

## SEROLOGICAL SURVEY FOR CANINE CUTANEOUS AND VISCERAL LEISHMANIASIS IN AREAS AT RISK FOR TRANSMISSION IN RIO DE JANEIRO WHERE PROPHYLACTIC MEASURES HAD BEEN ADOPTED

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*A serological survey for canine visceral (VL) and American cutaneous leishmaniasis (ACL) has been carried out during 1984-1989, to assess the effects of the prophylactic measures adopted in areas where there was a risk of transmission of the diseases in Rio de Janeiro. A previous serological survey (1982/83) had detected serum positive dogs as well as the human disease in these same areas. A total of 22,828 dogs have been examined in this last survey, 7,807 of which came from Campo Grande (VL and ACL area), 4,110 from Jacarepaguá (ACL area), 4,146 from Realengo, 3,879 from Bangu and 2,886 from Senador Camará, (three VL areas). The analysis of these results showed a notable reduction in the number of serum positive dogs, compared to those of the first survey of 1982/83 as follows: (a) in Campo Grande (VL and ACL) the infection rate of the first survey was 12.7%, against 0.62% of the second; (b) in Jacarepaguá (ACL) it decreased from 8.6% to 1.8% (c) in Bangu, Realengo and Senador Camará (VL) the rate decreased from 4.3% to 0.38%. The results indicate that this decrease was due to the prophylactic measures adopted in those areas.*

Key words: leishmaniasis – dog – indirect immunofluorescence – prophylaxis

The first cases of human American cutaneous leishmaniasis (ACL) in Rio de Janeiro were described by D'Utra e Silva in 1915, and there have been several other subsequent reports. In 1974 workers from Fundação Oswaldo Cruz (FIOCRUZ) indicated a high transmission rate of the ACL in Jacarepaguá, an area in the periphery of Rio de Janeiro (FIOCRUZ, 1974). Other studies in the same area have been carried out by Marzochi et al. (1980), Coutinho et al. (1981), and Souza et al. (1982). An important observation during these studies was that some dogs showed skin lesions due to *Leishmania (v) braziliensis* (Lopes et al., 1984).

Naturally infected dogs with ACL in America were first observed by Pedroso (1913) and Brumpt & Pedroso (1913) in the forest area of São Paulo State. Recent studies have demon-

strated the presence of infected dogs in areas endemic for cutaneous leishmaniasis, mainly in the southeastern and northeastern regions of Brazil (Dias et al., 1977; Araújo Filho, 1978; Barreto et al., 1984; Coutinho et al., 1985; Marzochi et al., 1985 a, b) suggesting their possible role as amplifying the disease transmission when a peridomestic cycle occurs (Sabrosa, 1981). *Lutzomyia intermedia* is suspected as the main vector in this area.

On the other hand, VL is a disease which appeared in Rio more recently. The first autochthonous case in this area was described by Salazar et al. (1979). Sabrosa et al. (1978) showed high densities of *Lu. longipalpis* in the regions of Bangu and Realengo, a new observation in the municipality. In these areas the presence of naturally infected dogs has also been recorded (Marzochi et al., 1985 a, b).

The first cases of VL in dogs in Brazil were found in Ceará by Chagas et al. (1938) and since then infected dogs have been observed in all States where the human disease has been diagnosed. In the domiciliary environment dogs

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This work was partially supported by CNPq and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Received 3 June 1991.

Accepted 27 September 1991.

are important reservoirs of the parasite, since the *Lu. longipalpis* is readily infected, due to the abundance of parasites in the skin lesions and blood of the infected animal (Deane & Deane, 1955).

Classical control measures such as DDT spraying, identification and elimination of infected animals, as well as the treatment of patients, represent the main strategy of preventing the infection of the human and canine populations. According to the World Health Organization (WHO, 1984), the elimination of infected dogs is recommended as one of the most effective measures in the control of VL transmission. In Rio de Janeiro a canine survey was carried out in 1982/83, in peripheral areas of the city where human ACL and VL cases had been diagnosed (Coutinho et al., 1985). The indirect immunofluorescence test (IF) was then utilized to identify serum positive animals. Based on the results of this survey, positive dogs were caught and eliminated by SUCAM. Other classical prophylactic measures such as therapy of human cases and spraying insecticides in the houses were also performed. The goal of the present paper is to verify the effectiveness of such measures, by means of a new serological survey carried out on dogs in the same areas previously studied.

#### MATERIALS AND METHODS

*Studied areas* – The sites we have studied have been those showing the highest prevalence of human ACL in the municipality of Rio de Janeiro (Marzochi et al., 1980; Sabroza, 1981), located on the Atlantic slope of the Pedra Branca mountain chain, in the district of Jacarepaguá (sites of Camorim, Rio Bonito, Piabas, Grota Funda, Vargem Grande, Vargem Pequena, Pau da Fome and Rio Pequeno).

This area has still some rural characteristics. Part of its population are involved in agriculture and raise domestic animals, such as hens and pigs, both for its own subsistence and trade.

In this area *Lu. intermedia* and *Lu. migonei* have been captured feeding on men and dogs, suggesting that both these sandflies might be participating in the transmission of ACL in Jacarepaguá. *Lu. longipalpis* has also been found, though at lower rates (1.1%) (Rangel et al., 1986; Lima et al., 1988).

Concerning VL, the areas we studied have been those at risk for human and canine VL transmission in the municipality (Marzochi et al., 1981, 1985a; Coutinho et al., 1985) located on the continental border of the Pedra Branca mountain chain, comprising of the suburbs of Bangu, Realengo, Senador Camará and Campo Grande (Fig. 1). In Campo Grande both VL and ACL have been diagnosed (Lima et al., 1981).

The people of these areas of VL transmission has a low income and are mostly concerned with agriculture. In the regions studied the presence of *Lu. longipalpis*, the only species of *Lutzomyia* spp so far incriminated in the transmission of VL in Brazil has been confirmed (Souza et al., 1981).

In both VL and ACL areas, most houses are located at the base of the hills, although some can be found in flatter, more urbanized areas.

The present canine serological survey was carried out in VL and ACL areas from January, 1984, to December, 1989.

*Serological diagnosis* – The method used to detect anti-*Leishmania* antibodies in the blood of dogs was the indirect immunofluorescence test (IF). Promastigote forms of a Brazilian strain of *Leishmania* phenotypically similar to *L. major* (MHOM/BR/76/JOF) (Momen et al., 1985) has been used as antigen. They were obtained from 7 day old culture in NNN medium enriched with RPMI and 5% of fetal calf serum.

Blood was collected on filter paper from a small cut made on the ear of each dog. In order to make a semi-quantitative test, an area was determined on the filter paper soaked with blood which, when eluted in 0.7 ml of PBS and kept at 4 °C overnight, corresponded to a dilution of 1:40 of serum (Coutinho et al., 1985). Titres > 40 were considered positive for leishmaniasis. Rabbit IgG anti-dog conjugated with fluorescein was provided by SUCAM-MS and was used throughout this work.

A total of 22,828 samples of dog blood were examined by the IF. The dogs examined were from the following areas: Campo Grande – 7,807; Realengo – 4,146; Bangu – 3,879; Senador Camará – 2,886; and Jacarepaguá – 4,110. These numbers refer to the years bet-

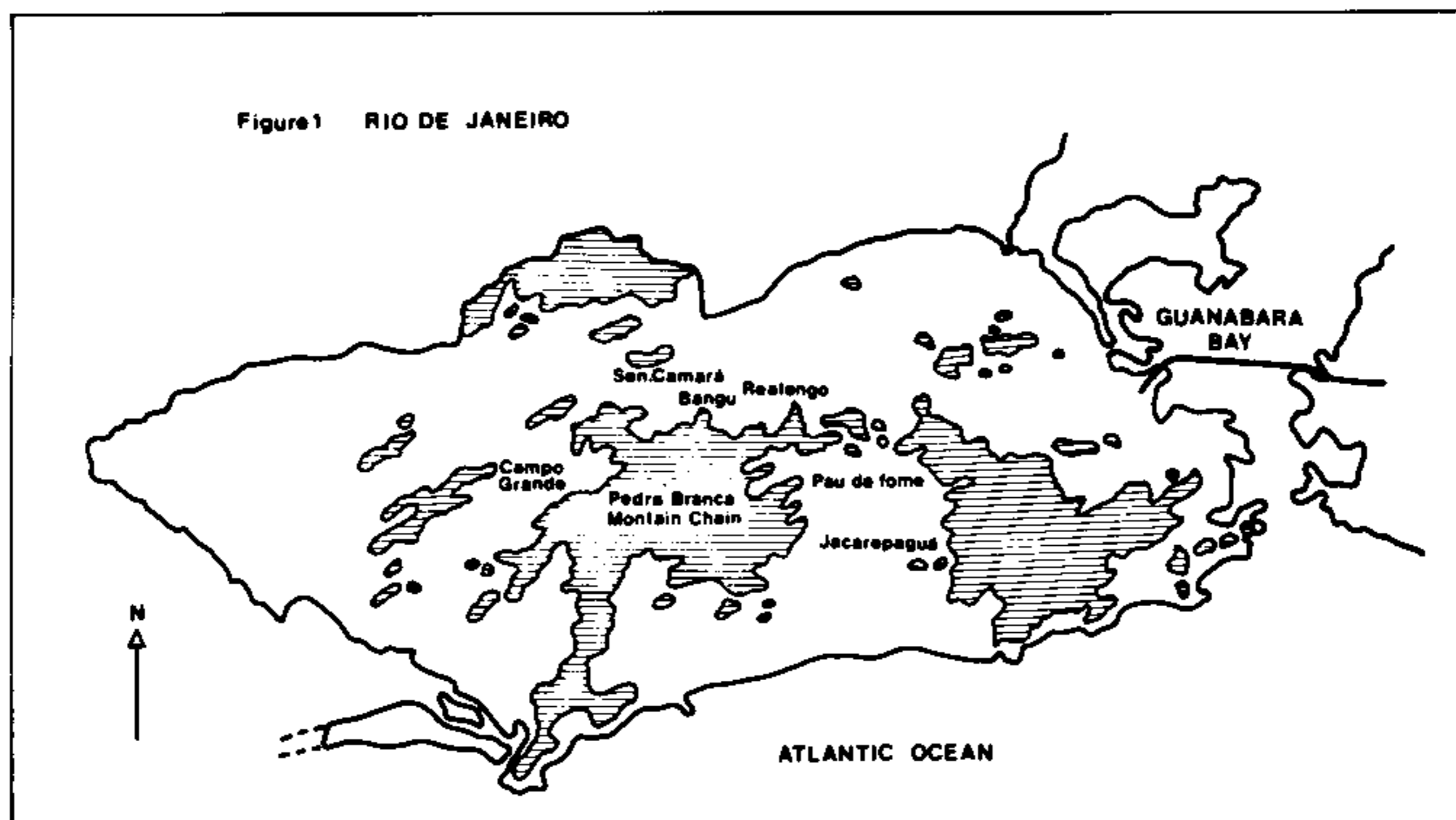


Fig. 1: municipality of Rio de Janeiro, showing the areas of human and canine visceral and american cutaneous leishmaniasis.

ween 1984 and 1989, except for 1985, which has not been included, due to the lack of data.

*Prophylactic measures employed* – Previous studies (Araújo Filho, 1978; Mayrink et al., 1979; Sabroza, 1981; Lima et al., 1988) and the results of our first canine survey carried out in the areas of VL and ACL, suggest that the transmission of both diseases occurs in and around the human dwellings. Thus SUCAM, during the period of 1983-89 undertook the prophylactic measures in VL and ACL areas, which consisted of: (a) active search for human and canine cases and sanitary education; (b) residences, where serum positive dogs were detected, were sprayed with DDT (2 g/m<sup>2</sup>) both inside and outside as well as in all outside buildings including animal shelters and other residences located within a radius of 100 m; (c) canine blood was collected in each of the areas for detection of serum-positive animals (Table II); (d) serum-positive dogs were removed and eliminated; and (e) patients with leishmaniasis were treated.

*Statistics* – The chi-square was used to determine differences in prevalence of serum-positives dogs.

## RESULTS

From January 1984 to December 1989, the size of the areas sprayed by SUCAM increased

in relation to the area previously studied (Coutinho et al., 1985) and as did the number of dogs studied.

Table I shows the distribution of the houses studied and/or sprayed with insecticide in areas at risk for VL and ACL transmission.

Table II and Figs 2, 3 and 4 show the results of the present survey for canine leishmaniasis. In all of the areas a clear decrease in the number of serum positive dogs was verified between 1982/83 (Coutinho et al., 1985) and 1984 ( $p < 0.01$ ). In VL areas the prevalence of dogs showing positive IF test during 1984-1989 was the smallest (0.38%) when compared with the other areas. Moreover, a significant decrease in the prevalence of positive dogs in VL and VL + ACL areas occurred much earlier (1984/1986) than in ACL area. The prevalences observed in 1989 were significantly much lower than that ones of 1984 in all the three areas studied. (Table II and Figs 2, 3, 4).

## DISCUSSION

An attempt was performed to evaluate the prevalence of serum positive dogs after the adoption of classical prophylactic measures based on the results of a canine serological survey in the areas of VL and ACL transmission in the municipality of Rio de Janeiro between 1982 and 1983. The adopted measures, as we

TABLE I

Number of houses sprayed and/or studied in areas at risk for visceral (VL) and/or American cutaneous leishmaniasis (ACL) in Rio de Janeiro

Years Areas	1984			1986			1987			1988			1989			Total		
	ST	S	(%)	ST	S	(%)	ST	S	(%)	ST	S	(%)	ST	S	(%)	ST	S	(%)
Bangu (VL)	3534	1175	(33.2)	4920	753	(15.3)	4420	498	(11.3)	4149	1671	(40.3)	3347	763	(22.8)	20370	4860	(23.9)
Realengo (VL)	1758	325	(18.5)	3827	823	(21.5)	3696	382	(10.3)	4374	717	(16.4)	3984	798	(20)	17639	3045	(17.3)
Senador Camará (VL)	2335	383	(16.4)	3039	507	(16.7)	4174	911	(21.8)	2364	704	(29.8)	1772			13684	2505	(18.3)
Sub-total (VL)	7627	1883	(24.7)	11786	2083	(17.7)	12290	1791	(14.6)	10887	3092	(28.4)	9103	1561	(17.1)	51693	10410	(20.1)
Campo Grande (VL + ACL)	4279	1508	(35.2)	8558	1172	(13.7)	6110	1124	(18.4)	6954	1990	(28.6)	12770	2736	(21.4)	38671	8530	(22.1)
Jacarepaguá (ACL)	2847	1213	(42.6)	2075	288	(13.9)	3710	2300	(62)	3198	680	(21.3)	4743	2069	(43.6)	16573	6550	(39.5)

ST: number of houses studied; S: number of houses sprayed; (-): area not sprayed; (%): number of houses sprayed of the total of houses studied.

TABLE II

Serological survey for canine leishmaniasis in areas of transmission risk in Rio de Janeiro

Areas	Number of dogs examined						Number of serum positive dogs <sup>a</sup> (%)					
	1984	1986	1987	1988	1989	Total	1984	1986	1987	1988	1989	Total
Bangu (VL)	451	502	937	1269	720	3879	16 (3.5)	2 (0.4)	0	4 (0.3)	1 (0.14)	23 (0.6)
Realengo (VL)	413	238	695	1471	1329	4146	0	0	7 (1)	2 (0.13)	2 (0.15)	11 (0.26)
Senador Camará (VL)	281	244	908	730	723	2286	4 (1.4)	0	2 (0.2)	2 (0.27)	0	8 (0.28)
Sub-total (VL)	1145	984	2540	3470	2772	10911	20 (1.7)	2 <sup>b</sup> (0.2)	9 <sup>c</sup> (0.35)	8 <sup>c</sup> (0.23)	3 <sup>c</sup> (0.10)	42 (0.38)
Campo Grande (VL + ACL)	667	1156	1554	1865	2565	7807	17 (2.5)	13 <sup>b</sup> (1.1)	7 <sup>b</sup> (0.45)	7 <sup>c</sup> (0.37)	5 <sup>c</sup> (0.2)	49 (0.62)
Jacarepaguá (ACL)	586	269	1166	938	1151	4110	23 (3.9)	9 <sup>c</sup> (3.3)	28 <sup>c</sup> (2.4)	9 <sup>b</sup> (0.96)	4 <sup>c</sup> (0.35)	73 (1.8)

a: indirect immunofluorescence test; b: prevalence significantly different ( $p < 0.01$ ) when compared with the former year; c: prevalence not significantly different ( $p > 0.05$ ) when compared with the former year; VL: areas at risk for transmission of visceral leishmaniasis. ACL: areas at risk for transmission of american cutaneous leishmaniasis.

have already mentioned, had already been successfully adopted in other regions of Brazil (Nery-Guimarães & Bustamante, 1954; Alencar & Cunha, 1963; Magalhães et al., 1980).

The present study demonstrates that there was a significant decrease in the prevalence of canine leishmaniasis in all of the areas studied, as indicated by a much lower serum positivity rates in dogs in 1989 (VL - 0.38%; VL + ACL - 0.62%; ACL - 1.8%) than observed in 1982/83 (Figs 2, 3, 4 and Table II).

In VL areas a remarkable decrease was observed in the number of positive dogs when comparing the first survey (4.3%) with the second survey 1984-1989 (0.38%). In Campo Grande, a VL and ACL transmission area, the prevalence reached 12.7% by the time of the first survey. After the adoption of the prophylactic measures this rate decreased significantly to 0.62% in 1989. With regard Jacarepaguá, an ACL area, the first survey showed an average of 8.6% positivity, while between 1984 and 1989 this rate decreased to 1.8%, showing that

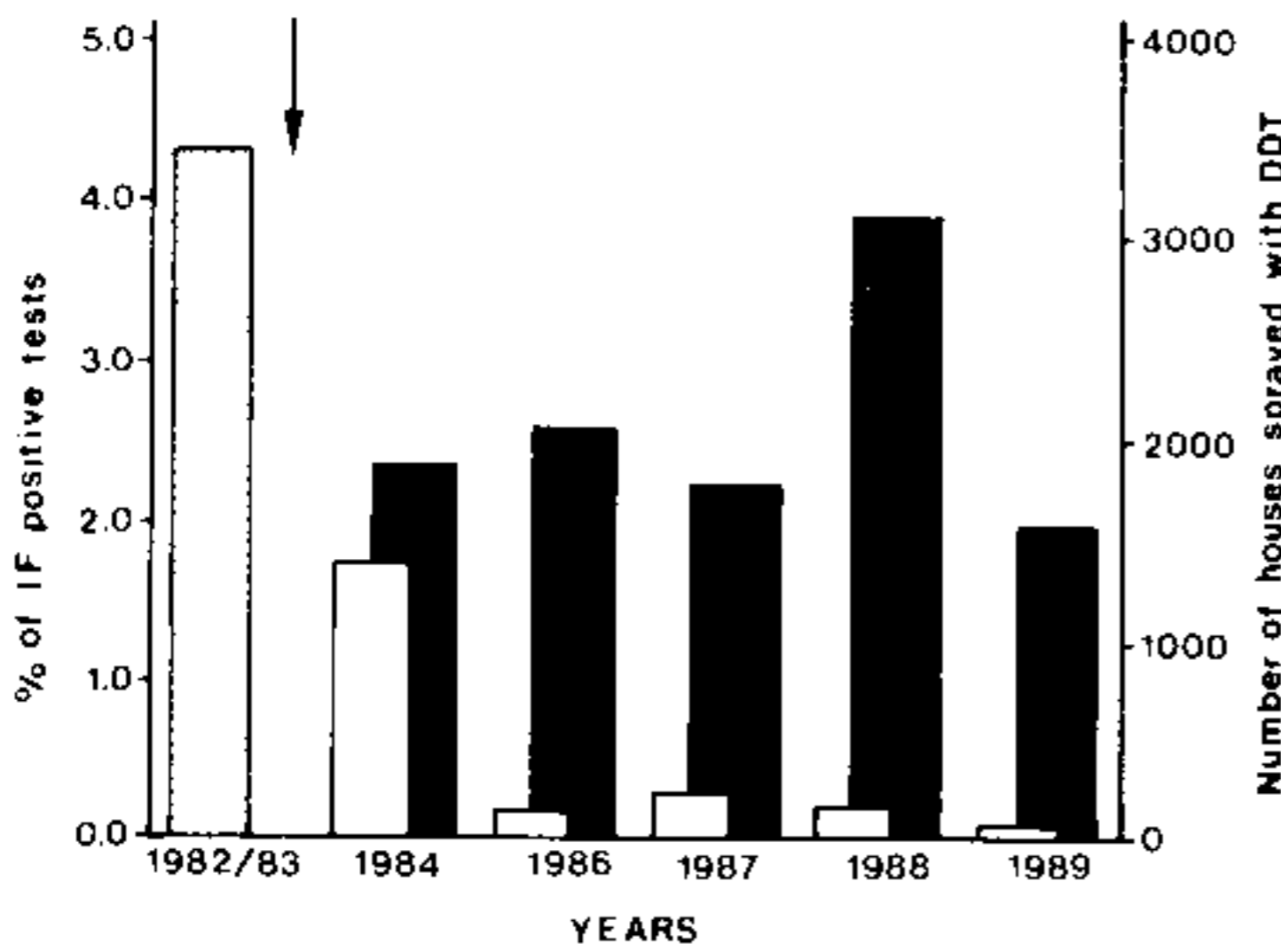


Fig. 2: serology for leishmaniasis using the indirect immunofluorescence (IF) test in dogs from the VL areas (Bangu, Realengo and Senador Camará): percentage of serum positive dogs in a previous survey (Coutinho et al., 1985) (□); percentage of serum positive dogs after the adoption of prophylactic measures (□); number of houses sprayed with DDT (2 g/m<sup>2</sup>) (■). The arrow indicates the beginning of DDT spraying.

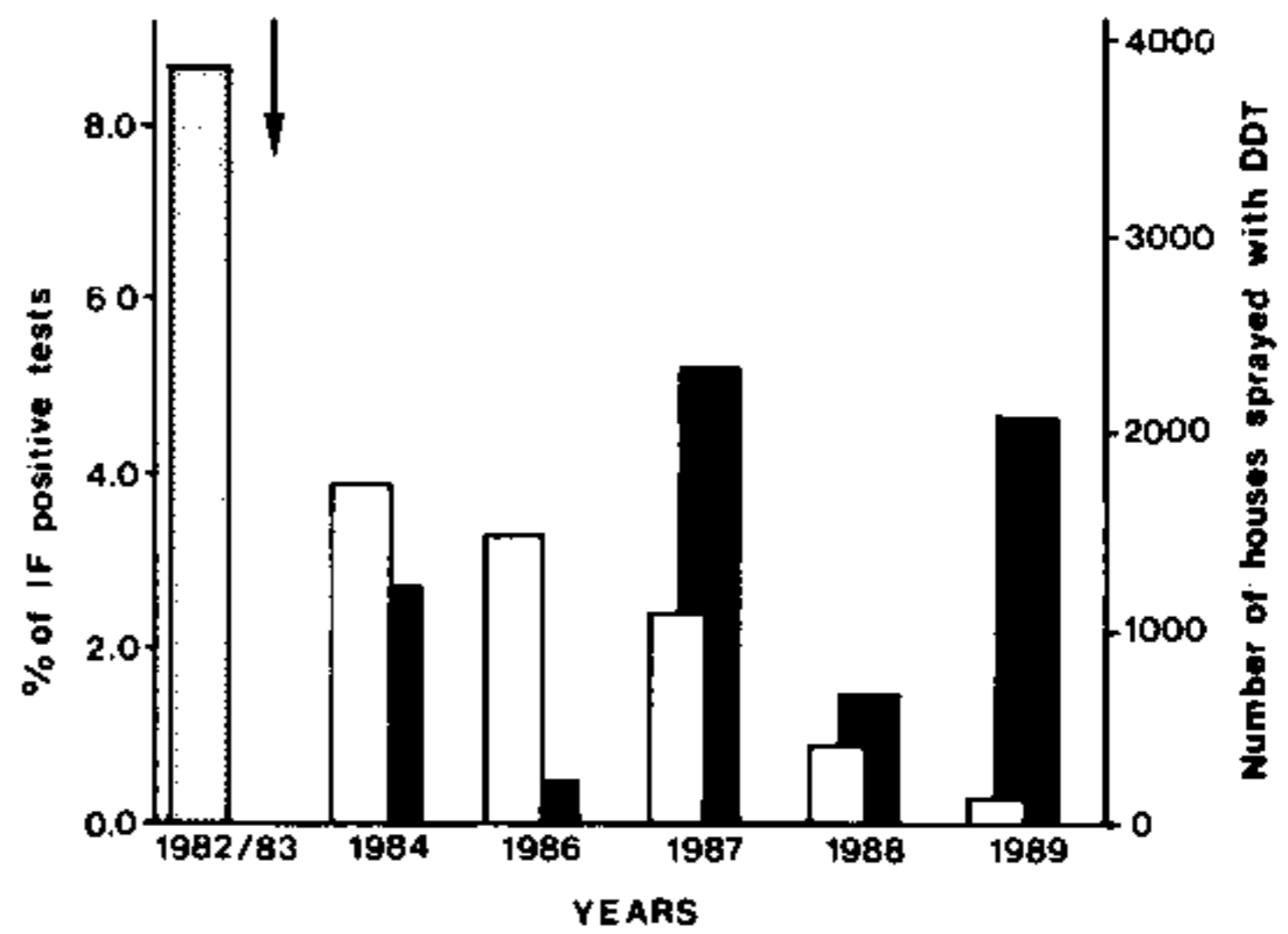


Fig. 4: serology for leishmaniasis by using the indirect immunofluorescence (IF) test in dogs from the ACL area in Jacarepaguá: percentage of serum positive dogs in a previous survey (Coutinho et al., 1985) (□); percentage of serum positive dogs after the adoption of prophylactic measures (□); number of houses sprayed with DDT (2 g/m<sup>2</sup>) (■). The arrow indicates the beginning of DDT spraying.

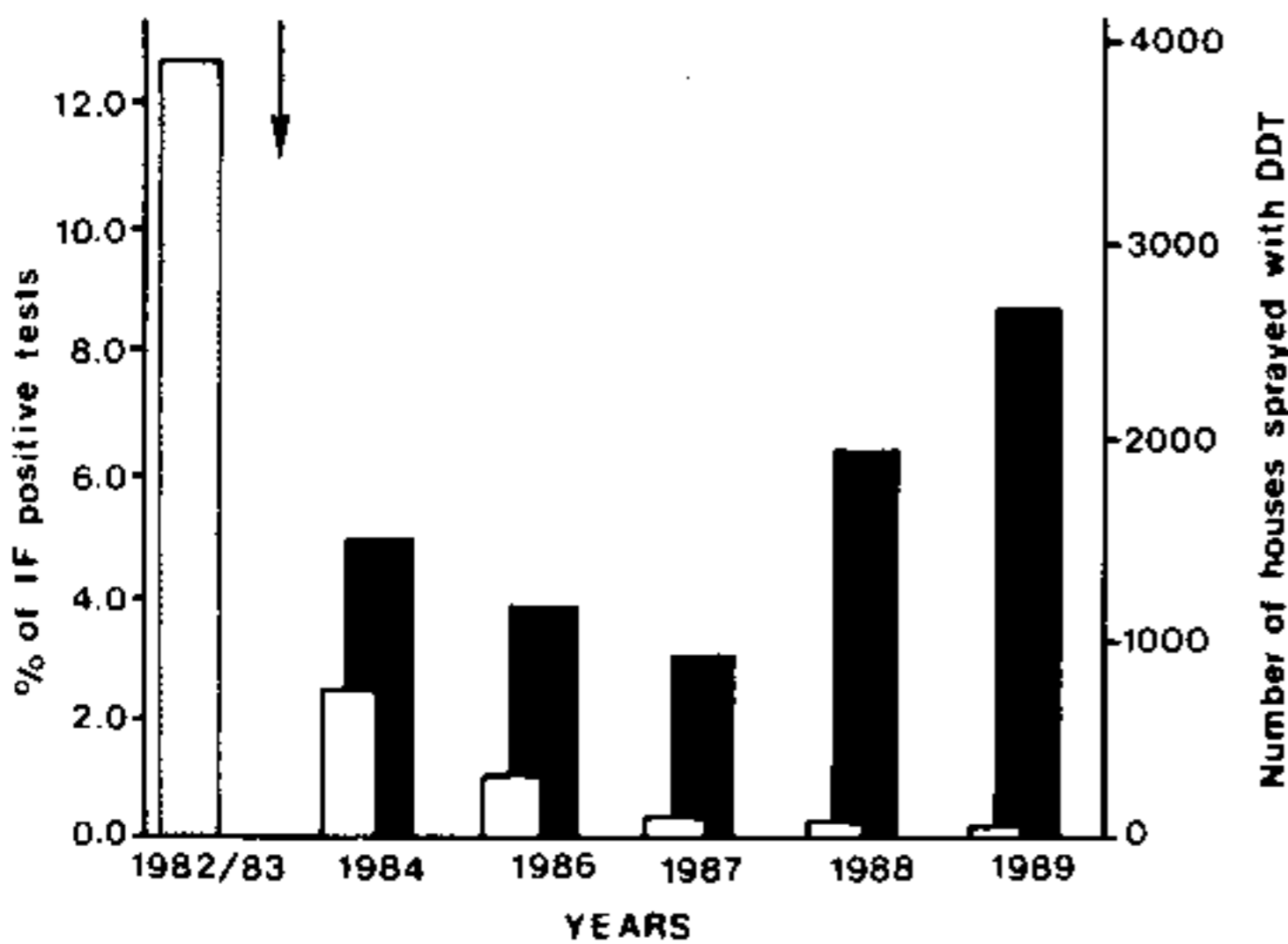


Fig. 3: serology for leishmaniasis using the indirect immunofluorescence (IF) test in dogs from the LV and ACL area in Campo Grande: percentage of serum positive dogs in a previous survey (Coutinho et al., 1985) (□); percentage of serum positive dogs after the adoption of prophylactic measures (□); number of houses sprayed with DDT (2 g/m<sup>2</sup>) (■). The arrow indicates the beginning of DDT spraying.

the infection rate decreased progressively after the DDT spraying. These data indicate the effectiveness of the prophylactic measures adopted by SUCAM, at least concerning canine infection.

In the present survey a dramatic reduction of serum positive dogs in VL + ACL areas (Figs 2, 3) has been observed, probably because the control activities were more intense in these areas, probably due both to the importance of dogs as VL reservoirs and to the severity of the disease for the human population. Table I and Fig. 4 show that the number of houses sprayed in the ACL areas was not so constant in the period 1984-89 as it was in the VL areas, where it was maintained at about 2,000 sprayed houses. These data may explain why there was no significant drop in ACL area during 1986 and 1987 (Fig. 4, Table II) and emphasize the importance of the control measures for sandflies. According to SUCAM-RJ, a significant decrease has been observed in the number of human cases in VL areas after the spraying (1984), terminating in a total absence of disease transmission between 1986 and 1989.

These results are in accordance with Alencar & Cunha (1963), who compared data of a survey of canine and human VL in some areas that had been treated with DDT and in others which had not, in the State of Ceará. They verified that the infection-rate was significantly lower in the former.

Magalhães et al. (1980), working in a VL area in the Rio Doce valley, State of Minas Gerais, observed that after adoption of prophylactic

lactic measures in several municipalities, it was possible to interrupt the transmission of the disease in the two last years of the survey (1978/79).

In the ACL transmission area of Jacarepaguá, the prophylactic measures adopted seem to have resulted in a decrease in the percentage of serum positive dogs; 1989 showed a remarkable decrease, although it did not reach the same low levels observed in VL areas. It also seems to have happened with human ACL, since the number of new cases registered by SUCAM in Vargem Grande (Jacarepaguá) decreased from 50 and 79 cases in 1982 and 1983, respectively, to 29 and 18 in 1988 and 1989, respectively, although there was no interruption in the transmission. These results may reflect what occurred in the whole area of Jacarepaguá where a small percentage of positive dogs were still present.

The role played by dogs in VL epidemiology is well-known, but this is not totally true for ACL. Some authors suggest that dogs may play an important role in the amplification of domiciliary and peridomiciliary transmission of ACL (Foratini et al., 1976; Sabroza, 1981). Gomes et al. (1990) however studied the possibility of the dog participating in ACL transmission in both forest and peridomiciliary environments in the region of the Vale da Ribeira (State of São Paulo) and verified that this animal did not play an important role in transmission of the disease from the forest environment to the peridomestic habitat.

The effects of DDT spraying on the phlebotomine fauna in Jacarepaguá must have contributed significantly to the decrease of transmission (Lima et al., 1988), together with the removal of serum positive dogs and the treatment of the human cases.

As conclusion, the use of DDT together with other classical control measures, plays an important role not only in VL, but also in ACL prophylaxis, in areas where sandfly vectors have adapted to a peridomestic habitat.

#### ACKNOWLEDGEMENTS

To Dr M. C. A. Marzochi for critical suggestions; A. C. L. Sobral for useful discussions and R. L. Pelegrino for secretarial work.

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