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LETTER TO THE EDITOR

VACCINATION OF DOGS AGAINST LEISHMANIA (VIANNIA) BRAZILIENSIS

Canine cutaneous leishmaniasis was first recorded in Brazil in the second decade of this century[3,17] from the State of São Paulo. Epidemiological studies in the Rio Doce Valley of the State of Minas Gerais[7,8] have shown that about 3 per cent of the dog population have active leishmanial lesions. In this focus of human American cutaneous leishmaniasis, both dogs and man are probably accidental hosts of **Leishmania (Viannia) braziliensis**. However, in a rural area in the municipality of Viana, State of Espírito Santo, 25 per cent of the dogs were found to be infected with **L. braziliensis**[9] and a close correlation between prevalence and distribution of human and canine infections was established[10]. A similar high level of canine infections has been reported from the municipality of Nova Iguaçu, State of Rio de Janeiro[1], where 20-25 per cent of the dog population are infected with this parasite. The prevalence of canine cutaneous leishmaniasis in the Viana and Nova Iguaçu foci is much higher than the 10 per cent infection rate amongst domestic dogs living on the western slopes of the Peruviana Andes[12], where the infective agent is **L. (V.) peruviana**. It is usually considered that canine infections constitute the major source of human *uta peruana*. The development of a prophyllactic vaccine against canine cutaneous leishmaniasis would be of value, at least in certain areas, in controlling human cutaneous and muco-cutaneous leishmaniasis. The vaccine developed for use in man[14], with or without **Corynebacterium parvum** as adjuvant, proved to be inadequate[15]. After receiving three weekly doses of vaccine, experimental dogs showed increases in IgG titres but developed skin lesions when challenged with promastigotes of **L. braziliensis**. The immunotherapeutic methods developed in Venezuela for the treatment of leprosy[5] and later modified to treat cases of human American cutaneous leishmaniasis[6] suggested us to use it as a means for protecting dogs against **L. braziliensis**. Various other vaccination schemes (Route, dose, interval between doses, booster, addition of adjuvants) were tested and these are the first results that suggest the possibility of success. The preliminary results obtained in the use of this new vaccine are recorded herein. Eight 4-month-old mongrel puppies were used in the experiments. Before vaccination, the puppies were treated with anthelminthics, vaccinated against parvovirus, leptospirosis and distemper, and tested with Montenegro antigen containing 200 μg/0,1 ml of protein nitrogen[2,11,13]. All animals had negative Montenegro reactions before being vaccinated. The vaccine was prepared from promastigotes of two WHO reference stocks of **Leishmania**: MHOM/BR/75/M2903 (**L. braziliensis**) and IFLA/BR/67/PH8 (**L. amazonensis**). The organisms were grown in LIT medium[4] and harvested in the stationary phase. Harvested promastigotes were autoclaved for 15 minutes at 120 lb/in^2. Autoclaved promastigotes were suspended in buffered saline to give a concentration of 600 μg of nitrogen/ml. The eight puppies were divided into three experimental groups: I) Four animals received a single intradermic injection of the vaccine, containing 600 μg of nitrogen/ml of autoclaved promastigotes (300 μg of nitrogen of each stock) plus 500 μg of viable, lyophilized BCG; II) Two animals were given a single intradermal injection of 500 UG of BCG; III) Two animals served as unvaccinated controls, and were given a single intradermic injection of buffered saline. Thirty days later, the animals were again tested with Montenegro antigen, and the results were read after 72 hours[15]. 2/4 in group I, 1/2 in group II, and 2/2 in group III had developed positive reactions to the antigen. These results suggest that it is necessary to use other methods to assess cellular hypersensitivity in dogs. In our observations (in press) on the use of Montenegro tests as indicators of cells producing delayed hypersensitivity in vaccinated individuals, histological studies showed that a single dose of Montenegro antigen induces sensibility, without inducing nodule formation in persons receiving placebos. Immedia-

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tely after reading responses to Montenegro antigen, the animals were challenged with 1×10^5 LIT cultured promastigotes of strain MCAN/BR/82/BH348 (**L. braziliensis** isolated from a dog living in the Rio Doce Valley), administered intradermally on the inner surface of the ear. Two months after challenge, four of the dogs developed leishmanial lesions at the sites of inoculation. These included both animals in group III, one of group II and one in group I. The dog belonging to group I that developed a lesion had given a positive Montenegro reaction before being challenged. The dogs were kept under observation for a further eight months and none of the remaining four developed lesions during this period. The negative animals included the two dogs belonging to Group I with an unchanged (negative) response to Montenegro antigen after immunization with the **Leishmania**/BCG vaccine and a dog of Group II that became Montenegro positive after receiving only BCG. Although no conclusions can be drawn from these preliminary observations, the results obtained justify further investigations on the utility of the **Leishmania**/BCG vaccine as a means of protecting dogs against infection by **L. braziliensis**. The results also suggest the need to develop a more sensitive Montenegro antigen to skin test dogs.

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