Leishmania species have long been considered to reproduce asexually. However, there is now a huge pile of data that indicate genetic material exchange between Leishmania species, by which they could better adapt to changing environmental conditions.

- We have previously reported the first confirmed hybrid Leishmania isolated from Turkey, which showed viralization in laboratory mice after inoculation into footpads.
- Recent clinical reports of cutaneous leishmaniasis (CL) cases from different provinces of Anatolia that are culture-negative and got no benefit from antimonial therapy may show that hybrids are not uncommon.
- We aimed to assess the genetic and proteomic diversity of autochthonous Leishmania isolates from CL patients.

### Materials and Methods:

- Twenty CL patients from two provinces, 10 from Hatay (Group 1) where both L. tropica and L. infantum are isolated in CL cases and 10 from Saniurfa (Group 2) where only L. tropica is isolated, were enrolled.
- Giemsa-stained smears of lesions were initially prepared, followed by inoculation to NNN medium and enriched medium, which was specially designed for Leishmania culture.

-A Real Time PCR protocol that targeted the ITS-1 region of Leishmania spp. was applied using both amastigotes and promastigotes, followed by DNA sequence analysis and isozyme analysis (for 1 sample each).

- Proteomic profiles of two groups were compared with MALDI-TOF and 2-dimensional electrophoresis (2DE). All isolates were inoculated into the right footpads of mice to assess their in vivo activities.

### Results:

- All isolates from Saniurfa were found to be L. tropica with Real-Time PCR and confirmed by sequence analyses and MALDI-TOF.
- They caused only cutaneous lesions in mice, just as two isolates from Hatay which were shown to be L. tropica and L. major.
- Four of the 8 remaining isolates from Hatay showed two peaks in RT-PCR concordant with L. tropica and L. infantum, and confirmed as L. infantum with sequence and isozyme analyses.
- Comparison of their proteomic profiles with the reference L. infantum strain with 2DE identified seven different proteins, after which they are named as L. infantum/L. tropica hybrids.
- Others were found as L. tropica but they had six different proteins compared to reference L. tropica strain (L. tropica/L. infantum hybrid).

### Discussion:

This is the first demonstration of proteomic differences between the hybrid and non-hybrid isolates of Leishmania spp. from Turkish CL patients. These different proteins may be involved in significant biochemical pathways and associated with viralization in mice. Further analyses are needed to unravel their roles in hybridization and pathogenesis of leishmaniasis in vivo.

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**References**


**Assessment of the Varieties between hybrid and non-hybrid Leishmania strains isolated from Cutaneous Leishmaniasis Patients in Turkey using Genotypic and Proteomic Methods and Mouse Model**

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**Materials and Methods**

- Twenty autochthonous CL cases with no history of recent travel to endemic sites were identified -> 10 from Saniurfa
- 10 from Hatay provinces!

The following methods were applied for all 20 samples in the study:

- Microscopy & Culture
- Real Time PCR followed by sequence analyses => ITS-2 region of Leishmania spp. was amplified, from lesion culture.
- 2D Electrophoresis & MALDI-TOF => To analyze genotypic and proteomic differences.
- Inoculation in Laboratory Animals => To assess the clinical outcome of infections in vivo.

**References**