Rev. Inst. Med. trop. S. Paulo
40 (1): 41-47, January/February, 1998

EPIDEMIOLOGICAL SURVEY ON CANINE POPULATION WITH THE USE OF IMMUNOLEISH SKIN TEST IN ENDEMIC AREAS OF HUMAN AMERICAN CUTANEOUS LEISHMANIASIS IN THE STATE OF RIO DE JANEIRO, BRAZIL

Elizabeth Gloria O. BARBOSA SANTOS(1), Mauro Célio A. MARZOCHI (1), Nilton Francisco CONCEIÇÃO (1), Célia Maria M. BRITO (1) & Raquel S. PACHECO (1,2)

SUMMARY

A survey for canine tegumentary leishmaniasis (CTL) has been carried out between 1986 and 1993 in seven endemic localities for American cutaneous leishmaniasis in the State of Rio de Janeiro. 270 dogs have been examined for their clinical aspects, the development of delayed hypersensitivity (DHS) with Immunoleish antigen and with immunofluorescent antibody research of IgG (IF). 28.2% of them had ulcer lesions and 3.3% had scars. The lesions consisted of single (39.5%) and mucocutaneous lesions (31.6%), multiple cutaneous (25.0%) and mucocutaneous lesions associated with cutaneous ulcers (4.0%). Twelve (15.8%) isolates from biopsies were analyzed by zimodeme and schizodeme and identified as *L. (V.) braziliensis*. The overall prevalence of canine infection that was evaluated with the skin test was of 40.5% and with IF it was of 25.5%. Both tests showed a high positive rate with relation to the animals with mucosal lesions, as in the case of human mucocutaneous leishmaniasis. The comparison of the two tests showed the skin test to have a better performance although there was no statistical difference ($p>0.05$) between them. The proportional sensitivity and specificity was of 84.0% and 74.0%, respectively. The Immunoleish skin test and IF are useful tools to be employed in CTL field epidemiological surveys.

KEYWORDS: Cutaneous leishmaniasis; Dogs; Survey; Skin test

INTRODUCTION

American cutaneous leishmaniasis (ACL) occurs between the South of the United States and the North of Argentina, thus constituting an important public health problem in Latin America (GRIMALDI et al., 1989). According to the report of the Brazilian Health Ministry (1996), ACL occurs in every State of Brazil except for the Federal District. ACL that is caused by *Leishmania (Viannia) braziliensis* shows distinct epidemiological profiles (MARZOCHI & MARZOCHI, 1994). In the northern region it is still a zoonosis that involves wild animals of the Amazon region. In the other regions, due to environmental and demographic changes, ACL occurs in the peripheries of the great centers and shows characteristics old colonizations that are close to secondary forests, in which domiciliary vectors and synantropic animals take part. The fact that infected dogs are frequently found in these endemic areas demonstrates the close relation between the presence of these animals and the occurrence of the human disease.

PEDROSO (1913) and BRUMPT & PEDROSO (1913) were the first ones to observe the natural infection of dogs in the State of São Paulo. Over the following years dogs were considered accidental hosts (PESSOA & BARRETO, 1948). However, after the outbreak of the 70s, a lot of authors have demonstrated the presence of infected dogs to be relatively frequent, especially in the Southeastern Region (ARAÚJO FILHO, 1978; DIAS et al., 1977; TOLEZANO et al., 1980). FALQUETO et al. (1986) have discovered a clear relation between the presence of infected dogs and the upsurge of new human cases, which suggested that the disease behaved as a zoonosis that was maintained by these animals.

PIRMEZ et al. (1988), in a clinical study that involved dogs that had been naturally and experimentally infected with *L. (V.) braziliensis*, have found out that these animals constitute the best model for the study of the human disease. PACHECO et al. (1986) have observed that the same *L. (V.) braziliensis* schizodeme was circulating in the human and canine population of ACL areas in the State of Rio de Janeiro. This fact is one more indicator that dogs take part as reservoirs in the peri-domestic epidemiologic cycle of ACL

In the end of the 70s, cutaneous leishmaniasis outbreaks took place in the State of Rio de Janeiro, at first in the District of Ilha Grande (ARAÚJO FILHO, 1978) and Jacarepaguá (MARZOCHI

(1) National School of Public Health, FIOCRUZ, Rio de Janeiro, RJ, Brazil
(2) Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, RJ, Brazil
Correspondence to: Elizabeth Gloria Barbosa Santos, Departamento de Ciências Biológicas, ENSP/FIOCRUZ, Rua Leopoldo Bulhões 1480, 6º andar, Manguinhos, 21041-210 Rio de Janeiro, RJ, Brasil. Fax: (021) 564-8985 - e-mail: ebsantos@manguinhos.fiocruz.ensp.br

et al., 1980) in the Municipalities of Angra dos Reis and Rio de Janeiro, respectivelly. In the following decade the outbreaks increased and new foci were discovered: the localities of Chatuba and Serrinha in the Municipality of Mesquita in the metropolitan area of Rio de Janeiro (OLIVEIRA NETO et al., 1988); Santa Isabel farm in São José do Vale do Rio Preto in the mountain region (MARZOCHI et al., 1991); Paraty in the Araújos island in the southern part of the State (SOUZA et al., 1991); and Braçanã do Norte District in the Municipality of Rio Bonito in the northern part of the State of Rio de Janeiro (County Secretariat of Health from Rio Bonito, personal communication).

Between 1986 and 1993 canine epidemiologic inquest were undertaken in the affected areas mentioned above. For this study, the delayed hipersensitivity skin test for veterinarian use, Immunoleish (MARZOCHI & BARBOSA SANTOS, submitted) was introduced as an auxiliary in the diagnosis of canine tegumentary leishmaniasis (CTL).

The aim of this study was to investigate the prevalence of the canine disease through cutaneous reaction and through the detection of anti-*Leishmania* flourescent antibodies. Furthermore, it contains a comparison between both reactions as screening for CTL diagnosis.

MATERIAL AND METHODS

The studied areas

The following seven localities in the State of Rio de Janeiro have been studied between the years of 1986 and 1993: the localities of Sacarrão and Pacuí (located on the Atlantic slope of Pedra Branca mountain chain) in District of Jacarepaguá in the Municipality of Rio de Janeiro; the areas of Serrinha and Chatuba, both of them situated on the Atlantic face of the Gericinó massif in the Municipality of Mesquita; Paraty, which lies in the Araújo island on the southern coast of the State; Santa Isabel farm in the Municipality of São José do Vale do Rio Preto the mountain region; and the District of Braçanã do Norte in the Municipality of Rio Bonito. (Table 1). The areas that were studied had rural characteristics, although they were close to urban centers. Part of the population cultivated bananas, vegetables, and raised pigs and chicken for subsistence.

In these localities, there was a high frequency of *Lu. intermedia* and *Lu. migonei* sandflies (RANGEL et al., 1984; RANGEL et al., 1990; SOUZA et al., 1991). The insects were captured with human baits on the inside walls and with light traps in chicken coops, pigsties and near the dog's shelter.

The animals

A total of 270 dogs were examined. Most of them were adult mongrel dogs that lived together with human ACL cases that had been treated or that were under treatment. 160 (59.3%) of the dogs were male and 110 (40.7%) female; 76 (28.2%) of them showed single or multiple skin or mucocutaneous lesions and 09 (3.3%) showed scars that suggested a prior *Leishmania* infection (Table 1).

Every dog had a clinical-epidemiological record which contained the following data: locality/city, address, owner's name, dog's name, sex, race, age, clinical observations, skin test, venous blood samples and biopsies of suspected lesions. The studies of the animals were made under verbal agreement with their owners.

Leishmania sp. research

During the epidemiological survey 36 samples of *Leishmania* sp. were isolated by biopsies of suspicious lesions. The fragments were harvested and kept over night at 4^0 C in a bottle with a solution of saline, streptomicin 100 µg and penicillin 1000 I.U. On the next day the fragments were transferred to an NNN medium that contained an a layer of liquid brain heart infusion and were incubated at 26^0 C. Two weeks later, each isolate was transferred to a Schneider's Drosophila medium that was supplemented with 20% heat-inactivated fetal calf serum at 26^0 C. Twelve (15.8%) stocks were characterized by isoenzyme analysis (MOMEN et al., 1985) and by restriction fragment lenght polymorphisms of kDNA analysis (PACHECO et al., 1986).

Laboratory Assays

The delayed hipersensitivity (DHS) cutaneous reaction with the use of the immunobiological Immunoleish was undertaken as it has been described by MARZOCHI & BARBOSA SANTOS (1988). The suspension that consisted of membrane fractions of *L. braziliensis* promastigotes was administered intradermically in a volume of 0.1 ml of the suspension that contained 200 mg of total protein in a merthiolated saline solution (1:10000) on the inside of one of the back limbs. The reading was undertaken after 48 hours and a cellular red cutaneous reaction of $\geq$ 5.0 mm was considered positive.

In the indirect immunofluorescence reaction (IF) for the detection of anti-*Leishmania* antibodies total blood of the femur vein of each animal was employed. IF was undertaken according to COUTINHO et al. (1985). Promastigotes of a Brazilian sample that was phenotypically similar to *L.major* (MHOM/BR/76/JOF) were used as antigen (MOMEM et al., 1985). The sample was obtained in a 5-day culture in a bifasic NNN medium that was enriched with BHI (Brain Heart Infusion) and supplemented with 10% inactivated fetal calf serum.

The samples of serum were diluted twofold in PBS saline (pH 7.2) after 1:40. The titers above 1:40 were considered positive for leishmaniasis. The immunoflourescent conjugate was offered by Biomanguinhos (FIOCRUZ). The conjugate was used in a 1:150 dilution. In each slide, positive and negative controls were tested.

Statistical analysis

Single variables were analyzed with the use of the chi-squared test (c^2) (FLEISS, 1973). In the paired comparison of the immunologic methods, the Kappa (k) agreement test (FLEISS, 1973) was employed. Sensitivity and specifity were calculated as follows: P (T_2 +/ T_1 -) and P (T_2 -/ T_1 -), respectively (FLEISS, 1973).

The chi-squared test was used in order to make the comparison between the frequency of the DHS and IF variables and the presence of dogs that had cutaneous lesions. In order to establish the type of association between positive or negative one it was calculated the Coefficient of Yule (BERQUÓ, 1981).

RESULTS

Clinical and epidemiological evaluation

Table 1 shows the result of the clinical/epidemiological enquiry that was undertaken among the 270 dogs of the localities of Sacarrão (56-20.7%), Pacuí (45-16.7%), Chatuba (33-12.2%), Serrinha (30-11.1%), Araújo island (16-5.9%), Santa Isabel farm (40-14.8%) and Braçanã do Norte District (50-18.6%). The clinical examination showed 76 (28.2%) of them to have cutaneous and mucocutaneous lesions and 09 (3.3%) to have ACL compatible scars. The ulcer lesions were detected among the animals: 30 (39.5%) had single lesions; 19 (25%) had multiple lesions; 24 (31.6%) had single mucocutaneous lesions and 3 (4%) had mucocutaneous lesions associated with cutaneous ones. The cutaneous ulcer lesions were found on their ears (43.8%), on their scrotum (18.4%) and on their snouts (37.8%).

The houses in which the dogs lived frequently had chicken coops, pigsties and a fruit storage. The population frequently referred to opossums and mice in the vicinity of the domiciles (about 70% of the interviews). Santa, Isabel farm was transformed into a poultry farm and its employees lived in masonry houses that were ceded by the owner; in these houses there were found dogs that took part in the inquest.

Leishmania identification

Out of 36 isolated suspicious lesions, 12 (15.8%) samples were identified as *L. (V.) braziliensis* through profiles of isoenzyme electrophoresis and restriction of kDNA.

Skin test and IF

The frequency of positive DHS (diameter ≥ 5.0 mm) and of *Leishmania* antibodies (titers ≥ 1:40) that were detected through IF among the 270 studied dogs were respectively of 105 (38.9%) and 66 (25.5%). There have been 11 (4.0%) losses due to lack of DHS and/or IF reading (Table 2). 144 (55.6%) of the dogs were negative (Table 3).

The diameter of the intradermic reactions varied between 5.0 and 18.0 mm and the titers of *Leishmania* antibodies varied between 1:40 and 1:320. The animals that had mucosal lesions were highly positive in both reactions: DHS ≥ 9.0 mm and IF ≥ 1:80.

On table 3 the results of the two tests as well as the co-positivity and the co-negativity of the responses to each test in relation to the other are represented in pairwise fashion. We have observed a poor level of agreement between the two tests ($k = 0.32$), although the rate of agreement was statistically significant ($z = 9.72$; $p < 0.01$).

TABLE 1

Epidemiological survey for tegumentary canine leishmaniasis in studied areas of transmission risk in Rio de Janeiro State.

Locality	Number of Animals					Number of Lesions				
	Total (%)	Male	Female	With lesion	With scars	Single Ulcer (%)	Multiple Ulcers (%)	Single Mucosa (%)	Associated Mucosal (%)	Total (%)
Sacarrão	56 (20.7)	31	25	15	-	05	04	03	02	14
Pacuí	45 (16.7)	21	24	14	-	05	03	09	-	17
Chatuba	33 (12.2)	17	16	16	05	05	08	02	-	15
Serrinha	30 (11.1)	19	11	10	-	03	03	05	-	11
Araújo	16 (5.9)	11	05	07	-	05	-	02	01	08
Sta Isabel	40 (14.8)	29	11	03	-	01	-	01	-	02
Braçanã	50 (18.6)	32	18	11	04	06	01	02	-	09
Total (%)	270 (100)	160 (59.3)	110 (40.7)	76 (28.2)	09 (3.3)	30 (11.1)	19 (39.5)	24 (25)	03 (4)	76 (100)

TABLE 2
Number of infective dogs examined by IF and DHS assays in the studied locality between 1986 - 1993.

Area	Total of Animals	Assays	
		IF (+)	DHS (+)
	(%)	(%)	(%)
Jacarepaguá	99	28	35
(Sacarrão/Pacuí)	(38.2)	(10.8)	(13.5)
Mesquita	65	21	42
(Chatuba/Serrinha)	(25.1)	(8.1)	(16.2)
Paraty	16	05	08
(Ilha do Araújo)	(6.2)	(1.9)	(3.1)
São José do Vale	32	01	05
do Rio Preto	(12.5)	(0.4)	(1.9)
(Santa Isabel Farm)			
Rio Bonito	50	10	15
(Braçanã do Norte)	(19.2)	(3.9)	(5.8)
Total	259⊗	66	105
(%)	(100)	(25.5)	(40.5)

⊗ 11 losses
$\chi^2 = 25.19$ $p < 0.001$

TABLE 3
Relation between the results obtained in the DHS and IF (IgG) reactions detected during the canine epidemiological survey.

Assays	IF (+)	IF (–)	TOTAL
	(%)	(%)	(%)
DHS (+)	56	49	105
	(21.6)	(18.9)	(49.5)
DHS (–)	10	144	154
	(3.9)	(55.6)	(58.5)
Total	66	193	259
(%)	(25.5)	(74.5)	(100)⊗

⊗ 11 losses
Co-positivity DHST/IF = 21.6% Sensitivity =84%
Co-negativity DHST/IF = 55.6% Specificity = 74%
$z = 9,72$ $k = 0,32$ $p < 0.001$

TABLE 4
Correlation between DHS and IF reactions in the detection of canine tegumentary disease among the studied animals.

Samples	DHS		IF	
	+	-	+	-
	(%)	(%)	(%)	(%)
Lesion (+)	53	6	45	14
n=59	(90)	(10)	(76)	(24)
Lesion (-)	52	148	21	179
n=200	(26)	(74)	(11)	(89)

Lesion (+)/DHS (+)
$\chi^2 = 76$
Q = 92.2%
$p < 0.001$

Lesion (+)/IF (+)
$\chi^2 = 58$
Q = 90.6%
$p < 0.001$

On table 4 the χ^2 test has been used to correlate the presence of a lesion in the animals and the positivity of the used tests. The results that were obtained showed no significant difference between the nominal positive values of DHS ($\chi^2 = 76$) and of IF ($\chi^2 = 58$) in relation to the presence of the skin lesions. It was estimated through the Coeficient of Yule that the level of correlation was positive in both, but slightly higher in the DHS reaction (Q=92.2% - IC =95%) than in the IF (Q=90.6% - IC =95%). Indeed, the sensitivity obtained in this study for the cutaneous test was of 90% whereas for the IF it was of 76% although the specificity that was found was of 74.0% and 90%, respectively. However, the use of both tests showed a proportional sensitivity of 84% and an specificity of 74 %.

DISCUSSION

Over the last decades ACL has been recorded in a growing number in many localities in the Brazilian States showing a different epidemiological classic pattern with transmission in wild areas (ARAÚJO FILHO, 1978; DIAS et al., 1977; MARZOCHI & MARZOCHI, 1994; OLIVEIRA NETO et al. 1988; TOLEZANO et al., 1980). In these areas the disease is found among adults and children of both sexes and among domestic animals, like dogs (AGUILAR ET AL., 1989; COUTINHO et al., 1985; FALQUETO et al., 1986) and horses (AGUILAR et al., 1989; VEXENAT et al., 1986; YOSHIDA et al., 1990). Furthermore, the sandfly population of the *Lu.*

intermedia species is bigger in a domestic and peri-domestic environment than in forests (GOMES et al., 1982) and *Lu. migonei*, due to its cinophily, might take part in the transmission chain of canine leishmaniasis (AGUIAR et al., 1993).

In the 70's there was a *L. (V.) braziliensis* ACL outbreak in the District of Jacarepaguá (in the city of Rio de Janeiro) and some years later in the cities of Mesquita, Paraty, São José do Vale do Rio Preto and Rio Bonito, in the State of Rio de Janeiro (MARZOCHI et al., 1980; MARZOCHI et al., 1991; OLIVEIRA NETO et al., 1988).

In the present study, a total of 12 isolates were identified as *L. (V.) braziliensis,* which is the only species that has been isolated in the State of Rio de Janeiro (GRIMALDI et al., 1989), except for a sole human case and other canine one in which the primary cutaneous lesion was caused by *L. (Leshmania) chagasi* (OLIVEIRA NETO et al., 1986).

Visceral leishmaniasis has been detected in Rio de Janeiro since 1977 (SALAZAR et al., 1979). Human autochthonous cases has been diagnosed in peri-urban areas of the city located on the continental (Pedra Branca and Gericinó Massifies) and littoral slopes (Pedra Branca Massif) (MARZOCHI et al., 1994), where is situated Jacarepaguá District. Even this it was not observed any case of canine visceral leishmaniasis in our study.

The epidemiological survey that was realized with a total of 270 dogs didn't show a statistically significant difference between the amount of animals per locality, sex or presence of lesions ($p< 0.05$). Ninety eight lesions were detected on hairless areas, as ears, scrotum and snout and consisted of single and mucosal ulcers (11.1% and 5.5%, respectively), multiple cutaneous lesions (8.8%) and mucosal lesions associated with cutaneous lesions (5.5%). These observations agree with those of FALQUETO et al., (1986) with respect to an endemic area in the State of Espírito Santo.

Scars that suggested *Leishmania* were observed on 5 and 4 dogs from Chatuba and Braçanã do Norte Districts, respectively. The canine disease seems to show seasonal changes between lesions in the winter and scars in the summer. ACL in the State of Rio de Janeiro usually presents a single cutaneous lesion. In human the proportion of oronasal mucosal lesions and reinfection is low (OLIVEIRA NETO et al., 1988). However, the parasite can persist and be reisolated from appropriately treated lesions even eight years after the treatment (SCHUBACH et al., 1997). Maybe the mitocondrial genomic pattern, with homogeneous characteristics, as found in this State (PACHECO et al., 1986), could represent a distinct biological behavior.

The prevalence of infected dogs which was detected in this study was of 25.5% for IFR and 40.5% for the Immunoleish skin test. Both reactions have shown a high rate of frequency as compared to other studies (AGUILAR et al., 1989; FALQUETO et al., 1986; MAYWALD et al., 1996; SILVEIRA et al., 1988). This result may indicate that the canine tegumentary infection, as well as kala-azar (ALENCAR, 1978), occurs more frequently than the human disease in the Brazilian endemic area.

In the present paper the screening precision for leishmaniasis canine infection of IF and of the skin test was compared. The k agreement of the two tests was statistically significant ($z = 9.72$; $p < 0.001$). The sensitivities of the methods were of 76.0% and 90.0% and the specificities were of 90% and 74%, respectively. However when both tests were used together their sensitivities and specificities were of 84.0% and 74.0% (Table 3).

Infection with *L. (V.) braziliensis* elicits cellular and humoral immune responses. Delayed hypersensitivity is measured by Montenegro test, which tends to render positive results even after treatment (PESSOA & BARRETO, 1948). However, the use of Montenegro antigen in dogs was innocuous (PIRMEZ et al., 1988). The first skin test for CTL diagnosis was prepared by MARZOCHI and BARBOSA SANTOS (1988) and was called Immunoleish (MARZOCHI & BARBOSA SANTOS, submitted). The performance of Immunoleish skin test shows it is a useful tool for CTL survey (SANTOS et al., 1994). TAFURI et al. (1993) compared Immunoleish and Leishvaci® skin tests through a histopathological study. The analysis has shown that Immunoleish has elicited a more intense and diffuse inflammatory reaction than Leishvacin®, which is consistent with the clinical test that was read among dogs that had been naturally infected by *L. (V.) braziliensis*. Furthermore the authors confirmed that the best time to read the Immunoleish skin test read was after 48 hours, which is the same as for the Montenegro skin test.

When comparing the IFI and the skin test as a screening test for CTL it was found out that the skin test showed higher agreement rates with relation to the dogs that had characteristic lesions ($\chi^2 = 76$, 95% IC) than IF ($\chi^2 = 58$, 95% IC) although a statistical difference has not been noticed ($p>0.05$).

The results reflect the high prevalence of tegumentary leishmaniasis infection and disease among dogs from the studied areas, which confirms the observations that these animals play an important part on peri-domiciliar and domestic transmission of ACL. The use of the Immunoleish skin test and IF is a useful tool to be employed on ACL field epidemiological surveys.

RESUMO

Inquérito epidemiológico em população canina utilizando o teste cutâneo immunoleish em áreas endêmicas de leishmaniose cutânea americana no Estado do Rio de Janeiro, Brasil.

Um inquérito epidemiológico em população canina foi realizado em 7 localidades endêmicas de Leishmaniose Tegumentar Americana (LTA) entre os anos de 1986 a 1993, no Estado do Rio de Janeiro. Duzentos e setenta cães foram examinados, segundo os parâmetros: clínicos, desenvolvimento

de hipersensibilidade tardia e dosagem de anticorpos por imunofluorescência indireta (IFI). 28,2% dos animais possuíam lesões e 3,3% eram portadores de cicatrizes compatíveis com infecção prévia de *Leishmania* sp. De um total de 98 lesões ulceradas detectadas, 39,5% eram cutâneas únicas, 25,0% lesões cutâneas múltiplas, 31,6% lesões de mucosa e 4,0% lesões de mucosa associadas a lesões cutâneas. Doze amostras (15,8%) colhidas de fragmentos de lesões cutâneas foram analisadas por zimodema e esquizodema e identificadas como *Leishmania (V.) braziliensis* . A prevalência da infecção canina avaliada pelo teste cutâneo e pela IFI foram respectivamente de 40,5% e 25,5%. A comparação entre as reações mostrou que o teste cutâneo foi superior na detecção da infecção e da doença canina, embora, sem diferença estatística significante (p> 0,05). A sensibilidade e a especificidade proporcional foram respectivamente de 84,0% e 74,0%. A utilização de ambas as reações, cutânea e sorológica mostraram-se úteis como instrumento de diagnóstico epidemiológico em áreas de LTC.

ACKNOWLEDGEMENTS

We thank Dr. Pedro Cabello for statistical analysis and Mrs. Betina von Staa for reviewing the English manuscript.

REFERENCES

1. AGUIAR, G.M.; MEDEIROS, W.M.; SANTOS, T.G. et al. - Ecology of sandflies in a recent focus of cutaneous leishmaniasis in Paraty, littoral of Rio de Janeiro State (Diptera, Psychodidae, Phlebotominae). **Mem. Inst. Oswaldo Cruz, 88**: 339-340, 1993.

2. AGUILAR, C.M.; RANGEL, E.F. & DEANE, L.M. - Cutaneous leishmaniasis is frequent in equines from an endemic area in Rio de Janeiro, Brazil. **Mem. Inst. Oswaldo Cruz, 81**: 471-472, 1986.

3. ALENCAR, J.E. - Leishmaniose visceral no Brasil. **Rev. Fac. Med. Univ. Fed. Ceará, 17**: 129-148, 1978.

4. ARAÚJO FILHO, N.A. - Epidemiologia da leishmaniose tegumentar na Ilha Grande, Rio de Janeiro. Estudos sobre a infecção humana, reservatórios e transmissores. Rio de Jnaeiro, 1978. (Dissertação de Mestrado - Universidade Federal do Rio de Janeiro).

5. BERQUÓ, E.S.; SOUZA, J.M.P. & GOTLIEB, S.L.D. - Bioestatística. São Paulo, E.P.U., 1981.

6. BRUMPT, E. & PEDROSO, A. - Pesquisas epidemiológicas sobre a leishmaniose Americana das florestas no Estado de São Paulo. **An. paul. Med. Cirurg., 28**: 97-136, 1913.

7. COUTINHO, S.G.; NUNES, M.P.; MARZOCHI, M.C.A. & TRAMONTANO, N. - A survey for American cutaneous and visceral leishmaniasis among 1,342 dogs from areas in Rio de Janeiro (Brazil) where the human disease occur. **Mem. Inst. Oswaldo Cruz, 80**: 17-22, 1985.

8. DIAS, M.; MAYRINK, W.; DEANE, L.M. et al. - Epidemiologia da leishmaniose tegumentar. 1. Estudo de reservatórios em área endêmca no Estado de Minas Gerais. **Rev. Inst. Med. trop. S. Paulo, 19:** 403-410, 1977.

9. FALQUETO, A.; COURA, J.R.; BARROS, G.C. et al. - Participação do cão no ciclo de transmissão da leishmaniose tegumentar no Município de Vianna, Estado do Espírito Santo, Brasil. **Mem. Inst. Oswaldo Cruz, 81**: 155-163, 1986.

10. FLEISS, J. - Statistical methods for rates and proportions. New York, John Wiley & Sons, 1973. p 146-153.

11. GOMES, A.C.; COUTINHO, S.G.; PAIM, G.V. et al. - Aspectos ecológicos da leishmaniose tegumentar americana. **Rev. Inst. Med. trop. S. Paulo, 32**: 105-115, 1990.

12. GRIMALDI JR., G.; TESH, R.B. & MCMAHON-PRATT, D. - A review of the geographic distribuition and epidemiology of leishmaniasis in the new world. **Amer. J. trop. Med. Hyg., 41**: 687-725, 1989.

13. MARZOCHI, M.C.A.; COUTINHO, S.G.; SABROZA, P.C. & SOUZA, W.J.S. - Reação de imunofluorescência indireta e intradermorreação para leishmaniose tegumentar americana em moradores nas áreas de Jacarepaguá (Rio de Janeiro). Estudo comparativo dos resultados observados em 1974 e 1978. **Rev. Inst. Med. trop. S. Paulo, 22:** 149-155, 1980.

14. MARZOCHI, M.C.A. & BARBOSA SANTOS, E.G.O. - Evaluation of a skin test on the canine mucocutaneous leishmaniasis diagnosis. **Mem. Inst. Oswaldo Cruz, 83:** 391-392, 1988.

15. MARZOCHI, M.C.A. & BARBOSA SANTOS, E.G.O. - Immunoprophylaxis in dogs and others domestic animals as an alternative method for the control of American visceral and tegumentary leishmaniasis. **Mem. Inst. Oswaldo Cruz** (submitted).

16. MARZOCHI, M.C.A.; MARZOCHI, K.B.F.; VIVÓRIO, K. et al. - Leishmaniose tegumentar americana por *Leishmania (Viannia) braziliensis* em Município da região serrana do Estado do Rio de Janeiro. **Rev. Soc. bras. Med. trop., 24 (suppl. 2)**: 100, 1991.

17. MARZOCHI, M.C.A. & MARZOCHI, K.B.F. - Tegumentary and visceral leishmaniasis in Brazil, Emerging anthropozoonosis and possibilities for their control. **Cadern. Saúde públ. (Rio de J), 10 (suppl. 2)**: 359-375, 1994.

18. MAYWALD, P.G.; MACHADO, M.I.; COSTA CRUZ, J.M. & GONÇALVES PIRES, M.R.J. - Leishmaniose tegumentar, visceral e doença de Chagas caninas nos Municípios do Triângulo Mineiro e Alto Paranaíba, Minas Gerais, Brasil. **Cadern. Saúde públ. (Rio de J.), 12**: 321-328, 1996.

19. MINISTÉRIO DA SAÚDE/FNS-CENEPI - Leishmaniose: distribuição de casos notificados em junho de 1995 e igual período de 1996, por Unidade Federada, Brasil. **Inf. epidem. SUS, 3:** 101, 1996.

20. MOMEN, H.; GRIMALDI JR., G.; PACHECO, R.S. et al. - Brazilian *Leishmania* stocks phenotypically similar to *Leishmania major* . **Amer. J. trop. Med. Hyg., 34**: 1076-1084, 1985.

21. OLIVEIRA NETO, M.P.; GRIMALDI Jr., G.; MOMEN, H. et al. - Active cutaneous leishmaniasis in Brazil, induced by *Leishmania donovani chagasi.* **Mem. Inst. Oswaldo Cruz, 81:** 303-309, 1986.

22. OLIVEIRA NETO, M.P.; PIRMEZ, C.; RANGEL, E. et al. - An outbreak of American cutaneous leishmaniasis (*Leishmania braziliensis braziliensis*) in a periurban area of Rio de Janeiro city, Brazil: clinical and epidemiological studies. **Mem. Inst. Oswaldo Cruz, 83:** 427-435, 1988.

23. PACHECO, R.S.; LOPES, U.G.; MOREL, C.M. et al. - Schizodeme analysis of *Leishmania* isolates and comparision with some phenotypic techniques. **In:** RIOUX J.A., ed. ***Leishmania* taxonomie et phlogenèse. Applications eco-épidemiologiques**. Montpellier, France, Colloque International CNRS/ INSERM, 1994. IMEE, 1986, p. 91-104.

24. PEDROSO, A.M. - Leishmaniose local do cão. **An. paul. Med. Cirurg., 1:** 33-39, 1913.

25. PESSOA, S.B. & BARRETO, M.P. - Leishmaniose Tegumentar Americana. Rio de Janeiro, Ministério de Educação e Cultura, Imprensa Nacional, 1948.

26. PIRMEZ, C.; COUTINHO, S.G.; MARZOCHI, M.C.A. et al. - Canine American cutaneous leishmaniasis: a clinical and immunological study in dogs naturally infected with *Leishmania braziliensis braziliensis* in an endemic area of Rio de Janeiro, Brazil. **Amer. J. trop. Med. Hyg., 38**: 52-58, 1988.

27. RANGEL, E.F.; SOUZA, N.A.; WERMELINGER, E.D. & BARBOSA, A.F. - Infecção natural de *Lutzomyia intermedia* Lutz & Neiva, 1912 em área endêmcia de leishmaniose tegumentar no Estado do Rio de Janeiro. **Mem. Inst. Oswaldo Cruz, 79**: 395-396, 1984.

28. RANGEL, E.F. & AZEVEDO, A.C.R. - Studies on sandflies fauna (Diptera: Psychodidae) in a foci of cutaneous leishmaniasis in Mesquita, Rio de Janeiro, Brazil. **Mem. Inst. Oswaldo Cruz, 85**: 39-45, 1990.

29. SANTOS, G.P.L.; PONTES, C.; MÁSPERO, R.C. et al. - Leishmaniose tegumentar americana (LTA) no Município de Paracambi, Estado do Rio de Janeiro. III. Inquérito canino. **Rev. Soc. bras. Med. trop., 27 (suppl.)**: 35, 1994.

30. SCHUBACH, A. ; CUZZI-MAYA, T.; OLIVEIRA, A.V. et al. - Leishmanial antigen detected in active lesions and ancient scars of American tegumentary leishmaniasis patients: diagnostic possibilities and immunopathological considerations. **Amer. J. trop. Med. Hyg.** (submitted).

31. SILVEIRA, T.G.; TEODORO U.; LONARDONI, M.V.C. et al. - Investigação sorológica em cães de área endêmica de leishmaniose tegumentar, no Estado do Paraná, sul do Brasil. **Cadern. Saúde públ. (Rio de J), 12**: 89-93, 1996.

32. SOUZA, M.B.; MARZOCHI, M.C.A. & CONCEIÇÃO, N.F. - Estudo preliminar da fauna flebotomínica da Ilha do Araújo, Município de Parati, Rio de Janeiro. **Rev. Soc. bras. Med. trop., 24 (suppl.)**: 106, 1991.

33. TAFURI, W.L.; RASO, P.; HERMETO, M.V. et al. - Estudo histopatológico comparativo do teste cutâneo em cães de área endêmica de leishmaniose tegumentar, utilizando dois antígenos: Leishvacin® e o P10.000. **Rev. Soc. bras. Med. trop., 26**: 11-14, 1993.

34. TOLEZANO, J.E.; MACORIS, S.A. & DINIS, J.M.P. - Modificação na epidemiologia da leishmaniose tegumentar no Vale do Ribeira, Estado de São Paulo, Brasil. **Rev. Inst. Adolfo Lutz, 40:** 49-54, 1980.

35. VEXENAT, J.A.; BARRETO, A.C.; ROSA, A.C.O. et al. - Infecção natural de *Equus asinus* por *Leishmania braziliensis braziliensis* , Bahia, Brazil. **Mem. Inst. Oswaldo Cruz, 81**: 237-238, 1986.

36. YOSHIDA, E.L.A.; CORREA, F.M.A.; MARQUES, S.A. et al. - Human and equine (*Equus caballus*) leishmaniasis due to *Leishmania braziliensis* (= *Leishmania braziliensis braziliensis)* in the south-west region of São Paulo, Brazil. **Mem. Inst. Oswaldo Cruz, 85**: 133-134, 1990.

Recebido para publicação em 09/09/1997
Aceito para publicação em 16/02/1998