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Short Communication: **Palestinian infantile visceral leishmaniasis caused by a genetic variant of *Leishmania infantum* belonging to a new zymodeme**

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Summary The parasites causing a Palestinian case of infantile visceral leishmaniasis (IVL) and those from four dogs from the Jenin District were identified serologically, biochemically and molecular biologically as *Leishmania infantum*, showing dogs act as a reservoir. The strain from the human case was distinct because of its unique 200-bp kDNA-polymerase chain reaction (PCR) component in its restriction fragment length polymorphism (RFLP) profile after digestion with the endonuclease *Rsa*I, and by the electrophoretic mobility of its malate dehydrogenase (MDH^{140}), designating it the reference strain of a new zymodeme of *L. infantum*, MON-281.

keywords *Leishmania infantum*, isoenzyme analysis, polymerase chain reaction, Palestinian Authority

Human infantile visceral leishmaniasis (IVL) caused by *Leishmania infantum* occurs in all the countries surrounding the Mediterranean Basin. Qubain *et al.* (1997) described a case of IVL from Bal'a, Tulkarm District, Palestine. Abdeen *et al.* (2002) described the epidemiology of human IVL in the nearby Jenin District. Fifty cases were diagnosed between 1989 and 1998 and 5.5% of the dogs screened were found to be seropositive. Isolation of a parasite stock from a case of IVL from the Jenin District at the end of that period gave the first opportunity of characterizing and comparing the parasite circulating there with those isolated from infected dogs from the area and from Israel.

In September 1999, a Palestinian male infant two and a half years old from the village of El-Yamoun, Jenin District, Palestine, was admitted to the Al-Watany Hospital, Nablus, after 2 months of fever and displaying anaemia and hepatosplenomegaly. Smears of his bone marrow stained with Giemsa's stain revealed amastigotes. Its culture in rabbit blood-agar slopes overlaid with Schneider's *Drosophila* medium (SDM) containing 10% foetal calf serum produced promastigotes – strain MHOM/PS/1999/LRC-L773.

Five strains of *Leishmania* were isolated from the lymph nodes of local dogs: four from the Jenin District, MCAN/PS/2000/SawalhaO25 (LRC-L806), MCAN/PS/2000/SawalhaK56 (LRC-L807), MCAN/PS/2000/SawalhaS47 (LRC-L808), MCAN/PS/2004/LRC-L1131 (LRC-L1131) and one from northern Israel, MCAN/IL/1996/LRC-L705, were included for comparison. As human cutaneous leishmaniasis (CL) caused by *L. tropica* and *L. major* also occurs in and just south of the Jenin District, respectively, local strains of *L. tropica*, MHOM/PS/2002/63JnF21 (LRC-L950) and *L. major*, IPAP/IL/1984/8A1 (LRC-L464) were used for comparison, as were the WHO reference strains of *L. donovani* MHOM/IN/1980/DD8 and *L. infantum* MHOM/TN/1980/IPT1.

Characterization was performed by: excreted factor (EF) serotyping (Schnur & Zuckerman 1977); enzyme electrophoresis using the standard 15 enzyme systems (Rioux *et al.* 1990); polymerase chain reaction (PCR)-based kinetoplast DNA (kDNA) analysis (Anders *et al.* 2002), generating restriction fragment-length polymorphism (RFLP) profiles (Abdeen *et al.* 2002) and nuclear DNA (nDNA) analysis using the internal transcribed spacer 1 (ITS1)–PCR (Schönian *et al.* 2003).

Strain LRC-L773 was EF sub-serotype B_2 as were all five strains from the dogs and the reference strain of *L. infantum*, IPT1. Its enzyme profile was almost identical to that of strain IPT1, the type strain of the zymodeme MON-1. Of the 15 enzymes, only one, malate dehydrogenase EC

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1.1.1.37, MDH, migrated more rapidly than that of the reference strain. This indicated that strain LRC-L773 was a strain of *L. infantum* but represented a new zymodeme of *L. infantum*, zymodeme MON-281. Strain LRC-L1131 was identical to strain IPT1. The 800-bp kDNA PCR product of strain LRC-L773 aligned with those of IPT1, *L. donovani* DD8, LRC-L705, -L807 and -L808. The kDNA PCR amplification product of the reference strain *L. major* LRC-L464 was only 650 bp in size (Figure 1a).

Figure 1b presents the RFLP pattern yielded by the 800-bp kDNA PCR product of strain LRC-L773 after its digestion with the endonuclease *Rsa*I, comparing it with those of the strain *L. infantum* IPT1 and three of the strains isolated from local dogs. All shared the three higher-molecular-weight bands. The lower-molecular-weight bands were polymorphic (Figure 1b, arrows). Strain LRC-L773 was different from strain IPT1, having an extra 200-bp band in its profile, also seen in the profiles of the three local dog strains. The RFLP profile of strain LRC-L773 also shared one extra 280-bp band with strain IPT1 from a human infantile case of VL. This band was absent from the profiles of the canine strains.

The ITS1–PCR produced a single band of approximately 300 bp for each of the strains tested (data not shown). The restriction pattern of the ITS1-PCR product of strain LRC-L773 was identical to those of the strain *L. infantum* IPT1 and the four strains isolated from the dogs (result for strain LRC-L1131 not shown). All six were different from the local strain of *L. tropica* LRC-L950 (Figure 1c). Strain LRC-L773 was identical to strain *L. infantum* IPT1 in EF sub-serotype (B_2), the electrophoretic mobilities of 14 of the 15 enzymes in its enzyme profile and, as previously shown, its permissively primed intergenic polymorphic (PPIP)-PCR profile type (*Li*A) (Abdeen *et al.* 2002). However, its MDH migrated more rapidly, MDH^{140}, than that of strain *L. infantum* IPT1 (MDH^{100}). It also differed from all the other known electrophoretic variants of MDH found among strains of *L. infantum* (Rioux *et al.* 1990; Pratlong *et al.* 2001). This alone designated strain LRC-L773 as the reference strain of a new zymodeme of *L. infantum*, zymodeme MON-281.

The RFLP profile generated from the kDNA PCR product of strain LRC-L773 was identical to that of strain *L. infantum* IPT1 in its four higher-molecular-weight components of 800, 540, 420 and 280 bp (Figure 1b). However, it also had a fifth lower-molecular-weight component of 200 bp, indicating genetic difference. The three higher-molecular-weight components in the profile were also shared by the RFLP profiles of strains LRC-L705, -L807 and -L808 from dogs. Their RFLP profiles also possessed the fifth lower-molecular-weight component seen in the RFLP profile of strain LRC-L773 that was absent in

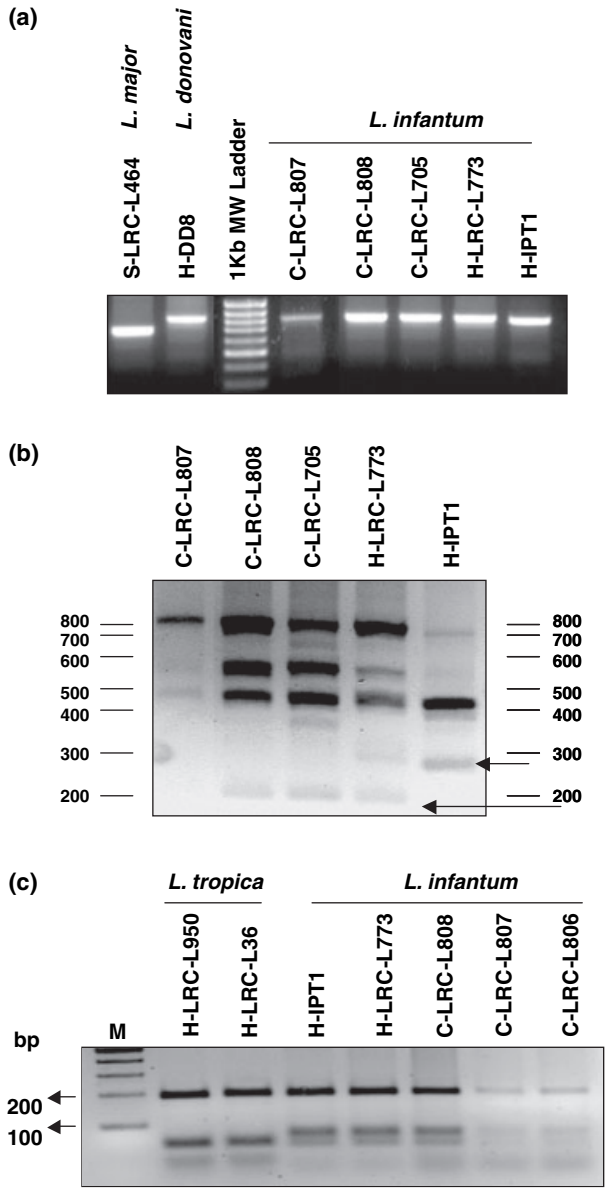

Figure 1 Comparison of (a) the kDNA PCR products, (b) RFLP profiles of the kDNA PCR products and (c) the ITS1-PCR products: H, human; S, sand fly; C, canine.

the RFLP profile of strain *L. infantum* IPT1 (Figure 1b). However, they lacked the fourth 280-bp component that the RFLP profile of strain LRC-L773 shared with the RFLP profile of strain *L. infantum* IPT1 (Figure 1b). The RFLP profile of strain LRC-L705 displayed an extra 700-bp component not seen in the RFLP profiles of the other strains in Figure 1b. Some of these genetic differences appear to correlate with the type of host and others with

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locality. However, one must be cautious in assuming such relationships even though one is dealing with kDNA which is known to be very variable (Rogers & Wirth 1987). The RFLP profile generated from the kDNA PCR product of strain LRC-L773 was also different from profiles of seven other Israeli strains of *L. infantum* from a human case of infantile VL and six dogs (Abdeen *et al.* 2002).

All strains of *L. infantum* from humans and dogs examined by EF serotyping have been EF sub-serotype B_2. All those examined by the PPIP-PCR have been PPIP-PCR type *Li*A (Abdeen *et al.* 2002). All those characterized by the size of their kDNA PCR amplification product and RFLP profile of their ITS1-PCR product were identical regarding these criteria.

The EF serotypes and DNA characters of strains LRC-L806, -L807 and -L808 indicated they were strains of *L. infantum*, suggesting that infected dogs are the medically important reservoir of human IVL in the Jenin District. Unfortunately, the frozen stabilates of these strains were lost and were not available for enzyme electrophoretic analysis. Strain LRC-L1131 isolated thereafter from another dog from the Jenin District was not from zymodeme MON-281 but belonged to the ubiquitous zymodeme MON-1, the only other zymodeme represented so far in the region encompassing Israel and the West Bank. The IVL case described was not exceptional despite the enzymological and genetic differences displayed by its parasite. Although these differences existed in parallel, the genetic differences are not likely to be the genetic basis of the difference seen in the electrophoretic mobility of the MDH of the strain.

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