Rapid Immunochromatographic Test for Serodiagnosis of Canine Leishmaniasis

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An rK39 immunochromatographic test and immunofluorescent-antibody test (IFAT) for serodiagnosis of canine leishmaniasis were evaluated. The two tests showed correlation for all but one of the sera obtained from 68 dogs confirmed as leishmaniasis cases and 40 dogs (22 healthy dogs and 18 dogs with other diseases) from areas where the disease is not endemic. Specificity was 100% for both tests, while sensitivity was 97% for the rapid test and 99% for IFAT.

Leishmania spp. are parasitic protozoa which cause human cutaneous, mucocutaneous, and visceral leishmaniasis (VL). VL has a mortality rate as high as 100% if left untreated and is spreading in several areas of the world due to increases in the numbers of AIDS victims and the frequency of Leishmania-human immunodeficiency virus coinfections (6). Dogs are the main domestic reservoir of VL caused by Leishmania infantum in the Mediterranean area and in Central and South America (1). Therefore, early diagnosis of this disease in dogs is essential for surveillance and control programs. A rapid immunochromatographic test for the qualitative detection of anti-Leishmania circulating antibodies by means of antigen-impregnated nitrocellulose paper strips has recently become available (13). This test employs the leishmanial recombinant antigen K39 (rK39), which is the product of a gene cloned from Leishmania chagasi and which contains a 39-amino-acid repeat conserved among viscerotropic Leishmania species (Leishmania donovani, L. infantum, and L. chagasi) (2, 5). This antigen was employed in an enzyme-linked immunosorbent assay (ELISA) for serodiagnosis of canine leishmaniasis, and the ELISA exhibited a good correlation with the immunofluorescent-antibody test (IFAT) (12).

The aim of this study was to evaluate the performance of this test for serodiagnosis of canine leishmaniasis and to compare the results with those obtained by IFAT.

Serum samples from 108 dogs were tested. These included 68 animals from an area of endemicity (Apulia, southern Italy) with parasitologically proven leishmaniasis and 40 negative-control dogs from an area where the disease is not endemic (Sweden) and which had never traveled outside this area. The negative controls, 22 healthy dogs and 18 dogs with other diseases, including sarcotic mange (n = 9), ehrlichiosis (n = 7), and borreliosis (n = 2), were tested to measure possible cross-reactivity.

The dipstick test (RapidTest; DiaSys Europe Ltd., Wokingham, United Kingdom) was performed according to the manufacturer’s instructions. Briefly, 20 μl of serum was added to the sample wells of the test devices, followed by 100 μl of buffer provided by the manufacturer with the kits. The test was read 10 min after the addition of the serum. The results were positive if two distinct red or pink lines appeared (one in the test region and another in the control region), they were negative when no red or pink lines appeared in the test region, and they were invalid if the control line failed to appear. IFAT was carried out according to an established method (8) using promastigotes of L. infantum zymodeme MON1 cultured in Tobi-Evans medium as the antigen. The parasitic cells were exposed to sera diluted (1:80 and 1:160) in phosphate-buffered saline, pH 7.2, in a moist chamber, washed, and then exposed to fluoresceinated rabbit anti-dog immunoglobulin G (diluted 1:40; Sigma-Aldrich Chemie, Munich, Germany; lot 68H9190); both incubations took place at 37°C for 30 min. Samples scored positive when they produced promastigote cytoplasmic or membrane fluorescence, with a cutoff dilution of 1:80. Each test included L. infantum-positive and -negative canine sera as controls. The presence of parasites in lymph node smears was detected by microscope in order to establish a definitive diagnosis of leishmaniasis in dogs. Popliteal lymph node samples were obtained by a nonaspiration technique (9) and then used to prepare smears stained with Giemsa.

Results demonstrated that IFAT was positive in 67 out of 68 dogs with proven Leishmania infections and that the rapid test was positive in 66 out of 68 infected dogs. One parasite-positive dog was found to be negative both by IFAT and by the dipstick test. Conversely, serum from a second parasite-positive dog was positive by IFAT (1:80 and 1:160) and negative by the dipstick test. Both tests were negative in the control group. On the whole a correlation between IFAT and the dipstick test was found in all but one of 108 examined sera.

From our results the immunochromatographic test showed 97% sensitivity and 100% specificity, while IFAT sensitivity was 99% and specificity was 100%.

Although the IFAT and rK39 dipstick tests are both accurate for the detection of anti-Leishmania antibodies, both techniques present advantages and disadvantages for the serodiagnosis of canine leishmaniasis. In fact, the IFAT is not only a qualitative assay, like the dipstick test, but is also a quantitative...
method, which makes it possible to evaluate anti-\textit{Leishmania} antibody titers produced by dogs. On the other hand, the interpretation of IFAT may be subjective, dependent on the skill of the operator, while the interpretation of the dipstick test is unequivocal even in the presence of lines of different intensities (from red to pink) in the test region clearly distinct from the background.

In another trial designed to evaluate the rK39 dipstick test for the diagnosis of dog leishmaniasis, Reithinger et al. (11) compared this test to ELISA and PCR assays and found that the dipstick test was associated with low specificity (61 to 75%), thus leaving a high proportion of dogs misdiagnosed as false positives, and 72 to 77\% sensitivity. Such discrepancy between the results of Reithinger et al. (11) and our results may be due to differences in the ability of the test to detect antibodies against different strains of \textit{L. infantum} from Brazil and from Europe, or to the different approaches used to estimate the true-negative and -positive populations in reference 11 and in the present work. In fact, Reithinger et al. (11) used both the criteria of PCR on the Buffy coat from peripheral blood and ELISA results to estimate the true numbers of infected and uninfected dogs, while in our study exclusion of the infection was based on epidemiological criteria (dogs from an area where leishmaniasis is not endemic) and the true-positive control included dogs with parasitologically confirmed infection. In any case, although there are many advantages to using molecular tools for the diagnosis of \textit{L. infantum}, PCR-based methodologies may exhibit low sensitivity for detecting infection in asymptomatic dogs and in dogs during follow-up after treatment (10).

In addition, our results on the serodiagnosis of canine leishmaniasis confirm those reported by Bern et al. (3), Sundar et al. (13), Jelinek et al. (7), Zijlstra et al. (14), and Brandonisio et al. (4), where the rK39 dipstick test used for the diagnosis of human VL had a specificity of 97 to 100\%.

Finally, the rK39 dipstick test proved to be a rapid, sensitive, and specific diagnostic test particularly useful in mass-screening surveys for assessing the spread of canine leishmaniasis and intervention campaigns in order to establish prophylaxis against vectors. The above-mentioned advantages are further compounded by the fact that the rK39 dipstick test does not require specific laboratory equipment, like immunofluorescence microscopy, and technological expertise (e.g., personnel trained to interpret IFAT results) and it is less expensive than IFAT.

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\textbf{REFERENCES}