Comparison of two commercially available serological rapid tests with the official screening test used to detect *Leishmania* seropositive dogs in Brazil

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ABSTRACT

Visceral leishmaniasis is a major public health problem in Brazil, where seropositive dogs are usually culled as part of the control program. Currently, a rapid immunochromatographic test (DPP® Leishmaniose Visceral Canina) is used as the official screening test. However, the production of this test has not been sufficient to attend the national demand, particularly in private laboratories. In this study, we compared the level of agreement between results obtained with the official screening test with two commercial rapid tests (*i.e.*, SNAP® Leishmania Test and Alere™ Leishmaniose Ac Test). By testing 95 serum samples of dogs from a visceral leishmaniasis-endemic area, we found a substantial agreement (Kappa = 0.77; $P < 0.0001$) between the official rapid test and SNAP® Leishmania Test and a fair agreement (Kappa = 0.26; $P < 0.0001$) between the official rapid test and Alere™ Leishmaniose Ac Test. In conclusion, SNAP® Leishmania Test should be considered as an equally reliable alternative to DPP® Leishmaniose Visceral Canina. The second commercial test, Alere™ Leishmaniose Ac Test, seems less reliable and could lead to a significant underestimation of the actual number of positive dogs during serological screenings in the framework of the Brazilian visceral leishmaniasis control program. Indeed, 16 dogs that were positive at both DPP® Leishmaniose Visceral Canina and SNAP® Leishmania Test were negative at Alere™ Leishmaniose Ac Test.

Keywords: visceral leishmaniasis, diagnosis, rapid test, blood, dogs

1. Introduction

Visceral leishmaniasis caused by *Leishmania infantum* is a major zoonosis in several countries around the world. Brazil is one of the six countries reporting more than 90% of the global human visceral leishmaniasis cases, with an average of 3,246 new cases annually (Alvar et al., 2012). Since the 1950s, the official visceral leishmaniasis control program in Brazil has been based on three pillars: (1) early diagnosis and treatment of human cases, (2) dog culling; and (3) vector control (Ministério da Saúde, 2006).

Until 2011, the elimination of dogs was based on results of an enzyme-linked immunosorbent assay ELISA (EIE LVC, Bio-Manguinhos, Brazil), as the screening test, and an immunofluorescence antibody assay (IFI LVC, Bio-Manguinhos, Brazil) as the confirmatory test (Ministério da Saúde, 2006). However, concerns regarding the sensitivity, specificity and practicality of these tests (Lira et al., 2006; Romero and Boelaert, 2010; Peixoto et al., 2015) led the Brazilian Ministry of Health to introduce the rapid immunochromatographic test DPP® LVC as the official screening test and

adopt the EIE LVC as the confirmatory test. Recent studies indicate that the criteria currently recommended by the Ministry of Health of Brazil (*i.e.*, DPP® LVC as the screening test and EIE LVC as the confirmatory test) may not be reliable and that the DPP® LVC should be used as the confirmatory test rather than EIE LVC (Coura-Vital et al., 2014; Laurenti et al., 2014). Nonetheless, the introduction of DPP® LVC increased the accuracy of the diagnosis, reducing false-positive results and the consequent elimination of false-positive dogs (Fraga et al., 2016).

The production of DPP® LVC has not been sufficient to meet the national demand. Moreover, the test is exclusively used by local public health authorities and official laboratories in Brazil, being not available in private veterinary laboratories. A recent study has suggested the rapid immunochromatographic test Alere™ Leishmaniose Ac Test as a possible alternative (Souza Filho et al., 2016). However, this tested presented a sensitivity of 85%, as calculated using combined results of serological and parasitological tests (Souza Filho et al., 2016). Moreover, the direct agreement between DPP® LVC and Alere™ Leishmaniose Ac Test was not assessed in the above-mentioned study.

In 2015, a new rapid ELISA test (SNAP® Leishmania Test, Idexx Laboratories) was registered by the Brazilian Ministry of Agriculture, Livestock and Food Supply. This rapid ELISA could also represent an alternative to DPP® LVC, considering its good performance in terms of sensitivity and specificity, as well as its reduced time required between sample application and results (*i.e.*, 6 min) as compared with Alere™ Leishmaniose Ac Test (*i.e.*, 20 min). In this context, we compared the level of agreement of the official screening test (DPP® LVC) with both SNAP® Leishmania Test and Alere™ Leishmaniose Ac Test.

2. Material and methods

2.1. Ethical approval

The study (project number 66/2014) was approved by the Animal Ethics Committee (CEUA) of the Aggeu Magalhães Research Centre, Oswaldo Cruz Foundation (Fiocruz), Pernambuco, Brazil.

2.2. Clinical samples

A total of 95 serum samples from dogs from the municipality of Goiana, Pernambuco, north-eastern Brazil, an area of active visceral leishmaniasis transmission, were used. The blood samples were collected in serum separator tubes and centrifuged at 2,000 *g* for 10 min to obtain serum. All serum samples were stored at –20°C until serological testing.

2.3. Serological tests

The serological tests (*i.e.*, DPP® LVC, SNAP® Leishmania and Alere™ Leishmaniose Ac Test Kit) were performed following the manufacturers' instructions. While these tests may be performed on blood, plasma and serum, all tests were performed using serum samples. A brief summary of some characteristics of each test is presented on Table 1.

As per manufacturers' instructions, results were interpreted as: negative (only positive control spot/line developed colour), positive (control spot/line and sample spot/line developed colour), and invalid result (control spot/line did not develop colour).

2.4. Data analysis

Confidence intervals (CI) of 95% were calculated for positivity rates. The agreement between the results obtained with the rapid tests was assessed using Kappa statistic, as follows: kappa < 0 (no agreement); 0–0.20 (slight); 0.21–0.40 (fair); 0.41–0.60 (moderate); 0.61–0.80 (substantial), and 0.81–1 (almost perfect agreement) (Landis and Koch, 1977). Statistical analyses were performed using BioEstat 5.3 (Instituto Mamirauá, Brazil).

3. Results and discussion

Out of 95 serum samples from dogs tested herein, 30 (31.6%; 95% CI: 22.2–40.9%) were positive using DPP® LVC, the official screening test recommended by the Brazilian Ministry of Health. This high positivity rate is in line with previous studies carried out in the same region, where dogs are usually highly exposed to the risk of *L. infantum* infection (Dantas-Torres et al., 2006).

Using the two commercial tests studied herein, 26 (27.4%; 95% CI: 18.4–36.3%) by SNAP® Leishmania Test and six (6.4%; 95% CI: 1.4–11.2%) by Alere™ Leishmaniose Ac Test. Kappa statistics revealed a substantial agreement between DPP® LVC and SNAP® Leishmania Test (Kappa value = 0.77, $P < 0.0001$) (Table 2), but a fair agreement between DPP® LVC and Alere™ Leishmaniose Ac Test (Kappa value = 0.26, $P < 0.0001$) (Table 3).

The DPP® LVC and SNAP® Leishmania Test detected a higher number of seropositive dogs as compared with Alere™ Leishmaniose Ac Test. This apparent lower sensitivity of the Alere™ Leishmaniose Ac Test is in disagreement with its claimed sensitivity reported in the manufacturer's instructions (*i.e.*, 97.2%) and in line with a recent study, which reported a 85% sensitivity using parasitological examination as gold standard (Souza Filho et al., 2016).

Altogether these results indicate that the SNAP® Leishmania Test may be a more reliable alternative to DPP® LVC than Alere™ Leishmaniose Ac Test. Moreover, it suggests that the use of Alere™ Leishmaniose Ac Test could lead to an underestimation of the actual number of positive dogs during serological screenings in the framework of the visceral leishmaniasis control program in Brazil. These findings reemphasize the difficulty to compare results obtained from different epidemiological surveys, using different tests. Moreover, these results also point out that veterinary practitioners dealing with dogs with suspicious visceral leishmaniasis should interpret the results of qualitative serological tests cautiously, always in conjunction with detailed clinical and anamnestic data (Solano-Gallego et al., 2011).

A recent study has confirmed that the use of the DPP® LVC improved the diagnosis of canine visceral leishmaniasis by reducing the detection of false-positive dogs (Fraga et al., 2016). The present study indicates that the SNAP® Leishmania Test may be a reliable alternative to the DPP® LVC in order to complement the demand for rapid tests in the visceral leishmaniasis control program in Brazil as well as in private veterinary laboratories.

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Competing financial interests

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Table 1
Summary of serological tests used in this study as supplied in the manufacturers' instructions

Features	DPP® LVC	SNAP® Leishmania Test	Alere™ Leishmaniose Ac Test
Batch	152TV0003Z / 158TV012Z	JL728	003/15-2104DA006
Test type	Immunochromatography	ELISA	Immunochromatography
Antigen	rK28	Purified *Leishmania* antigen [a]	rK28
Sensitivity	100%	96.3%	97.2%
Specificity	87.5–91.7%	99.2%	99.8%
Time to results	10 min	6 min	20 min
Automation capacity	Not reported	Available [b]	Not reported

[a] Derived from *Leishmania infantum* promastigotes prepared by sonic disruption, filtration, and diethylaminoethyl column purification (Ferroglio et al., 2007).
[b] More information at: http://www.idexx.co.uk/smallanimal/inhouse/vetlab/snapshotdx.html

Table 2
Agreement between the official test (DPP® LVC) and SNAP® *Leishmania* Test (Kappa = 0.77, $P < 0.0001$)

TR DPP® LVC	SNAP® *Leishmania*		Total
	Pos	Neg	
Pos	22	8	30
Neg	1	64	65
Total	23	72	95

Table 3
Agreement between the official test (DPP®) and Alere™ Leishmaniose Ac Test (Kappa = 0.26, $P < 0.0001$)

TR DPP® LVC	Alere™ Leishmaniose Ac test		Total
	Pos	Neg	
Pos	6	24	30
Neg	0	65	65
Total	6	95	95