P-15

Unlocking *Leishmania* Diversity in Majorca: kDNA Insights

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Background

PCR-based methods targeting kDNA minicircles have provided new insights into *Leishmania* species distribution and host interactions, the approach yielding 16 *Leishmania infantum* genotypes to date [1]. This study aimed to obtain new information about *L. infantum* diversity by analyzing hypervariable regions of kDNA minicircles using state-of-the-art SNP and *in silico* RFLP assays.

Materials and Methods

Human samples (14 skin, 3 bone marrow, and 1 blood) obtained from patients diagnosed with cutaneous or visceral leishmaniasis by microscopy and PCR (VIASURE *Leishmania* Real-Time PCR Detection Kit, Certest Biotec S.L.) were molecularly identified using the ITS-1 region [2]. DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) for skin, and EZ1 DSP DNA Blood Kit (Qiagen) for bone marrow and blood samples. For the phylogenetic study, kDNA minicircles were targeted using the primers described by Cortes *et al.* [1] followed by DNA sequencing. Genotypes were assigned by comparative SNP analysis using ClustalW and *in silico* RFLP via Restriction Mapper (http://www.restrictionmapper.org/) [3].

Results and discussion

All samples were identified as *L. infantum*. SNP analysis yielded 11 genotypes, 7 described for the first time (Table 1), while RFLP grouped all samples into genotypes B (33.3%) and F (66.7%). The globally distributed genotype B is predominant in the Mediterranean Basin and South America [4], unlike genotype F, which is reported only rarely in Mediterranean regions. Therefore, the observed prevalence of genotype F in the Balearic Islands, where it causes cutaneous and visceral leishmaniasis, indicates an insularity effect.

Table 1: Genotypes based on kDNA single nucleotide polymorphisms (SNP) compared to the KX098509 reference sequence.

SNP Genotype	26	55	64	80	122	252	253	317	322	326	328	353	366	387	388	389	412	Nº of samples	RFLP genotype
KX098509	A	A	G	A	C	A	G	A	G	C	G	T	A	A	-	A	C		
G1	·	·	·	·	·	·	·	·	·	·	·	·	·	·	-	·	·	3	B
G18	G	·	A	·	A	G	·	·	·	·	·	C	G	G	-	·	·	3	F
G20	G	·	A	·	A	G	·	·	·	·	·	C	G	·	-	-	·	3	F
G22	G	·	A	·	A	G	A	·	·	·	·	C	G	·	-	-	·	1	F
G32*	G	·	A	·	A	G	·	·	·	·	·	C	·	·	-	·	·	1	F
G33*	·	·	·	·	·	·	·	G	·	·	·	·	·	·	-	·	·	2	B
G34*	G	·	A	·	A	G	·	·	·	·	A	C	G	·	-	-	·	1	F
G35*	G	·	A	·	A	G	·	·	·	T	·	C	·	-	A	·	·	1	F
G36*	G	·	A	·	A	G	·	·	·	·	·	C	·	G	-	·	·	1	F
G38*	G	·	·	·	A	·	·	·	A	·	·	·	·	·	-	·	·	1	B
G39*	G	·	A	·	A	G	·	·	·	·	·	C	G	G	G	·	·	1	F

(*) Genotypes described for the first time. Identical nucleotides (.) and nucleotide deletions (-) with respect to the reference sequence for each position.

Conclusion

The discriminative power of kDNA minicircle analysis provided phylogenetic insights into *L. infantum* in an endemic area. SNP analysis was more informative than RFLP, classifying the 18 samples into 11 versus 2 genotypes.

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References

1. Cortes, S., Mauricio, I., Almeida, A., *et al.* Application of kDNA as a molecular marker to analyse *Leishmania infantum* diversity in Portugal. Parasitol Int. 2006; 55(4) : 277–283.
2. Tomás-Pérez, M., Hide, M., Riera, C., *et al.* Multilocus microsatellite typing of *Leishmania infantum* isolates in monitored Leishmania/HIV coinfected patients. Parasit Vectors 2015 ; 8(1) : 1 – 9.
3. Ortuño, M., Latrofa, M. S., Iborra, M. A., *et al.* Genetic diversity and phylogenetic relationships between *Leishmania infantum* from dogs, humans and wildlife in south east Spain. Zoonoses Public Health 2019 ; 66 (8) : 961 – 973.
4. El Hamouchi, A., Ejghal, R., Hida, M., *et al.* Intraspecific genetic variability in a population of Moroccan *Leishmania infantum* revealed by PCR RFLP of kDNA minicircles. Acta Trop. 2017 ; 169 : 142–149.