American Cutaneous Leishmaniasis in Pernambuco, Brazil: Eco-epidemiological Aspects in 'Zona da Mata' Region

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American cutaneous leishmaniasis (ACL) has been increasing in Pernambuco, thus becoming an important problem for Public Health. The incindence is predominant in the region called 'Zona da Mata', in the east of this state. This region corresponds geographically to the primitive area of the Atlantic forest. In order to characterize the eco-epidemiology expression of ACL in this region, two localities situated in the municipalities of Amaraji e Cortes have been selected by the criterion of higher incindence of human cases. Five stocks of patients were characterized and identified on the basis of enzyme profiles as a new variant of Leishmania (V.) braziliensis. *A survey of wild and domestic animals was carried out by means of a parasitological and serological diagnosis. Through the analysis of the spleen and liver imprints, were detected amastigotes compatible with* Leishmania *in five* Nectomys s. squamipes, *five* Bolomys l. pixuna, *two* Rattus r. alexandrinus *and one* Rattus r. frugivorus. *For two years we carried out monthly sandflies captures using CDC light traps as well as manual captures.* Lutzomyia whitmani *was predominant, which accounted for 97.4% of the total. These data indicate a strong evidence on the vector and the potential reservoirs of* L. braziliensis *in this region.*

Key words: cutaneous leishmaniasis - *Leishmania braziliensis* - *Lutzomyia whitmani* - mammalian reservoirs - eco-epidemiology

In the last five years, American cutaneous leishmaniasis (ACL) has been increasing in Pernambuco, thus becoming an important problem for Public Health. Pernambuco is located in the Northeast Region of Brazil, where ACL is endemic and accounts for more than half of the notified cases in this country. Between 1989 and 1991, 1,604 cases were notified in Pernambuco by the Fundação Nacional de Saúde, Ministry of Health. The majority of these cases occur in the region called 'Zona da Mata', with 64.2% of the cases. This region corresponds geographically to the primitive area of the Atlantic forest, one of the most important primary rain forest in Brazil, ranging out on the east coast from the northeast down to the south of the country.

Zona da Mata was setlled during the colonization process and economic formation of Brazil and is characterized in Pernambuco by the exploitation of sugar-cane, the main agricultural activity. Other supplementary agricultural activities are also exploited in the region: manioc, corn, beans, bananas and other regional fruits.

In order to characterize the ecology of ACL in this region, in 1991 we started a study in the area of Zona da Mata where the largest quantity of human cases was verified during this period. This area includes part of the municipalities of Amaraji and Cortes, where 329 cases were notified between 1989 and 1991. Two neighboring localities (Refrigério and Tranqüilidade) were chosen for this study, for also showing the largest incidence during this period, with 127 cases.

CHARACTERISTICS OF THE STUDY AREA

Refrigério and Tranqüilidade are located between 8° 29' S and 35° 35' W, southeast of Pernambuco (Fig. 1). Both localities have similar characteristics regarding vegetation, geo-physical composition and socio-economic profile. Refrigério has 295 inhabitants while Tranqüilidade has 210. Most people work in agriculture, specially in the sugar-cane plantation. Besides this predominant vegetation, there is still some remainders of the Atlantic forest (Fig. 2), manioc and banana plantations, as well as those of regional fruits (Fig. 3). Most workers are males from 15 to 35

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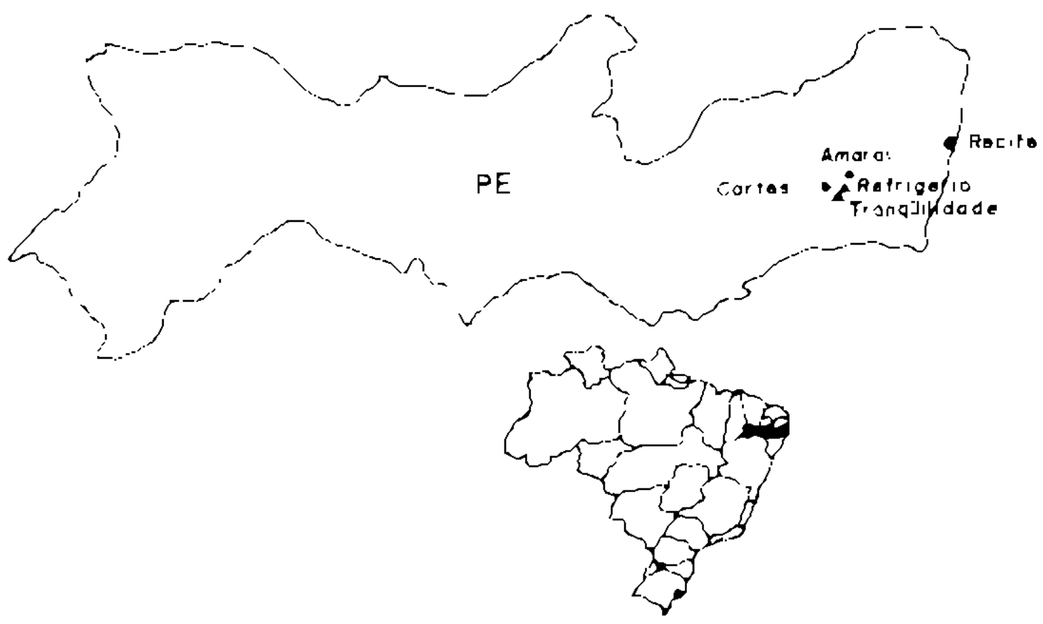

Fig. 1: geographical localization of the study area in the State of Pernambuco, Brazil.

years old. There are 92 houses among which brick houses, stables and two schools.

DIAGNOSIS CRITERIA FOR PATIENTS

Diagnosis was done through clinical appearence of the lesions, microscopic detection of amastigotes in lesion aspirate and biopsy smears, isolation of the parasite in culture media and positive Montenegro intradermal reaction. Parasite isolation was done by lesion aspirate and cultivation in NNN plus Schneider's medium supplemented with 10% inactivated fetal calf serum and streptomyicin 250 µg/ml. Five stocks were isolated, characterized and identified on the basis of enzyme profiles as a new variant of *Leishmania (V.) braziliensis* (Felinto de Brito et al. 1993). The diagnosed cases were predominantly of localized lesions.

EPIDEMIOLOGICAL SURVEY

Montenegro skin test - It was applied to all the population using reference Leishmanin antigen, produced by Dr Wilson Mayrink's team of the Universidade Federal de Minas Gerais and distributed by Fundação Nacional de Saúde. Twenty nine percent of the population was reactive (induration ≥ 5mm) shown by Delayed-type hipersensitivity (DTH). Fourteen patients in undergoing treatment for leishmaniasis at the time were not submitted to the test in order to avoid exarcebation of the lesions.

Indirect immunofluorescence assay - A serological survey was performed utilizing *L. (V.) braziliensis* reference strain (MHOM/BR/75/M2903) antigen for the reaction. The index of positivity was 26%, with the 1/40 pattern of serum dilution for the trial.

SAND FLIES

For two years (1991-1992) we carried out monthly captures in both localities by using CDC light traps as well as manual captures. The collections were made in the domicile, peridomicile and extradomicile of both localities. The domicile corresponding to the inside area as well as to the outside walls of the house. The peridomicile area included the chicken pen, the stable and the near fruit trees. The extradomicile consisted of the vegetation area greater than 100 m from the

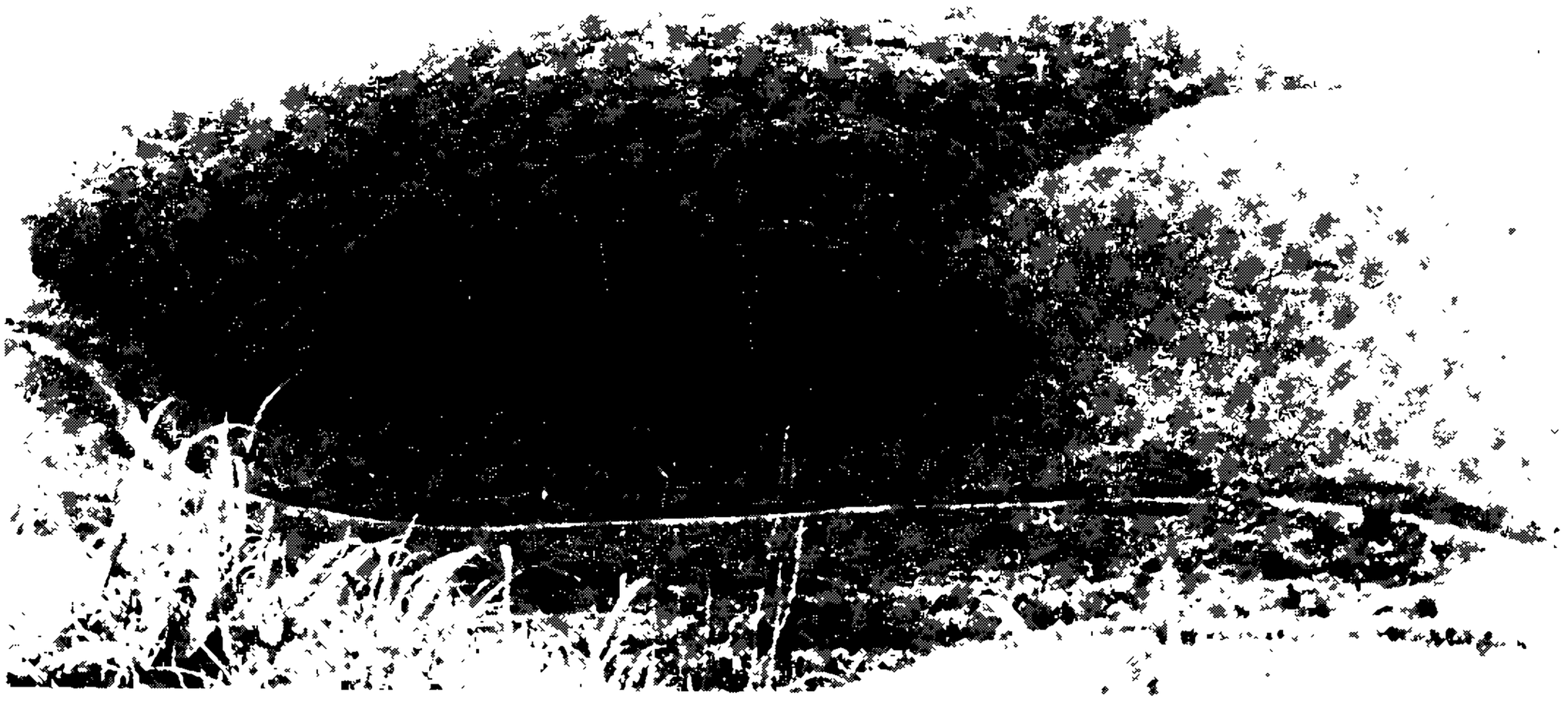

Fig. 2: remainders of the Atlantic forest in the sugar-cane plantation area present in both localities.

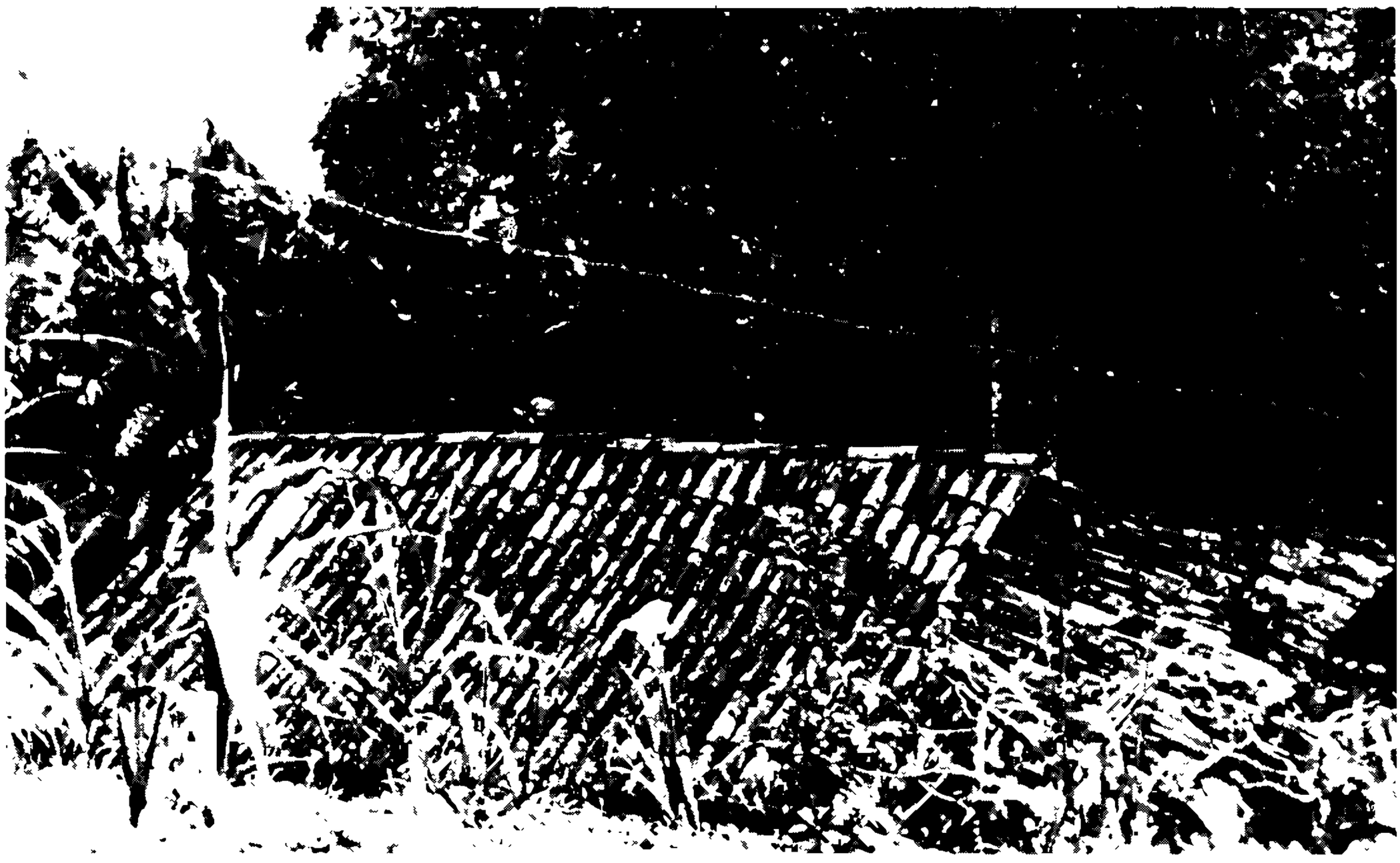

Fig. 3: typical housing in the region. Aspects of the regional fruits cultivation in the area around the house.

TABLE I

Sandflies collected in Tranqüilidade and Refrigério localities (1991-1992)

Species	Male	Female	Total	%
Lu. whitmani	18570	17197	35767	97.4
Lu. quinquefer	195	112	307	0.8
Lu. evandroi	136	67	203	0.6
Lu. aragoai	35	9	44	0.1
Lutzomyia sp.	264	129	393	1.1
Total	19200	17514	36714	100.0

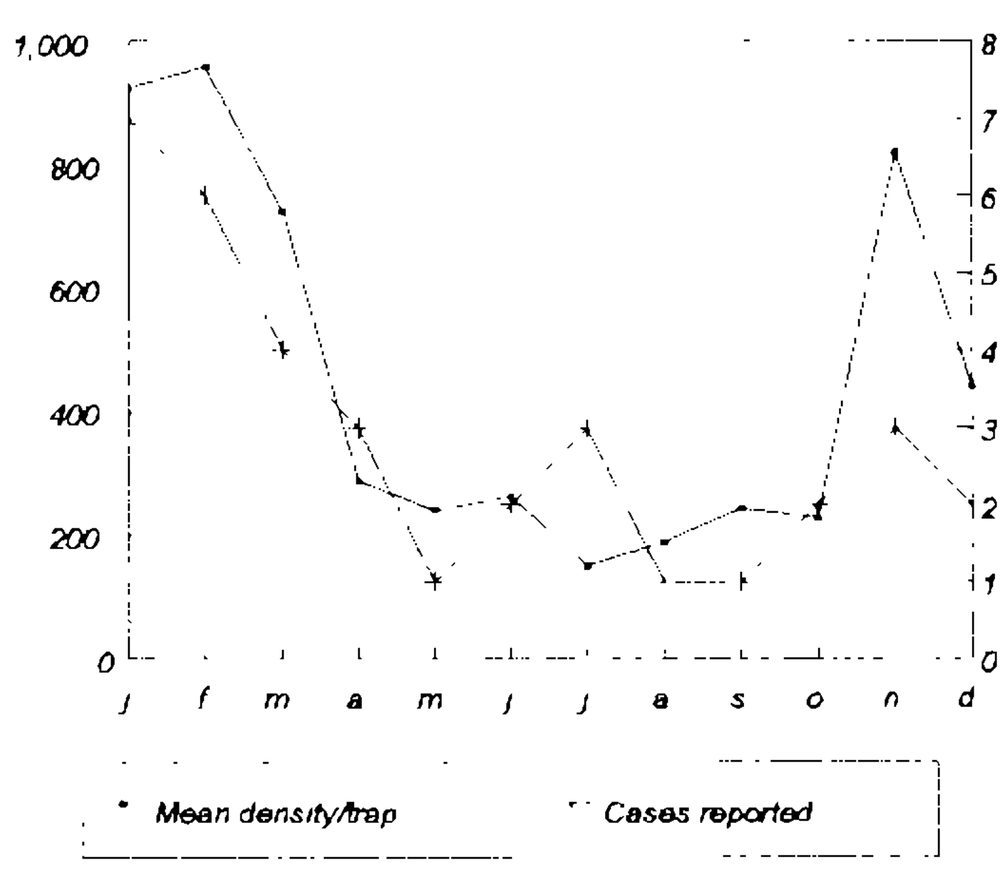

Fig. 4: monthly density of *Lutzomyia whitmani* in relation to ACL cases reported in the Refrigério locality.

TABLE II

Distribution of species in relation to capture local

Species	Domicile No.	Domicile %	Peridomicile No.	Peridomicile %	Extradomicile No.	Extradomicile %
Lu. whitmani	443	1.3	3304	9.2	32020	89.5
Lu quinquefer	0	0	25	8.1	282	91.9
Lu evandroi	11	5.4	93	45.8	99	48 8
Lu aragoai	0	0	21	47 7	23	52.3
Lutzomyia sp.	36	9 2	131	33.3	226	57 5

houses. Among the species captured and identified (Table I), we found a predominance of *Lutzomyia whitmani* (97.4% of the total). The results concerning the place of capture are shown in Table II. The extradomicile was the predominant place for capturing *Lu. whitmani*. The relation of the mean monthly variation density of *Lu. whitmani* to the number of cases reported showed that the highest density in the period corresponded to the appearance of some more human cases in both localities (Figs 4, 5).

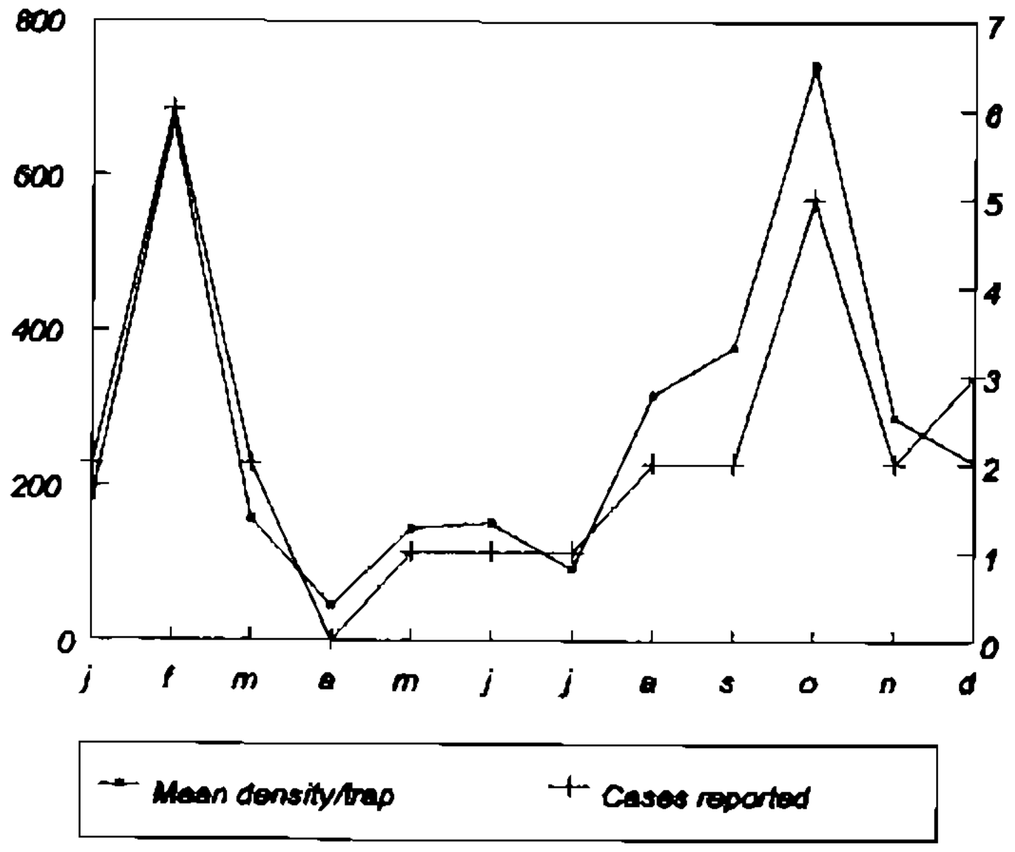

Fig. 5: monthly density of *Lutzomyia whitmani* in relation to ACL cases reported in the Tranqüilidade locality.

DOMESTIC AND WILD ANIMALS

The search for the reservoir in both localities took nine months. We used 50 standard traps for small rodents and 15 traps for larger animals. The traps were set 3,425 times in the domicile and in the extradomicile area. The latter corresponding to sugar-cane, manioc, corn, banana plantations and remainders of the Atlantic forest. The traps were set 725 times in the domicile area and 2,700 in the extradomicile. The total number of animals captured and identified was 478 comprising: 407 small rodents and 71 marsupials (Table III). In the extradomicile area 302 rodents and 70 marsupials were found and 105 rodents and one marsupial in the domicile. All the animals were sacrificed for research and isolation of *Leishmania* through blood smear, spleen and liver imprint, spleen and liver samples cultures in NNN plus Schneider's medium supplemented with 10% inactivated fetal calf serum and streptomycin 250 µg/ml, as well as innoculation of spleen and liver macerates into hamster. In the imprints of 13 animals (five *Nectomys s. squamipes*, five *Bolomys l. pixuna*, two *Rattus r. alexandrinus* and one *Rattus r. frugivorus*) we detected amastigotes compatible with *Leishmania* (Table IV). Unfortunatelly all cultures were contaminated and we were unable to isolate the parasite for identification. The hamsters were negative for infection.

A study of the dog population was carried out on all 139 dogs in both localities; 18.5% were positive for anti-*Leishmania* antibodies, shown by indirect immunofluorescence assay, however none of the animals presented lesions, to the contrary, they all presented a normal physical appearance.

TABLE III

Domestic and wild animals captured for *Leishmania* sp. research

Species	Total captured	% total
Nectomys s. squamipes	145	30.3
Bolomys l. pixuna	115	24.0
Rattus r. frugivorus	95	19.9
Didelphis paraguayensis	35	7.3
Rattus r. alexandrinus	23	4.9
Monodelphis d. domestica	20	4.2
Marmosa sp.	16	3.3
Oryzomys lamia	13	2.8
Oryzomys subflavus	6	1.3
Nectomys sp.	6	1.3
Holochilus sciures	4	0.8
Total	478	100.0

TABLE IV

Species examined and suspected of *Leishmania* infection by amastigotes presence in spleen and liver imprints

Species	Total examined	Amastigote (*Leishmania?*) in imprint
Nectomys s. squamipes	128	5
Bolomys l. pixuna	103	5
Rattus r. frugivorus	86	1
Didelphis paraguayensis	33	0
Rattus r. alexandrinus	21	2
Monodelphis d. domestica	18	0
Marmosa sp.	12	0
Oryzomys lamia	6	0
Oryzomys subflavus	5	0
Nectomys sp.	4	0
Holochilus sciures	4	0
Total	420	13

CONCLUSIONS

Similar to other endemic regions of ancient colonization in Brazil, ACL shows similar characteristics in Pernambuco in the process of adaptation to the modified conditions in the natural space of zoonosis (Mayrink et al. 1979, Jones et al. 1987, Gomes et al. 1989, Falqueto et al. 1991). The most striking difference between the area towards the southeast of Brazil and other endemic areas in the northeast, such as Bahia and Ceará, is the massive distribution of sugar-cane plantations in Pernambuco. The results obtained

so far suggest some conclusions on the ACL eco-epidemiology in this area : (1) the probable vector is *Lu.whitmani* due to its predominance in this area. This specie has already been incriminated in other areas of northeast, such as Ceará (Azevedo et al. 1990) and Bahia (Vexenat et al. 1986); (2) the domicile is not relevant as a risk factor for ACL in this area, for the amount of *Lu. whitmani* found was not significant in these places. Transmission probably occurs in the peri or extradomicile; (3) the results found in relation to the reservoirs suggest the ocurrence of natural enzootic focus in this area due to the identification of amastigotes compatible with *Leishmania* in 13 rodents, which had peridomiciliary and forest habitats (specially in *Nectomys s. squamipes* and *Bolomys l. pixuna*). Furthermore, the variant isoenzymatic profile of *L. (V.) braziliensis* contribute to this hypothesis; (4) we could not find direct evidence of the role of the dog in a possible peridomestic cycle.

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