RESEARCH NOTE

Putative Reservoirs of Leishmania amazonensis in a Sub-andean Focus of Bolivia Identified by kDNAPolymerase Chain Reaction

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From 1994 to 1996, an outbreak of leishmaniasis was described in Cajuata and surrounding communities in Inquisivi province, La Paz Department, Bolivia; eight strains were isolated from patients with cutaneous ulcers and characterized by isoenzyme typing using 11 loci. All of these stocks were genetically related to *Leishmania amazonensis*. In the current work, new ubiquitous primers L1:5'-CCT ACC CAG AGG CCT GTC GGG-3' L2:5'-TAA TAT AGT GGG CCG CGC AC-3', purchased from Genset laboratory (Paris, France) were designed from the minicircle sequence of MHOM/BR/75/M2904 *L. braziliensis* strain (MHL de Bruijn & DC Barker 1992 *Acta Tropica* 52: 45-

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58) to amplify variable regions of kDNA minicircles. These primers generate polymorphic multi-banding patterns for all *Leishmania* sp. and other Kinetoplastidae, Trypanosoma cruzi, T. rangeli and T. brucei sp. Three probes were generated from major polymerase chain reaction (PCR) bands derived from strains of L. mexicana (MNYC/ BZ/62/M379), L. chagasi (MHOM/BR/74/PP75) and L. braziliensis (MHOM/BO/90/CG) species (SF Brenière et al. 1996 International Workshop on Molecular Epidemiology and Evolutionary Genetics of Pathogenic Microorganisms, CDC, Atlanta, SF Brenière et al. 1997 Medicina 55 Suppl. III: 81). The heterogeneity of the *Leishmania* sp. was investigated by hybridization of these probes to membrane-bound PCR products obtained from a large set of Leishmania strains previously characterized by isoenzyme typing (F Guerrini 1993 Genétique des Populations et Phylogénie des Leishmania du Nouveau Monde, PhD Thesis, University of Montpellier II, France, 111 pp.). These probes were specific of their respective Leishmania complex.

During September 1996, 42 mammals were captured near dwellings and in citrus plantations: 12 Didelphis marsupialis (MSP), 2 Micoureus cinerea (MSP), 14 Akodon spp. (ROD), 8 Oligoryzomys spp. (ROD), 1 Oryzomys spp. (ROD), 2 Rhipidomys leucodactylus (ROD), 2 Conepatus chinga rex (CAR) and 1 Histiotus velatus (CHT). For each mammal, a piece of skin, liver and spleen were ground together with sterile PBS in a tissue grinder and the extracts inoculated in the hind feet of hamsters. Only one stock was isolated from a C. chinga rex and was characterized by isoenzyme analysis (8 loci) and kDNA-PCR as belonging to the *L. braziliensis* complex (Fig. A, B, lanes 25 and 26). A blood sample of each mammal was tested by kDNA-PCR. Thirtyfive percent of the samples gave highly polymorphic multi-banding patterns. After Southern blot and hybridization with the three different probes, four samples from 1 Akodon spp., 2 Oligoryzomys spp., and 1 *C. chinga rex* (mentioned above) were only recognized by the *L. mexicana* complex probe (Fig. A, B, lanes 5, 7, 8 and 15). The profiles of kDNA-PCR from the three rodents were very similar and were also recognized by kDNA-PCR products of a patient strain isolated from this focus and previously characterized as belonging to L. amazonensis. The kDNA-PCR profile of C. chinga rex was different from the three others, presenting weaker hybridization with the L. mexicana complex probe and did not hybridize with the kDNA-PCR products from the patient strain. As the stock isolated from this mammal belongs to the L. braziliensis complex, this animal appears to be in-

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fected by two *Leishmania* belonging to different complexes. All the other PCR samples were not recognized by any of the *Leishmania* complex probes and similarly did not hybridize with PCR products from *T. cruzi*.

These results showed that some mammals are putative reservoirs of *Leishmania*. As the primers used are ubiquitous and amplify a large range of Kinetoplastidae, the majority of the studied mammals could have been infected by parasites other than T. cruzi and Leishmania of the three complexes tested. The strong hybridization of the PCR products of the three rodents with the L. mexicana probe and PCR products from a patient strain support the hypothesis that Akodon spp. and Oligoryzomys spp. are reservoirs of L. amazonensis at this focus. Akodon spp. and Oligoryzomys spp. represented 56% and 32% respectively of captured rodents and their infection rates reached 7% and 25%. Moreover at this focus, the sandfly, Lutzomyia nuñeztovari anglesi is an abundant species and three strains were isolated and typed by isoenzyme. All three were genetically closely related to L. amazonensis and one presented the same genotype as the strain isolated from a patient. An infected sandfly gut from another L. nuñeztovari anglesi

specimen was kDNA-PCR tested and the amplification products were recognized only by the L. braziliensis complex probe. Although no human strain of L. braziliensis complex was isolated, parasites belonging to the L. braziliensis and L. mexicana complexes co-exist in this area. Very few data are available on reservoirs of the L. mexicana complex. Leishmaniasis due to these parasites occurs more commonly as outbreaks, and human lesions mostly cure spontaneously (A Barral 1991 Am J Trop Med Hyg 44: 536-546, BL Herwaldt et al. 1992 J Inf Dis 165: 518-527). Nevertheless, in different New World foci, species belonging to various orders of mammals including dogs, rodents and carnivors have been infected by parasites of the L. mexicana complex (FJ Andrade-Narvaez et al. 1990 Trans R Soc Trop Med Hyg 84: 219-220, RD Kreutzer 1990 Am J Trop Med Hyg 43: 90-136, RN Johnson et al. 1992 Am J Trop Med Hyg 46: 282-287). In Ecuador, T Mimori et al. (1989 Am J Trop Med Hyg 40: 154-158) reported that single isolates from Sciurus vulgaris (ROD), Potos flavus (CAR) and Tamandua tetradactyla (EDE) were identified as L. amazonensis. In the Cajuata focus, the putative reservoirs corresponded to the most frequently captured mammals.

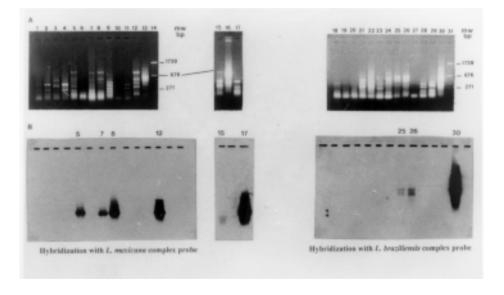


Fig.- A: ethidium bromide stained 1.5% agarose gel comparing kDNA-PCR products examined in this study. Products from reference strains of *Leishmania donovani* complex, strain MHOM/BR/79/L101 are shown in lanes 11 and 28; products from *L. mexicana* complex, MNYC/BZ/62/M379 are in lanes 12, 17, and 29; and *L. braziliensis* complex, MHOM/BO/90/CG in lanes 13, 16 and 30. Products from mammal blood samples were as follows: rodents (lanes 1-5, 7, 8, 19-24), *Conepatus chinga rex* lanes 6 and 15, marsupial (lane 18). Products from isolated strain of *C. chinga rex* (lanes 25 and 26) and Paraguayan *Leishmania* sp. (lane 9). Water template (lanes 10, 27); molecular weights Puc 19/Ras I (lanes 14, 31). B: hybridization patterns of these products with the three *Leishmania* complex specifc probes; only positive hybridizations are presented; hybridization with M379 probe, corresponding to *L. mexicana* (lanes 1-17); hybridization with CG probe corresponding to *L. braziliensis* complex (lanes 18 to 31). These products were negative with other probes except when corresponding to strain control.