

SUMMARY OF THESIS*

RODRIGUES, Eduardo Henrique Gomes – Validação de abordagens moleculares para o diagnóstico da leishmaniose tegumentar americana em Pernambuco. Recife, 2000. (Dissertação de Mestrado – Departamento de Saúde Coletiva/Centro de Pesquisas Aggeu Magalhães/Fundação Oswaldo Cruz).

VALIDATION OF MOLECULAR APPROACHES FOR THE DIAGNOSIS OF AMERICAN CUTANEOUS LEISHMANIASIS IN PERNAMBUCO STATE

The control of leishmaniasis depends on the availability of accurate and sensitive methods for the detection of *Leishmania* species. In the present study, two molecular approaches were evaluated for diagnosing American cutaneous leishmaniasis (ACL) in patients from endemic areas of Pernambuco State. One of the approaches is genus specific whereas the other is specific for the *Viannia* subgenus. The DNA detection limits were determined and the sensitivity and specificity calculated, for comparison with conventional diagnostic tests. The DNA detection limit of the PCR specific for the *Viannia* subgenus was 10 fg of total DNA, whereas this was 1 pg for the genus specific PCR. Diagnosis of ACL was performed on the basis of clinical-epidemiological criteria associated to positive reaction in at least one of the following tests: direct microscopy examination, histopathological examination and culture. Based upon these criteria, from the 98 patients initially diagnosed as ACL exclusively on clinical-epidemiological basis, 87 were considered ACL cases, and used for the sensitivity calculation. Thirty one patients with cutaneous lesion caused by other agents were included as controls for the calculation of specificity. The genus specific PCR was positive in 60/67 biopsies

from LTA, resulting in a sensitivity of 89.6%, whereas the PCR specific for the *Viannia* subgenus was positive in 82/87 cases of LTA, resulting in a sensitivity of 94.3%. However, these differences were not significant. Taken together with other observations, these results suggest that the *Viannia* subgenus is the only causative agent of ACL in Pernambuco State. Both PCRs showed 100% specificity. The diagnosis by direct observation of the parasite, histopathological examination, culture and indirect immunofluorescence was positive in 68.2%, 89.9%, 44.0% and 81.3% of the LTA cases, respectively. Since working with non radioactive material is clearly more convenient in endemic underdeveloped regions, a few preliminary experiments were carried out with PCR/hybridization with cold probes. In conclusion, the evaluated molecular methods showed high sensitivity and specificity, one of them being able to detect the subgenus *Viannia*. The simplification of sample collection and processing, and in-house preparation of reagents can transform the PCR into a low-cost technology appropriate for use in situations of limited resources, providing epidemiological information concerning the parasite identification that are relevant for the planning of control strategies.

*This thesis is available at the Library of the Instituto de Medicina Tropical de São Paulo