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Evaluation of a Rapid Device for Serological Diagnosis of *Leishmania infantum* Infection in Dogs as an Alternative to Immunofluorescence Assay and Western Blotting

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In this study, we compared a rapid immunochromatographic test (Speed Leish K; BVT Groupe Virbac, La Seyne sur Mer, France) with an indirect immunofluorescence assay (IFAT) and Western blotting (WB) for the detection of *Leishmania infantum* antibodies in dogs. A total of 250 serum samples were collected from 125 *L. infantum*-positive and 125 *L. infantum*-negative dogs. Among the positive samples, 81 were strongly positive at low IFAT dilutions, while 44 were low-reactivity sera (IFAT titers, 1:40 to 1:80). The sensitivity and specificity of the Speed Leish K were 96.3% and 100%, respectively, compared with those of the IFAT. When IFAT low-reactivity sera (titers, 1:40 or 1:80) were tested with the Speed Leish K, using WB results as a reference, the sensitivities were 93.75% for sera with a 1:80 titer and 73.33% for sera with a 1:40 titer, and the specificity was 100%. The Speed Leish K is easy to use and performs well, so it can be considered a quick and reliable tool for the diagnosis of *L. infantum* infection in dogs.

Visceral leishmaniasis is a protozoan zoonosis caused by *Leishmania infantum*. It is transmitted by sandflies of the *Phlebotomus* and *Lutzomyia* genera in the Old and New World, respectively. The domestic dog *Canis familiaris* is the reservoir of this parasite, which is endemic in the Middle East, in many tropical and subtropical areas of the world, and in Mediterranean areas of Europe, where the seroprevalences range from 1.7% to 48% (1, 2). Human visceral leishmaniasis (HVL) is a neglected disease, even though the WHO considers it one of the top 10 diseases, and at the end of the 1990s, it was estimated that about 12 million people were infected and 350 million were at risk of acquiring the infection (see http://www.who.int/docstore/water_sanitation_health/vectcontrol/ch07.htm).

The spread of canine leishmaniasis (CanL) in the continental regions of Europe, such as northern Italy (3, 4) and Germany (5), represents a risk to human health. Autochthonous cases of HVL were recently reported in an area where CanL is newly endemic (6), and surprisingly, a high prevalence of infection was also found in asymptomatic people from northern Italy (7), where the infection was first reported in dogs in the late 1990s (3).

Considering the relevance of CanL to public health, a quick and accurate diagnosis represents the main tool for effectively managing clinical cases in dogs and minimizing the risk for human beings. In fact, serological tests represent the first step in CanL diagnosis and although an immunofluorescence assay (IFAT) is the most used test both for epidemiological studies and in clinical practice (8), a number of enzyme-linked immunosorbent assays (ELISAs) and direct agglutination tests (DATs) have been developed and are available for use in diagnostic laboratories or clinic testing (9, 10, 11).

Although the IFAT response is considered unequivocal for serum titers of <1:40 (negative) or ≥1:160 (positive), it is ambiguous for titers of 1:80 and 1:40, which are considered less reactive (12). Western blotting (WB) has proven to be more sensitive than an IFAT (13, 14); however, it cannot be used routinely, and apart from research, it is applied mainly to validate other techniques (11).

Some rapid tests developed especially for dogs were shown to be highly sensitive and specific (11, 15), while some immunochromatographic tests developed for and used in human medicine did not show high sensitivity or specificity when used for dogs (16, 17). A rapid, sensitive, and specific diagnostic test might be relevant not only for mass-screening surveys but also for routine in-clinic diagnosis, because the rapid and cost-effective detection of infected dogs is a key point in the control of infection and can greatly reduce the risk of infection transmission.

As few data on the immunochromatographic test in CanL diagnosis are available, we compared a commercial rapid immunochromatographic test (Speed Leish K) with an IFAT and WB for the serological diagnosis of CanL and evaluated its sensitivity and specificity.

MATERIALS AND METHODS

Samples. Blood samples were collected from the radial veins of 250 dogs from November 2010 to February 2011 in three veterinary clinics located in three areas (Liguria Region, Asti Province, and Aosta Valley Region in northwestern Italy) where CanL is traditionally or newly endemic. There were 81 positive samples (IFAT titer, ≥1:160), 44 doubtful samples (25 with an IFAT titer of 1:40 and 19 with an IFAT titer of 1:80), and 125 negative samples (IFAT titer, <1:40). Blood was allowed to clot and was centrifuged. The resulting serum was separated, frozen, and stored in single vials at −20°C until testing. The IFAT was carried out as reported previously (18), and Western blotting (WB) was carried out as described by Ferroglio et al. (12) on sera with doubtful results (IFAT titers, 1:40 and 1:80) to evaluate the performance of the Speed Leish K on IFAT low-reactivity sera. Samples were considered positive by WB when at least two bands of 169, 115, 66, or 33 kDa could be detected (12). The Speed Leish K canine *Leishmania* antibody test kit (BVT Groupe Virbac, La Seyne sur

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TABLE 1 Comparison of Speed Leish K and IFAT results for *L. infantum* infection diagnosis in 81 seropositive (IFAT titer, ≥1:160) and 125 seronegative (IFAT titer, ≤1:20) dogs[a]

Incubation time and Speed Leish K result	No. of samples with IFAT titer of:		Concordance index (%)	*k*
	≥1:160	≤1:20		
20 min			98.54	0.97
Positive	78	0		
Negative	3	125		
30 min			99.00	0.98
Positive	79	0		
Negative	2	125		

[a] The diagnostic capacity of the Speed Leish K was evaluated for unequivocally positive (IFAT titer, ≥1:160) or negative (IFAT titer, ≤1:20) serum samples. The Speed Leish K results after incubation for 20 or 30 min are reported.

TABLE 2 Comparison of the Speed Leish K and Western blotting results for the diagnosis of *L. infantum* infection in 44 dogs with equivocal IFAT titers (i.e., 1:40 or 1:80)[a]

IFAT titer	Incubation time and Speed Leish K result	No. of samples that were:	
		WB positive	WB negative
1:40	20 min		
	Positive	8	0
	Negative	7	10
	30 min		
	Positive	11	0
	Negative	4	10
1:80	20 min		
	Positive	15	0
	Negative	1	3
	30 min		
	Positive	15	0
	Negative	1	3

[a] Samples yielding IFAT titers of 1:40 and 1:80 were analyzed separately. Results of these assays after 20 or 30 min of incubation are reported.

Mer, France) is a dipstick device which detects anti-*Leishmania infantum* antibodies through an immunochromatographic principle. The capture antigen is a complex of recombinant kinesins. Kinesins are sensitive and specific for the diagnosis of visceral leishmaniasis (19, 20). The test kit was stored at room temperature. Testing was carried out according to the manufacturer's instructions, and the result was read after 20 min. Another reading at 30 min was also performed to evaluate if a 10-min delay in reading (which is possible when the test is carried out under field conditions) can alter the sensitivity or specificity of the test.

Statistical analysis. Agreement (*k*) among the tests and the evaluation of their sensitivities and specificities were calculated using Win Episcope version 2.0 software.

RESULTS

The results of the IFAT, WB, and the Speed Leish K are summarized in Tables 1 and 2. Table 1 shows the comparative data between the Speed Leish K and the IFAT. In Table 2, we report the results of the Speed Leish K and WB for sera with doubtful IFAT results.

Using the IFAT results as the reference standard (Table 1), we found the sensitivity and specificity of the Speed Leish K to be 96.30% (95% confidence interval [CI], 92.18 to 100.00%) and 100.00% (95% CI, 100.00 to 100.00%), respectively, with a high degree of agreement (concordance index = 98.54%; $k = 0.97$) at the 20-min reading and 97.53% (95% CI, 94.15 to 100.00%) and 100.00% (95% CI, 100.00 to 100.00%), respectively, at the 30-min reading, with an agreement (*k*) of 0.98. When sera with doubtful IFAT results (1:40 and 1:80 titers) were tested by WB and the Speed Leish K (Table 2), the Speed Leish K showed a good resolution capacity for 1:80 titer sera, with a sensitivity of 93.75% (95% CI, 81.88 to 100.00%), a specificity of 100.00% (95% CI, 100.00 to 100.00%), and good agreement ($k = 0.83$). When the Speed Leish K was compared with WB to test sera positive at a 1:40 IFAT dilution, its sensitivity and specificity were 53.33% (95% CI, 28.08 to 78.88%) and 100% (95% CI, 100.00 to 100.00%), respectively, with a *k* value of 0.48 for the 20-min reading, and the sensitivity increased to 73.33% (95% CI, 50.95 to 97.71%) with a *k* value of 0.69 for the 30-min reading.

We noted that reading the dipstick at 30 min did not produce any false-positive results among the negative results, which indicates very good test specificity and slightly improved performance for low IFAT-positive sera (titer, 1:40).

DISCUSSION

The clinical severity of CanL and the role that dogs play as reservoir hosts make the monitoring and surveying of *L. infantum* in this species a fundamental action in the effort to prevent the spread of this infection (1, 21, 22). This is particularly true because of the large variability in clinical symptoms and the presence of asymptomatic but still-infectious dogs (21, 23).

Although PCR is now a common diagnostic tool that is available to many veterinary practitioners, it cannot be used routinely in clinical medicine or in wide field surveys in many countries where CanL is endemic. Moreover, highly sensitive PCR protocols have a low positive predictive value in detecting this disease (24).

In fact, serological methods remain the main tools for CanL diagnosis in veterinary clinics and mass-screening surveys. Among them, the IFAT is still considered the reference test, even though it has some drawbacks due to the subjective interpretation of results that are often not repeatable in different laboratories (8) and with the use of different cutoff values in each laboratory.

Some authors have suggested the use of WB in the diagnosis of CanL (13, 14). However, this technique requires a good technical background, it is limited to research laboratories, and it is not applicable in routine diagnosis.

The need for a rapid serological test is evident from the numerous attempts to develop one in the past few decades (11, 15, 17, 20, 25, 26, 27, 28, 29). In comparison with other studies, in which immunochromatographic dipstick tests for *L. infantum* were compared to ELISAs and PCRs (16) or to DATs (17, 29), and apart from the data of de Lima et al. (30), who found a 91.5% sensitivity and a 94.7% specificity in an immunochromatography test based on the K39 antigen, the agreement between the immunochromatographic dipstick and other classical laboratory techniques (IFAT and WB) seems to be higher with the Speed Leish K, which performs similarly to a rapid ELISA-based test such as the Snap (11). Moreover, it has been demonstrated that the anti-kinesin antibody test does not detect antibodies induced by vaccination with LiESP/QA-21, a vaccine composed of purified excreted/secreted proteins (ESPs) from *Leishmania infantum* (31). This makes the Speed Leish K suitable for the diagnosis of leishmaniasis in vaccinated animals, as the presence of antibodies is indicative of contact with the parasite.

In conclusion, our results show that the Speed Leish K, compared with the IFAT and WB, is simple to use and rapid. It showed

good sensitivity and specificity for a reliable diagnosis of *L. infantum* infection in dogs, especially for assessment of the infection status of animals for which the IFAT is not conclusive. For sera with doubtful IFAT results (titer, 1:80), the specificity was 100%. For IFAT-positive sera to a 1:40 dilution, the sensitivity shown by the Speed Leish K was 53.33% at a normal (20-min) reading and increased to 73.33% at a 30-min reading. From a practical point of view, it must be considered that only 2% of dogs with a 1:40 titer at first diagnosis develop CanL (32). The Speed Leish K showed good sensitivity compared with that for WB and good specificity for doubtful IFAT samples, especially with IFAT-positive sera at a titer of 1:80. Reading the dipstick at 30 min did not produce any false-positive results among the negative results, which indicates very good test specificity and slightly improved performance with sera with low IFAT-positive titers (1:40 titer).

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