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Workshop**: Improving treatment of Leishmaniasis based on species differentiation**. 29th and 30th of January 2010

This workshop was initiated within TropNet Europe and organized by Dr. Johannes Blum (MD) and Dr Ingrid Felger of the Swiss Tropical Institute in Basel. The main purpose of the workshop was to form a platform of experts to share knowledge and expertise on treatment and diagnosis of leishmaniasis as for now this is scattered over different centres. During this kick-off meeting 15 medical doctors and diagnostic experts from 6 European countries exchanged views on species differentiation, treatment and monitoring the effectiveness of treatment.

In many European centres, imported cases of leishmaniasis have become more frequent over the past years, due to increased travel to risk areas. Standardized species identification and treatment protocol are warranted to provide the patients with the best possible treatment.

The broad availability of PCR allows a rapid determination of species. Each species has a different sensibility to the different antileishmanial drugs. Therefore a species specific treatment approach has been evaluated for many species and is widely applied in many centres. The importance of species differentiation for guiding adequate treatment, in particularly for New World leishmaniasis, has been documented recently (Schwartz *et al.*, 2006) (Victoir *et al.*, 2003) (Blum *et al.*, 2004). As the Leishmania species influences the outcome of systemic first line treatment with pentavalent antimonials, parasite species identification is of high clinical relevance. In particular in the setting of travel clinics, where the origin of an infection often cannot be assigned to a specific location, and more sophisticated species identification than just the geographical one is required.

A number of molecular typing techniques are available, many of them targeting different marker genes eliminating the need to establish in vitro cultures. The common principle of typing Leishmania species is PCR-amplification of a polymorphic gene such as rDNA ITS, hsp 70, kDNA, mini exon, gp63 and others. Many assays include restriction digests resulting in a species-specific pattern of bands.

Molecular diagnosis is straight forward for most of the species; however, accurate differentiation is more difficult in closely related species such as the New World species belonging to the *L. braziliensis* complex. Interlaboratory comparisons have shown that genotyping results may vary for different marker genes (Marfurt *et al.*, 2003).

During the meeting, it was agreed that sequencing data are the most reliable in species determination. It is not clear however which target or combination of targets should be used to obtain the most accurate results. Each of the participants will perform DNA sequencing of a target on a panel of 96 samples from Montpellier to access the discriminatory value of the different targets.

Furthermore, non-species typing should be evaluated in elucidating the differences in for example miltefosine treatment efficacy of *L. braziliensis* cutaneous disease that are observed in different endemic regions and for example in the therapeutic differences that are found in two separate genotypes of *L. major*.

A new prospective study proposal was discussed, such a study should include species-typing and strain-typing to reveal the correlation of species and strains on success and failure of treatment in cutaneous leishmaniasis. A standardized list of clinical criteria will be made in the coming months to describe the lesions before and after treatment.

In conclusion this workshop made a first step in forming a diagnostic and clinical platform that will offer the possibilities to harmonize and improve the diagnosis and treatment of leishmaniasis in European centres. Hopefully the group will meet again in Paris in the end of September do discuss the outcome of the sequencing data and the list of clinical criteria to describe lesions before and after treatment.

Reference List

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4. Victoir,K, S De Doncker, L Cabrera, E Alvarez, J Arevalo, CA Llanos, D Le Ray & JC Dujardin. 2003 Direct identification of Leishmania species in biopsies from patients with American tegumentary leishmaniasis. Transactions of the Royal Society of Tropical Medicine and Hygiene **97**: 80-87.