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Comparative evaluation of the DPP® CVL rapid test for canine serodiagnosis in area of visceral leishmaniasis

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ABSTRACT

We investigated the performance of the DPP® canine visceral leishmaniasis (CVL) rapid test, a novel immunochromatographic assay launched by BioManguinhos (Brazil), which was recently included in the new Brazilian protocol for screening CVL in serological surveys. The present study compared the DPP® with the ELISA and IFA produced by BioManguinhos (Brazil) both with *L. major*-like antigens and with in-house tests using *Leishmania infantum chagasi* (in-house ELISA and in-house IFA). We analyzed the sera from clinically symptomatic ($n = 47$) and asymptomatic ($n = 38$) infected dogs from an endemic area of CVL, as well as from healthy ($n = 18$) dogs, in addition to the sera of dogs ($n = 81$) infected with other pathogens. The DPP® and the in-house ELISA showed a sensitivity of 90.6% and 94.1%, respectively, and specificity of 95.1% and 97.5%, respectively, and both presented cross-reactivity only with the sera of dogs with babesiosis, 44% for the DPP® and 22% for the in-house ELISA. The clinical groups were detected equally by the two assays. The ELISA BioManguinhos, IFA BioManguinhos, and in house-IFA showed a good sensitivity, 90.6%, 96.5% and 89.4%, respectively, but very low specificity, 77.8%, 69.1% and 65.8%, respectively, due to the high cross-reactivity with the sera from the animals harboring other pathogens. The in-house ELISA provided the highest accuracy (95.8%), followed by the DPP® (92.7%), ELISA BioManguinhos (84.3%), IFA BioManguinhos (83.1%), and in-house IFA (78.0%). The simultaneous use of the DPP® and ELISA BioManguinhos reached a sensitivity of 99.1% and 82.1% when used sequentially. In conclusion, the DPP® performed well as serological test for CVL, and detected both asymptomatic and symptomatic dogs in equal proportions. Although its sensitivity is not ideal yet, discarding the IFA and including the DPP® improved the accuracy of the new Brazilian CVL diagnostic protocol, particularly of detecting truly infected dogs. Moreover, considering the higher specificity of DPP® (95.1% vs 77.8%), positive predictive value (95.1% vs 81.1%) and positive likelihood value (18.3% vs 4.1%) in comparison with the ELISA BioManguinhos, the use of DPP® as a confirmatory test instead of a screening test is suggested.

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1. Introduction

Visceral leishmaniasis (VL) is a zoonotic disease in Brazil caused by the digenetic protozoan *Leishmania infantum chagasi*, which is primarily transmitted by the *Lutzomyia longipalpis* vector. The disease affects humans and wild and domestic animals. The domestic dog presents an important skin parasitism that favors infection of the sand fly and is considered the main reservoir of the parasite (Lainson and Shaw, 1987). From the epidemiological point of view, the disease in canines must be considered because it is more prevalent than the disease in humans, usually precedes the occurrence of human cases, and because of the high competence of infected dogs, including those that are asymptomatic, to transmit the parasite to the vector (Marzochi et al., 1985; Laurenti et al., 2013).

The Brazilian program for controlling leishmaniasis is based on the early diagnosis and treatment of human cases, the spraying of insecticides in areas where human cases are diagnosed and the elimination of seropositive dogs to reduce the sources of infection for sand flies. However, several studies have questioned the effectiveness of the actions taken against the domestic reservoir; as the serology is not accurate enough to detect the infection in dogs, the impact on human transmission is limited, and canine euthanasia is costly (Paranhos-Silva et al., 1996; Dietze et al., 1997). Therefore, a more accurate diagnosis is important both for preventing the unnecessary culling of uninfected dogs and to reduce the presence of infected animals in the environment (Ferreira et al., 2008). Among the methods most applied for the serological diagnosis, the indirect fluorescence assay (IFA) is used quite frequently in epidemiological studies (Alvar et al., 2004). Its sensitivity and specificity range between 60% and 100% (Almeida et al., 2005; da Silva et al., 2013), but cross-reactivity with the antibodies of dogs infected with other diseases has been reported (Zanette et al., 2014). The enzyme-linked immunosorbent assay (ELISA) is also commonly used for the diagnosis of canine visceral leishmaniasis (CVL). This technique allows the simultaneous analysis of a large number of samples and the use of crude, soluble, purified or recombinant antigens.

Despite the wide variety of diagnostic methods available in Brazil, the ELISA (screening assay) and the IFA (confirmatory assay), both of which with *L. major*-like antigens and produced by BioManguinhos (Fiocruz, Rio de Janeiro, Brazil), were recommended by the Ministry of Health to assess the canine seroprevalence until 2011. However, aiming to enhance the accuracy of the serodiagnosis, the government published a Technical Note introducing the DPP® CVL rapid test, a new rapid assay launched by BioManguinhos (Fiocruz, Rio de Janeiro, Brazil) that uses the rK26/rK39 fusion protein as an antigen, recommending the DPP® as a screening test and the ELISA-*L. major*-like as a confirmatory assay for diagnosing CVL (Grimaldi et al., 2012; Coura-Vital et al., 2013).

There are few studies comparing the performance of DPP® with other serological tests, considering the CVL clinical groups and the dogs with potentially cross-reactive infections. Thus, the purpose of this study was to evaluate the performance of the DPP® in the CVL diagnosis through its comparison with the ELISA and the IFA BioManguinhos and also with the in-house ELISA and IFA using a homologous antigen. The cross-reactivity was tested using the sera of dogs infected with *Babesia canis vogeli*, *Dirofilaria immitis*, *Trypanosoma cruzi*, *Ehrlichia canis*, *Neospora caninum*, *Toxoplasma gondii* and *Toxocara canis*. In addition, the impact of the introduction of the DPP® into the new Brazilian CVL diagnostic protocol was also investigated.

2. Materials and methods

Population of the study: Mongrel dogs referred to the Center for Zoonosis Control of Araçatuba (21°12′32″S and 50°25′58″W), Northwestern São Paulo state, a municipality highly endemic for visceral leishmaniasis, with different ages and gender, destined for euthanasia were enrolled in this study. Eighty-five dogs with a positive parasitological diagnosis by immunohistochemistry were divided into two groups, one composed of 47 dogs presenting clinical signs of leishmaniasis, such as weight loss, lymphadenomegaly, hepatosplenomegaly, pale mucous membranes, skin lesions, onychogryphosis, epistaxis and keratoconjunctivitis, designated the symptomatic group, and another group composed of 38 dogs without external clinical signs, with serum protein <8.5 mg/dL and serum creatinine within normal limits, according to the International Renal Interest Society (IRIS, 2006), designated the asymptomatic group.

For the specificity evaluation, 81 sera from dogs born in a VL-free area of Brazil were tested: 18 from healthy animals (negative controls) and 63 (cross-reaction controls) from 9 dogs naturally infected with *B. canis vogeli* presenting pale mucous membranes, anemia, intraerythrocytic inclusions of *Babesia* sp. and anti-*Babesia* antibodies determined by IFA, with titers ranging from 400 to 800; 17 dogs naturally infected with *Ehrlichia* presenting thrombocytopenia, *Ehrlichia* morulae within the macrophages on capillary blood smears and antibodies detected by a commercially available kit (SNAP®3Dx®, IDEXX Laboratories Inc., Westbrook, ME, USA); 9 dogs naturally infected with *T. gondii* presenting clinical signs of the disease and anti-*T. gondii* antibodies detected by IFA, with titers ranging from 1:128 to 1:1024; 6 dogs presenting clinical signs of *N. caninum* infection, with anti-*N. caninum* antibodies with titers ranging from 400 to 800 by IFA; 4 dogs co-infected with *T. gondii* and *N. caninum* with compatible clinical signs and serological confirmation; 3 dogs infected with *D. immitis* presenting with parasites on thick blood smears; 9 dogs infected with *T. canis* confirmed by PCR and 6 dogs experimentally infected with *T. cruzi* (Colombian strain) with chronic cardiomyopathy and anti-*T. cruzi* IgG antibodies determined by IFA, with titers ranging from 160 to 640.

This study was submitted and approved by the Ethical Committee for the use of Animals of the Medical School of the University of São Paulo, under protocol 296/10. All of the procedures were conducted according to the guidelines of the COBEA – Brazilian College of Experimentation in Animals.

Parasitological diagnosis: Immunohistochemistry was used as the gold standard to define canine infection by *Leishmania* spp. All the dogs with parasites in both the