Endorsement

Title of thesis:

Diagnosis of Visceral Leishmaniasis Using the Polymerase Chain Reaction and Restriction Fragment Length Polymorphic analysis of the Kinetoplast DNA Minicircles

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ABSTRACT

In the Old World, Visceral Leishmaniasis (VL) is caused by the *Leishmania donovani* (*L. donovani*) complex. In the Middle East, VL is mostly caused by the sub-species *Leishmania donovani infantum* (*L. infantum*) with the host reservoir being dogs and wild canids. A kinetoplast DNA based polymerase chain reaction (PCR) technique was employed for the diagnosis of *Leishmania* parasites, utilizing the Uni21/Lmj4 primer pair, originally designed to amplify the full *Leishmania major* (*L. major*) minicircle DNA. This primer pair amplifies the minicircles of all the Old World *Leishmania* species, including *Leishmania tropica* (*L. tropica*) and *Leishmania aethiopica* (*L. aethiopica*) as well as the *L. donovani* complex. The minicircles of the different species have similar sizes, ranging between 650-900 bp. An investigation utilizing the restriction fragment length polymorphic analysis (RFLP) was carried out to known minicircle kDNA sequences using a group of different restriction endonucleases (RsaI, MboI, BanII, HpaII) which cut the DNA PCR products of *L. infantum*, and *L.donovani* isolates. In this project discrimination between local and distant regional isolates based on different restriction profiles was achieved successfully. The same group of endonucleases gave more complicated patterns showing variation even between the isolates from the same region, when amplified by a high fidelity DNA polymerase. A field survey was conducted in the village of El -Yamun located in a visceral leishmaniasis focus in the Jenin district of the West Bank. Fifty-nine blood samples were collected from domestic dogs, during a time interval of one-month including two samples from Ramallah region. All samples were screened for anti -leishmanial antibodies by the

enzyme - linked immunosorbent assay (ELISA). Five out of 59 (8.5%) were seropositive, and one borderline. All the seropositive and borderline dogs were biopsied. Parasites were successfully cultured from three dogs out of four seropositive dogs biopsied and both RFLP of kDNA and the excretion factor serology characterized the strains as *L. infantum*. The PCR method utilized in this study will be of great value if optimized to work directly on clinical samples of any kind this will provide fast and reproducible diagnostic tool.