LEPTOSPIROSIS: AN UPDATE

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CATHERINE BOVENS DVM, CertSAM, MRCVS looks at diagnostic tests for leptospirosis before considering treatments and reminding practitioners of health implications for humans handling patients

LEPTOSPIROSIS is a zoonotic bacterial disease caused by pathogenic species of the spirochete bacteria *Leptospira*. It is a re-emerging disease worldwide[1,4] and is probably the most widespread and prevalent zoonotic disease in the world, with recent increases in human cases[2,4].

Disease incidence or outbreaks are more likely to occur during periods of higher rainfall or flooding[1,3,5,6]. The bacteria can remain viable for several months in moist soil or stagnant water[1,5]. Dogs usually become infected via contact with soil or water contaminated with urine from infected animals (such as rodents, foxes, hedgehogs or other dogs) or, more rarely, via direct contact with infected animals[1,3,5]. Working dogs, dogs living in rural environments and dogs having access to rivers or lakes are more at risk, but any dog can be affected, including dogs living in a suburban or urban environment[1,3,5].

When to suspect leptospirosis

The classical presentations of leptospirosis are acute renal failure and/or acute hepatopathy[1,4,5]. However, there are many other presentations where leptospirosis should be part of the differential diagnosis (Table 1). These may occur separately or in variable combinations.

Dogs with leptospirosis may present with polyuria/polydipsia and/or haematuria, which are not

always associated with azotaemia[1,4]. Affected dogs can develop chronic hepatitis or chronic kidney disease[1,4,5]. Interstitial pneumonia and/or pulmonary haemorrhage may develop, causing clinical signs of tachypnoea, dyspnoea and coughing. In one study, 62 per cent of dogs with leptospirosis had respiratory signs and 70 per cent had pulmonary abnormalities on thoracic radiographs[7].

Leptospirosis should also be considered as a potential differential diagnosis in dogs with pyrexia of unknown origin, coagulopathies, uveitis and/or abortion[1,4,5]. It is important to consider leptospirosis can develop even in dogs vaccinated against the disease.

How to test for leptospirosis

Serology with MAT

Leptospira organisms are classified into serogroups. Each serogroup contains several serovars that share common antigens. The classical test for leptospirosis is serology with the microscopic agglutination test (MAT), which tests the serum for agglutinating antibodies against a panel of serovars. Frequently tested serovars include Icterohaemorrhagiae, Canicola, Bratislava, Hardjo, Pomona and Grippotyphosa.

Positive titres can develop after vaccination against leptospirosis, including titres for non-vaccinal serovars. Vaccinal titres are usually 1:800 or less, although titres as high as 1:3200 have been reported following vaccination, usually for short durations[4,5,8-10]. Vaccinal titres generally decline to low levels after three to four months following vaccination, but may persist for longer in some cases[1,4,5,8].

A single MAT titre ?1:800 to 1:1600 is generally considered to be consistent with a diagnosis of leptospirosis when associated with consistent clinical signs[3,4]. A lower or negative result does not exclude leptospirosis. Lower titres may indicate infection depending on the duration of clinical signs and the dog's vaccinal status; a diagnosis algorithm was recently published to help interpret MAT titres[9]. False-negative results can occur, particularly in the first one to two weeks of illness (prior to seroconversion) or if the infecting serovar is not included in the panel of serovars tested[1,5]. In dogs with positive or equivocal MAT results, or in dogs with an acute clinical presentation and negative serology results, blood and/or urinary PCR is indicated (see below).

A repeat serology can also be performed after two to four weeks; a four-fold change in titre confirms a diagnosis of leptospirosis[1,3-5]. However, a stable titre does not exclude leptospirosis, especially if the initial titre was high, as the titre may have already been at its maximum at the first test and then will remain high for several months[5]. The initial magnitude in titre or change in titre after two to four weeks does not correlate with the severity of the clinical illness[5].

While the highest titre may represent the infecting serovar, this is not reliable as there can be cross-reactivity between serovars. The highest titre may be toward a different serovar than the one

causing the infection[1,3]. Determination of the infecting serovar is useful for epidemiological studies, but does not affect treatment for an affected individual.

Immunofluorescence antibody test (IFA)

The IFA test commercially available in the UK (Biobest Laboratories) detects antibodies against the sheath antigen shared by pathogenic leptospires[11]. It does not allow determination of the infecting serovar. In a small clinical trial including limited numbers of dogs, this test detected all non-vaccinated dogs challenged with *Leptospira* and none of the vaccinated dogs[12].

This test is less costly than the MAT and results can be obtained more rapidly. However, data on the test's sensitivity and specificity in the field is not available. This test is unlikely to be as sensitive as PCR[4]. False positives may also occur with non-specific binding of the antibody[4].

PCR

PCR can be performed on whole blood and/or urine samples to detect DNA from leptospires. It does not allow determination of the infecting serovar. PCR can confirm the diagnosis of leptospirosis in acute cases when the serology may still be negative[4,5]. It is also useful to confirm the diagnosis in vaccinated dogs with a single positive MAT titre or in dogs with equivocal MAT results. PCR results are often available more rapidly than serology. MAT serology should also ideally be performed in all cases. Vaccination does not appear to affect the results of PCR; no dogs were positive on whole blood PCR shortly following vaccination in a study[10].

In the first 10 days of infection, the organism numbers are higher in the blood; after that, the numbers are higher in urine[1,3,5]. If the stage of infection is unclear, PCR should be performed on both blood and urine. False-negative results can occur with recent antibiotic treatment[1,3,5], so samples for PCR should be collected prior to antibiotic treatment. A negative result does not exclude leptospirosis in dogs having received antibiotics prior to sample collection[1,5]. Although PCR generally has a high sensitivity, false-negative results may occur when organism numbers are low[1,4]. False-positive results are unlikely to occur[4]; however, positive urinary PCR results will occur in asymptomatic carrier dogs, so a positive result does not always indicate clinical disease[1,5]. A study performed by the University of Dublin showed seven per cent of the tested dogs from shelter and university hospital environments were positive on PCR assessment of their urine, indicating asymptomatic carrier status with urinary shedding was relatively common in these populations[13].

• Fluorescent in-situ hybridisation (FISH) for the detection of leptospires can be performed on tissue samples, such as renal or hepatic biopsies. It is very sensitive and specific, but requires invasive tissue biopsies and is not commercially available.

• Silver staining of tissue samples and urine dark-field microscopy can be used to visualise leptospires, but these tests are poorly sensitive and not commonly performed.

Treatment for leptospirosis

The mainstay of treatment for leptospirosis is antibiotics. In severely ill animals, intravenous antibiotics are recommended; appropriate choices include intravenous amoxicillin-clavulanate (20mg/kg IV every six to eight hours) or penicillin G (25.000-40.000 units/kg IV every 12 hours)[1,5]. Intravenous cefuroxime is not effective against leptospirosis.

Amoxicillin-clavulanate and penicillin G are effective in resolving clinical illness, but do not reliably eliminate organisms from the kidneys and will not prevent development of a chronic renal carrier state[1,5]. Urinary shedding of live leptospires can persist for months in chronic renal carriers, so preventing this is essential.

Once the dog is clinically better, a two to three-week course of doxycycline (5mg/ kg orally every 12 hours) is recommended to clear the renal carrier state[1,3-5]. In dogs with mild illness, doxycycline can be used orally from the start and is then the only antibiotic required[1,3,5].

Supportive treatments (such as intravenous fluids, antiemetics, gastroprotectants and hepatoprotectants) are also frequently required, depending on the clinical presentation. Treatment with glucocorticoids has been shown to reduce mortality in humans with severe pulmonary leptospirosis; this requires further evaluation in dogs similarly affected[14]. Glucocorticoids may be beneficial in cases with severe secondary thrombocytopaenia[4] or severe secondary immune-mediated haemolytic anaemia, but may also cause a worsening of the condition due to immunosuppression; their role in such presentations requires further evaluation.

Urinary PCR is useful following treatment to ensure the organisms have been eliminated from the kidneys. A positive result does not always mean viable leptospires are present in the urine as dead leptospires may be detected following antibiotic treatment[1]. False negative results may occur as urinary shedding of leptospires can be intermittent[5]. However, PCR is the best test available for urinary shedding[1,5]. For safety reasons it is recommended to observe appropriate handling precautions in case of a positive result and to consider further antibiotic treatment with doxycycline until the PCR becomes negative.

Dogs sometimes develop a renal carrier state without developing any clinical signs of leptospirosis. For this reason and because leptospirosis is a serious zoonotic disease, treatment of all other dogs in the household or in-contact dogs is recommended with a two-week course of doxycycline[1].

Health and safety measures and handling precautions

In the hospital

As leptospirosis is a serious zoonotic disease, it is essential to have a high index of suspicion for leptospirosis in all dogs with potentially consistent clinical signs.

Appropriate precautions should be taken to handle any potentially affected dogs until leptospirosis can be excluded. Viable leptospires are present in the blood, urine and tissues of dogs with clinical leptospirosis[1]. The infection can also be transmitted via bite wounds[15]. While complete isolation of potentially affected dogs is not necessary as the organism is not airborne or very resistant, strict handling precautions for the dog and in-contact practice equipment such as bedding, food and water bowls should be observed.

Pregnant or immunosuppressed members of staff should not have any contact with the dog, its cage or kennel, blood or urine from the dog, or any in-contact equipment.

Potentially affected dogs should be barrier nursed strictly (using disposable long-sleeved gowns and gloves) to reduce the risk of disease transmission to veterinary staff and to other dogs; similar precautions should be observed when handling their bedding and in-contact practice equipment. Separate instruments such as thermometers and stethoscopes should be used.

A labelled clinical waste bag should be used to collect any potentially infected material. Care should be taken to avoid needle-stick injuries and other blood contact. Goggles and face masks should be used when there is a risk of spraying or aerosolisation of blood or urine, such as when washing the cage or kennel, changing urinary collection bags or collecting blood samples.

A urinary catheter should be placed in dogs with urinary incontinence to reduce contamination of the environment, and an appropriate disinfectant should be injected in urinary collection bags prior to disposal.

Dogs without a urinary catheter should be walked frequently in an area that is not accessible to other dogs and that can be easily decontaminated. A 10 per cent bleach solution or another appropriate disinfectant can be used to treat areas where the dog urinates[1]. Pressure washing of cages should be avoided as it could create urine aerosolisation. Staff should wear protective equipment to handle dirty bedding. Normal machine washing of bedding will inactivate leptospires[1]. Movements of the dog in the hospital should be minimised. If the dog has to be moved to another part of the clinic, such as for imaging, a trolley should be used and disinfected after use.

Out-patient dogs

In dogs treated as outpatients, owners should be instructed to avoid contact with the dog’s urine and to use disposable gloves when cleaning urine. Areas used for toileting should be limited to those strictly necessary, away from standing water and, if possible, in a private garden where no other animals or people have access. Areas where the dog urinates should be treated as described previously.

Strict hand washing is recommended after handling the dog. The dog should not be allowed in areas where food is stored or prepared. Contact with immunosuppressed people, pregnant women,

children, and people or other dogs outside of the household has to be avoided. Handling precautions for the potentially affected dog should be observed until tests for leptospirosis come back negative or, in cases of confirmed leptospirosis, until the end of urinary shedding.

Humans (including veterinary staff) having contact with an affected or potentially affected dog should see their GP immediately if they develop any signs of illness, particularly fever and 'flu-type signs, and mention the possibility of exposure to leptospirosis. Any in-contact dogs should be closely monitored at home and presented immediately to their vet if they develop any signs of illness.

Prevention of leptospirosis

Leptospirosis is endemic to the UK[16]. Almost all dogs are walked outdoors, where there is a potential risk of exposure to the disease. Vaccination is the most significant tool available to prevent the disease. It also has a role to play as a public health measure to prevent development of asymptomatic renal carrier dogs that excrete live leptospires in their urine.

Most available vaccines in the UK are effective at controlling clinical disease and preventing mortality. Only some claim to be able to reduce renal excretion following challenge, which is an important property in reducing the spread of this zoonotic disease[17]. Effective protection has not been shown to persist beyond 12 months[4],[17]. It is therefore appropriate to recommend vaccination against leptospirosis annually in all dogs in the UK.

Available vaccines contain inactivated strains of *Leptospira*. Canine leptospirosis vaccines have label claims to immunise against specific serovars and will not cross-protect against serovars from different serogroups. This is important as cases of leptospirosis do occur in vaccinated dogs; in such cases a non-vaccinal serovar is usually the cause of the disease. Most vaccines are licensed to immunise against the serovars Icterohaemorrhagiae and Canicola.

A tetravalent vaccine (Nobivac L4) was launched in the UK in 2013 that offers immunity to serovars in the four major serogroups (Icterohaemorrhagiae, Canicola, Australis, Grippotyphosa) that have been implicated in cases of canine leptospirosis in Europe[16]-[18]. This vaccine is likely to play an important role in the prevention of leptospirosis in the UK as there is a suspicion that cases of leptospirosis may have been caused by the serovar Bratislava in the south-west based on serological evidence (non-published data), and as this serovar has been isolated from dogs in the UK with nephritis or reproductive problems[16].

The antibody titres produced following vaccination are usually low and generally decline after three to four months following vaccination[1],[5],[8]. However, MAT titres cannot be used to predict protection against infection, and dogs can be protected against experimental challenge even when there are no or very low detectable circulating serum antibodies on MAT[5].

Dogs having recovered from leptospirosis should also be vaccinated as they are likely to remain susceptible to infection with other serogroups than the one which caused their disease, and as the duration of immunity following infection is unknown[1].

Can cats be affected?

The role of healthy carrier cats as a source of contamination, as well as the role of leptospires as a pathogen in cats, are probably underestimated.

Antibodies against *Leptospira* species are common in the feline population, with a prevalence ranging from zero per cent to 48 per cent of tested cats[5,19,20]. Urinary shedding of *Leptospira* has been demonstrated in asymptomatic cats with outdoor exposure[21,22].

Cats mostly become infected through transmission from hunting rodents. Experimental infection of cats results in mild disease with histological evidence of renal and hepatic inflammation[20]. Although clinical leptospirosis is rare in cats, several cases have been reported in the literature. The most common manifestation reported is renal disease due to an interstitial nephritis caused directly by the bacteria[20]. A case series of three cats with leptospirosis reported that all cats suffered from renal failure, while liver disease was not present[23]. The disease may be underdiagnosed as cats are rarely tested for leptospirosis.

There is no vaccine against leptospirosis in cats.

• The author wishes to point out her residency in small animal medicine was funded by MSD Animal Health, which produces the Nobivac L4 vaccine.

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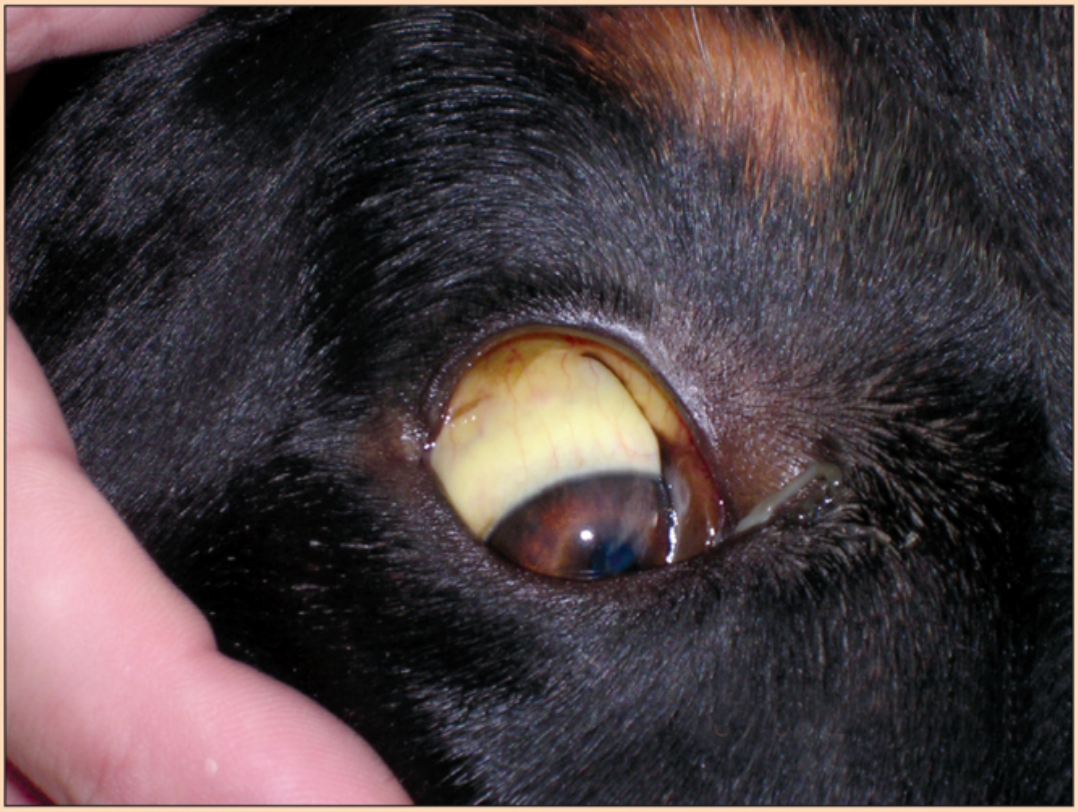
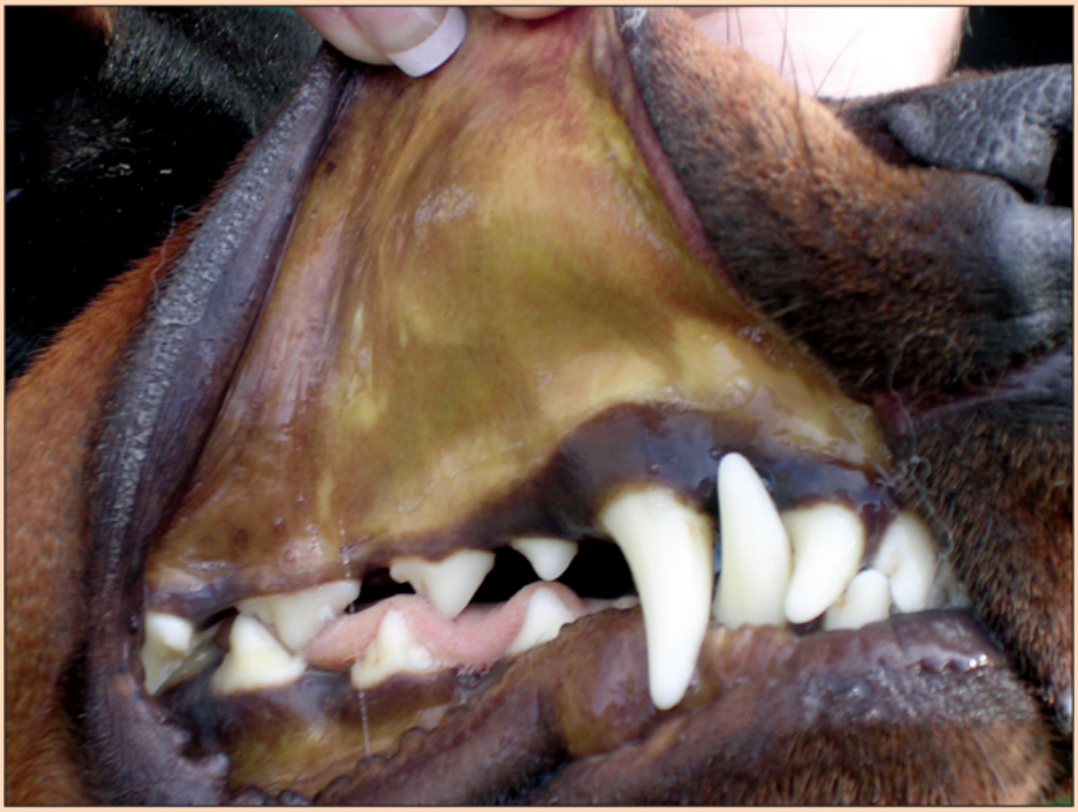
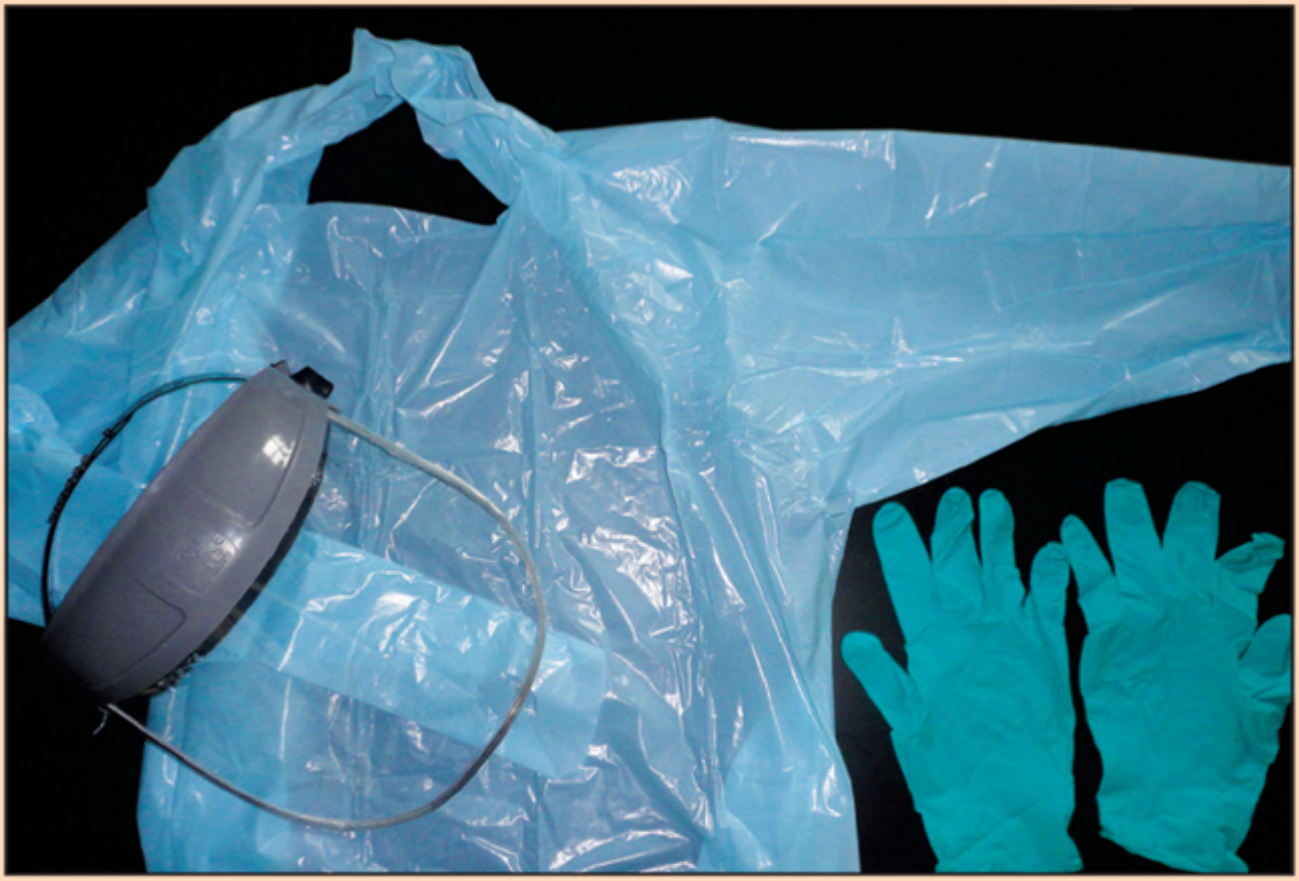
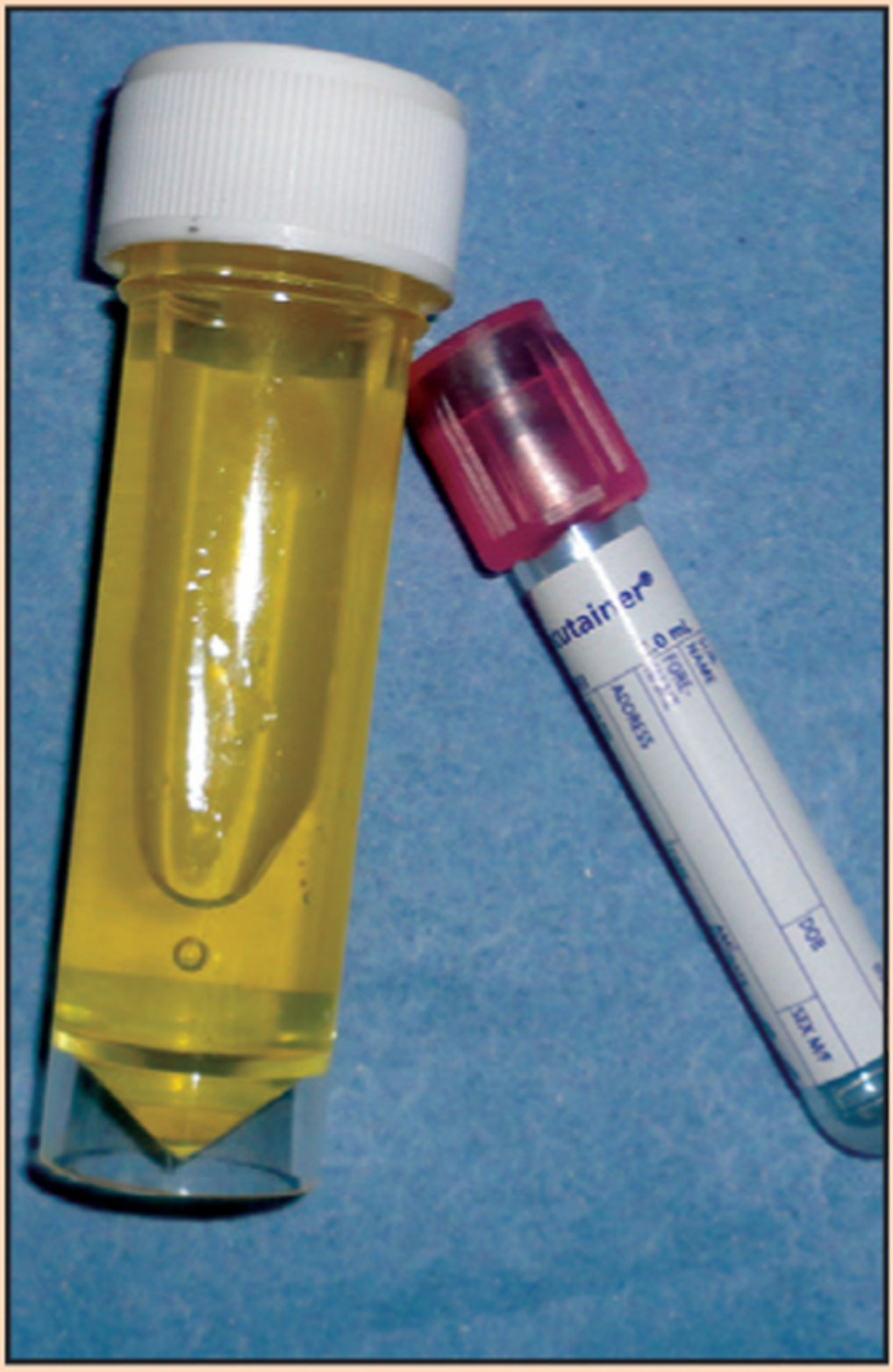




Top and above. Icterus in a dog.



Strict barrier nursing of potential leptospirosis cases is essential to avoid transmission to veterinary staff and other dogs.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Serology and PCR in blood and urine play a role in the diagnosis of leptospirosis.

Urinary signs may include acute kidney injury, chronic kidney disease, polyuria/polydipsia (with or without azotaemia) and/or haematuria
Acute or chronic hepatopathy or hepatic failure – Ultrasonographic findings can include a diffusely hyperechoic liver, liver masses or target lesions
Pyrexia
Generalised muscle discomfort, stiffness
Tachypnoea, dyspnoea and/or coughing due to interstitial pneumonia and/or pulmonary haemorrhage
Bleeding due to thrombocytopenia or coagulopathy (including petechiae, ecchymoses, haematemesis, melena, and/or epistaxis)
Uveitis
Myocarditis (with reduced cardiac systolic function and/or cardiac arrhythmia)
Intestinal intussusceptions
Anaemia (due to haemorrhage or immune-mediated haemolytic anaemia)
Abdominal pain (due to renal or hepatic inflammation or intestinal intussusceptions)
Peripheral lymphadenomegaly
Vasculitis with peripheral oedema, effusions (pleural, pericardial and/or peritoneal)
Abortion and infertility
Rare: meningitis (presented as neck or spinal pain, reluctance to move)
Rare: neurological signs of central nervous system disease, such as ataxia, vestibular disease, obtundation (either due to infarcts in the central nervous system or to the leptospirosis directly affecting the central nervous system)

Table 1. Possible clinical presentations of leptospirosis in dogs