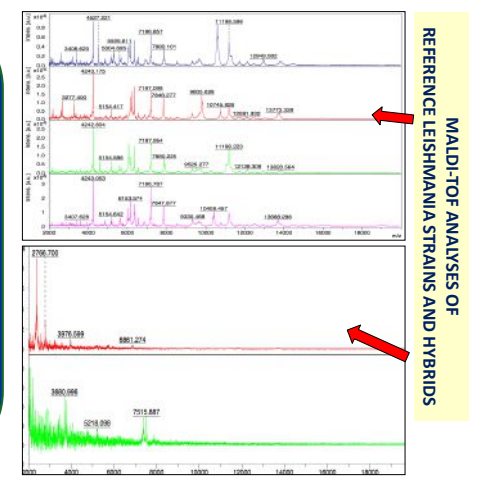


Assessment of the Varieties between hybrid and non-hybrid *Leishmania* strains isolated from Cutaneous Leishmaniasis Patients in Turkey using Genotypic and Proteomic Methods and Mouse Model

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Abstract

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* isolates from Turkey, which showed visceralization 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 Şanlıurfa (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 isoenzyme 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 Şanlıurfa 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 isoenzyme 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* hybrids). These eight isolates caused life-threatening visceralization in mice.

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 visceralization in mice.* Further analyses are needed to unveil their roles in hybridization and pathogenesis of leishmaniasis in vivo.

Introduction

❖ By developing hybrid strains, the microorganisms may adapt to changing environmental conditions, including their vectors and hosts, which helps their survival in nature by gaining new genotypic characteristics.

❖ We have previously isolated *Leishmania* hybrids in Turkey, with different clinical manifestations, and diagnostic and therapeutic aspects.

❖ New methods, such as PCR and MALDI-TOF have brought in the opportunity for detailed diagnosis of *Leishmania* species, with information about their genotypes and protein profiles.

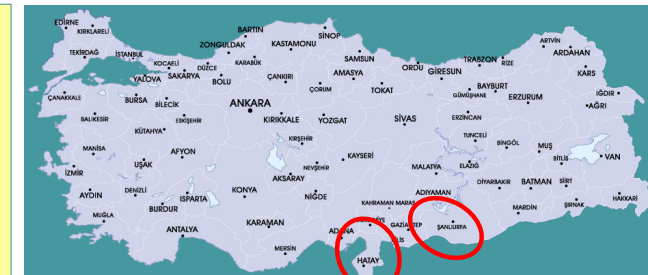
❖ Here, we aimed to assess the genetic and proteomic diversities of autochthonous *Leishmania* isolates from CL patients, as well as their clinical behaviors in laboratory animal models.

Materials and Methods

❖ Twenty autochthonous CL cases with no history of recent travel to endemic sites were identified =>

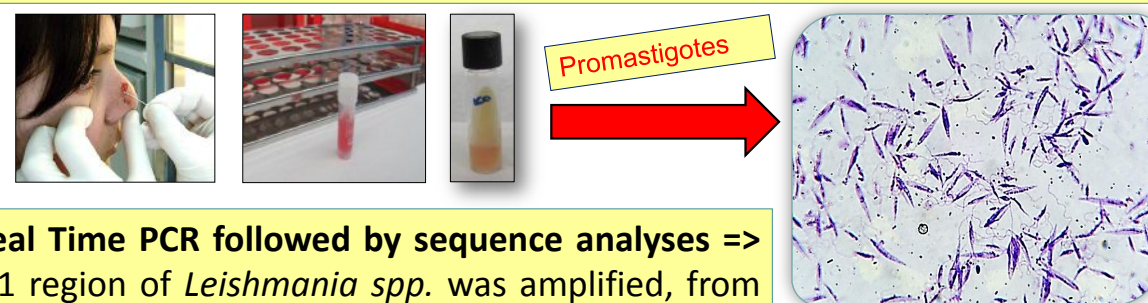
10 from Şanlıurfa

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-1 region of *Leishmania* spp. was amplified, from lesion and culture.

▪ **2D Electrophoresis & MALDI-TOF =>** To analyze genotypic and proteinic differences.

▪ **Inoculation in Laboratory Animals =>** To assess the clinical outcome of infections in vivo.

▪ **Reference strains of *L. tropica*, *L. major*, *L. donovani* and *L. infantum* were used for comparing the results.**

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Results

Despite the distance between the two cities is not high (almost 350 km), there is an obvious difference between the genotypes, proteinic composition and clinical manifestations of Hatay and Şanlıurfa.

Code of the Isolate*	Clinical Findings in Mice	Real-Time ITS1 PCR (from lesion sample)	Real-Time ITS1 PCR (from culture sample)	Giemsa-stained Smear of the Mouse Sample	Culture of the Mouse Sample	DNA Sequence	Isoenzymes	MALDI-TOF
MHOM/TR/2012/HU19	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU20	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU31	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU38	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU40	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU24	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU41	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU26	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU27	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/HU29	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-7	<i>L. tropica</i>
MHOM/TR/2012/MK32	C	<i>L. major</i>	<i>L. major</i>	KL+	KL+	<i>L. major</i>	MON-103	<i>L. major</i>
MHOM/TR/2012/MK11	C	<i>L. tropica</i>	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-312	<i>L. tropica</i>
MHOM/TR/2012/MK08	C/V	<i>L. tropica</i> / <i>infantum</i> 2 peaks	<i>L. tropica</i> / <i>infantum</i> 2 peaks	VL and KL +	VL and KL +	<i>L. infantum</i>	MON-319*	N/A
MHOM/TR/2012/MK33	C/V	<i>L. tropica</i> / <i>infantum</i> 2 peaks	<i>L. tropica</i> / <i>infantum</i> 2 peaks	VL and KL +	VL and KL +	<i>L. infantum</i>	MON-319*	N/A
MHOM/TR/2012/MK10	C/V	<i>L. tropica</i> / <i>infantum</i> 2 peaks	<i>L. tropica</i> / <i>infantum</i> 2 peaks	VL and KL +	VL and KL +	<i>L. infantum</i>	MON-319*	N/A
MHOM/TR/2012/MK05	C/V	<i>L. tropica</i> / <i>infantum</i> 2 peaks	<i>L. tropica</i> / <i>infantum</i> 2 peaks	VL and KL +	VL and KL +	<i>L. infantum</i>	MON-1	N/A
MHOM/TR/2012/MK03	C/V	<i>L. tropica</i>	<i>L. tropica</i>	VL and KL +	VL and KL +	<i>L. tropica</i>	MON-7	N/A
MHOM/TR/2012/MK04	C/V	<i>L. tropica</i>	<i>L. tropica</i>	VL and KL +	VL and KL +	<i>L. tropica</i>	MON-54	N/A
MHOM/TR/2012/MK06	C/V	<i>L. tropica</i>	<i>L. tropica</i>	VL and KL +	VL and KL +	<i>L. tropica</i>	MON-54	N/A
MHOM/TR/2012/MK09	C/V	<i>L. tropica</i>	<i>L. tropica</i>	VL and KL +	VL and KL +	<i>L. tropica</i>	MON-313	N/A
TURKEY'S REFERENCE STRAINS								
MHOM/TR/2009/MK01	C/V	-	<i>L. tropica</i>	VL ve KL +	VL ve KL +	<i>L. tropica</i>	MON-315	N/A
MHOM/TR/2010/MK02	C/V	-	<i>L. tropica</i>	VL ve KL +	VL ve KL +	<i>L. tropica</i>	MON-314	N/A
MHOM/TR/2009/CBU11	C/V	-	<i>L. tropica</i>	VL ve KL +	VL ve KL +	<i>L. tropica</i>	MON-315	N/A
MHOM/TR/2009/HU07	C	-	<i>L. tropica</i>	KL+	KL+	<i>L. tropica</i>	MON-313	<i>L. tropica</i>

HU => Şanlıurfa isolates; MK: => Hatay isolates; CBU: Manisa isolate

✓ Some of the samples from Hatay were shown to be hybrids.
 ✓ Proteinic differences may cause different clinical manifestations in mice.

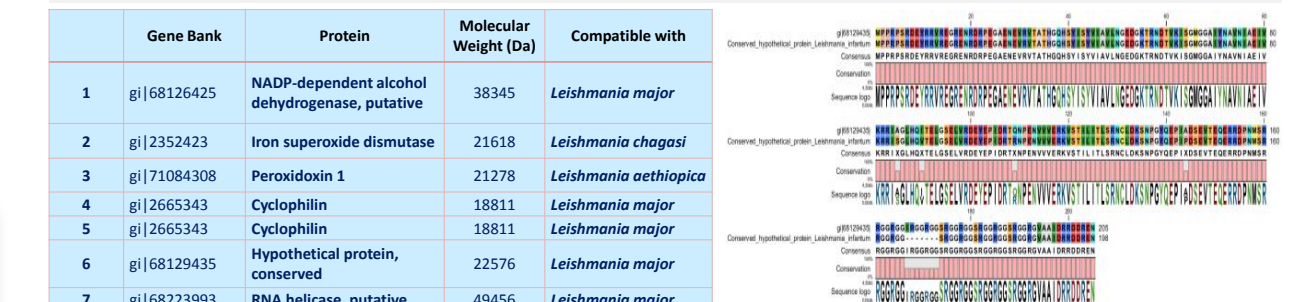


Figure 2. List of unique *Leishmania* proteins from Hatay identified by 2D gel electrophoresis (LEFT); conserved hypothetical protein is present in *L. infantum/L. tropica* hybrids. This diagram shows its similarities to *L. major*'s and differences from *L. infantum*'s (RIGHT).

Conclusions

❖ This is the first report of unique proteins in hybrid and non-hybrid *Leishmania* isolates in Turkey, which may be responsible for the visceralization behaviour of some *L. tropica* isolates, etc.

❖ Despite the isolates from Şanlıurfa showed total similarity to reference *L. tropica* strain, Hatay isolates showed obvious varieties in terms of species, RT-PCR, MALDI-TOF, sequence analyses and clinical manifestations *in vivo*.

❖ *Further research is needed to unveil the roles of these proteins in the visceralization of the parasites and the unique clinical outcomes, which may sometimes harden their diagnosis with routine methods.*