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ORAL PRESENTATION

DIAGNOSIS – EXPERIMENTAL AND CLINICAL

SYSTEMATIC COMPARISON OF FIVE GENOTYPIC MARKERS FOR SPECIES DISCRIMINATION IN LEISHMANIA

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Summary

Background: Several genetic markers have been used for discriminating medically important *Leishmania* species, using a variety of different techniques. As commercial standardized tests are not available, methods are in general validated only for a specific region or country, but a systematic comparison of different markers on a global scale has so far not been undertaken.
Methods: In the context of a recently established European network on the harmonisation of diagnostic and clinical management of the leishmaniases, called **LeishMan** (leishman.tropnet.eu), we analysed a total of 75 *Leishmania* isolates from 30 countries and belonging to 17 species. From all these, the sequences of the following genetic markers were determined and analysed: the heat-shock protein 70 gene (*hsp*70); the internal transcribed spacer 1 of the ribosomal DNA array (ITS1); the spliced leader 7SL; and the mini-exon (ME). In addition, the sequences of 7 genes were concatenated for multilocus sequence typing (MLST). Clusters of parasite isolates were established on the basis of pairwise sequence comparisons, and the obtained groups were compared amongst all markers. Finally, the established groups were evaluated against species typing on the basis of multilocus enzyme electrophoresis (MLEE).
Results: Results from the different genetic markers were remarkably congruent, as almost no contradictory results were obtained. There was, however, a difference in the resolution of the groups: some markers discriminated down to the species complex level, while others permitted a finer distinction down to the exact species level. As for the comparison with MLEE, there was a near-to-perfect agreement with all genetic markers. Previously identified MLEE species such as *L. archibaldi* and *L. chagasi* were not evidenced in our genetic analysis, while on the other hand we identified a distinct lineage of the *L. braziliensis* species complex.
Conclusion: The fact that different markers result in congruent species typing is encouraging, and confirms the basically clonal reproduction mode of *Leishmania*. Also, because of their agreement molecular typing proved a valid alternative to the cumbersome MLEE technique, by many still considered the gold standard in *Leishmania* species typing. Given its high resolution and general applicability on clinical samples, *hsp*70 sequences could be used for routine species typing worldwide.