO

Casos humanos de Leishmaniose Visceral e Leishmaniose Tegumentar têm sido diagnosticados em áreas periféricas da cidade do Rio de Janeiro. Os parasitas isolados têm sido identificados respectivamente como *Leishmania donovani* e *Leishmania braziliensis braziliensis*. Foi feito um inquérito para Leishmaniose entre 1.342 cães provenientes daquelas áreas utilizando-se a pesquisa de anticorpos pela imunofluorescência indireta. Dos cães examinados, 616 eram provenientes de áreas de ocorrência de casos humanos de Leishmaniose Visceral, 373 de área de Leishmaniose Tegumentar humana e os restantes 353 de uma região (Campo Grande) onde além de casos humanos de Leishmaniose Visceral, alguns casos de Leishmaniose Tegumentar haviam sido diagnosticados. A prevalência de anticorpos para *Leishmania* entre os cães provenientes de área de Leishmaniose Tegumentar foi significativamente maior que a observada nas áreas de Leishmaniose Visceral (8,6% vs. 4,3%; $p < 0,02$). Interessante é que a maior prevalência foi observada entre os cães da área onde ambas as doenças ocorreram (12,7%).

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