

RESEARCH NOTE

Phase I and II Open Clinical Trials of a Vaccine Against *Leishmania chagasi* Infections in Dogs

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Visceral leishmaniasis occurs in tropical and subtropical parts of the world and is most commonly found in rural areas. In the Americas, more than 90% of the cases have been recorded in Brazil. The disease can be controlled by treatment of all human cases, elimination of infected dogs and application of insecticide to the walls of dwellings and peridomestic buildings (PA Magalhães et al. 1980 *Rev Inst Med Trop São Paulo* 22: 197-202). After applying these measures, constant vigilance must be exercised. Control measures must be applied again as soon as there is evidence of the reactivation of the transmission cycle.

As alternative control measures some authors have emphasized the importance of immunopro-

phylaxis for canine visceral leishmaniasis (CVL) (MCA Marzochi et al. 1985 *Mem Inst Oswaldo Cruz* 80: 349-357, L Monjour et al. 1985 *CR Acad Sc Paris* 301: 803-806). Observations in Europe, however, have produced contradictory results. The vaccine used by Monjour (*loc. cit.*) and D Frommel et al. (1988 *Infect Immun* 56: 843) protected mice against *Leishmania mexicana* and *L. major*, and was found to stimulate the production of neutralizing antibodies when given to dogs (S Dunan et al. 1989 *Parasite Immunol* 11: 397-402). A similar vaccine incorporating *L. infantum* (semi-purified and lyophilized) was used in a pilot study of domestic dogs in an endemic area of CVL (BV Ogunkolade 1988 *Vet Parasitol* 28: 33-41). Surprisingly, vaccinated dogs were found to be more susceptible to infection than the controls. In Brazil, W Mayrink et al. (1990 *Rev Inst Med Trop São Paulo* 32: 67-69) found that dogs can be partially protected against cutaneous leishmaniasis by a vaccine prepared from a single stock of *L. braziliensis*.

Presently, this line of study has been developed to explore protection of dogs against infection with *L. chagasi*. In order to evaluate the safety (phase I) and immunogenicity/efficacy (phase II) of this vaccine against CVL, we carried out experiments in dogs with experimental challenge of promastigotes of *L. chagasi* (strain MHOM/BR/72/BH46) after immunization.

Thirty one 4 month-old laboratory-reared mongrel dogs of both sexes were immunized against parvovirus, leptospirosis, distemper, parainfluenza and hepatitis and treated with mebendazol for intestinal helminthic infections.

The *Leishmania* vaccine was composed of methylolated sound-disrupted promastigotes of *L. braziliensis*, strain MCAN/BR/72/C348 (Mayrink *loc. cit.*). The promastigotes were cultured in NNN/LIT media (EP Camargo 1964 *Rev Inst Med Trop São Paulo* 6: 43-100). The flagelates were submitted to ultra-sound during 1 min at 40 watts, in an ice bath. The process was repeated three times, at 1 min intervals. Total nitrogen content was then determined and the extracts were diluted in saline mixed with thimerosal (1:10,000), adjusting the final concentration to 240µg of total N/ml. Bacillus Calmete Guérin (BCG - Fundação Ataufo de Paiva, Rio de Janeiro) was added as an adjuvant.

The phase I trial was carried out on 12 non-immune dogs, and the aim was to evaluate the kinetics of the inflammatory skin reaction to the vaccine and BCG and to determine localized and systemic side effects. Four groups composed of three dogs each were used. In Group I dogs received an injection of vaccine containing 600µg protein mixed with 400µg of BCG. Animals in groups II,

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TABLE
Parasitological and immunological observations on 19 dogs challenged with promastigotes of *Leishmania chagasi* after immunization with a vaccine against visceral leishmaniasis

Group	Dog	Parasite isolation ^a	Stimulation index of lymphocyte proliferation assay ^b	Reciprocal of IFAT titres ^c	Conclusion (infection)
Vaccinated	1	Negative	14.3	Negative	No
	2	Negative	13.0	Negative	No
	3	Negative	5.6	Negative	No
	4	Negative	3.3	Negative	No
	5	Negative	5.6	Negative	No
	6	Negative	3.3	Negative	No
	7	Negative	6.6	Negative	No
	8	Positive	1.9	1:160	Yes
	9	Negative	3.9	Negative	No
Control	10	Negative	4.7	Negative	No
	11	Negative	1.0	1:360	Yes
	12	Negative	1.7	1:640	Yes
	13	Negative	2.1	1:360	Yes
	14	Positive	2.1	1:1280	Yes
	15	Positive	1.7	1:160	Yes
	16	Positive	1.3	1:2560	Yes
	17	Negative	0.8	1:640	Yes
	18	Positive	0.7	1:1280	Yes
	19	Positive	0.8	1:1280	Yes

a: final results obtained on the day of the animals' necropsies; b: results obtained after the third dose of the vaccine; c: IFAT= immunofluorescent antibody test.