**FP5: The *Leishmania* genotyping project.**

**Co-ordinator: M.Miles, LSHTM**

The broad aim of the project was devise ***"diagnostic and epidemiological markers for tracking of endemic and resurgent European leishmaniasis"***.

***Objectives*** of the project:
1. Amplification and sequencing of selected *Leishmania* DNA targets that might provide epidemiological tools.
2. Construction of comparative phylogenies based on the sequence data.
3. Identification of new genotypic markers.
4. Evaluation of the specificities and sensitivities of the markers with different assay procedures and on clinical and veterinary samples.
5. Pilot application of the genotypic markers to epidemiological questions, such as: the extent of genetic isolation of *Leishmania* populations in European endemic foci.
6. Construction of a database and website to carry outputs of the project.

**The Partners:**
London School of Hygiene and Tropical Medicine, London, UK.
Leopold Instituut voor Tropische, Antwerp, Belgium.
Institut für Mikrobiologie und Hygiene, Charité Campus Mitte, Berlin, Germany.
Institute of Parasitology, Czech Academy of Sciences, Ceske Budejovice, Czech Republic.
Instituto de Higiene e Medicina Tropical, Lisboa, Portugal.
Instituto de Salud Carlos III, Ctra. Madrid, Spain.
Universite Montpellier, Monpellier, France.
Hellenic Pasteur Institute, Athens, Greece.

The project was divided into **10 workpackages** and four principal phases, which overlapped.
In the **preparatory phase** panels of *Leishmania* reference strains and isolates were assembled, grown in bulk, DNA extracted and distributed to relevant partners.
The **second phase** focused firstly, on the sequencing of the DNA targets, including housekeeping genes encoding enzymes used in multilocus enzyme electrophoresis (MLEE), a few antigen coding genes, intergenic regions, and markers detected through random amplification of polymorphic DNA (RAPD), and secondly, on the development of microsatellite methods. The sequence data from the enzyme genes were used to examine the genetic basis of isoenzyme mobility differences seen by MLEE, and sequences from all targets were incorporated into phylogenetics analysis.
In the **third phase** the most promising genotypic markers were assessed as epidemiological tools, and procedures for using the markers were compared.
In the **fourth phase** pilot epidemiological studies were conducted in Spain, Portugal and Greece.

Here is a summary of some of some of the **outputs** of the project:
1. Amplification and sequencing of 10 single copy housekeeping genes, encoding enzymes used in MLEE, for a panel of European and some non-European *Leishmania donovani* complex isolates. This has revealed genetic polymorphisms that are not detected by MLEE, explained many mobility differences on MLEE, provided characteristics that split the

zymodeme MON1, which predominates in Europe, and provided the basis for high resolution alternatives to MLEE.
2. Provision of a panel of microsatellite markers for the *L. donovani* complex, including a panel that splits the zymodeme MON1, for high resolution epidemiological analysis. Microsatellite markers have been applied to more than 200 isolates.
3. Enhanced understanding of the molecular epidemiology of leishmaniasis in Spain, including the Balearic Islands, in Portugal and in Greece, encompassing canine leishmaniasis, and HIV-associated leishmaniasis.
4. Multifactorial robust phylogenetic analysis of the *L. donovani* complex.
5. Training of several PhD students.

**Finally:**
The Leishgenotyping project demonstrated a " generous spirit of true and open collaboration and synergy between partners, without confrontation and without competition."
"Strengthens the view that such international networks can have a genuine impact on scientific progress and the improvement of public health and, through their strong consensus, can have a realistic influence on policy development, both within and beyond Europe."
Generated the optimism to apply for FP6 funding.

**Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy**

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