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Short Communication

Assessment of serological tests for the diagnosis of canine visceral leishmaniasis

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ABSTRACT

An immunoenzymatic assay (ELISA), an indirect immunofluorescence antibody test (IFAT) with different antigens (ELISA-*Leishmania chagasi*, ELISA-*L. major*-like, IFAT-*L. chagasi* and IFAT-*L. major*-like), and an immunochromatographic test were assessed for the diagnosis of canine visceral leishmaniasis (CVL). Serum samples from 144 dogs from an endemic area for visceral leishmaniasis in the municipality of Rio de Janeiro were tested. The sensitivities of the serological tests were 93%, 100%, 73%, 60% and 93%, with specificities of 87%, 92%, 77%, 96% and 92% for the ELISA-*L. major*-like, ELISA-*L. chagasi*, IFAT-*L. major*-like, IFAT-*L. chagasi* and the immunochromatographic test, respectively. ELISA-*L. chagasi* was the best test for the diagnosis of CVL, but the immunochromatographic test could be a useful alternative as it offers simple and rapid diagnosis without the need for a specialized laboratory.

The Brazilian Ministry of Health recommends an immunoenzymatic assay (ELISA) and an indirect immunofluorescence antibody test (IFAT) for the diagnosis of canine visceral leishmaniasis (CVL). The results of these tests are used as criteria for the culling of seroreactive dogs in surveillance and control programs for visceral leishmaniasis (VL) (Ministério da Saúde, 2006). However, the accuracy of these tests is controversial and culling does not appear to contain the spread of the disease (Alves and Bevilacqua, 2004; Silva et al., 2005). Recently, there has been interest in validating immunochromatographic tests for the diagnosis of CVL due to the need for rapid and reliable diagnostic tools for the detection of early-infected dogs, thus allowing for effective control interventions. One of these immunochromatographic tests is the DPP (Dual-Path Platform), which has emerged as an alternative for the diagnosis of CVL (Grimaldi et al., 2012).

The use of homologous antigens could improve the specificity of serological tests. The IFAT and ELISA kits currently distributed to the public service in Brazil use the *Leishmania major*-like antigen, but replacing this with an *L. chagasi* antigen is under discussion as homologous antigens give better performance and, in Brazil, CVL is caused by *L. chagasi* (Laurenti, 2009). The objective of this study was to evaluate the IFAT, ELISA and DPP assays in serum samples from seroreactive dogs from a VL endemic area in the municipality of Rio de Janeiro, Brazil. The study was approved by the Ethics Committee on Animal Research of the Oswaldo Cruz Foundation (CEUA/FIOCRUZ, Process L-023/06.).

We assessed serum samples from 144 dogs with titers $\geqslant 40$ by IFAT on blood collected onto filter paper (eluate) identified by the leishmaniasis control program (Epidemiology Service of Rio de Janeiro). Blood samples were collected by venipuncture of cephalic or jugular veins and placed in tubes without anticoagulant. Serum samples were stored at −20 °C. Each serum sample was tested using two IFAT protocols. The first used the IIF-Canine-Visceral-Leishmaniasis kit (Bio-Manguinhos), which contained promastigote forms of *L. major*-like (MHOM/BR/76/JOF) antigen (IFAT-*L. major*-like). The second protocol (IFAT-*L. chagasi*) was performed with the same kit, but the antigen was composed of promastigote forms of *L. chagasi* (MHOM/BR/74/PP75). The IFAT protocols were performed following the manufacturer's instructions.

The samples were also tested with two ELISA protocols: (1) the EIE-Canine-Visceral-Leishmaniasis kit (Bio-Manguinhos), which employs a soluble antigen from promastigote forms of *L. major*-like (ELISA-*L. major*-like), and (2) the same kit, but with the antigen from promastigote forms of *L. chagasi* (ELISA-*L. chagasi*). Both ELISA protocols followed the manufacturer's instructions. The DPP assay (Bio-Manguinhos), which uses the recombinant antigens K26 and K39, was also conducted according to the manufacturer's instructions.

Parasitological culture with etiologic identification was used as the reference test, as previously reported (Silva et al., 2011). Dogs with isolation of *L. chagasi* ($n = 15$) were defined as the positive group, while dogs without isolation of *L. chagasi* or with isolation of other protozoans (*L. braziliensis* and *T. caninum*) ($n = 129$) were

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Table 1
Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for the indirect immunofluorescence (IFAT), immunoenzymatic assay (ELISA) and immunochromatographic assay (DPP) tests.

Parameters	Serologic tests/antigens used				
	IFAT		ELISA		DPP
	L. major-like	*L. chagasi*	*L. major*-like	*L. chagasi*	rK26/rK39
Sensitivity (%)	73 (48–89)	60 (36–80)	93 (70–99)	100 (80–100)	93 (70–99)
Specificity (%)	77 (69–83)	96 (91–98)	87 (80–92)	92 (86–96)	92 (86–95)
PPV (%)	27	64	45	60	58
NPV (%)	96	95	99	100	99

Confidence interval, 95%.

used as the negative group. Percent sensitivity and specificity were calculated using the following equations:

$$\text{Sensitivity} = [\text{number of true positives}/(\text{number of true positives} + \text{number of false negatives})] \times 100$$

$$\text{Specificity} = [\text{number of true negatives}/(\text{number of true negatives} + \text{number of false positives})] \times 100$$

Of the 144 serum samples tested, 39% (56/144) were seroreactive for *Leishmania* spp., regardless of the test or antigen used. Overall, 28%, 10%, 22%, 17% and 17% of serum samples were positive in the IFAT-*L. major*-like, IFAT-*L. chagasi*, ELISA-*L. major*-like, ELISA-*L. chagasi* and DPP, respectively. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are presented in Table 1.

Due to the importance of domestic dogs in the transmission cycle of VL, there is a great interest in improving the sensitivity and specificity of tests used in the diagnosis of VL. Although the samples used in this study were obtained from dogs with previous positive serological diagnosis (IIF-Canine-Visceral-Leishmaniasis performed by the Epidemiology Service of Rio de Janeiro for the removal seroreactive dogs), the results showed a significant disagreement regarding the accuracies of the tests. This may be partially explained by the type of samples employed: eluate (in the initial serological test) and serum (in the present study), as previously reported by others (Figueiredo et al., 2010a). In the present study, all 144 eluate samples that were processed and analyzed at the Epidemiology Service of Rio de Janeiro were seroreactive for leishmaniasis by IFAT; however, when the same samples were reevaluated in our laboratory with the same IFAT kit, using serum samples, the results could not be confirmed. Like Figueiredo et al. (2010b), we suggest that IFAT using blood samples collected on filter paper should not be used for epidemiological surveys due to problems related to sample collection, transportation, storage and operational difficulties (lack of calibration, validity of the conjugate, etc.).

The IFAT and ELISA kits use the *L. major*-like strain, which is not the agent responsible for VL in Brazil. Laurenti (2009) mentioned that the use of homologous antigens would increase the specificity of these tests and in the samples we tested, considering the positive results of parasitological culture, the IFAT-*L. major*-like assay showed higher sensitivity than the IFAT-*L. chagasi* assay. Nevertheless, the ELISA-*L. major*-like and ELISA-*L. chagasi* assays gave higher sensitivity values than the IFAT. These results showed the superiority of the ELISA when compared to IFAT, regardless of the antigen used.

In our study, the DPP assay showed higher sensitivity than the IFAT-*L. major*-like kit that is currently distributed to the public services. Additionally, cross-reaction was not verified in the serum from dogs parasitized by *L. braziliensis* and *Trypanosoma caninum*. Our results showed higher specificity of the ELISA-*L. chagasi* assay when compared to the ELISA-*L. major*-like assay; however this difference does not seem to be significant enough to suggest a complete change in the production line due to the operational difficulties when producing the *L. chagasi* antigen on a large scale.

Based on these results, the ELISA-*L chagasi* was the best test for the diagnosis of CVL; however, the number of dogs in the positive reference group was too low (15/144) to reach definitive conclusions. We suggest that the DPP assay is used as an alternative serological test, because of its similar results to the ELISA-*L chagasi* test, ease of storage and transportation, and the ability to reach a simple and rapid diagnosis without the need for a specialized laboratory.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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