

MONTENEGRO SKIN TESTS IN DOGS EXPERIMENTALLY INFECTED WITH *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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Human cutaneous leishmaniasis due to *Leishmania (Viannia) braziliensis* is distributed throughout much of tropical America. Within this vast area, the parasite has been isolated from several species of wild mammals (see review by R. Lainson & J. J. Shaw, 1987, *The Leishmaniasis in Biology and Medicine*, Vol. I: 1-120, Academic Press). In addition, in several countries, especially Brazil (M. Dias et al., 1977, *Rev. Inst. Med. Trop. São Paulo*, 19: 403-10; W. Mayrink et al., 1981, *Trans. R. Soc. Trop. Med. Hyg.*, 75: 757-8; S. Coutinho et al., 1985, *Mem. Inst. Oswaldo Cruz*, 80: 17-22), the high incidence of canine infections suggests that domestic dogs could be reservoirs or link hosts for the human disease.

Diagnosis of *L. braziliensis* in dogs is normally based on the clinical appearance of skin lesions, the presence of amastigotes in impression smears prepared from such lesions, isolation of strains in hamsters inoculated with triturated fragments of lesions and/or the use of indirect immunofluorescent tests to assess humoral responses. The delayed hypersensitivity skin test devised by J. Montenegro (1926, *Arch. Dermat. & Syph.*, 13: 187) has proved a useful diagnostic tool for distinguishing between American Cutaneous Leishmaniasis and other pathological skin conditions in humans. However, in dogs, the use of Montenegro antigen to diagnose *L. braziliensis* infection has given inconsistent results (C.

Pirmez, 1988, *Amer. J. Trop. Med. Hyg.*, 38: 52-58; M. Marzochi & E. Barbosa-Santos, 1988, *Mem. Inst. Oswaldo Cruz*, 83: 391-92). It is clear that on the cellular immunological processes of dogs infected with *L. braziliensis* require further critical study. The present communication records preliminary results.

Observations were made on 16 four-month-old puppies that had previously been treated with anthelmintics and immunized against distemper, parvovirus, parainfluenza, hepatitis and leptospirosis. Six animals were used as uninfected controls. The other 10 were inoculated intradermally in the inner surface of the right ear with 1×10^5 promastigotes belonging to stock MCAN/BR/73/C348 of *Leishmania braziliensis*. The flagellates were grown in the NNN/LIT culture medium described by C. da Costa et al. (1983, *Rev. Farm. Bioq.*, 5: 7-12) and harvested in the stationary phase. Within four months of infection, all experimental dogs developed ulcerated lesions at the site of inoculation. Amastigotes were detected in impression smears prepared from all of the lesions.

Skin tests were carried out using an antigen similar to the human antileishmaniasis vaccine described by W. Mayrink et al. (1979, *Trans. R. Soc. Trop. Med. Hyg.*, 73: 385-387). The antigen was prepared from killed promastigotes taken from five stocks of *Leishmania*: MHOM/BR/60/BH6; MHOM/BR/71/BH49; MHOM/BR/73/BH121; IFLA/BR/67/PH8; MHOM/BR/70/M1176. None of the stocks used in the preparation of the antigen "cocktail" had been isolated from dogs. One of the stocks (MHOM/BR/70/M1176) belongs to the subgenus *Viannia*, but it can be placed

This work was partially supported by CNPq, FAPEMIG, FINEP, UFMG and UFOP.

*Research Fellows CNPq.

Received 29 August 1991.

Accepted 9 October 1991.

in the *L. guyanensis* complex of J. Rioux et al. (1990, *Ann. Paras. Hum. Comp.*, 65: 111-125). Four months after the experimental animals had been infected, all dogs (including controls) were intradermally injected with 0.1 ml antigen containing 200 µg protein. Seventy-two hours later, all the infected dogs had positive reactions to the antigen, with indurated areas varying in size from 0.7 to 24 mm². None of the controls reacted to the antigen.

The kinetics of the responses to the antigen were studied in three infected and three control dogs. In these animals, the antigen was given intradermally in six different places on the ventral surface of the abdomen. A biopsy was taken from each of these inoculation sites, the first after 6 h, and the remaining five after 12, 24, 48, 72 and 96 h respectively. The biopsied material was 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.

In infected dogs, an inflammatory process was histologically apparent within 6 h of infection with the antigen. The inflammatory process progressively increased in intensity, reaching its maximum in biopsies taken 72 h after administration of the antigen, and then

diminishing once more in 96 h biopsies. The histopathology of the inflammatory sequence in infected dogs, and the virtual absence of such a process in biopsies taken from controls, parallels observations by W. Mayrink et al. (1989, *Rev. Inst. Med. trop. São Paulo*, 31: 256-261) on human subjects tested with a Montenegro antigen containing 25 µg protein per 0.1 ml (M. Melo et al., 1977, *Rev. Inst. Med. trop. São Paulo*, 19: 161-164). The antigen used for human skin testing is unreliable in diagnosis of *L. braziliensis* infection in dogs (C. Pirmez et al. *loc. cit.*). An improved diagnostic method for canine cutaneous leishmaniasis was developed by M. Marzochi & E. Barbosa-Santos (*loc. cit.*) using a P10.000G subcellular fraction obtained from *L. braziliensis* promastigotes and tested at a concentration of 200 µg of protein per 0.1 ml. The antigen used in our study, however, commends itself for a number of reasons: it is simple to prepare; it has 8x greater than that of the Montenegro antigen used for skin tests on human subjects; it gives responses that can be read after 72 h, and it presents histological congruence with inflammatory processes in humans. This suggests that we have obtained not only a useful tool for the diagnosis of *L. braziliensis* infection in dogs but also a means for evaluating the epidemiological significance of such canine infections.