THE RELEVANCE OF CHARACTERIZING LEISHMANIA FROM CUTANEOUS LESIONS. A SIMPLE APPROACH FOR ISOLATION

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We report a simple method for obtaining material which proved successful in isolating parasites in the majority of cases investigated without contamination. Patients received 2% xylocaine, infiltrated in the area of the lesion as a local anesthetic. Half a milliliter of sterile saline was injected directly into the lesions with a 21 G needle, using a 10ml syringe. The needle was removed with gentle rotary movements while aspirating the blood-stained saline. The material was then placed in NNN agar slants of the following formulation (Bacto agar 3.5g; sodium chloride 1.5g; Tryptose peptone 1.25g; distilled water 225ml, plus 25% defibrinated rabbit blood) overlaid with 1ml of modified LIIT medium (sodium chloride 4.0g; 10X concentrated RPMI 1640 liquid culture medium and 10X concentrated medium 199, 10ml of each; penicillin 100 IU/ml; streptomycin 100 μg/ml and distilled water to 1,000 ml). Cultures were kept at 25°C and examined then twice weekly. Negative cultures for 4 weeks after seeding, were transferred to new NNN slants and observed for a period of two months. In some patients a biopsy of the lesion border was also taken and the material was macerated and cultured in the same medium.

Cultures from aspirated material were performed in 54 patients with leishmaniasis and were positive in 33 of them (61.1%). Positivity was much higher in the group with cutaneous lesions (CL; 25 positives out of 33 patients or 75.7%) than in patients with mucocutaneous lesions (MCL; 8 positives out of 18 cases or 44%). Isolates were characterized by a panel of monoclonal antibodies (courtesy of Dr. G. Grimaldi, FIOCRUZ, Rio de Janeiro) being 13 of the L. mexicana complex (11 from CL cases and 2 from MCL patients; 20 of the L. braziliensis complex (14 CL, 6 MCL). In 21 cases it was not possible to isolate parasites and the diagnosis of leishmaniasis was made by Monte- Negro reaction and/or immunofluorescence assay to Leishmania antigen (17 cases) or by histopathological examination (4 cases).

In 24 patients a comparison between aspirated and macerated lesion material was made. In these cases aspirated material gave 15 positive cultures (62%) without bacterial or fungal contamination. Cultures from macerated material were positive on 12 occasions (50%), but 3 exhibited bacterial contamination reducing the percentage of useful cultures to 37.5% (9/24 cases).

The high positivity and low contamination rate obtained with the technique described here should prove to be an important instrument in isolating Leishmania stocks from cutaneous lesions.

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