Leishmaniasis in Brazil. XXII: Characterization of *Leishmania* from man, dogs and the sandfly *Lutzomyia longipalpis* (Lutz & Neiva, 1912) isolated during an outbreak of visceral leishmaniasis in Santarém, Pará State

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**Abstract**

During epidemiological studies on an outbreak of visceral leishmaniasis in Santarém, Pará State, north Brazil, isolates of *Leishmania* from two children, three dogs and six naturally infected specimens of the sandfly *Lutzomyia longipalpis* were compared, biochemically, by starch-gel enzyme electrophoresis. They have proved to be indistinguishable from each other, and from a reference strain of *Leishmania chagasi* Cunha & Chagas, 1937 from a case of human visceral leishmaniasis from Bahia State, north-east Brazil, on their enzyme profiles for ASAT, ALAT, PGM, GPI, MDH and MPI. *Lu. longipalpis* is the principal, and possibly the only vector to man in the Amazon Region of Brazil.

**Introduction**

In recent publications (LAISON et al., 1984, 1985) we discussed the epidemiology of Amazonian visceral leishmaniasis, with particular reference to the role of the sandfly *Lutzomyia longipalpis* as the vector in a severe outbreak of the disease in the small town of Santarém, Pará. During field studies in the latter locality, isolates of *Leishmania*, highly viscero tropic in the hamster, were made from four cases of human infection, five dogs and 16 naturally infected specimens of *Lu. longipalpis*.

On morphology, and behaviour in the sandfly, in vitro blood-agar culture and the hamster, we considered the parasite from all three hosts to be *L. chagasi*, the only causal agent of human visceral leishmaniasis so far recognized in Latin America.

The present paper records our comparison of the enzyme profiles of a number of these isolates with our reference strain of *L. chagasi*, by starch-gel electrophoresis.

**Materials and Methods**

*Leishmania* isolates

Codes for these follow the recommended labelling system of the World Health Organization Expert Committee on the Leishmaniasises (ANON., 1984). Our reference strain of *L. chagasi* MHOM/BR/74/PP75 from man, Bahia State, north-east Brazil, has previously been used for similar comparison of viscero tropic leishmaniasis from other parts of Brazil, Central America and the Old World (LAISON et al., 1981); to characterize *L. chagasi* from foxes in Pará; and to differentiate this parasite from the three other important aetiologic agents of (cutaneous) leishmaniasis in the Amazon Region of Brazil, namely *L. mexicana amazonensis*, *L. braziliensis braziliensis* and *L. b. guyanensis* (SILVEIRA et al., 1982).

The Santarém isolates examined here were as follows: MHOM/BR/84/M8235, MHOM/BR/84/M8299 from man; MCAN/BR/84/M8280, MCAN/BR/84/M8363, MCAN/BR/84/M8364, from dogs; ILON/BR/84/M8190, ILON/BR/84/M8194, ILON/BR/84/M8197, ILON/BR/84/M8198, ILON/BR/84/M8352, ILON/BR/84/M8361 from the sandfly *Lu. longipalpis*.

**Cultivation, lysate preparation and electrophoresis**

In our hands, *L. chagasi* is a difficult parasite to maintain in vitro. Difco blood-agar medium (WALTON et al., 1977) has proved to be the best tried to date for primary isolation: the number of flagellates steadily declines with sub-culture, however, and many isolates have subsequently died out completely. We have overcome this problem to some extent by culturing only from very heavily infected hamster liver and spleen (four to five-month-old infections), using 10 or more tubes of medium, and pooling the contents of these when they are harvested at the maximum phase of growth, 7 to 10 days later.

For preparation of lysates, their use in starch-gel electrophoresis and enzyme abbreviations, see MILES et al., (1980); in the present study we have used the enzymes ASAT, ALAT, PGM, GPI, MDH and MPI.

**Results and Discussion**

Fig 1. summarizes the results, showing that the six enzymes failed to indicate any significant differences between the isolates of *L. chagasi* from man, dogs and *Lu. longipalpis* in the Santarém focus of visceral leishmaniasis, and our reference strain of the parasite from man in Bahia.

It is, of course, difficult to suggest how many enzymes need to be used before one can conclude that any two organisms are the same. Nevertheless, from our previous biological and biochemical evidence and the present results, we conclude that the organisms isolated from foxes (*Cerdocyon thous*), dogs, man and *Lu. longipalpis* in the Marajó and Santarém foci of visceral leishmaniasis in Pará are the same, and that the parasite is indistinguishable, on the same criteria, from that we refer to as *L. chagasi*, responsible for visceral leishmaniasis of man in the major endemic areas of Ceará and Bahia.

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Fig. 1. Enzyme profiles of Leishmania chagasi from man, dogs and the sandfly Lutzomyia longipalpis from Santarém, Pará State, north Brazil. Reading from left to right, the parasites under comparison are: MHOM/BR/74/PP75, reference strain of L. chagasi from man, Bahia, Brazil; MHOM/BR/84/M8235, MHOM/BR/84/M8299, man, Santarém, Pará, Brazil; MCAN/BR/84/M8280, MCAN/BR/84/M8363, MCAN/BR/84/ M8364 from dogs, Santarém; ILON/BR/84/M8190, ILON/BR/84/M8194, ILON/BR/84/M8197, ILON/BR/84/M8198, ILON/BR/84/M8332, ILON/BR/84/M8361, from the sandfly Lu. longipalpis; and MHOM/BR/74/PP75, reference strain of L. chagasi from man, Bahia.


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