RESEARCH NOTE

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

J Telleria, MF Bosseno*, T Tarifa, R Buitrago, E Martinez, M Torrez, F Le Pont*, SF Brenière*/+**

Universidad Mayor de San Andrés, Instituto Boliviano de Biología de Altura, CP 641, La Paz, Bolivia
*UMR CNRS/ORSTOM No. 9926: "Génétique Moléculaire des Parasites et des Vecteurs", ORSTOM, BP 5045, 34032 Montpellier Cédex 01, France
**UMSA, Colección Boliviana de Fauna, La Paz, Bolivia

Key words: *Leishmania amazonensis* - *Leishmania* complex - reservoirs - Sub-andean region - polymerase chain reaction - Bolivia

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*: 45-58) 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. Thirty-five 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-

This investigation received financial support from the French Ministry of Foreign Affairs and UNDP/Word/Bank/WHO, Special Program for Research and Training in Tropical Diseases, grant no. 940902.
+Corresponding author. Present address: Instituto de Investigaciones Biomédicas, Departamento de Inmunología, Ciudad Universitaria, AP 70228, CP 04510, México DF. E-mail: breniere@servidor.unam.mx
Received 15 December 1997.
Accepted 20 October 1998

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.

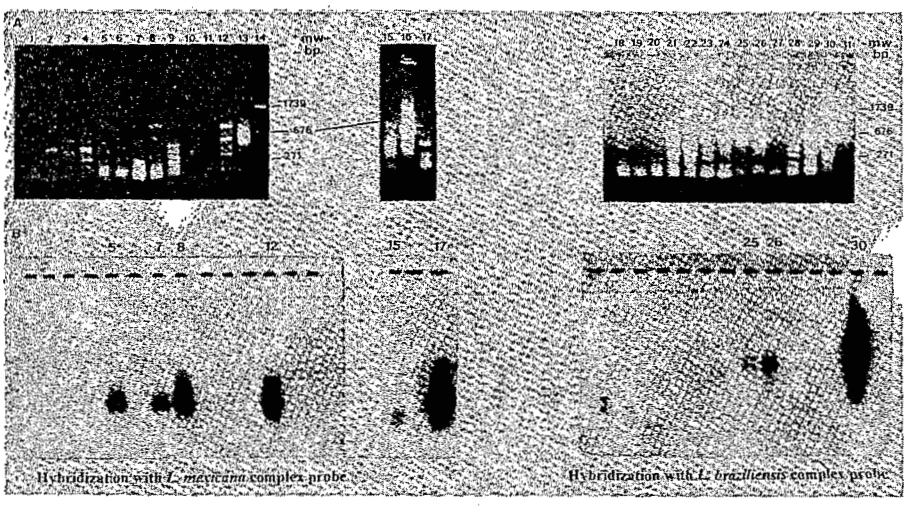

Fig.- A: ethidium bromide stained 1.5% agarose gel comparing kDNA-PCR products examined in this study. Products from reference strains of *Leishmania donovani* complex, strain MHOM/BR/79/L101 are shown in lanes 11 and 28; products from *L. mexicana* complex, MNYC/BZ/62/M379 are in lanes 12, 17, and 29; 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.

MEMÓRIAS DO INSTITUTO OSWALDO CRUZ