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AN OUTBREAK OF AMERICAN CUTANEOUS LEISHMANIASIS *(LEISHMANIA BRAZILIENSIS BRAZILIENSIS)* IN A PERIURBAN AREA OF RIO DE JANEIRO CITY, BRAZIL: CLINICAL AND EPIDEMIOLOGICAL STUDIES

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*From July 1984 to September 1986, 105 cases of American cutaneous leishmaniasis were studied in a locality closely situated to an urbanized area of the city of Rio de Janeiro, Brazil. Settlement in this area was established at least 20 years ago but the first cases were noted six months prior to the beginning of this study. Cases were almost exclusively cutaneous and ulcerated, with one to six months of evolution. Montenegro's skin tests were positive in all cases and anti-*Leishmania *antibodies were detected by indirect immunofluorescence test in 74.3% of the patients. Parasites were demonstrated in 69.5% of cases. Domestic animals were easily found infected: 32% of the examined dogs and 30.8% of the examined equines were positive to the presence of* Leishmania *in cutaneous ulcerated lesions. Parasite isolates from human, dog and equines were immunologically characterized and identified as* L. b. braziliensis. *73.0% of the sandfly population were* Lutzomyia intermedia *mainly caught on human baits and on domestic animals.*

Our observations suggest that this is an area of recent established L. b. braziliensis *infection and that transmission probably occurs indoors or outdoors close to the houses.*

Key words: American cutaneous leishmaniasis – outbreak – peridomiciliar transmission

American cutaneous leishmaniasis (ACL) is present at least since the beginning of the century in Rio de Janeiro state (D'Utra e Silva, 1915; Aragão, 1927) with several outbreaks described at different sites of the state, all related with new settlements established in recently deforested areas. However, in the last twenty years or so, the disease has showed an epidemiological behaviour different from that initially studied and now it is possible to observe the disease near the metropolitan region of great cities in areas of ancient colonization, where forest had been cleared long time ago (Araujo F., 1978; Mayrink et al., 1979; Tolezano et al., 1980).

With the recent techniques of molecular characterization and identification of the causative species of *Leishmania*, the clinical and epidemiological studies of a new focus regain importance. In Rio de Janeiro, for instance the only subspecies causing ACL, as detected so far by our group (Grimaldi et al., 1987) is *Leishmania braziliensis braziliensis*, although we have previously described one case of cutaneous leishmaniasis induced by *L. donovani chagasi* (Oliveira-Neto et al., 1986).

In July 1984 several cases of cutaneous leishmaniasis were registered in the out-patients unit of our hospital, all proceeding from the locality of Mesquita at the municipality of Nova Iguaçu which integrates Rio de Janeiro's metropolitan region. Visiting this location we were able to register more cases. In this study, we report the major clinical and epidemiological aspects of this outbreak.

MATERIAL AND METHODS

The area under study: the municipality of Nova Iguaçu is situated at 22°45'37" latitude South and 43°26'52" longitude West, about 35 km of Rio de Janeiro's city centre, from which it is by the Madureira hill (Fig. 1). The highest point is Gericinó Peak, with 887 metres. Geologically, the hill is made up of ancient pre-cambrian rocks, mostly granite and gneiss. The weather is hot and humid with mean temperature of 23.8°. The pluviometric precipitation is high (1.200 mm), and rainy season is between November and February during the hot, moist summer.

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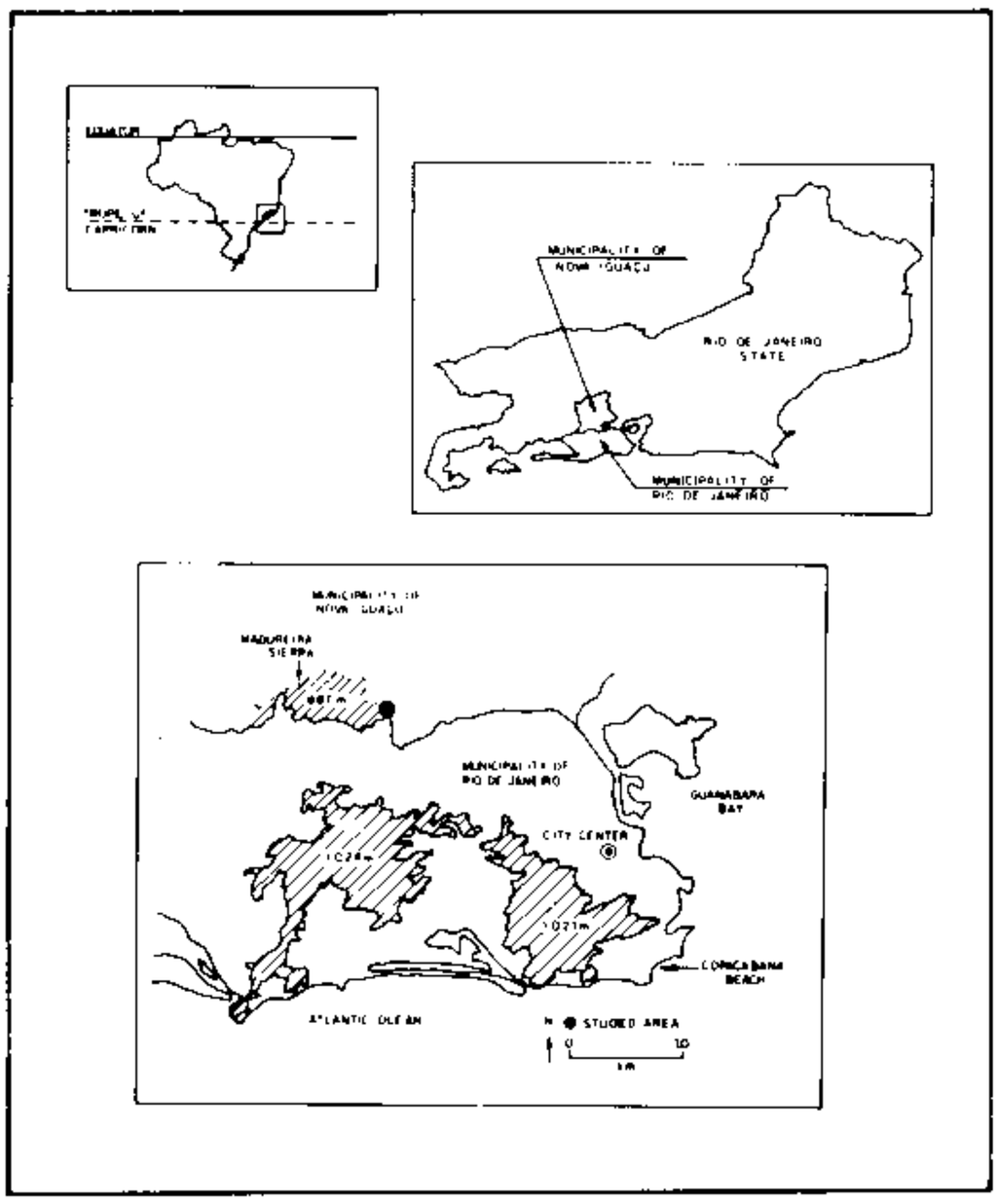

Fig. 1: map of Rio de Janeiro state (upper left), Rio de Janeiro city (upper right) and relation of the studied area with metropolitan region (bottom).

The leishmaniasis outbreak was detected at the atlantic side of Madureira hill and localized at the first slopes, at the immediate vicinity of urbanized areas (Fig. 2). Steeps and bold passages conduct to the hill tops. Alongside these passages, the area is intensily deforested and human settlement is established by way of miserable shacks and hovels, without latrines, electricity or piped water supply. The inhabitants are poor people that work the land for small plantations and orchards.

Human infection: in the beginning of this study – July to December 1984 – inhabitants were examined in a house-to-house visit for cutaneous and/or mucosal lesions. Each individual was submitted to: *Montenegro's test* – parasite antigens (leishmanin) containing 40 μg of proteic nitrogen/ml was obtained from the Institute of Biological Sciences, Federal University of Minas Gerais, Brazil. The degree of skin response was measured 48 hours after intradermal injection of 0.1 ml of the antigen at the anterior face of forearm. An enduration of 5 mm or more (in the larger diameter) was considered positive; *Serological test* – an indirect immunofluorescence test (IFAT) was used to detect anti-*Leishmania* antibodies. Promastigotes of a Brazilian *Leishmania* strain (MHCM/BR/76/JOF) phenotipically similar to *L. major* (Momen et al., 1985) were used as antigen. A rabbit anti-human immunoglobulin fluorescent IgG (Cappel Labs, USA) was used as conjugates diluted 1:20. Titres were expressed as the reciprocal of the highest serum dilution giving positive promastigote fluorescence. The dilutions of 1:45 or more were considered positive.

Fig. 2: the affected area (→) is located between the two hills (at the center right) extending till the forested mountain.

Patients selected among these individuals or by spontaneous demand were further evaluated in the out-patients unit of Evandro Chagas' Hospital, where they were also submitted to: *smears* from active lesions were stained by the classical Leishman method for demonstration of parasites; *biopsies:* tissue samples from the active border of the lesions were obtained in most of the patients after local anesthesia with xylocain 2%. These fragments were divided and processed for: Paraffin embedding and Haematoxylin & Eosin staining for histopathological examination, and cultivation of *Leishmania* parasites in an enriched blood-agar medium (NNN) containing modified liver infusion tryptose (LIT) liquid medium as supernatant (Camargo, 1964).

The area was kept under vigilance during 27 months. Many of the initially non-affected individuals developed lesions during the course of the studied period. They were also included in this study and submitted to the same procedures describe above.

Domestic animals infection (canine and equine): a census of dogs was made in the community. Each animal was clinically examined and submitted to a blood collection for serological test using the same immunofluorescence technique but now with a rabbit anti-dog immunoglobulin fluorescent antibody as conjugate, kindly supplied by Dr. Mario Camargo (Inst. Med. Trop. – USP São Paulo). Cutaneous and/or mucosal lesions were also biopsied and processed as already described.

Equines present in the locality were also studied by means of smears, biopsies of skin ulcers and culture of *Leishmania* parasites as previously described (Aguilar et al., 1986).

Parasite identification: Leishmania stocks isolated from patients and dogs were immunologically characterized and identified using species and subspecies specific monoclonal antibodies (MoAb) using indirect radioimmune binding assay (RIA) or immunofluorescence technique (Grimaldi et al., 1987). The MoAb used in this study, specific for members of the *Leishmania mexicana, L. braziliensis,* and *L. donovani* complexes, have already been described (Jaffe et al., 1984; Mc Mahon-Pratt et al., 1985; Grimaldi et al., 1987).

Search for phlebotomine vectors: during six consecutive months (October 1984 to March 1985) weekly captures of sandflies were made from 6 to 9 p.m. in different situations: simultaneously on human baits inside houses and outdoors close to the houses; on animal baits around the houses; with CDC light traps simultaneously in the vicinity of houses and in plantations.

Statistical analysis: Qui square test was used to compare number of lesions, time of evolution and titers of anti-*Leishmania* antibodies found by means of IFAT.

RESULTS

Human infection: during July 1984, 137 individuals were studied, 18 of which with exclusively cutaneous lesions.

Results of the IFAT and Montenegro's test on these 137 inhabitants are shown in Table I.

TABLE I

Results of Montenegro's test and immune fluorescence antibody test (IFAT) on 137 individuals examined during an outbreak of ACL in Nova Iguaçu, Rio de Janeiro (1984)

	No of individuals examined	IFAT				Montenegro's test			
		non reactive*		titres ⩾45		negative		positive	
		No.	%	No.	%	No.	%	No.	%
Withouth lesions	119	115	96.6	4**	3.4	113	95.0	6***	5.0
With lesions	18	5	27.8	13	72.2	0	0	18	100.0

* Non reactive at 1:45 dilution.
** The 4 cases were also positive for Montenegro's test.
*** These individual never presented skin lesions during 27 months period of observation.

The area was kept under vigilance for 27 months from July 1984 to September 1986. As shown in Fig. 3 other cases appeared and a total of 105 cases were detected during this period. The greatest number appeared in April 1986; 38 of them being among the healthy individuals when previously examined.

Among the cases studied, 60 (57.2%) were males and 45 (42.8%) females. Forty-six (43.8%) patients were under 15 years of age. There was not a statistically significant difference in the distribution of the disease between males and females having more or less than 15 years old.

All cases were of the cutaneous and ulcerative form. Initially the ulcer is shallow and afterwards the sore slowly extends, sometimes discharging a scanty ichorus material. The ulcer extended by the erosin of its perpendicular sharp-cut and jagged edge, is surrounded by a congestive and infiltrated area (Fig. 4a). The mean size of ulcers was about 2-3 cm (diameter), but in some instance they occupied an area larger than the average. The ulcers were single in 78 patients (74.3%) and multiple in the remaining 27 (25.7%). Lesions were mostly situated on uncovered and unhairy parts, like hands, feet, arms, legs. Location on face was also common (20%), three of which on eyelids (Fig. 4b). Scalp was rarely affected (2 cases). The evolution of these lesions was between 1 to 6 months (average of 3.5 months), 61 (58.0%) having up to three months of evolution.

Montenegro's test was positive in all patients (100%). IFAT showed anti-*Leishmania* antibodies titers in 78 (74.3%) patients. Patients with multiple lesions (Fig. 4c), had a statistically significant higher titres than those with single lesion ($p < 0.05$) (Table II). However, there were not statistically significant differences in the distribution of titres with regard to the period of evolution or the demonstration of the parasite in the lesion.

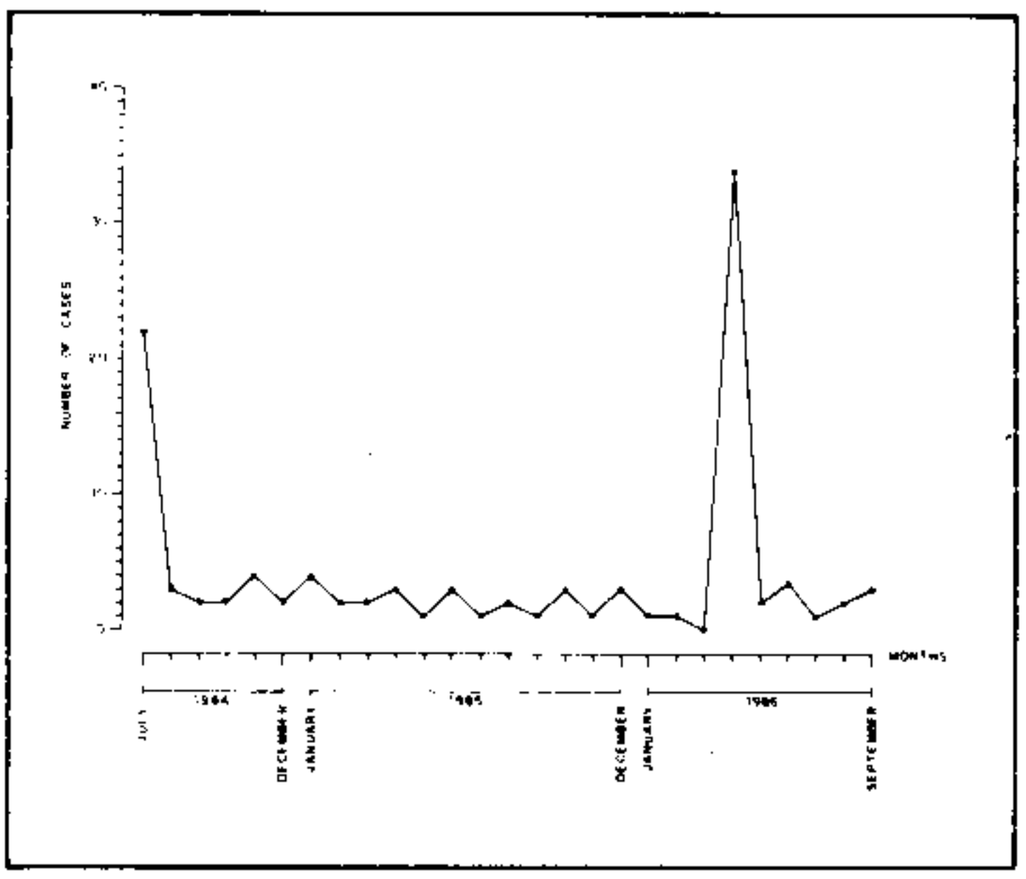

Fig. 3: number of human cases detected from July 1984 to September 1986.

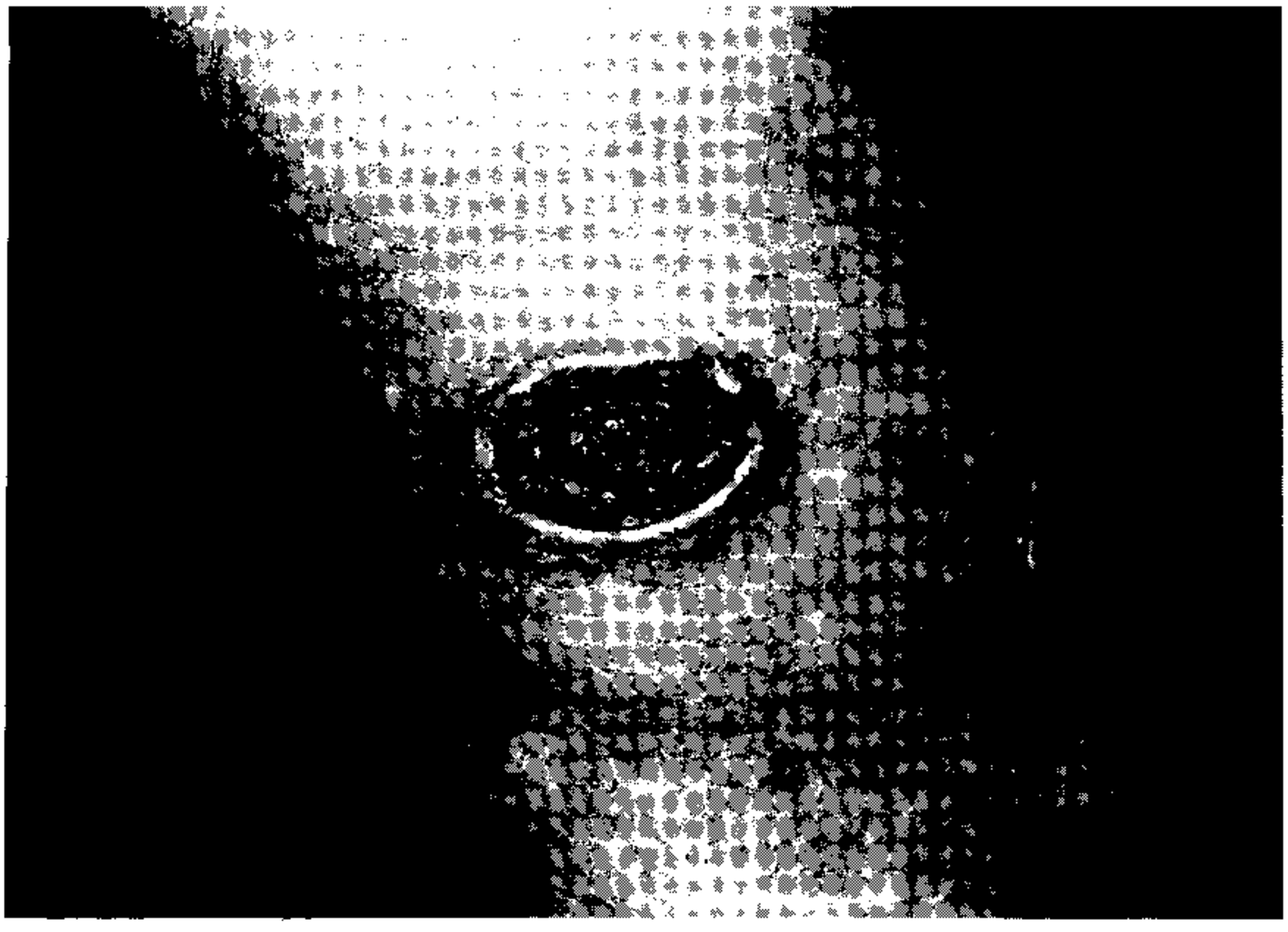

Fig. 4a: typical ACL ulcerated cutaneous lesion.

Fig. 4b: ulcerated cutaneous lesions located on both eyelids and nose.

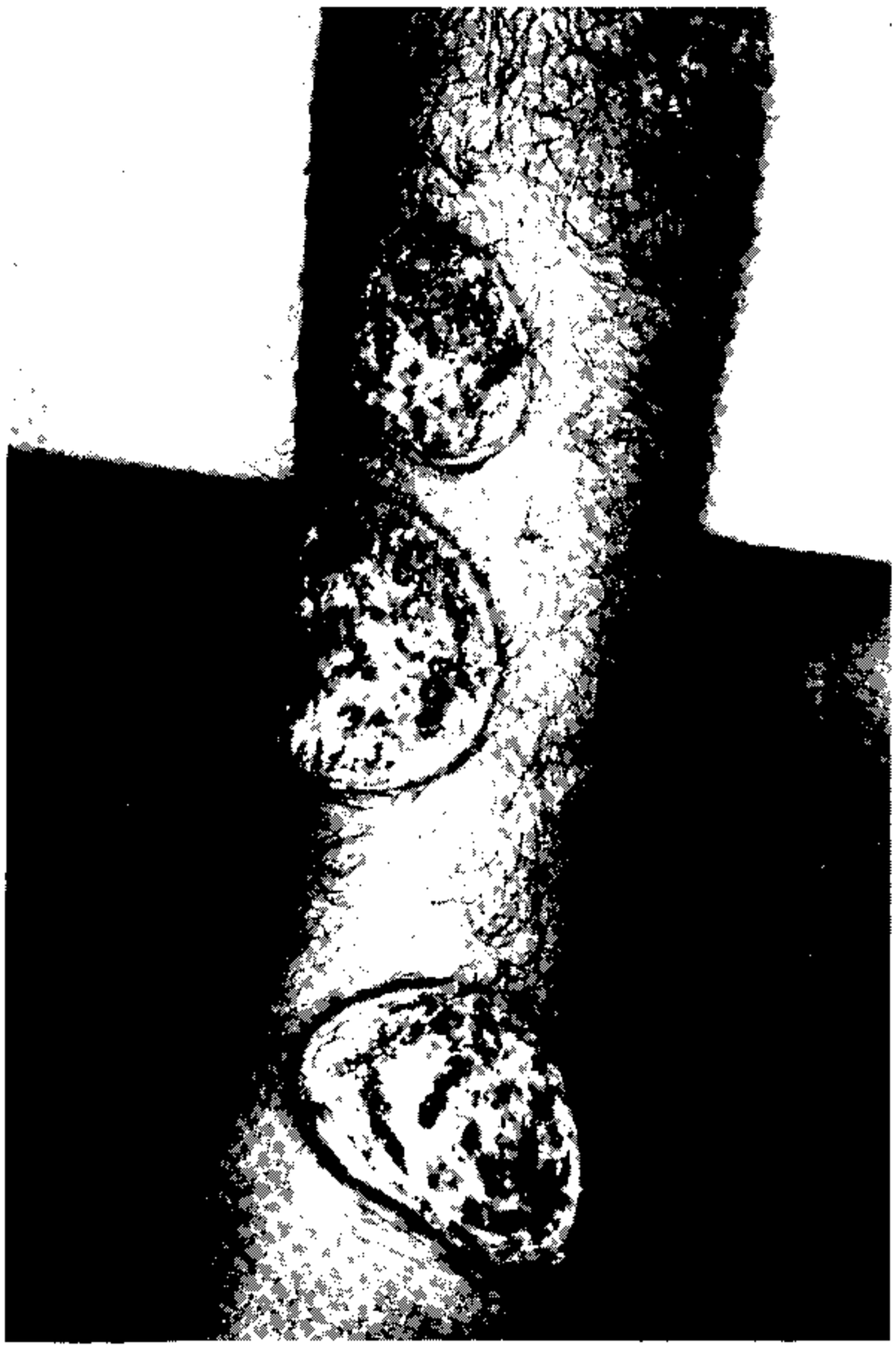

Fig. 4c: multiple skin ulcers on lower limb.

TABLE II

Correlation between number of lesions and antibody titres (IFAT) on 105 cases of American Cutaneous Leishmaniasis

Titres	Number of lesions			
	Single		Multiple	
	No.	%	No.	%
Non reactive* or titres $<1:90$	66	84.0	15	55.5
High titres ($\geq 1:90$)	12	16.0	12	44.5

* Non reactive at 1:45 dilution.

Parasites were demonstrated in 73 cases (69.5%) using at least one of the methods employed. Leishman stained inprints of smears, performed in all cases, were positive in 57.1% of them. Parasite cultivation and histopathological examination, performed in 70 patients, were positive in 48.6% and 38.5% cases respectively. All three methods were performed in 67 patients. However, in lesions with less than three months of evolution, 85.3% of the cases showed positive results using one or more of the three employed methods, in contrast with the results found in those patients with longer evolution (33.3%). The difference among positive results using on, two or three methods were not statistically significant.

Histopathological examination showed a diffuse infiltrate of lymphocytes and plasma cells associated, in 80% of the cases, with a granulomatous reaction. Fibrinoid degeneration of the connective tissue was a strinking finding involving small vessels, in 24% of the cases. Parasites were found in 38.5% of the cases, always scarce and localized in the vicinity of necrotic areas.

Infection of domestic animals: eight out of 25 dogs (32%) examined in the area had single, ulcerated and exclusively cutaneous lesions (Fig. 5). Sites of involvement were the ears (56.%), footpads (33%) and scrotum (11%). Parasites were isolated by culture in all cases. Histopathological examination showed a diffuse plasma cell infiltration which were associated with a granulomatous reaction only in two cases. Three dogs belonged to families which had at least one member with ACL, and three other dogs belonged to families in which human cases appeared after canine leishmaniasis had been diagnosed.

Ten out of the 26 equines examined had ulcerated lesions. Parasites were demonstrated by Leishman stained smears in 8 animals (30.8%).

Fig. 5: ulcers on the internal face and border of the ear in an infected dog.

Parasite characterization: isolates from 26 human, 2 canine and 1 mule lesion were characterized. All were identified as *L. b. braziliensis* by monoclonal antibodies using either RIA or IF. Furthermore, these *Leishmania* isolates did not cross-react with a large panel of anti-*L. mexicana* and *L. donovani* species-specific MoAb.

Phlebotomine vectors: sandfly population was evaluated during 6 consecutive months, with a total of 204 hours of capture. Of the 3806 specimens collected, 73.0% were *Lu. intermedia* mainly caught on human baits, inside houses and outdoors close to the houses, where they were collected in equines. We could not demonstrate any infected phlebotomine by dissecation procedure.

DISCUSSION

Except for the Amazon basin, where the epidemiological pattern is the same to that described in the begining of the century (Laison, 1983), ACL is at present characterized, in several places, by outbreaks in small settlements in which the primary forest has been cleared for agricultural development (Tolezano et al., 1980; Araujo F^{o}, 1978).

Among the numerous persons with active cutaneous lesions, only one patient with mucosal involvement was detected (a child not included in our statistics because had been previously treated in an another hospital). This suggests that cutaneous leishmaniasis had been recently stablished in the area.

Although there was a little difference in age or sex prevalence, a great number of children and women were affected, suggesting that transmission might occur in the peridomiciliar area. Women were dedicated to domestic works and men were in general engaged with agriculture activities. Sanfly population in this area is dominated by *Lu. intermedia* which has been found mainly in the surroudings of the houses. Although no *Leishmania* parasites were isolated from the sandflies, intradomiciliar transmission may be suspected since female sandflies were collected, in some instances, inside houses. In addition, eyelids lesions were found in three children a site that suggests that the sting was performed during the sleep.

Domestic animals were easily found infected in the studied area. This was not surprising since dogs and equine infection have been increasingly reported in areas that closely resemble the one of the present study (Aguilar et al., 1984; Dias et al., 1977; Falqueto et al., 1986; Pirmez et al., 1988). These findings suggest that if domestic animals are not the only one reservoir in this area, they might represent at least an ampliflying ecosystem for the maintenance of the disease. However, wild animals cannot be ruled out as reservoirs in this area since a systematized searching for such infected animals remains to be performed.

Clinically, the disease presents itself as a single ulcerated and exclusively cutaneous lesion, mainly in lower and upper limbs. Principal tools for diagnosis were clinical aspects of the lesion, epidemiological data and positive skin test. Parasite demonstration using histopathological examination was always difficult, even in recent lesions. IFAT evaluation showed a significant correlation between positive titres and the presence of active cutaneous lesions. Highest titres were also significantly found in cases with multiple lesions when compared with those with single lesions.

Another criterium for diagnosis was the characteristic response to specific therapy. All the patients were treated with pentavalent antimonial (results not shown) where 80% of these cases showed good response to therapy with healing after a 30-day series. The remaining 20% of the patients needed a longer course of therapy.

Subclinic infection probably exists in this area, since six cases (5%) with positive Montenegro's test, four of which presenting also specific antibodies titres against *Leishmania*, developed no skin lesions during the whole period of observation.

The bulk of data reported (Nery-Guimarães, 1955; Junqueira, 1969, Araújo F^{o}, 1978; Marzochi et al., 1980; Souza et al., 1982; Pirmez et al., 1988), together with our present findings lead us to conclude that ACL in Rio de Janeiro is usually a single and ulcerated cutaneous lesion caused, till now, only by *L. b. braziliensis* (with one exception already mentioned — Oliveira Neto et al., 1986). Mucosal forms are rare. Response to antimonial

pentavalent therapy is nearly always good with only one 30-days series. Cases occur through outbreaks situated, in the last years, in peri-urban areas where domiciliar transmission seems to be frequent.

RESUMO

Um surto de leishmaniose cutânea americana (*Leishmania braziliensis braziliensis*) numa área periurbana da cidade do Rio de Janeiro, Brasil: estudos clínicos e epidemiológicos – De julho de 1984 a setembro de 1986, 105 casos de leishmaniose cutânea foram estudados numa localidade situada na imediata vizinhança da área urbanizada da região metropolitana da cidade do Rio de Janeiro. A ocupação do sítio deu-se aproximadamente há 20 anos, mas os primeiros casos foram registrados somente seis meses antes do início de nosso estudo.

Os casos eram quase que exclusivamente cutâneos, da forma clínica ulcerada com um e seis meses de evolução. O teste de Montenegro foi positivo em todos os casos e anticorpos anti-leishmania foram detectados por imunofluorescência indireta em 74,3% dos pacientes. A demonstração do parasito foi obtida em 69,5%. Animais domésticos infectados foram facilmente encontrados: 32% dos cães examinados e 30,8% dos equinos mostravam presença de leishmânia em lesões ulceradas.

Parasitos isolados, tanto de casos humanos como de cães e equinos, foram imunologicamente caracterizados e identificados com *L. b. braziliensis*.

Da população de flebotomíneos encontrados 73% eram de *Lutzomyia intermedia* capturados principalmente com iscas humanas e de animais domésticos.

Nossas observações sugerem que esta é uma área de estabelecimento recente da infecção por *L. b. braziliensis* e que a transmissão ocorre provavelmente tanto no peridomicílio como no interior das habitações.

Palavras-chaves: leishmaniose cutânea americana – surto – transmissão peridomiciliar

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