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RESEARCH NOTE

EVALUATION OF A SKIN TEST ON THE CANINE MUCOCUTANEOUS LEISHMANIASIS DIAGNOSIS

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In endemic areas of American Cutaneous Leishmaniasis (ACL) caused by *Leishmania braziliensis braziliensis* in the southeast of Brazil, the domestic dog seems to be an important link in the epidemiological chain of this disease (Coutinho et al., 1985, *Mem. Inst. Oswaldo Cruz, 80:* 17-22; Lopes et al., 1984, *J. Parasitol., 70:* 89-98; Marzochi et al., 1985, *Mem. Inst Oswaldo Cruz, 80:* 347-349 & Pirmez et al., 1988, *Am. J. Trop. Med. Hyg., 38:* 41-47). The frequent occurence of canine disease, co-exististing with the human disease in the same area, corroborates this hypothesis (Marzochi et al., 1985, *Mem. Inst. Oswaldo Cruz, 80:* 347-349 & Pirmez et al., 1988, *Am. J. Trop. Med. Hyg., 38:* 41-47).

The diagnostic of infection in dogs is normally achieved by clinical examination, by demonstration of the protozoan, and by serology using the indirec immunofluorescence test (IFT) (Coutinho et al., 1985, *Mem. Inst. Oswaldo Cruz, 80:* 17-22; Marzochi et al., 1985, *Mem. Inst. Oswaldo Cruz, 80:* 347-349 & Pirmez et al., *Am. J. Trop. Med. Hyg., 38:* 41-47). So far, the demonstration of the delayed type hypersensitivity (DTH) in infectd dogs with conventional antigens by the classical Montenegro's skin test has not been succefull (Pirmez et al., 1988, *Am. J. Trop. Med. Hyg., 38:* 41-47).

In this study the authors have used the P10000 G fraction obtained from the subcellular fractionation of *Leishmania b. braziliensis* according to the method previously reported (Barbosa-Santos et al., 1986, *Mem Inst. Oswaldo Cruz, 81*, Suppl: 146). Briefly the *L. b. braziliensis* ABS strain was cultivated in liver-infusion tryptose (LIT) medium at 24 °C for 10 days. The promastigotes were collected from the culture medium by centrifugation (6,000 G for 10 min). Iodoacetamide (Sigma) and Phenylmethyl-sulphonylfluoride (Sigma) were added to the homogenate which was lysed by various cicles of freezing and thawing, and centrifuged at 10.000 G for 60 min. to yield the particulate and the soluble fraction. The protein content in the pellet (P10000) was measured by the Lowry's method.

L. b. braziliensis P10000 (100 μg, 200 μg and 400 μg of protein) was inoculated intradermally in the groin of 3 dogs experimentally infected with *L. b. braziliensis*, 11 dogs naturally infected in the *L. b. braziliensis* transmission area (Camorim – Jacarepaguá, Rio de Janeiro, RJ, Brazil) and 12 controls from an area where leishmaniasis does not occur (Parque Carlos Chagas, RJ).

The best result was obtained with 200 μg of protein per-inoculum. The DTH and IFT were both positive in 100% of the first 2 animal groups. The DTH induration varied between 5 and 22 mm in the diameter and the IFT was positive with titres above 40. All the control animals were negative to both DTH and IFT.

In an epidemiological survey performed in another endemic area of ACL (Sacarrão – Jacarepaguá, Rio de Janeiro, RJ, Brazil) 48 dogs were studied by clinical examination, DTH and IFT. The DTH consisted of an intradermal inoculation in the groin of 200 μg of the P10000 protein-antigen in saline merthiolate 1:10000. The skin test (DTH) evaluated after 48 h was positive (induration $\geqslant$ 5 mm) in 18 dogs (36.5%), which 11 ones (22.9%) had ulcerated lesions and 7 (14.6%) had healed lesions. Positive IFT (titres $\geqslant$ 40) was observed in 11 dogs (22.9%) but only 9 (18.8%) have showed ulcerated lesions. Of the 30 dogs (62.5%) without lesions or scars, 2 dogs (4.2%) had

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TABLE

	DTH (+)	(%)	DTH (–)	(%)	Total	(%)
IFT +	9*	(18.8)	2***	(4.2)	11	(22.9)
IFT –	9**	(18.8)	28***	(58.3)	37	(77.0)
	18	(36.5)	30	(62.5)	48	(100.0)

* All the dogs showed ulcerated lesions.
** Two dogs with ulcerated lesions and 7 with scars.
***Dogs without lesions or scars.

positive IFT and negative DTH. The others 28 dogs (58.3%) had both negative IFT and DTH (Table).

The proposed intradermal test is quick and sensitive for the identification of dogs with active or healded ACL. This study opens new perpectives for both individual diagnosis and epidemiological surveys of canine ACL such as in the selection of dogs for the experimental and field trial of vacines.

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