

RESEARCH NOTE

Cutaneous Leishmaniasis in Venezuela Caused by Infection with a New Hybrid between *Leishmania (Viannia) braziliensis* and *L. (V.) guyanensis*

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American cutaneous leishmaniasis (ACL) has a relatively high prevalence and constitutes a serious health problem in Venezuela. The disease is endemic and widely distributed in the country in nearly all states (JV Scorza et al. 1985, Leishmaniasis in Venezuela, p. 283-296. In KP Chang & RS Bray (eds) *Leishmania*, vol.1, Elsevier, Amsterdam). More studies, however, are needed to better determine the epidemiological aspects of the various leishmaniasis in the country, including their geographical distribution, etiological agents, zoonotic reservoirs and insect vectors.

Several *Leishmania* species are circulating in this country and have been indicated as responsible for different clinical forms of the disease (G Grimaldi et al. 1989 *Am J Trop Med Hyg* 41: 687-725). An extensive study was carried out regarding the etiological agent of cutaneous leishmaniasis in western Venezuela (R Bonfante-Garrido et al. 1992 *Trans R Soc Trop Med Hyg* 86: 141-148),

where *L. (Leishmania) venezuelensis* and *L. (Viannia) braziliensis* were characterized. In the same study evidence for hybrid parasites between *L. (V.) braziliensis* and *L. (V.) guyanensis* were found in this region. The disease is a problem in other regions and its etiology needs to be investigated as well.

We have isolated several leishmanial strains from patients with ACL, representing a wide geographical distribution of Venezuela (Table). The isolates were characterized by isoenzyme electrophoresis. Procedures for growing the promastigotes and for preparation of samples have been already described (H Momen et al. 1985 *Am J Trop Med Hyg* 34: 1076-1084). Electrophoresis was carried out in agarose gel and the characterization was based on the profile for 18 loci (E Cupolillo et al. 1994 *Am J Trop Med Hyg* 50: 296-311). The isolates were compared with World Health Organization reference strains for *L. (V.) braziliensis* and *L. (V.) guyanensis*.

The presence of *L. (V.) braziliensis* was confirmed in this country. Parasites presenting an identical profile to *L. (V.) guyanensis* were detected. Among the isolates we found a new enzymatic variant (IOC/Z 67) of *L. (V.) braziliensis*, differing from the reference strain of *L. (V.) braziliensis* by the pattern for the enzymes PEPD and NH. Some isolates produced a profile with alleles identical to *L. (V.) braziliensis* variant (IOC/Z 67) for some loci and identical to *L. (V.) guyanensis* for other loci. Those isolates presented heterozygotic patterns for the loci 6PGDH and NH2 suggesting that they could be hybrid parasites with the putative homozygotic bands corresponding to those of zymodeme (IOC/Z 67) of *L. (V.) braziliensis* and *L. (V.) guyanensis* (Fig.).

We characterized parasites from several endemic foci in Venezuela and the following *Leishmania* types were found: *L. (V.) guyanensis*, *L. (V.) braziliensis* enzymatic variant (IOC/Z 67) and *L. (V.) guyanensis/L. (V.) braziliensis* hybrid.

The *Leishmania* types could not be correlated with clinical manifestation as we found the same type producing either cutaneous or mucosal lesion (*L. (V.) braziliensis*) and cutaneous or "esporotricóide" lesion (*L. (V.) guyanensis*). The hybrids parasites were associated with the classical cutaneous form of the disease.

Hybrids between *Leishmania* parasites have already been studied in other endemic regions. In the Old World the presence of hybrids between *L. (L.) major* and *L. (L.) arabica* (isolated from wild animals in a zoonotic focus) was reported based on phenotypic and genotypic characteristics (D Evans et al. 1987 *Parassitologia* 29: 165-173, JM Kelly et al. 1991 *Mol Biochem Parasitol* 46: 253-264). In

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the New World hybrids were observed between *L. (V.) braziliensis* and *L. (V.) panamensis* among human isolates in Nicaragua (MDarce et al. 1991 *Trans R Soc Med Trop Hyg* 85: 58-59, AA Belli et al. 1994 *Parasitol* 109: 435-442, HA Noyes et al. 1996 *Am J Trop Med Hyg* 55: 98-105) and between *L. (V.) braziliensis* and *L. (V.) guyanensis* in Lara State, Venezuela, infecting humans, dogs and *Lutzomyia ovallesi* (Bonfante-Garrido et al. *loc. cit.*, H Momen et al. 1994, Population Genetics of *Leishmania* in the New World, p. 187-198. In AN Bhaduri et al. (eds) *Current Trends in Leishmania Research*, New Delhi), by isoenzyme analysis. The latter parasite has hybrid alleles different from the *L. (V.) guyanensis/L. (V.) braziliensis* hybrid described in

this report. In Peru hybrids were found between *L. (V.) braziliensis* and *L. (V.) peruviana* as demonstrated by molecular karyotype (JC Dujardin et al. 1993 *Ann Soc belge Méd Trop* 73: 101-118).

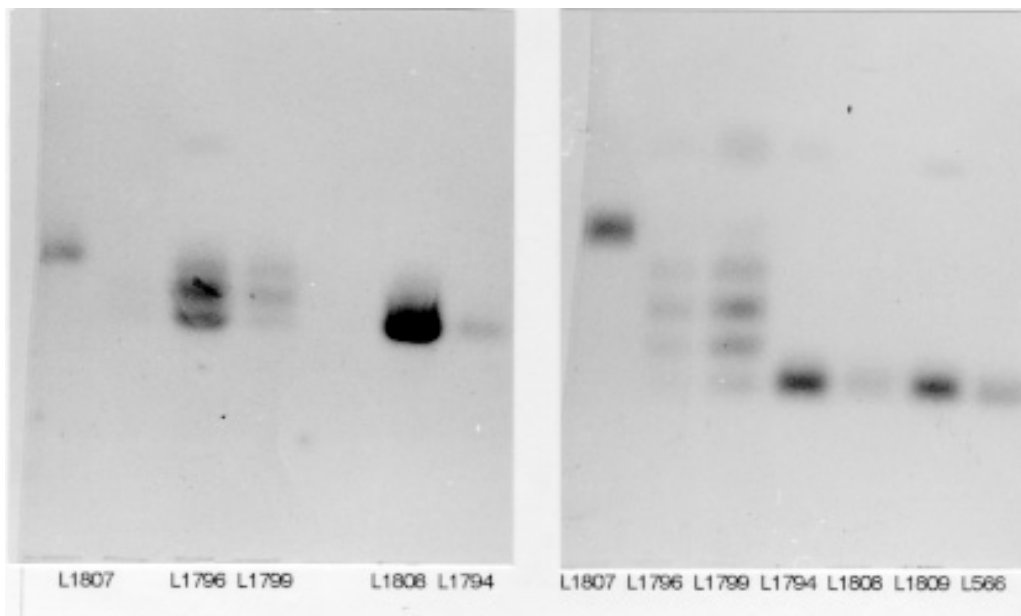
In conclusion, these data do not prove that recombination occurs in *Leishmania*. However, it reinforces the idea that sexual reproduction may occur in *Leishmania*, but at a level as yet undefined. The rarity of this phenomenon may reflect the fact that progeny from occasional sexual reproduction within clonally reproducing populations may become detectable only when the hybrid phenotype confers a selective advantage. This process may be occurring in several endemic foci in Venezuela.

TABLE
Origin and identification of *Leishmania* strains characterized in this study

IOC	International code	Species	Geographic origin
L1794	MHOM/VE/84/CX-3	<i>L. (V.) braziliensis</i>	Miranda, Guatopo
L1796	MHOM/VE/84/CJ-8	Hybrid var ^b	Tachira
L1799	MHOM/VE/87/GJ-13	Hybrid var ^b	DF, El Junquito
L1800	MHOM/VE/84/GM-14	Hybrid var ^b	Miranda, Guarenas
L1803	MHOM/VE/84/JE-18	<i>L. (V.) guyanensis</i>	Sucre
L1804	MHOM/VE/83/LO-19	<i>L. (V.) braziliensis</i> var ^b	Miranda, Osuma
L1805	MHOM/VE/85/MB-21	<i>L. (V.) guyanensis</i>	Miranda, Charallave
L1807	MHOM/VE/87/PJ-26	<i>L. (V.) guyanensis</i>	Anzoategui, Guanape
L1808	MHOM/VE/87/PD-27	<i>L. (V.) braziliensis</i>	Bolívar, Gran Sabana
L1809	MHOM/VE/85/RA-28	<i>L. (V.) braziliensis</i>	Portuguesa, Acarigua
L1810	MHOM/VE/85/RN-29	Hybrid var ^b	Yaracuy, Yaritagua
L565 ^a	MHOM/BR/75/M4147	<i>L. (V.) guyanensis</i>	Pará
L566 ^a	MHOM/BR/75/M2903	<i>L. (V.) braziliensis</i>	Pará

a: World Health Organization reference strains used to compare with the new isolates

b: *L. (V.) braziliensis* var = IOC/Z 67, Hybrid var = Hybrid between *L. (V.) braziliensis* var and *L. (V.) guyanensis*



Enzymatic profile of some *Leishmania* isolates. Fig. 1: enzymatic staining for 6PGDH, Fig. 2: enzymatic staining for NH.