ANALYSIS OF THE ANTIGENS OF *LEISHMANIA DONOVANI* AND AN ASSESSMENT OF THEIR POTENTIAL USE IN IMMUNODIAGNOSIS

BY

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DECLARATION

This dissertation is my original work and has not been presented for a degree in any other University.

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This dissertation has been submitted for examination with our approval as University Supervisors.

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SUMMARY

In any attempt to improve sensitivity and efficacy of serodiagnostic tests in visceral leishmaniasis, it has become increasingly important to study the antigenic reportoire of Leishmania donovani, the causative organism.

Three strains of L. donovani promastigotes were cultured in RPMI 1640, supplemented with foetal calf serum and antibiotics, at 26°C. Late log phase/early stationary phase promastigotes were harvested and the parasites disrupted by freeze - thawing. Crude and soluble antigens were made, which were stored, in aliquots, at -70°C.

The antigens were analysed by SDS - PAGE separation and immunoblots were carried out on sera from thirty kala-azar patients admitted to the Clinical Research Centre (Kenya Medical Research Institute, Nairobi), before treatment up to 12 months after treatment, so as to identify the particular antigens involved in immunity and could be of use in immunodiagnosis.

There was no difference in the antigenic patterns and antigenicity between the three strains of L. donovani studied. Individuals cured of kala-azar did not develop antibodies that differed in specificity to those undergoing treatment.

Patterns of antigen reactivity were compared by using sera from individuals with malaria, tuberculosis, schistomiasis and hydatidosis infections. Sera from individuals with these infections did not recognise L. donovani antigens.

Trypanosomiasis sera recognised the 22-30 kD, 40 kD, 49 kD, 56 kD, 60 kD, 63 kD, 65 kD, 66 kD, 82 kD and 110 kD.

L. donovani antigens of 66-74 kD region were recognised by all individuals with kala-azar. This region was found to contain at least three distinct bands. These bands were eluted from SDS-PAGE gels and used to diagnose L. donovani infection by ELISA (Enzyme-linked immunosorbent assay). Whereas the crude gave false positive results with other infection sera, the eluted 66-74 kD protein was 100% specific and sensitive in the diagnosis of visceral leishmaniasis.