

# Evaluation of SNAP<sup>®</sup> Lepto in the Diagnosis of Leptospirosis Infections in dogs: Twenty two Clinical Cases

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## ABSTRACT

Leptospirosis affects many mammalian species, including both humans and dogs. It is caused by spirochetes of the species *Leptospira interrogans sensu lato* and is a

common zoonotic bacterial infection with worldwide significance.<sup>1-3</sup> The purpose of this study was to evaluate the correlation between *Leptospira* species antibody detection using the SNAP<sup>®</sup> Lepto test, an enzyme-linked immunosorbent assay (ELISA), and the microscopic agglutination test (MAT). A total of 162 serum samples

from dogs with a differential diagnosis of leptospirosis were evaluated with the MAT and the SNAP® Lepto to detect antibodies to *Leptospira*. A subset of whole blood and urine samples (n=59) were also tested on *Leptospira* real-time polymerase chain reaction (PCR) assay. On initial presentation, the SNAP® Lepto was positive in 15/22 confirmed leptospirosis cases, 4/9 suspected leptospirosis cases and 20/131 dogs with other illnesses. The MAT was positive (1:100 titer or greater) in 18/22 confirmed leptospirosis cases, 6/9 suspected leptospirosis cases and 29/131 dogs with other illnesses. The PCR was positive in 5/22 confirmed leptospirosis cases. The overall agreement with the clinical diagnosis of leptospirosis was 80% for the SNAP® Lepto and 78% for MAT. The results of this study support the utility of the SNAP® Lepto test in assisting in the diagnosis of leptospirosis in dogs with clinical signs consistent with infection.

## INTRODUCTION

Early diagnosis of leptospirosis and prompt administration of appropriate antimicrobial treatment is critical due to the zoonotic and progressive nature of the disease. Dogs suspected of leptospirosis have variable clinical presentations, and the course of the disease can range from subclinical infection to an acute, fatal disease that is characterized by multi-organ dysfunction.<sup>3,5</sup> Current methods for diagnosis of leptospirosis include MAT, ELISA(SNAP® Lepto, IDEXX Laboratories, Inc., Westbrook, ME), PCR and histopathology.

Due to the difficulty in culturing *Leptospira* from blood or urine, it is not readily performed in clinical veterinary medicine.<sup>4</sup> The most common diagnostic test used today to aid in the diagnosis of canine leptospirosis is the MAT, which detects antibodies capable of agglutinating cultured organisms.<sup>5</sup> Due to the complexity in performing the MAT, this test is only performed in commercial diagnostic laboratories.

A second serologic test is available, the SNAP® Lepto, an ELISA test that detects antibodies to a major outer membrane protein,

LipL32.<sup>6</sup> There are inherent limitations in the performance of serologic tests for the diagnosis of leptospirosis. In the early stages of infection, dogs may frequently have negative serology results.<sup>3,5,7</sup> It is for this reason that the current recommendation for MAT testing is to submit acute and convalescent samples, looking for a 4-fold change in titers in order to make a definitive diagnosis.<sup>3</sup> In addition, serologic tests for leptospirosis, including both MAT and SNAP® Lepto, may not reliably differentiate infection from vaccination.<sup>6</sup> Real-Time PCR testing is also available to aid in the diagnosis of leptospirosis in dogs. The detection of leptospiral DNA in either blood, urine, or tissue samples is most useful in acute phases of infection when assays for antibody detection may be negative.<sup>3</sup> A PCR positive result in any of these sample types is considered diagnostic for a *Leptospira* infection in patients presenting with clinical signs consistent with leptospirosis. Although positive PCR results indicate the presence of DNA and will confirm infection, a negative result does not necessarily rule out infection due to the limitations of PCR testing and the variable course of *Leptospira* infections. Due to the limitations of both serology and PCR, the variable nature of the infection and the potential for acute onset of clinical signs, serology, and PCR should be used in combination to test dogs suspected of leptospirosis. The purpose of this study was to evaluate the SNAP® Lepto test in the diagnosis of canine leptospirosis in 22 confirmed, clinical cases.

## MATERIAL AND METHODS

### Sample Populations

Serum, whole blood, and urine (where possible) were obtained as part of the routine standard of care from 162 dogs presenting to veterinary referral hospitals<sup>b,c</sup> with a clinical suspicion of leptospirosis. Any remaining samples were donated to the study with consent from the owner.

### Microscopic Agglutination Test

The MAT was performed at a diagnostic laboratory.<sup>a</sup> All samples had MAT performed

**Table 1.** Criteria used to classify the clinical canine population having a differential diagnosis of leptospirosis.<sup>4,5</sup>

Category	Description
Confirmed Leptospirosis	<ul style="list-style-type: none"> <li>• PCR positive for <i>Leptospira</i> in blood or urine on initial testing OR</li> <li>• MAT titer of 1:800 or greater on initial testing with no history of <i>Leptospira</i> vaccination OR</li> <li>• MAT titer of <math>\geq 1:3200</math> on initial testing with a previous history of <i>Leptospira</i> vaccination or an unknown vaccine history OR</li> <li>• A 4-fold increase in MAT titer between acute and convalescent samples</li> </ul>
Suspected Leptospirosis	None of the above categories could be satisfied, an alternative cause of illness could not be identified, the patient demonstrated an appropriate response to treatment, and the attending veterinarian's clinical diagnosis was leptospirosis
Other Illness	Patient was diagnosed with a disease that was not leptospirosis

for the following serovars: Pomona, Canicola, Icterohaemorrhagiae, Grippityphosa, Bratislava, and Autumnalis. Samples were considered positive if the serum agglutinated at 1:100 dilution. Samples that did not demonstrate agglutination with any of the serovars tested at a 1:100 serum dilution were characterized as negative.

**SNAP® Lepto**

The SNAP® Lepto test is an in-clinic ELISA licensed by the USDA for the detection of *Leptospira* spp antibodies. The presence or absence of antibody was determined by visual interpretation, comparing color intensities of the sample spot with the background color intensity of the flow matrix in the result window of the assay. The presence of color in the sample spot indicated that a sample was positive for antibody to *Leptospira*. A colorless sample spot indicated that a sample did not have detectable levels of antibody to *Leptospira*. The positive control spot must be blue for the ELISA to be valid.

**Leptospira PCR**

*Leptospira* real time PCR was performed by a national reference laboratory<sup>a</sup> on paired whole blood and urine samples for a subset (n=59) of clinically ill dogs presenting to veterinary referral hospitals with a differential diagnosis of leptospirosis. A PCR positive result in either sample type was considered diagnostic for a *Leptospira* infection in patients presenting with clinical signs consistent with leptospirosis.

**Diagnosis**

All patients were classified into one of three categories by the attending veterinarian:

1. Confirmed leptospirosis
2. Suspected leptospirosis, or
3. Other illness (no leptospirosis).

rosis

2. Suspected leptospirosis, or
3. Other illness (no leptospirosis).

Criteria for classification are shown in Table 1.<sup>4,5</sup>

**Statistical Methods**

The positive and negative percent agreement values obtained with the LipL32 ELISA in relation to the MAT results performed at a national reference laboratory<sup>a</sup> were calculated using SAS® 9.4. All confidence intervals (Clopper-Pearson) are two-sided and calculated as 95% confidence intervals.

**RESULTS AND DISCUSSION**

The presenting signs of leptospirosis in dogs can vary depending on severity of illness, infecting strain, and patient's immune status.<sup>3</sup> Veterinarians often suspect leptospirosis in dogs when signs are consistent with renal or hepatic failure.<sup>3</sup> In this study, the most common presenting signs, physical exam

**Table 2.** Most common clinical signs, physical exam findings and laboratory abnormalities of patients having a differential diagnosis of leptospirosis.

Clinical Signs	All Patients (n=162)	Confirmed Leptospirosis (n=22)
Lethargy	131	21
Anorexia	129	21
Vomiting	114	21
Polyuria/Polydipsia	61	9
Diarrhea	58	11
<b>Physical Exam</b>		
Dehydration	85	8
Icterus	49	10
Fever	30	3
CRT >2 seconds	28	6
Dyspnea/Tachypnea	17	2
<b>Laboratory Abnormalities</b>		
Increased liver enzymes	104	12
Increased BUN	91	21
Increased creatinine	86	21
Abnormal urinalysis	78	12
Leukocytosis	50	12
Thrombocytopenia	47	10

findings, and laboratory abnormalities of the canine patients selected based on having a differential diagnosis of leptospirosis are summarized in Table 2.

The population of dogs consisted of 88 females (12 intact) and 74 males (12 intact) with an age range from 3 months to 16 years (median age 7 years). All patients (n=162) were evaluated by both MAT and the SNAP® Lepto test at the time of initial examination. For a subset of patients (n=59), *Leptospira* PCR was also performed. Patients were categorized according to the established criteria (Table 2), resulting in 22 cases of confirmed leptospirosis, 9 cases of suspected leptospirosis and 131 dogs with

other illnesses.

On initial presentation, the SNAP® Lepto was positive for 15/22 confirmed leptospirosis cases, 4/9 suspected leptospirosis cases, and 20/131 dogs with other illnesses. The MAT was positive (1:100 titer or greater) in 18/22 confirmed leptospirosis cases, 6/9 suspected leptospirosis cases, and 29/131 dogs with other illnesses. The MAT titers in the dogs with other illnesses ranged from 1:100 to 1:800 with a median titer of 1:200. These lower MAT values are in contrast to those seen in dogs in the confirmed leptospirosis category with single MAT values ranging from 1:12,800 to 1:102,400, with the exception of one dog at 1:1600. Nine of the confirmed leptospirosis cases had convalescent samples collected; four had previously tested positive on SNAP® Lepto and repeated as positive, 2 remained negative, and 3 seroconverted from negative to positive within 11 days.

The population of clinically ill dogs contained 31 that had previously received *Leptospira* vaccination, 78 with an unknown history of vaccination, and 53 that had not previously been vaccinated. The SNAP® Lepto was positive for 14/31 dogs that had been vaccinated (1 confirmed and 1 suspected of leptospirosis), 14/78 dogs that had an unknown vaccine history ( confirmed and 1 suspected of leptospirosis), and 11/53 dogs that had not previously been vaccinated (10 confirmed and 1 suspected of leptospirosis).

The MAT test was positive for 17/31 dogs that had been vaccinated (2 suspected

of leptospirosis), 19/78 dogs that had unknown vaccine history (6 confirmed and 2 suspected of leptospirosis), and 17/53 dogs that had not previously been vaccinated (12 confirmed and 2 suspected of leptospirosis). It is important to note that all dogs in the diagnosis category of other illness that were not vaccinated tested negative by both SNAP® Lepto and MAT. As discussed earlier, serologic tests for leptospirosis, including both MAT and SNAP® Lepto may not reliably differentiate infection from vaccination.<sup>6</sup> In this study, performance of MAT and the SNAP® Lepto test were comparable across these subsets of patients.

The 22 confirmed leptospirosis cases were broadly distributed among the four criteria used for classification. Four out of the 22 confirmed dogs were PCR positive for *Leptospira* in blood or urine on initial testing. All had corresponding MAT negative results. In this group, only one dog was positive on the SNAP® Lepto, and this particular dog had a history of *Leptospira* vaccination one year prior to clinical presentation. The dogs in this category were likely in the acute phases of the infection, and these results highlight the utility of the PCR during this stage.

In the next confirmed leptospirosis category, 8/22 dogs had a MAT titer of 1:800 or greater on initial testing, with no history of *Leptospira* vaccination and 7/22 of those dogs were also positive on the SNAP® Lepto, with MAT values ranging from 1:1600 to >1:102,400. In the four dogs that had MAT titer of  $\geq$ 1:3200 on initial testing with either a previous history of *Leptospira* vaccination or an unknown vaccine history, 4/22 were positive on SNAP® Lepto. The MAT values in this group of dogs ranged between 1:12,800 and 1:102,400. In the last category of confirmed leptospirosis cases, 6/22 dogs had a 4-fold increase in MAT titer between acute and convalescent samples. Of these dogs, 2/6 were SNAP® Lepto negative on initial presentation and all six were positive on convalescent testing. These results highlight the importance of convales-

cent serologic testing in a patient suspected of leptospirosis. The overall agreement with a clinical diagnosis of leptospirosis was 80% for the SNAP® Lepto and 78% for MAT.

The utilization of ELISA technology in developing a diagnostic tool for leptospirosis presents an opportunity for a more rapid, sensitive, and convenient test. The results of this study indicate that the SNAP® Lepto test provides a tool for rapid assessment of *Leptospira* antibody status, providing information that may increase the index of suspicion for leptospirosis in an unvaccinated dog or a dog with an unknown vaccine history. Providing a serologic test for leptospirosis that is easily accessible to practitioners at the point-of-care facilitates the diagnosis of a zoonotic and potentially fatal disease to ensure that appropriate therapeutic interventions are initiated and that adequate precautions are taken to reduce the risk of transmission. The results of this study also highlight the importance of using both serology and PCR in testing samples from dogs suspected of leptospirosis and that as with other serologic assays, results of the SNAP® Lepto test should be interpreted in the context of clinical findings, vaccination history, and other diagnostic test results.<sup>8</sup>

## FOOTNOTES

- a IDEXX Reference Laboratories, Inc., 1 IDEXX Drive, Westbrook, ME 04092
- bVeterinary Specialty and Emergency Center, Levittown, PA
- cPort City Veterinary Referral Hospital, Portsmouth, NH
- dPortland Veterinary Specialists, Portland, ME
- eAnimal Medical Center, New York, NY

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