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Letter to the Editor

Rapid test for the serodiagnosis of acute canine leptospirosis

Acute leptospirosis in dogs – Weil's disease – is a life-threatening condition with zoonotic potential and which requires laboratory testing for accurate diagnosis. We report the development and evaluation of the first – patient side diagnostic test for acute canine leptospirosis which is based on the detection of *Leptospira*-specific immunoglobulin M antibodies. For serum samples from dogs with laboratory confirmed acute leptospirosis from the Netherlands the sensitivity was 100% (95% Confidence interval [CI], 76.7–100). The specificity was 95.3% (95% CI, 88.8–98.3) and all positive control dogs had a history of recent vaccination.

Suspicion of Weil's disease (acute icteric leptospirosis) in dogs requires rapid laboratory confirmation, because prompt intervention is essential for the survival of the patient (André-Fontaine, 2006; Davies, 2008; Maele van de et al., 2008; Levett, 2001). The causative pathogenic leptospires can be isolated from a wide range of – healthy – animal species of which many function as maintenance hosts – reservoirs – (Levett, 2001). Rodents and other small mammals are the maintenance hosts for the *Leptospira* pathogenic to humans and domestic animals. Dogs can host *Leptospira* belonging to a range of serogroups, but particularly the members of the serogroups Icterohaemorrhagiae and Canicola may cause severe acute icteric disease (Hartman, 1984), although subclinical infections occur on wide scale (Rojas et al., 2010; Houwers et al., 2010). Hence agent detection has limited diagnostic value. Canine leptospirosis is considered a public health issue because of the risk of transmission to dog owners and caretakers.

The most commonly applied serological test, the Microscopic Agglutination Test (MAT), which requires specific laboratory facilities and expertise, detects agglutinating antibodies and paired serum samples with an interval of at least ten days are needed to diagnose acute infection. ELISA for the detection of *Leptospira*-specific immunoglobulin M (IgM) antibodies presents a reliable test for diagnosing acute canine leptospirosis in a single sample as specific IgM antibodies quickly rise during the early stage of the infection (Hartman, 1984), but also requires laboratory facilities. To enable prompt diagnoses at the point of care – patient side –, we developed and evaluated a simple and rapid, user-friendly assay detecting canine IgM against all pathogenic serovars, the rapid canine leptospirosis test.

This so-called lateral flow assay consists of a plastic device containing a composite assay strip flanked at one end by a reagent pad and an absorption pad at the other (Smits et al., 2001; Eapen et al., 2002). The strip contains two lines, a test line consisting of a *Leptospira* specific antigen and a control line consisting of purified anti-dog IgM. The reagent pad holds dried conjugate consisting of colloidal gold-labeled anti-dog IgM antibody. The antigen was prepared by heat-extraction and proteolytic digestion of cultured pathogenic leptospiral strain Wijnberg of serovar Copenhageni. The rapid test is performed by adding 5 μl of neat serum to the sample pad followed by the immediate addition of 130 μl of running fluid (PBS containing 1.67% bovine serum albumin and 3% Tween 20). The result is read after 10 min by visual inspection for staining at the test and control lines. A valid test result is obtained if staining of the control line is observed. The test line may stain at different intensities, and a positive result was subjectively rated 1+ when staining was weak, 2+ when staining was moderate, 3+ when staining was strong and 4+ when staining was very strong (Fig. 1). Very weak (+/-) staining was considered negative. To secure stability of the bio-components, individual devices are sealed airtight in a moisture resistant protective foil together with a small bag containing dried silica. This acute canine leptospirosis Test-it™ device is available from the Royal Tropical Institute/Life Assay Diagnostics.

Performance of the rapid test was evaluated with several sets of dog serum samples. For validation of the diagnostic accuracy a total of 130 sera were taken from the serum banks of the Veterinary Microbiological Diagnostic Centre (VMDC) and the clinical chemistry laboratory (UVDL) of the Faculty of Veterinary Medicine of Utrecht University, the Netherlands. These included 23 samples from dogs with laboratory confirmed – IgM ELISA positive – acute leptospirosis and 107 samples from a random selection of dog sera which were submitted for other reasons than suspicion of leptospirosis and with accompanying clinical information that did rule out the likelihood of the presence of an infectious disease. VMDC's routinely used IgM/IgG specific indirect ELISA was used as reference test. The antigen consisted of an extract from strain Wijnberg (serovar Copenhageni) and the procedure was adapted from the original description by Hartman et al. (1983). Samples with a

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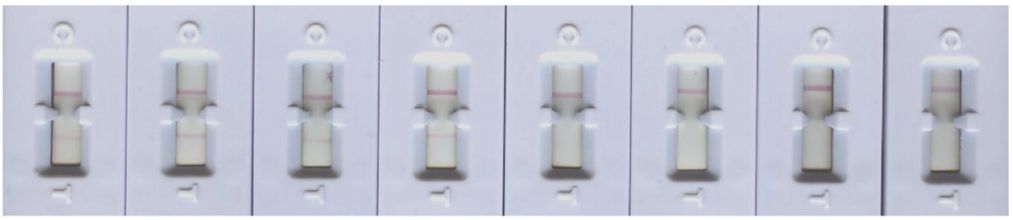

	Dogs with clinical signs of leptospirosis				Dogs without clinical signs of leptospirosis			
Sample No.	1	2	3	4	5	6	7	8
IgM ELISA titre	1:1.280	1:640	1:1.280	1:2.560	0	0	0	0
IgG ELISA titre	1:320	1:160	1:640	1:1.280	0	0	0	0
MAT titre[1]	1:80[2]	0	1:160[3] 1:320[4]	1:160[4] 1:640[5]	0	0	0	0
Rapid test result	2+[6]	2+	2+	2+	N[7]	N	N	N

Fig. 1. Examples of Rapid Canine leptospirosis test results.
Canine leptospirosis rapid test results for serum samples from dogs from The Netherlands with (sample no. 1–4) and without (sample no. 5–8) clinical leptospirosis were read 10 min after the addition of the sample and the running fluid. C, control area with control line. T, test area with test line.
1. Titers ≥1:160 are indicated; 2. strain Jez Bratislava, serovar Bratislava; 3. strain Kantorowic, serovar Icterohaemorrhagiae; 4. strain Wijberg, serovar Copenhageni; 5. strain Poi, serovar Poi; 6. Staining intensity of antigen line (1+, weak: 2+, moderate; 3+, strong; 4+, very strong); 7. N, negative.

clear IgM titer and an equal or lower IgG titer were considered indicative of acute infection, thus positive.

The rapid test was positive with all 23 samples from the confirmed acute leptospirosis cases: sensitivity 100% (95% confidence interval [95% CI], 76.7–100). It reacted negative with 102/107 (95.3% specificity; 95% CI, 88.8–98.3) of the healthy control samples. However, according to the subsequent information provided by the veterinary surgeons, all five positive dogs appeared to have been vaccinated within 5 months prior to sampling and this may have biased the specificity value. To rule out cross-reactivity with other infectious agents, 59 serum samples from dogs with various other infectious diseases than leptospirosis including leishmaniasis, distemper and demodicosis together with 40 samples from healthy dogs taken from a serum bank at the University of Trás-os-Montes e Alto Douro in Portugal were tested: 97/99 (98.0%; 95% CI: 92.9–99.8) were negative while the two samples with a positive result were from a dog with leishmaniasis and the other one was healthy.

In addition, to asses the performance of the rapid test in different geographic settings, possibly with other prevailing serovars, 75 dog serum samples from the School of Veterinary Medicine of the University of the West Indies in Trinidad & Tobago were tested of which 50 were from clinically suspected cases of leptospirosis and 25 samples from stray dogs without obvious signs of disease. Clinically suspected cases were defined by the presence of various signs including fever, anorexia, vomiting, dehydration and jaundice. The rapid test was positive in 39/50 (78.0%; 95% CI, 63.7–88.0) of the clinically suspected cases and 1/25 (4%) of the healthy dogs. Also, 103 serum samples from stray dogs from Mexico (provided by the Veterinary Faculty of the University of Yucatan) were tested and only 2 (1.9%) were found positive. All sera except a few of the Dutch and all Portuguese were also tested with the MAT as performed locally according to the guidelines provided by the World Health Organization (WHO) in *Human Leptospirosis*: *Guidance for Diagnosis, Surveillance and Control* (WHO, 2003) with locally relevant strain panels (Adesiyun et al., 2006; Jimenez-Coello et al., 2008). All strains used were provided by the Royal Tropical Institute (KIT), the Netherlands. Seroprevalences as determined with MAT were relatively high with a locally varying range of reactive strains, but with predominance of serovar Copenhageni in the Netherlands and Trinidad & Tobago, and serovar Canicola in Mexico. Note that dogs from the latter two countries were never vaccinated. Still seroprevalence based on the MAT for the stray dogs from Trinidad & Tobago and from Mexico was 52.0% (13/25) and 51.5% (51/103), respectively. Antibodies detected by MAT in these single samples did not correlate with the IgM as detected with rapid test or ELISA. This is consistent with the above notion that MAT has limited diagnostic value for canine leptospirosis and that in many cases reactivity in the MAT is reflecting sub-clinical infections.

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Finally, urine and blood samples from dogs from Trinidad & Tobago were subjected to culturing as described elsewhere (Suepaul et al., 2010); a total of 16/75 (21.3%) dogs were positive with nine yielding serovar Copenhageni. The latter dogs were all clinically suspected which is consistent with the notion that clinical leptospirosis in dogs is predominantly caused by members of serogroup Icterohaemorrhagiae.

In conclusion, the rapid test reported here for the first time offers the option for reliable point of care – patient side – diagnostic testing for clinically suspected acute leptospirosis in dogs. Positive results in non-suspected dogs may occur as a result from previous vaccination or from acute but sub-clinical infection.

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