EVALUATION OF A SKIN TEST ON THE CANINE MUCOCUTANEOUS LEISHMANIASIS DIAGNOSIS

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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 cycles 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 ≥ 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 ≥ 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
TABLE

<table>
<thead>
<tr>
<th></th>
<th>DTH (+)</th>
<th>(%)</th>
<th>DTH (-)</th>
<th>(%)</th>
<th>Total</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFT +</td>
<td>9*</td>
<td>(18.8)</td>
<td>2***</td>
<td>(4.2)</td>
<td>11</td>
<td>(22.9)</td>
</tr>
<tr>
<td>IFT -</td>
<td>9***</td>
<td>(18.8)</td>
<td>28***</td>
<td>(58.3)</td>
<td>37</td>
<td>(77.0)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>(36.5)</td>
<td>30</td>
<td>(62.5)</td>
<td>48</td>
<td>(100.0)</td>
</tr>
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</table>

* 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 healed ACL. This study opens new perspectives for both individual diagnosis and epidemiological surveys of canine ACL such as in the selection of dogs for the experimental and field trial of vaccines.

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