

## HUMAN, CANINE AND EQUINE (*EQUUS CABALLUS*) LEISHMANIASIS DUE TO *LEISHMANIA BRAZILIENSIS* (= *L. BRAZILIENSIS BRAZILIENSIS*) IN THE SOUTH-WEST REGION OF SÃO PAULO STATE, BRAZIL

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The first epidemic of American cutaneous leishmaniasis (ACL) in São Paulo coincided with the start of railway construction in the north-west of the State in 1908; several cases of the disease, known as Bauru ulcer, were described from patients working in the deforestation of areas where sandfly vectors abounded. At that time, the endemic area comprised one-third of the State (L. F. Sampaio, 1951, *Rev. bras. Med.*, 18: 717-721). Environmental changes, usually resulting from human activities, often modified the occurrence of the disease and new endemic areas were reported. These areas included the north-east (O. P. Forattini et al., 1972, *Rev. Saúde públ.*, S. Paulo, 6: 103-105; J. E. Tolezano et al., 1980, *Rev. Inst. Adolfo Lutz*, 40: 49-54) and south coastal regions (O. P. Forattini et al., 1973, *Rev. Saúde públ.*, S. Paulo, 7: 447-452). Natural infections of both sylvatic mammals and phlebotomine sandflies were detected in São Paulo and the parasites included species of the *Leishmania braziliensis* and *L. mexicana* complexes (S. B. Pessoa & J. O. Coutinho, 1941, *O Hospital*, Rio de Janeiro, 20: 25-35; O. P. Forattini et al., 1972, *Rev. Saúde públ.*, S. Paulo, 6: 255-261 and 431-433; E. L. A. Yoshida et al., 1985, *Rev. Inst. Med. trop. S. Paulo*, 27: 172). Dogs have also been found infected with *L. braziliensis* (G. Grimaldi et al., 1987, *Amer. J. Trop. Med. Hyg.*, 36: 170-187) and were implicated as a possible domestic reservoir for the maintenance cycle of this parasite in those endemic

areas (J. E. Tolezano et al., loc. cit.). Here we extend these studies, detecting some more cases of human infection associated with this leishmanial species and the results of our research on naturally infected domestic and wild mammal reservoirs of this parasite in the south-west region of São Paulo.

Itaporanga and Piraju, the two endemic municipalities where the patients originated and the domestic and sylvatic mammals were captured, are located in the Paranapanema river basin, in the south-west region of São Paulo State, Brazil (respectively at 23°11'44" and 23°42'13" S; 49°22'54" and 49°29'22" W). The physical geography of this region is similar to that already described for other endemic areas of ACL in Brazil, where the original forest environment has been altered (G. Grimaldi et al., 1989, *Amer. J. Trop. Med. Hyg.*, 41: 687-725).

During the two outbreaks of ACL in São Paulo, in 1981 and 1987, we examined 13 confirmed human cases of the disease (9 from Itaporanga and 4 from Piraju). Diagnosis was based on clinical appearance of the lesion, the positive Montenegro intradermal reaction, the presence of leishmanial parasites in lesion biopsy samples, and/or the isolation of the parasite from tissue fragments. Parasite isolation was achieved either by culturing in biphasic medium *in vitro* or inoculation in hamsters.

A search for domestic and wild mammal reservoirs of *Leishmania* sp. was conducted in the areas of this study. The methods employed were inoculation of both peripheral blood samples and tissue fragments (skin, liver and spleen) into culture medium and laboratory animals (hamsters, rats and mice), following

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recommended procedures (M. Dias et al., 1977, *Rev. Inst. Med. trop. S. Paulo*, 19: 403-310). Of 185 wild animals, belonging to several different mammalian orders and species [*Didelphis marsupialis* (28); *D. albiventris* (45); *Lutreolina crassicaudata* (1); *Rattus r. rattus* (93); *Balomys lasiurus* (8); *Cricetidae* (1); *Tupinambis* sp. (6); *Nasua nasua* (2); and *Procyon cancrivorus* (1)], 32 samples showed positive results for the presence of trypanosomatids other than *Leishmania* sp. when using Giemsa-stained blood smears; 27 of which were isolated by inoculation of peripheral blood in rats. On the other hand, although 51 out of the total animals examined showed evidence of skin alteration, we have failed to demonstrate leishmanial parasites in the lesions by using either the stained smear technique or other recommended procedures such as cultivation *in vitro* and inoculation of laboratory animals (925 hamsters) with biopsied material. However, of 30 dogs examined 2 (6.6%) as well as one equine (*Equus caballus*) had ulcer or skin hypochromic lesion, from which leishmanial parasites were isolated. Parasite identification was made by serodeme analysis using *Leishmania* species-specific monoclonal antibodies and zymodeme analysis

using isoenzyme electrophoresis as described (G. Grimaldi et al., 1987, loc. cit.; H. Momen et al., 1985, *Amer. J. Trop. Med. Hyg.*, 34: 1076-1084). All 13 isolates [10 from humans (6 from Itaporanga and 4 from Piraju), 2 from cases of canine and 1 from equine leishmaniasis (from Itaporanga)] were found to belong to the same serodeme and zymodeme and by comparison with reference strains the stocks were identified as *L. braziliensis*.

In conclusion, no evidence of a natural enzootic foci of the infection, despite our continuous search for wild reservoirs, was determined in this study; however, cases of human (13), canine (2) and equine (1) cutaneous leishmaniasis associated with this species were detected. These data support other investigations, showing that although ACL is basically a zoonosis, not all forms of the disease are associated with forests. Thus, ACL due to *L. braziliensis* may also occur in old well established communities, in non-forested areas, where the maintenance cycle seems to involve domestic animal reservoirs and phlebotomine sandflies with peridomiciliary habitats (for review see G. Grimaldi et al., 1989, loc. cit.).