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Research Note

DELAYED HYPERSENSITIVITY SKIN-TEST USING LEISHVACIN® FOR EPIDEMIOLOGICAL SURVEY OF CANINE CUTANEOUS LEISHMANIASIS IN A RURAL AREA OF MINAS GERAIS STATE, BRAZIL

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Since the initial report of J. Montenegro (1926, *An. Fac. Med. São Paulo, 1:* 323-330), the delayed hypersensitivity skin-test (DHST) employing extract of *Leishmania* has been successfully used for distinguishing among American cutaneous leishmaniasis (ACL) and other human dermatologic pathologies. Unfortunately, Montenegro antigen standardized by M. N. Melo et al. (1977, *Rev. Inst. Med. trop. São Paulo, 19:* 161-164) has given inconsistent results as a method for diagnosis of canine infections by *Leishmania (Viannia) braziliensis* (C. Pirmez et al., 1988, *Am. J. Trop. Med. Hyg., 38:* 41-47).

Most recently, two other different antigenic preparations have encouraged the use of DHST as a diagnostic tool for canine cutaneous leishmaniasis (CCL). The first was P10,000G, a sub-cellular fraction of *L. braziliensis* promastigotes (E. G. O. Barbosa-Santos et al., 1986, *Mem. Inst. Oswaldo Cruz, 81:* 146), which could allow the diagnosis of CCL in field trials with success (M. C. A. Marzochi & E. G. O. Barbosa-Santos et al., 1988, *Mem. Inst. Oswaldo Cruz, 83:* 391-392). The second was Leishvacin® (BioBRÁS, Brazil), a suspension of killed promastigotes primarily employed as a human anti-leishmaniasis vaccine (W. Mayrink et al., 1979, *Trans. R. Soc. Trop. Med. Hyg., 73:* 385-387), used as a DHST for CCL diagnosis under experimental conditions (O. Genaro et al., 1992, *Mem. Inst. Oswaldo Cruz, 87:* 163-164).

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This preliminary study was conducted aiming to evaluate the efficacy of DHST using Leishvacin® for CCL diagnosis under field conditions.

Eighty-eight dogs living in an endemic area for ACL in Virginópolis, Minas Gerais State, Brazil, were studied. In this area, no cases of American tripanosomiasis or visceral leishmaniasis have been reported in the recent years. Leishvacin® was prepared as previously described (W. Mayrink et al., *loc. cit.*), employing killed promastigotes of five stocks of *Leishmania*. The intradermal injection was done with 200 µl protein/dog at the inner side of the left thigh of the animals as standardized by E. G. O. Barbosa-Santos et al. (*loc. cit.*). Seventy-two hours later, the two greater diameters of cutaneous induration were measured. This is the maximal time of cellular infiltration at the site of Leishvacin® injection in dogs affected by the disease (O. Genaro et al., *loc. cit.*). A biopsy was done at each of these inoculation sites, fixed in 10% formalin buffered to pH 7.2, and embedded in paraffin wax. Sections were cut at 5 µm, and stained with haematoxylin and eosin.

Twenty-seven (30.7%) of the dogs studied presented positive cutaneous reactions to Leishvacin® (mean diameter of induration ≥ 5 mm). This prevalence of positive DHST, as in humans (M. C. A. Marzochi et al., 1980, *Rev. Inst. Med. trop. São Paulo, 22:* 149-155), contrasts with the clinical and parasitological diagnosis, which showed a lower rate of canine infection: only five animals were infected (5.7%). The histological picture of biopsied

sites showed a typical delayed hypersensitivity reaction as observed by O. Genaro et al. (*loc. cit.*). No local complications because of Leishvacin® injection were observed.

In the present study, the proportion of dogs showing positive DHST was about fivefold that of confirmed cases of CCL. Therefore, DHST employing either Leishvacin® or P10,000G (E. G. O. Barbosa-Santos et al., 1986, *Mem. Inst. Oswaldo Cruz, 82:* 164; M. C. A. Marzochi & E. G. O. Barbosa-Santos, *loc. cit.;* M. C. A. Marzochi, 1992, *J. Bras. Med., 63:* 82-104), revealed a prevalence of CCL higher than was clinically evident, perhaps due to abortive and/or subclinical infections. However, 2/5 of our confirmed cases of CCL showed a negative DHST. This finding contrasts with the experimental observations of O. Genaro et al. (*loc. cit.*) which observed high sensitivity and specificity for DHST using Leishvacin® in dogs experimentally infected with *L. braziliensis* and normal animals from non-endemic areas. It is not probable that such difference may be due to recent *Leishmania* infections, which, in humans, result in negative DHST despite the presence of the parasite (T. Furtado, 1980, *An. Bras. Dermatol., 65:* 81-86). Thus, the occurrence of false-positive responses in the remaining dogs should not be discarded. In view of these results, the use of DHST in studies of the cellular immune processes in natural canine infection by *L. braziliensis* and in its clinical follow-up needs to be evaluated in larger populations.

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p. 303 – Fig. 5

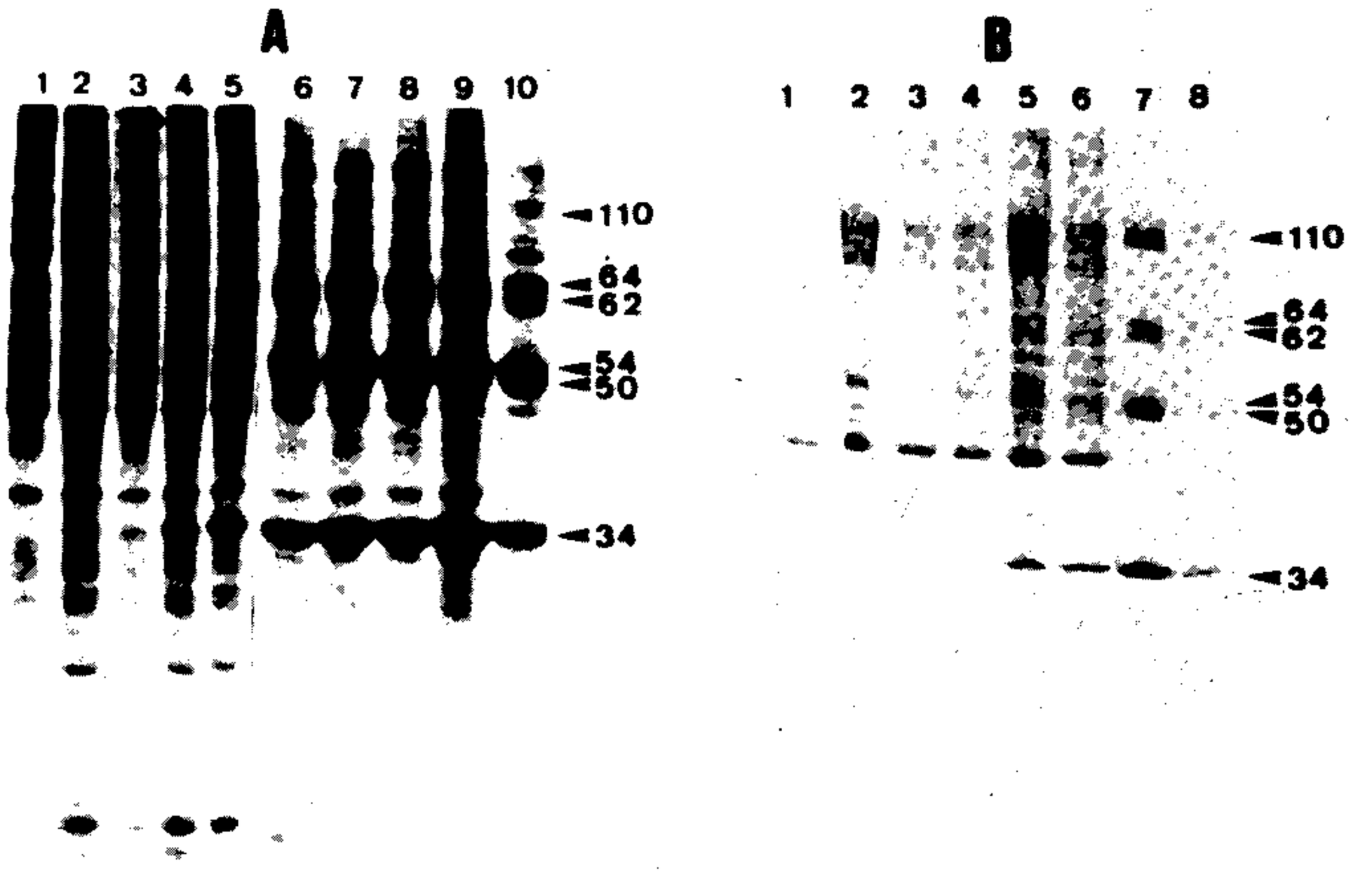

Fig. 5: time course of Mayaro virus protein synthesis. The cells were pulse-labelled for 1 hr with 30 uCi/ml of 35S-methionine at different times. The cells were lysed with sample buffer, SDS-polyacrylamide electrophoresis was performed, and dried gels were autorradiographed. A – *Aedes albopictus* cells: lane 1, mock infected cells; lane 2 to 10, respectively 1, 3, 5, 8, 12, 14, 30, 45 and 50 hr post-infection. B – BHK-21 cells; lane 1, mock infected cells; lane 2 to 8, respectively, 1, 3, 6, 8, 10, 25 and 46 hr post-infection.