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Research Note

DIFFERENCES BETWEEN *LEISHMANIA (LEISHMANIA) CHAGASI*, *L. (L.) INFANTUM* AND *L. (L.) DONOVANI* AS SHOWN BY DNA FINGERPRINTING

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There are several hypotheses relating to the origin of *Leishmania (L.) chagasi*. R. Lainson & J. J. Shaw, (1979, p. 1-116. In W. H. R. Lumsden & D. A. Evans (eds) *Biology of the Kinetoplastida*, Academic Press, London) suggested that visceral leishmaniasis in South America might be caused by both indigenous and imported parasites. R. Killick-Kendrick et al. (1980, *Ann. Trop. Med. Parasit., 74:* 563-565) concluded that the parasite may well have been transported in dogs accompanying Spanish and Portuguese Conquistadores. Furthermore, evidence was provided to support the notion that the New World vector *Lutzomyia longipalpis* was indeed susceptible to infection with *L. (L.) infantum*. More recently R. Lainson et al., have given their reasons for regarding *L. (L.) chagasi* as indigenous to the Americas, following their finding of a high rate of inapparent infection in Amazonian foxes (1987, *Trans. R. Soc. Trop. Med. Hyg., 85:* 517; 1990, *Mem. Inst. Oswaldo Cruz, 85:* 135-137). Biochemical (enzymes), serological (monoclonal antibodies) and molecular biology techniques, on the other hand, have till now shown *L. (L.) chagasi* and *L. (L.) infantum* to be very similar and, for these reasons, some authors regard the parasites as being a single species (R. Killick-Kendrick et al., 1980, *Ann. Trop. Med. Parasitol., 74:* 563-565; G. Grimaldi et al., 1987, *Ann. Trop. Med. Hyg., 36:* 270-287; J. A. Rioux et al., 1990, *Ann. Parasit. Hum. Comp., 65:* 111-115).

The commercial exploitation of hypervariable DNA sequences has recently been highlighted by the elegant work of A. Jeffreys et al. (1985, *Nature, 316:* 176-179) who used a simple repetitive DNA sequence for the fingerprinting of human DNA. DNA fingerprints have proven a powerful method for individual identification, establishing family relationships and in forensic science. During our studies on the sequence organization of the *Leishmania* genome, we have identified and characterized several highly repeated DNA sequences which may be associated with telomeric sites of the *L. (L.) donovani* genome (J. Ellis & J. Crampton, 1988, *Molec. Biochem Parasitol., 29:* 9-18). Simple

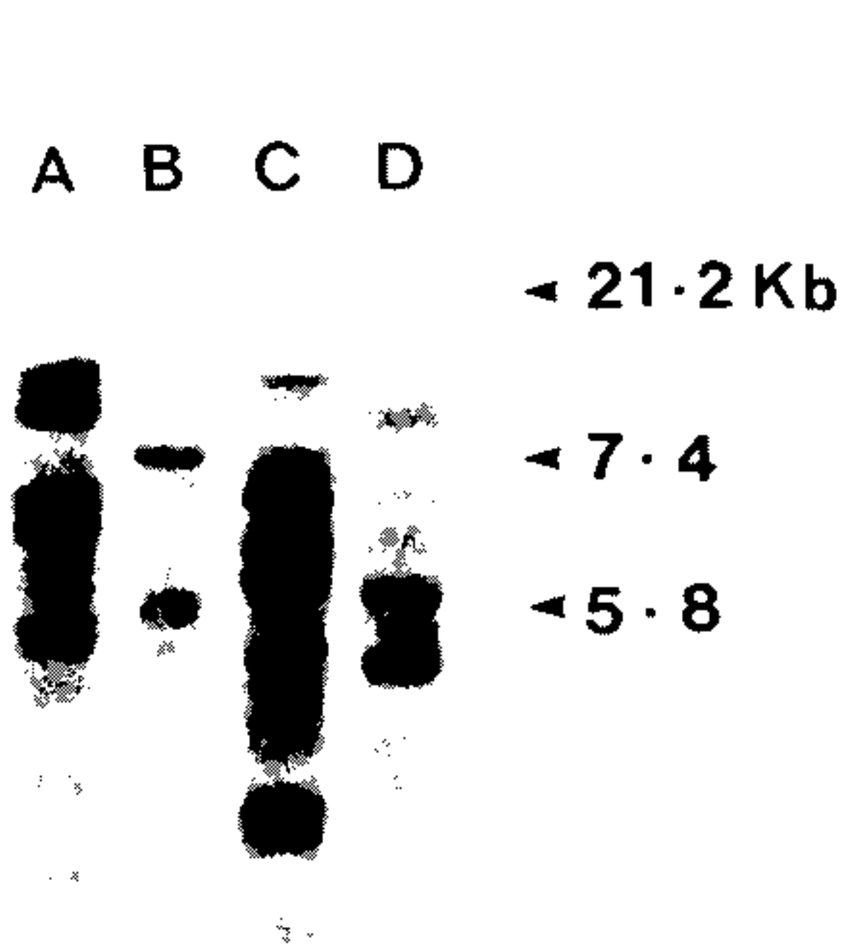

Fig. 1: fingerprinting of *Leishmania* DNAs. *L. (L.) chagasi* strain LV474 (Lanes A and C) and *L. (L.) major* strain LV561 (Lanes B and D) DNA was digested with the restriction enzymes EcoRV (Lanes A and B) and XhoI (Lanes C and D) and probed with pRs2A. Sizes are in kilobases.

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TABLE

Isolates of *Leishmania* studied by DNA finger-printing

Liverpool and other codes	WHO code	Parasite	Host	Country of origin
LV474	MHOM/BR/74/PP75	*L. (L.) chagasi*	Man	Brazil
LV561	MHOM/IL/67/JERICHO II	*L. (L.) major*	Man	Israel
LV9	MHOM/ET/67/HU3	*L. (L.) donovani* s.l.	Man	Ethiopia
DD8	MHOM/IN/80/DD8	*L. (L.) donovani*	Man	India
LEM235	MHOM/TN/80/IPTI	*L. (L.) infantum*	Man	Tunisia
M4192	MHOM/BR/76/150406	*L. (L.) chagasi*	Man	Brazil

repetitive DNA sequences at such sites are frequently hypervariable and exhibit restriction fragment length polymorphisms (E. H. Blackburn, 1984, *Cell, 37:* 7-8). We asked whether such a highly repetitive DNA sequence could be used for the DNA fingerprinting of *Leishmania* strains and species and in particular whether such a technique could indicate significant differences between *L. (L.) chagasi* and *L. (L.) infantum.* A summary of our results is presented here.

Genomic DNA from a variety of *Leishmania* isolates were digested with restriction enzymes and subsequently electrophoresed through a 1% agarose gel. Southern Blots were then hybridised with a ^{32}P-labelled, recombinant plasmid DNA probe, pRs2A, containing a cloned repetitive sequence (J. Ellis & J. Crampton, 1989, p. 413-419. In D. Hart *Leishmaniasis: The current status and new strategies for control.* Plenum Press, N. Y.). After high stringency washing of the nitrocellulose filter (0.1 x SSC, 65 °C) and low temperature autoradiography, a complex pattern of bands or "fingerprint" was obtained which was different for each of the isolates analysed. Fig. 1 shows an example of the patterns obtained with *L. (L.) chagasi* (LV474) and *L. (L.) major* (LV561) DNA. This probe may therefore prove useful to identify, classify and confirm the division of *Leishmania* isolates into species by the patterns of hybridisation visualised.

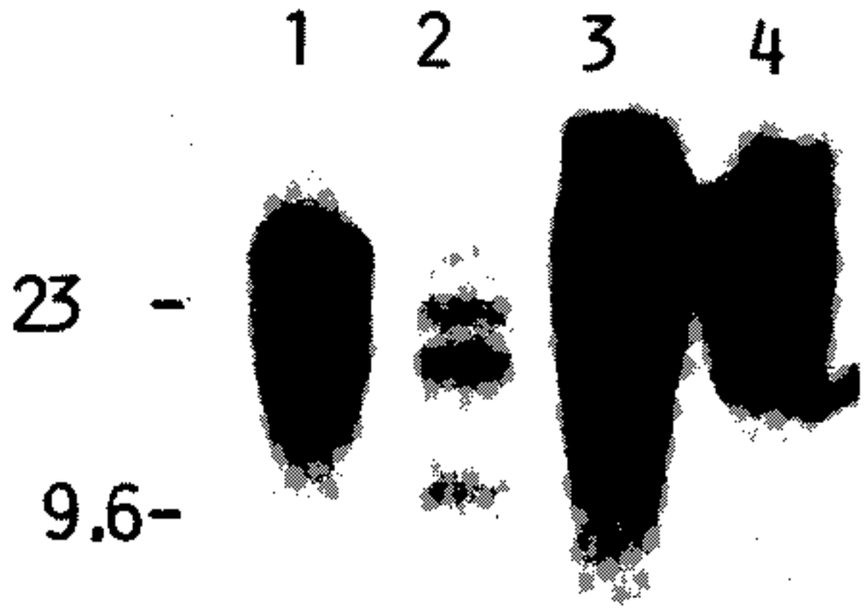

Fig. 2: fingerprinting of Old and New World strains of the *L. donovani* complex. HindIII digests of DNA from the Old World strains LV9 (Lane 1), DD8 (Lane 3) and LEM235 (Lane 4) and a New World strain M4192 (Lane 2) of *L. (L.) chagasi* probed with pRs2A. Sizes are in kilobases.

Leishmania (L.) chagasi strain (LV474) (Fig. 1) showed a very high level of dispersion of the repetitive DNA sequence recognized by probe pRs2A. The same high level of dispersion was also shown for another isolate of *L. (L.) chagasi* (M4192) with the same restriction enzymes. In a second experiment the same fingerprinting probe was used to compare the hybridisation patterns obtained with DNA from Old and New World parasites of the *donovani* complex and an example of the result is shown in Fig. 2. Hybridization of the probe pRs2A to

isolates of the Old World species *L. (L.) donovani* (DD8, India), *L. (L.) donovani* s.l. (LV9, Ethiopia) and *L. (L.) infantum* (LEM235, Tunisia) occurred exclusively to high molecular weight restriction fragments. This appears to be true of all Old World parasites of the *donovani* complex so far examined in this way. Clearly, the organization of this sequence in the *L. (L.) chagasi* genome, and therefore of the genome itself, is very different from that of the Old World *Leishmania*. Whilst these preliminary studies need to be interpreted with caution before any conclusions can be drawn regarding their taxonomic or phylogenetic importance, they do appear to give some support to the view that *L. (L.) chagasi* and *L. (L.) infantum* should be separated at some taxonomic level. Clearly, we need to compare more isolates of these parasites by the same method, and we are hoping to do this in the near future.