

Canine leptospirosis – a changing disease

NEW:

IDEXX *Leptospira* spp. RealPCR™

Two sample types – one price!

Aetiology, epidemiology and pathogenesis

Leptospirosis is a global zoonosis caused by spirochaetes of the genus *Leptospira*. Over 300 *Leptospira* serovars have been identified to date, some pathogenic and others saprophytic. The disease can infect over 150 different species of mammal.

The classification of *Leptospira* serovars has changed more than once over time and is currently based on both antigen/antibody tests, relying on the identification of particular lipopolysaccharides in the bacterial wall, and molecular diagnostic methods.

Canine leptospirosis is currently caused primarily by serovars of *Leptospira interrogans* and *Leptospira kirschneri*. Before the bivalent vaccine was introduced, the disease in the dog was most commonly associated with *L. Icterohaemorrhagiae* and *L. Canicola*. For a number of years now it has been assumed that *L. Grippityphosa*, *L. Autumnalis*, *L. Bratislava* and *L. Pomona* can also play a role in canine leptospirosis.



Transmission

Leptospirosis is transmitted via direct or indirect bacterial contact with mucous membranes or skin lesions. Contaminated urine plays a crucial role in transmission. Infected animals shed spirochaetes in their urine which then contaminate the surroundings. Even asymptomatic dogs can shed the bacteria in their urine for extended periods (sometimes over months to years). In moist environments, *Leptospira* can survive and remain infectious for several weeks or even months, especially in stagnant water at temperatures of 0 to 25°C. Leptospirosis is in fact a seasonal disease, occurring more frequently at warm and humid times of year.

Clinical symptoms

The clinical picture triggered by *Leptospira* can differ greatly in expression and severity, depending on the host's immune status, the virulence of the serovar and the level of infection. The incubation period is generally around 5 to 7 days. During this time, the bacteria multiply in the bloodstream. The antibodies then produced eliminate them from the bloodstream after 7 to 10 days but they become established mainly in the kidneys.

Some dogs display only mild clinical symptoms, or indeed experience only a subclinical infection. Others develop severe to extreme forms characterised by renal and/or hepatic insufficiency, fever, uveitis, pulmonary haemorrhages, vasculitis, pancreatitis and coagulopathies. A particularly severe form of the disease is the clinical form accompanied by pulmonary haemorrhages (leptospiral pulmonary haemorrhagic syndrome, LPHS). Lethargy, anorexia, vomiting, polyuria and polydipsia are the usual clinical symptoms. Less common symptoms include fever, abdominal pain, icterus, muscle stiffness, uveitis, dyspnoea and clinical signs of coagulopathy.

In the per-acute form of leptospirosis, death can occur within a few days. In the acute form, symptoms include fever, shivering and muscle pain, often followed by a decline in sensory perception, dehydration, vomiting and circulatory collapse. Coagulopathies may be accompanied by vomiting, bloody

diarrhoea, epistaxis and petechiae. This form may prove fatal even before the onset of renal or hepatic insufficiency. Many of the symptoms listed above occur in the sub-acute form. The most common signs are polyuria, polydipsia and respiratory symptoms. In addition, acute renal insufficiency caused by tubulointerstitial kidney damage can lead to oliguria and anuria. Once this stage has been overcome, renal function may either return to normal or animals may be left with chronic renal insufficiency with varying degrees of compensation. If liver disease (chronic active hepatitis or chronic hepatic fibrosis) is also a factor, patients may display symptoms of hepatic insufficiency including anorexia, weight loss and effusions in body cavities.

Clinico-pathologic findings

Anaemia, leukocytosis characterised by neutrophilia and left shift, lymphopenia and thrombocytopenia are the most common findings on the complete blood count. If these are accompanied by signs of kidney pain or acute renal damage and/or hepatic insufficiency, leptospirosis should be a primary consideration.

Infection of the renal tubules causes acute interstitial nephritis with tubular dysfunction, sometimes accompanied by acute tubular necrosis. Rises in blood urea nitrogen, creatinine and liver enzymes, hyperbilirubinaemia and electrolyte disturbances are the most common biochemical changes. Liver impairment may lead to hepatic necrosis. Electrolyte disturbances are the result of vomiting and diarrhoea. Low specific gravity and markers of tubular injury - including glycosuria, proteinuria, granular casts and haematuria - are often present on urinalysis. Possible coagulation abnormalities include longer prothrombin time (PT) and activated partial thromboplastin time (aPTT), as well as elevated fibrinogen levels.

Serology

The micro-agglutination test (MAT) remains the gold standard for detecting *Leptospira* antibodies and determining titres. In this test, different dilutions of the serum concerned are mixed with *Leptospira*; the presence of antibodies is then

established on the basis of the agglutination reaction. The MAT can be used to detect both IgM and IgG. There is more cross-reactivity between the different *Leptospira* serovars in the initial stages of infection. This is because IgM does not have high specificity, especially if it belongs to the same serogroup. These possible cross-reactions become much less important as the disease progresses. Dogs with leptospirosis may present with clinical symptoms before antibodies can be detected, especially in the first week of infection. On the other hand, there is a high prevalence of subclinical infections resulting in the persistence of antibodies. Lastly, vaccination will also produce MAT titres detectable over several months. However, these resulting titres are usually low. To interpret antibody titres correctly, therefore, it is essential to take the patient's clinical picture into account.

A MAT titre of 1:800 or greater in a dog displaying symptoms consistent with leptospirosis is a strong indication of disease. Because the dog may appear to be seronegative in the first week of infection, it is recommended that the MAT be repeated once or twice after 7 to 15 days before classing the patient as seronegative. A fourfold rise in antibody titre is a strong indication of infection. Nevertheless, seroconversion may be weaker in the presence of ongoing antibiotic therapy. Antibody titres below 1:400 are usually attributed to prior infections or to vaccination. In these cases, the positive result of the antibody test should change very little, if at all, if the test is repeated.

Molecular diagnosis

The real-time PCR for *Leptospira* is a highly specific and extremely sensitive diagnostic tool. The diagnostic advantage of PCR over serology occurs primarily during the early stages of infection, i.e. before antibodies can be detected. It is also useful for detecting urinary shedding of leptospires. In the first stage of infection (covering approximately the first week post-infection), *Leptospira* nucleic acid can be detected in the **blood**. In the second stage, from around 7 days p.i., *Leptospira* nucleic acid can be detected in the **urine**. Because the precise time of infection is generally unknown, simultaneous PCR testing of blood and urine is recommended. The IDEXX *Leptospira* spp. RealPCR™ test detects the *Leptospira*-specific gene LipL32. This gene codes for a protein in the leptospire's outer membrane, which is associated with their pathogenicity. A positive result for the IDEXX *Leptospira* spp. RealPCR™ test therefore indicates the presence of pathogenic leptospiral nucleic acid in the sample material. A positive PCR result for a urine sample does not necessarily mean that the animal is clinically infected; it may be a sub-clinical carrier and a shedder of the bacteria. Because urinary shedding of leptospires is intermittent, a negative PCR result does not rule out a diagnosis of leptospirosis and repeated testing may be necessary.



To increase diagnostic validity in clinically infected dogs, PCR testing should be carried out on both blood and urine samples. Samples should be taken before antibiotics are administered. The real-time PCR for *Leptospira* allows early and accurate diagnosis and appropriate management of individual patients.

Interpreting results

Detection of *Leptospira* DNA by PCR: blood positive, urine negative

The dog is infected with *Leptospira*. A positive blood result and negative urine result may occur within the first 7 days after infection. Leptospiraemia develops within a few days of infection. A negative urine result is indicative of either a stage prior to urinary shedding, or of intermittent shedding. Specific therapy is indicated. In addition, a follow-up PCR is recommended after 7 to 15 days.

Detection of *Leptospira* DNA by PCR: blood and urine both positive

The dog is infected. Positive results for both blood and urine samples are rare but may occur in the first few weeks of infection; the stages of bacteraemia and urinary shedding may overlap. Positive urine samples should be regarded as potential sources of infection for other animals and humans. Specific therapy is indicated. In addition, a follow-up PCR is recommended after 7 to 15 days.

Detection of *Leptospira* DNA by PCR: blood negative and urine positive

The dog is infected. A negative blood result and positive urine test result in a dog with clinical signs of infection suggests that the animal was infected at least one to two weeks prior to sample collection. Asymptomatic carriers of *Leptospira* may shed bacteria in their urine for a period of weeks to months. Urine samples testing positive on PCR should be regarded as potential sources of infection for other animals and humans. In addition, a follow-up PCR is recommended after 7 to 15 days.

Detection of *Leptospira* DNA by PCR: blood and urine negative

The dog is probably not infected. A dog with negative test results for blood and urine is probably not infected if the samples were collected prior to antibiotic therapy. Bear in mind that the detection of *Leptospira* spp. DNA in blood is limited to the early stages of infection and that urinary shedding may be intermittent. A follow-up PCR and detection of specific antibodies by MAT are therefore recommended. However, a PCR test may also be negative if the pathogen concentration is below the limit of detection or if new variants of the pathogen are not detectable.



In view of the potentially fatal consequences of inappropriate treatment and the risk of transmission to humans, the prompt use of multiple diagnostic procedures is strongly recommended. PCR tests should therefore be included in the basic diagnostic panel (blood tests, clinical biochemistry, urinalysis, coagulation profile if necessary), possibly in combination with antibody detection and titre determination by MAT. Whatever the method used, the presence of leptospires cannot be ruled out if a first test result proves negative. This is why the test should be repeated after a few days or weeks.

Treatment*

Antibiotic therapy is key to specifically treating leptospirosis. If leptospirosis is suspected, antibiotics should be initiated as soon as possible after diagnostic samples have been collected, even prior to confirmation of the diagnosis. Doxycycline (administered orally) or penicillin and its derivatives (i.e. ampicillin or amoxicillin administered orally or intravenously) are the antibiotics of choice for initial treatment. These drugs terminate a leptospiraemia within 24 hours, which in turn limits or prevents urinary shedding and transmission of the micro-organism to healthy animals. To treat renal infection and to prevent dogs from becoming carriers, doxycycline should be administered for three weeks, twice daily at a dose of 5 mg/kg, as soon as oral administration is possible. The rather uneven clinical picture and pathology of canine leptospirosis and its many different complications often call for specific symptomatic and supportive therapy. This includes, for example, fluid therapy to support and promote renal function and to correct electrolyte disturbances and acid-base abnormalities. Most dogs with leptospirosis are polyuric and polydipsic, so urinary output should be monitored closely. Symptomatic treatment of gastrointestinal disorders, hepatic and pulmonary symptoms, coagulopathies, pain and fever may also be required.



Prognosis

The prognosis is improved if the patient is assessed promptly and correctly. This is done using the basic diagnostic panel (especially blood tests, clinical biochemistry and urinalysis) and specific tests (PCR and MAT). Without specific therapy, permanent renal damage is more common and the disease is more likely to be fatal. With early diagnosis and appropriate treatment, the survival rate for dogs with acute renal insufficiency is approximately 80 %. To ensure that patients are monitored correctly, the basic diagnostic panel should be run every 24 or 48 hours.

*Country-specific regulations related to the usage of drugs as well as the individual situation of the patient must be considered.



Important notice: two sample types – one price!

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For the above reasons, it makes sense to conduct PCR tests on both EDTA-blood and urine. With immediate effect, therefore, we are underpinning *Leptospira* diagnosis by offering both tests for the price of one. This means that you can submit **EDTA-blood and urine** for PCR *Leptospira* testing and we will only charge for one test, if both sample types are submitted in one order at the same time.

