

## Short Communication

# Evaluation of DPP<sup>®</sup> and SNAP<sup>®</sup> Rapid Tests for diagnosis of *Leishmania infantum* canine infections

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### Abstract

**Introduction:** Visceral leishmaniasis is a disease that affects humans, wildlife, and domestic species. Since dogs play a key role in urban *Leishmania* spp. transmission, the Brazilian government maintains the Monitoring and Control Program of Visceral Leishmaniasis (VLMCP) in endemic regions, which promotes awareness campaigns aiming to enhance the control of the infection. The VLMCP recommends the Dual Path Platform (DPP<sup>®</sup>) canine visceral leishmaniasis test (Bio-Manguinhos, Brazil) for screening and enzyme-linked immunosorbent assay to confirm the infection. The DPP<sup>®</sup> test is produced and distributed by the Health Ministry to the Municipal Health Centers responsible for the local VLMCP. The test is not available to all the clinics, forcing some veterinarians to use other rapid tests for screening and diagnosis of this disease in their daily routine. **Methods:** The present study was conducted to compare the performance of the DPP<sup>®</sup> and SNAP<sup>®</sup> tests using sera from the dogs with confirmed infections of *L. infantum* and from the dogs with no previous testing, residing in areas with a low *Leishmania* infection. **Results:** There was 97.0% agreement between the two tests. Sensitivity and specificity of the SNAP<sup>®</sup> test were 96.3% and 100%, respectively. Agreement between both the antibody tests and the parasitological detection methods was 96.8%. The DPP<sup>®</sup> test had 95.8% sensitivity and 100% specificity. **Conclusions:** The SNAP<sup>®</sup> and the DPP<sup>®</sup> tests were virtually equivalent in terms of detection of canine antibodies against *L. infantum*, and both the tests demonstrated high and similar levels of sensitivity and specificity.

**Keywords:** Antibody. Canine visceral leishmaniasis. Diagnosis. Zoonosis.

### INTRODUCTION

Visceral leishmaniasis is a zoonotic disease of global importance, affecting humans, wildlife, and domestic species. It is estimated that 700,000 to 1 million new human cases occur every year, causing 20,000 to 30,000 deaths worldwide<sup>1</sup>. Since dogs play a key role in urban *Leishmania* spp. transmission, surveillance on the prevalence of canine visceral leishmaniasis (CVL) is of paramount importance for the success of the official programs to control the spread of these parasite species.

CVL presents variable signs and symptoms that are often similar to other diseases, making clinical diagnosis difficult<sup>2-4</sup>. This fact makes laboratory, serological, or parasitological diagnosis essential for confirmation of the disease<sup>5,6</sup>. As majority

of the dogs infected by the parasite are asymptomatic, the Brazilian government maintains the Visceral Leishmaniasis Monitoring and Control Program (VLMCP) in endemic regions, which promotes awareness campaigns aiming to enhance the control of the infection<sup>7</sup>.

The VLMCP is based on the diagnosis and early treatment of human cases, the use of pesticides in domestic environments that have presented human cases, and the removal and culling of seropositive dogs. When testing the dogs for infection, the VLMCP recommends immunochromatography using the Dual Path Platform (DPP<sup>®</sup>) CVL rapid test (Bio-Manguinhos, Rio de Janeiro, Brazil) for screening and the enzyme-linked immunosorbent assay (ELISA) for confirmation of infection<sup>7</sup>.

The DPP<sup>®</sup> visceral leishmaniasis test is a qualitative rapid test that applies a combination of recombinant antigens from *Leishmania infantum* to detect specific antibodies<sup>8</sup>. The DPP<sup>®</sup> test has shown specificity ranging from 87.8% to 98.6% and sensitivity between 90.6% and 98% using confirmed positive samples<sup>9,10</sup>.

DPP<sup>®</sup> for the diagnosis of CVL is produced and distributed by the Health Ministry to the Municipal Health Centers

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responsible for the local VLMCP. The product is not available to other establishments. Therefore, researchers as well as veterinary practitioners who operate outside the VLMCP do not have access to these tests, forcing them to use other rapid tests for screening and diagnosis of this disease in their daily routine.

One of the most commonly used rapid tests in the daily routine of veterinary practitioners is the immunoenzymatic SNAP® Canine *Leishmania* Antibody Test (IDEXX Laboratories, Inc., Westbrook, Maine, USA), which uses purified antigens of *L. infantum* promastigote. A few studies have been conducted on the efficacy of this test in Brazil. Most of these studies have not used a parasitological diagnostic test for comparison, which makes it difficult to determine its specific characteristics relative to the standard parasitological tests. A comprehensive evaluation of the anti-*Leishmania* antibody test in comparison with parasitological diagnosis as the gold standard test showed 94.7% sensitivity and 93.6% specificity<sup>11</sup>. The information provided by the manufacturer reports 96.3% sensitivity and 99.2% specificity<sup>12</sup>.

The present study was conducted to increase the knowledge about the performance of the DPP® and SNAP® *Leishmania* antibody tests and to compare the results obtained with these tests using sera from Brazilian dogs with confirmed infections of *L. infantum* and sera from dogs residing in non-endemic areas or areas with a low *Leishmania* infection.

## METHODS

Seven hundred and twenty-seven canine serum samples obtained during surveillance or research activities in Brazil and maintained at the Laboratório de Imunomodulação e Protozoologia do Instituto Oswaldo Cruz (Authorizations LW-16/10 and LW-33/11 - CEUA/Oswaldo Cruz Foundation) were used for these evaluations.

Samples were simultaneously tested with the DPP® and the SNAP® *Leishmania* antibody tests for detection of antibodies against *L. infantum* by two technicians blinded to the status of the sample. The serum samples were taken from the freezer in batches of approximately 50 samples and monitored until they reached room temperature. Each test was carried out according to the manufacturer's recommendations and was always performed by the same operator.

Five hundred and forty-one samples were used for testing the agreement between the two tests, including 19 samples from the endemic areas, 12 from the non-endemic areas, and 510 from the areas with occasional occurrence of CVL (eastern region of the state of Rio de Janeiro and communities surrounding a natural private reserve in the state of Mato Grosso).

Parasitological confirmation of infection status was obtained using parasite isolation in bone marrow samples, polymerase chain reaction for infected dogs, or by obtaining negative serological tests by indirect immunofluorescence assay and commercial ELISA from naïve dogs from the non-endemic areas of the city of Rio de Janeiro, RJ. One hundred and sixty-four samples from the infected dogs were used to compare the parasitological analytical methods with the SNAP® antibody test, and 72 out of those 164 samples were used for DPP®

comparisons. Additional samples from 22 naïve dogs were also included for the comparison of the two tests with parasitological analytical methods.

The results of the two tests were compared using agreement analysis to establish the serological parameters of sensitivity and specificity, and the observed and expected agreement indicators<sup>13-15</sup>. The accuracy was expressed by the kappa ( $\kappa$ ) index by inferring more rigor in relation to general agreement indicators, taking into account the proportions of the expected and observed agreement. The scale proposed by Fleiss & Cohen<sup>16</sup> was used for the interpretation of the kappa index. A value of less than 0 indicated no agreement; 0.0 - 0.20, poor agreement; 0.21 - 0.40, weak; 0.41 - 0.60, moderate; 0.61 - 0.80, substantial, and 0.81 - 1.0 indicated almost perfect agreement.

## Ethics approval

Ethics approval was obtained from the Animal Use Committee of the Oswaldo Cruz Foundation (numbers LW-16/10 and LW-33/10).

## RESULTS

Among the 541 samples used to determine the agreement between the two *Leishmania* antibody tests, 525 were consistent, showing gross agreement of 97.0% (Table 1). The agreement of the tests' results was almost perfect ( $\kappa = 0.821$ ). All the 16 samples with discordant results were from the dogs in areas with only an occasional incidence of canine *Leishmania* infection.

The evaluation of the SNAP® test showed that all the 22 samples from the naïve dogs were correctly determined as negative. However, among the samples from the infected dogs, 3.7% (6/164) were determined as negative (Table 2), showing a sensitivity of 96.3% (95% CI: 91.8% - 98.5%) and a specificity of 100% (95% CI: 87.3% - 100%). The gross agreement between the SNAP® *Leishmania* antibody test and the parasitological detection methods was 96.8% and it was deemed as an almost perfect agreement ( $\kappa = 0.862$ ).

Among the 94 samples included for the evaluation of the DPP® test, the samples from all the 22 naïve dogs were correctly determined as negative. However, among the samples from the infected dogs, three (4.2%) were determined to be negative (Table 3), showing a sensitivity of 95.8% (95% CI: 87.5% - 98.9%) and specificity of 100% (95% CI: 87.3% - 100%). The

**TABLE 1:** Agreement of results of canine serum reactivity for *Leishmania infantum* analyzed with DPP® and SNAP® *Leishmania* antibody detection tests .

|       |          | DPP®     |          | Total |
|-------|----------|----------|----------|-------|
|       |          | Positive | Negative |       |
| SNAP® | Positive | 42       | 3        | 45    |
|       | Negative | 13       | 483      | 496   |
| Total |          | 55       | 486      | 541   |

**DPP®:** Canine Visceral Leishmanianis Rapid Test (Bio-Manguinhos, Rio de Janeiro, Brazil); **SNAP®:** SNAP® Canine Leishmania Antibody Test.

**TABLE 2:** Comparison of the results of serum reactivity for *Leishmania infantum* using the SNAP® *Leishmania* antibody test with parasitological detection methods.

|       |          | Parasitological |          | Total |
|-------|----------|-----------------|----------|-------|
|       |          | Positive        | Negative |       |
| SNAP® | Positive | 158             | 0        | 158   |
|       | Negative | 6               | 22       | 28    |
| Total |          | 164             | 22       | 186   |

**TABLE 3:** Comparison of the results of serum reactivity for *Leishmania infantum* using the DPP® canine visceral leishmaniasis rapid test with parasitological detection methods.

|       |          | Parasitological |          | Total |
|-------|----------|-----------------|----------|-------|
|       |          | Positive        | Negative |       |
| DPP®  | Positive | 69              | 0        | 69    |
|       | Negative | 3               | 22       | 25    |
| Total |          | 72              | 22       | 94    |

gross agreement between the DPP® test and parasitological detection methods was 96.8%, with a kappa index of 0.915 (95% CI: 91.2% - 100%), indicating almost perfect agreement. The three samples from the infected dogs that tested negative in the DPP® antibody test were among the six samples that also tested negative in the SNAP® antibody test. Among the other three samples tested negative by the SNAP® test, there was only one sample in which the SNAP® did not detect antibodies but the DPP® did. The other two samples were analyzed only by the SNAP®.

## DISCUSSION

Despite using different methodologies and different antigens, there was almost perfect agreement between the SNAP® and DPP® test results, with  $\kappa = 0.821$  (82.1%). It is interesting to note that all the 16 samples with conflicting results between the two tests originated from the dogs residing in areas with only occasional occurrences of CVL. Therefore, the possibility of interference from other infectious agents in these areas, including *Trypanosoma cruzi*, *Trypanosoma caninum*, *Toxoplasma gondii*, *Neospora caninum*, *Babesia canis*, and *Ehrlichia canis*, as previously observed<sup>11,17,18</sup>, must be considered. This possibility is reinforced by the reported 47% canine seroprevalence of parasites transmitted by ticks in the eastern region of Rio de Janeiro<sup>19</sup> and 80% seroprevalence in the surrounding area of the natural reserve in the state of Mato Grosso (unpublished data).

Among the six samples in which the results obtained by the SNAP® were false negative, three samples had the same false negative result with the DPP®. Two out of the six false negatives were analyzed only with the SNAP®, which precluded

a comparison between the tests. It was determined that there actually was only one sample for which the DPP® test correctly identified the positive status of the sample that was declared negative by the SNAP® test. There is a possibility that the antibody levels in some or all of the six samples with false negative results may have declined during longer periods of storage.

In previous studies, the DPP® test showed specificity ranging from 87.8% to 98.6% and sensitivity between 90.6% and 98% using confirmed positive samples<sup>9,10</sup>. However, despite showing high levels of sensitivity among clinically symptomatic dogs, sensitivity of the DPP® test to identify the *Leishmania* infection in asymptomatic dogs was only 47% in one of the studies<sup>13</sup>. In a systematic review and a meta-analysis of the data from 25 studies, Peixoto et al<sup>20</sup> concluded that the ELISA tests using crude antigens and the DPP® tests have moderate accuracy (83% [ 95% CI: 78%-88%] sensitivity and 73% [ 95% CI: 70%-75%] specificity).

The SNAP® Canine *Leishmania* Antibody Test was designed to diagnose infections by *L. infantum*. It was evaluated in a large population of dogs, including 283 dogs positive for CVL attributed to *L. chagasi*, 86 clinically healthy dogs from a non-endemic area, and 31 dogs infected with other infectious and parasitic agents, to determine whether the infection by *L. chagasi* would also be identified by this test<sup>10</sup>. The sensitivity of the SNAP® test was 94.7% and the specificity was 90.6% in that study. When the results from the dogs with confounding diseases were excluded, specificity increased to 100%. Results obtained in another study have also demonstrated that the SNAP® test provided an acceptable alternative to the official DPP® screening test for CVL diagnosis<sup>21</sup>.

The results of the present study showed that the SNAP® and the DPP® tests were equivalent in terms of detection of canine antibodies against *L. infantum*. Since the agreement between the two tests was almost perfect and the performance was confirmed to provide high and similar levels of sensitivity and specificity, the SNAP® rapid antibody test is a convenient and reliable alternative to the standard DPP® screening test for the veterinary practitioners to use for screening canine samples for *Leishmania* antibodies.

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## Conflict of Interest

The authors declare no conflict of interest.

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