RESEARCH NOTE

Outbreak of Cutaneous Leishmaniasis in the Rio Doce Valley, Minas Gerais, Brazil

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American cutaneous leishmaniasis (ACL), widely endemic throughout the territory, is a considerable public health problem, especially in the North, Northeast and Southeast parts of Brazil. In the Southeast region, the eco-epidemiology of cutaneous leishmaniasis in the Rio Doce Valley of the State of Minas Gerais, Brazil had been studied since the early 1960's. However, information on epidemic outbreaks is scarce in this region. In 1991 an outbreak of cutaneous leishmaniasis was detected in the municipality of Virginópolis, Rio Doce Valley. The present paper describes some clinical and epidemiological findings of this outbreak and the possible factors accounting for its occurrence.

This study was carried out throughout April and July 1991 in Virginópolis municipality, at 18°49'24"S and 42°42'19"W (680m altitude). Virginópolis is located about 178km from Belo Horizonte, in the mid-northeastern part of the State in Rio Doce Valley. The work was limited to a community known as "São Bento". This region is geographically similar to that already described for other endemic areas of ACL in Rio Doce Valley, where the original forest environment has been modified (W Mayrink et al. 1979 *Ann Trop Med Hyg 73*: 123-137). In the study area, the agricultural population is prevalent and most of the houses are surrounded by secondary tropical forest. The people cultivate mainly coffee, bananas, sugar-cane and other tropical cultures after felling the surrounding secondary forest. The livestock include dogs, horses, cattle, pigs and domestic fowls. In this area, no cases of American trypanosomiasis or visceral leishmaniasis were reported in the last years.

Active search for skin lesions was started in April 1991. Questionnaires were prepared to record demographic and clinical data. After a house-to-house survey, 373 inhabitants distributed in 93 houses were interviewed and clinically examined. A search for previous suggestive skin scars and evidence of mucosal disease was made. Soon after, delayed hypersensitivity skin-test (DHST) was done with a standard antigen produced by the Department of Parasitology, Institute of Biological Sciences, Federal University of Minas Gerais, as outlined by MN Melo et al. (1977 *Rev Inst Med Trop São Paulo 19*: 161-164). This antigen (100 µl, 25 µg of protein) was injected intradermally in the volar surface of the forearm. Children under 1 year-old were not skin tested. After 48 hr, the long and short axes were measured to the nearest millimeter. A DHST with a mean diameter induration less than 5 mm was assumed to be negative. All subjects presenting a positive skin-test gave blood samples for immunofluorescence antibody-test (IFAT).

All patients presenting active suspected lesions were submitted to a punch biopsy from the edge. This skin fragment was used for stained-Giemsa printing slides and histopathological examination. In some patients, needle aspirates obtained near the edge of lesion were cultured in diphasic blood-agar medium with an overlay of LIT medium plus antibiotics (penicillin 100 U/ml and streptomycin 50 µg/ml). Parasites isolated were identified by isoenzyme typing, using starch-gel electrophoresis employing ASAT, ALAT, GPI, PGM, MDH, G6PDH and 6PGDH enzymes.

A resident medical officer in this community was not possible but one person who had received short-term paramedical training was acting as a volunteer for leishmaniasis treatment after diagnosis establishment by one of us. All patients with leishmaniasis were treated with intramuscular meglumine antimoniate (Glucantime®): 20 mg of Sb^{+5}/kg of body weight. The maximum daily dose

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was 850 mg of pentavalent antimony. Meglumine antimoniate was applied over ten days, followed by a ten-day interval without treatment. After this 10-day interval, the patients were clinically examined to decide if a further 10-day treatment was needed.

Forty-six cases of cutaneous leishmaniasis were detected among the 373 inhabitants surveyed, with a disease rate of 123/1,000 inhabitants. The human disease/house relation was near 1:2. All these cases were autochthonous as no migrations were reported by the inhabitants. As shown in Table I, almost 24% of the cases were detected in the population under 10 years. Aside this, all age groups were affected. Furthermore, no sex differences in the distribution of the disease were observed. Subjects with 20 years-old were those with the highest risk of disease, as almost 50% of the ACL cases occurred in this age group. Among the patients, no mucosal involvement was found upon clinical examination. The average number of skin lesions was 1.6 (ranging between 1 and 7 lesions), being 42.5% in the lower limbs, 25.9% in the upper limbs, 24.2% in the head/neck and 7.4% in the trunk. Eyelid lesions were found in two patients. The average time of lesion evolution was 1.5 months, ranging from 0.5 months to 7 months. Forty-one (89.1%) out of 46 patients showed a positive DHST. Four humans isolates were identified as *Leishmania braziliensis* by isoenzyme typing.

Sixteen inhabitants were not skin tested. From remaining, 57/311 (18.3%) subjects showed a positive DHST without history or clinical evidence of cutaneous leishmaniasis. In these subjects, with a probable sub-clinical or abortive infection, only five (1.6%) showed a positive IFAT (titters 1:40). Nine of them (2.9%) were children under 15 years-old. Thus, considering the patients with ACL, the general rate of positive human skin-tests was 103/357 (28.9%).

Sandfly captures were carried out between 6:00 p.m. and 9:00 p.m., during a three-day period in April 1991. The captures consisted of collections in peri-domestic sites (up to 10m distance from houses) using human bait and CDC light traps in domestic animal shelters. These captures were done next to houses with larger numbers of human leishmaniasis cases. Standard taxonomic keys were used for the identification of sandfly species.

Among 108 sandflies collected, six species were taxonomically confirmed: *Lutzomyia misionensis*, *Lu. monticola*, *Lu. whitmani*, *Lu. migonei*, *Lu. quinquefer* and *Lu. pessoai* (Table II). Females of *Lu. whitmani* were caught biting man at dusk but *Lu. monticola* showed the highest man-bite rate and the most pronounced attraction to light.

A search for suspected infected dogs was also conducted in the area. All dogs were submitted to a full ectoscopic examination. In addition, all dogs were submitted to a DHST (MCA Marzochi & EGO Barbosa-Santos 1988 *Mem Inst Oswaldo Cruz 83*: 391-392) as previously standardized by O Genaro et al. (1992 *Mem Inst Oswaldo Cruz 87*: 163-164). The results were measured after a 72-hour period and the positivity criterion was as described above for human patients. Biopsies were taken from active suspected lesions after barbituric general anaesthesia. The sample obtained by biopsy was halved: the first half was ground in normal saline and the suspension inoculated in the snouts of golden hamsters; the second half was used to make Giemsa-stained touch preparations aiming to search for amastigotes.

Five (5.7%) out of 88 dogs examined had active skin ulcers, from which *Leishmania* was isolated and identified as *L. braziliensis* by isoenzyme

TABLE I

Age and sex distribution of the human cases in an epidemic area of cutaneous leishmaniasis surveyed in Virginópolis municipality, Minas Gerais, Brazil, April 1991

Age group years	Males	Females	Both sex	%
0 - 10	8	3	11	23.9
11 - 20	7	5	12	26.0
21 - 30	2	3	5	10.9
31 - 40	2	3	5	10.9
41 - 50	5	3	8	17.4
≥ 51	1	4	5	10.9
Total	25	21	46	100

TABLE II

Sandflies caught in an epidemic area of cutaneous leishmaniasis surveyed in Virginópolis municipality, Minas Gerais, Brazil, April 1991

Sandfly species	Males	Females	Both sexes	%
Lu. misionensis	0	24	24	22.2
Lu. monticola	0	43	43	39.8
Lu. monticola ?	0	8	8	7.5
Lu. whitmani	5	10	15	13.9
Lu. migonei	6	5	11	10.2
Lu. quinquefer	2	2	4	3.7
Lu. pessoai	0	1	1	0.9
Lu. (Psychodopygus) ssp.	0	1	1	0.9
Not identified (n. sp. ?)	0	1	1	0.9
Total	13	95	108	100

electrophoresis analysis. Nevertheless, 27/88 (30.7%) dogs presented a positive DHST (MV Hermeto et al. *Mem Inst Oswaldo Cruz 88*: 635-636), showing a previous sensitization against *Leishmania*. All the canine cases were autochthonous for the study area.

This outbreak had a marked peri-domiciliary transmission feature as extensively discussed by AC Gomes (1992 *An Bras Dermatol 67*: 55-60) and MCA Marzochi (1992 *J Bras Med 63*: 82-104). It should be noted that the prevalence of positive DHST was very similar between humans and dogs (28.9% and 30.7%, respectively), showing a high exposure to *Leishmania*. Nevertheless, the circumstances causing the onset and subsequent interruption of the Virginópolis' outbreak are not clear. By the time the study visits were accomplished, active transmission was occurring. However, soon after all the patients had been treated with pentamonial no cases of cutaneous leishmaniasis could be detected for a two-year period. Further studies were required concerning the role of humans and dogs played in some endemic areas of Brazil, also their relevance in establishing outbreaks in favourable areas, as predicted by Marzochi (*loc. cit.*). In the particular case of Virginópolis, considering that human and canine cases were autochthonous, it is very difficult to establish with certainty the bio-ecological components that initiate the outbreak.

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