REVIEW

European consensus statement on leptospirosis in dogs and cats

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Leptospirosis is a zoonotic disease with a worldwide distribution affecting most mammalian species. Clinical leptospirosis is common in dogs but appears to be rare in cats. Both dogs and cats, however, can shed leptospires in the urine. This is problematic as it can lead to exposure of humans. The control of leptospirosis, therefore, is important not only from an animal but also from a public health perspective. The aim of this consensus statement is to raise awareness of leptospirosis and to outline the current knowledge on the epidemiology, clinical features, diagnostic tools, prevention and treatment measures relevant to canine and feline leptospirosis in Europe.

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INTRODUCTION

Leptospirosis is a zoonotic disease with a worldwide distribution affecting most mammalian species (Bharti *et al.* 2003). Clinical leptospirosis is common in dogs but appears to be rare in cats (André-Fontaine 2006, Arbour *et al.* 2012). Both dogs and cats, however, can shed leptospires in their urine without showing clinical signs of the disease (Rojas *et al.* 2010, Fenimore *et al.* 2012, Llewellyn *et al.* 2013, Rodriguez *et al.* 2014). This is problematic as it can lead to exposure of humans. The control of leptospirosis, therefore, is important not only from an animal but also from a public health perspective. At the same time, dogs may serve as indicators of the presence of leptospires in specific environments.

In 2011, a small animal consensus statement on leptospirosis was published by the American College of Veterinary Internal Medicine, outlining the current opinion on leptospirosis, with a focus on canine leptospirosis in North America (Sykes *et al.* 2011). However, there are important differences in the epidemiology and vaccine availability between North America and Europe (Ellis 2010). Moreover, in recent years, the leptospiral pulmonary haemorrhage syndrome (LPHS) has emerged as a life-threatening complication of canine leptospirosis in some areas of Europe, whereas so far, there are fewer reports of LPHS from North America (Schweighauser and Francey 2008a, Kohn *et al.* 2010, Sykes *et al.* 2011, Tangeman and Littman 2013).

In September 2012, an expert panel was gathered by the International Society of Companion Animal Infectious Diseases (ISCAID) to discuss important aspects of canine leptospirosis in Europe and to develop a peer-reviewed, European consensus statement for practitioners. The aim of this consensus statement was to raise the awareness about leptospirosis and to outline the current knowledge on the epidemiology, clinical features, diagnostic tools, prevention and treatment measures relevant to canine and feline leptospirosis in Europe.

LEPTOSPIRA: THE PATHOGEN

Leptospirosis is caused by infection with pathogenic spirochaete bacteria of the genus *Leptospira*. Leptospires are Gram negative, highly motile, elongated, helically coiled bacteria. The organism can be differentiated from other spirochaetes by their distinct hook or question mark–shaped ends (Faine *et al.* 1999) (Fig 1). The fairly complex taxonomy of the genus *Leptospira* is outlined in Table 1. The terms commonly used in the serological classification of leptospires are defined in Table 2.

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Table 1. Classification and Nomenclature of *Leptospira spp*

To understand the rather complex taxonomy of leptospires, it is useful to look back into the history of *Leptospira* typing. Originally, the genus *Leptospira* was divided into two species:

- *Leptospira interrogans* sensu lato (pathogenic strains)
- *L. biflexa* sensu lato (saprophytic, non-pathogenic strains)

This division was based on the phenotypic and growth characteristics as well as the pathogenicity of the organism. For example, saprophytic strains grow in the presence of the purine analogue 8-azaguanine and at low ambient temperatures (11–13°C), whereas pathogenic strains do not. More extensive phenotypic criteria, such as chemical properties and activities, that are commonly used for subclassification of other bacteria are largely unsuitable for *Leptospira*. Before the development of molecular typing methods, further subclassification into serovars was, therefore, almost exclusively based on serological determination of differences in the carbohydrate component of the leptospiral lipopolysaccharide using specific antisera (Faine et al. 1999). Antigenically related serovars were then grouped into serogroups. Currently, more than 250 known pathogenic serovars have been identified belonging to 24 serogroups (Ko et al. 2009).

More recently, genotypic classification based on DNA hybridization has defined 20 species of *Leptospira* including 9 pathogenic, 6 saprophytic and 5 intermediate species, and new species are being added as they are discovered. Unfortunately, the genetic classification of *Leptospira* species does not entirely correlate with the serological classification because serovars of the same serogroup may belong to different genomic species. However, the serological classification is still widely used. Different serovars are considered to be adapted to specific reservoir hosts. Thus, their recognition is important from an epidemiological perspective.

The accepted nomenclature is the name of the genus, followed by species name, followed by serovar, followed by strain (if appropriate). Genus and species are italicized, with the serovar name not italicized and with an upper case first letter.

For example: — *Leptospira interrogans* serovar Australis
— *Leptospira biflexa* serovar Patoc

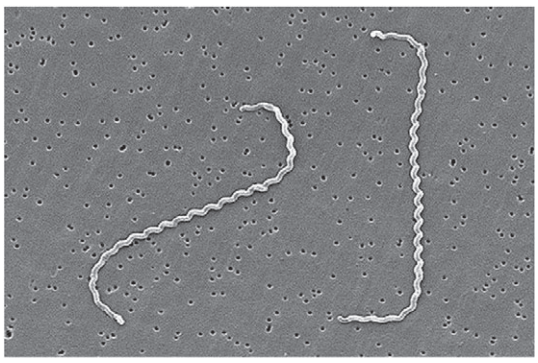

FIG 1. Scanning electron micrograph of *Leptospira interrogans* strain RGA. Image source: Public Health Image Library CDC/NCID/Rob Weyant (http://phil.cdc.gov/phil/details.asp)

Table 2. Definitions

Serovar	Member of the genus *Leptospira*, which reacts with a specific monoclonal antiserum. Antisera are specific to immunogenic carbohydrate antigens of leptospiral lipopolysaccharide.
Serogroup	Group of antigenically closely related leptospiral serovars. Members of the same serogroup agglutinate when incubated with patient serum containing antibodies to one serovar of the same serogroup.
Strain	Specific isolate of a defined leptospiral serovar

EPIDEMIOLOGY

Leptospires can survive for months in water and moist soil (Alexander 1975). Incidental hosts become infected either by direct contact of mucous membranes or broken skin with the urine from infected animals or by indirect contact with contaminated soil or surface water, and can develop acute, severe disease (Levett 2001) (Fig 2). In contrast, reservoir hosts generally do not show any clinical signs after infection with pathogenic *Leptospira* but can harbour leptospires in their renal tubules for prolonged periods of time from which they are shed into the environment via urine (Fig 3). Small rodents are considered the most important reservoir hosts. However, it is likely that every known species of rodent, marsupial, or mammal, including humans, can act as reservoir host for pathogenic *Leptospira* (Faine *et al.* 1999, Ganoza *et al.* 2010). A number of known relationships between reservoir hosts and host-adapted leptospiral serovars are listed in Table 3.

Dogs have been known to be hosts for pathogenic leptospires for over 80 years (Klarenbeek 1933). While infection was most commonly associated with the presence of antibodies to the serogroups Canicola and Icterohaemorrhagiae, it is now clear that dogs are susceptible to infection with a wide range of serovars. Based on the available antibody prevalence data, the major serogroups to which dogs in Europe seroconvert to are Icterohaemorrhagiae, Grippotyphosa, Australis, Sejroe and Canicola (Ellis 2010). Seroconversion of dogs to the serogroup Grippotyphosa is common in continental Europe, but appears to be rare in the UK and Ireland. This might be explained by the distribution of relevant reservoir hosts (Ellis 2010).

Leptospirosis is considered a seasonal disease, with human and animal outbreaks being linked to heavy rainfall or flooding (Faine *et al.* 1999, Ward 2002). A recent study assessing the seasonality of canine leptospirosis in four different regions in the USA showed that seasonal patterns are region-dependent, and supports a link between the amount of rainfall and the occurrence of leptospirosis in dogs (Lee *et al.* 2014). Similarly, the number of acute leptospirosis cases per month was correlated with the average monthly temperature (r^2 0.73, $P<0.001$) and the average rainfall (r^2 0.39, $P<0.001$) in a cohort of 256 dogs from Switzerland that were presented to a referral hospital (Major *et al.*

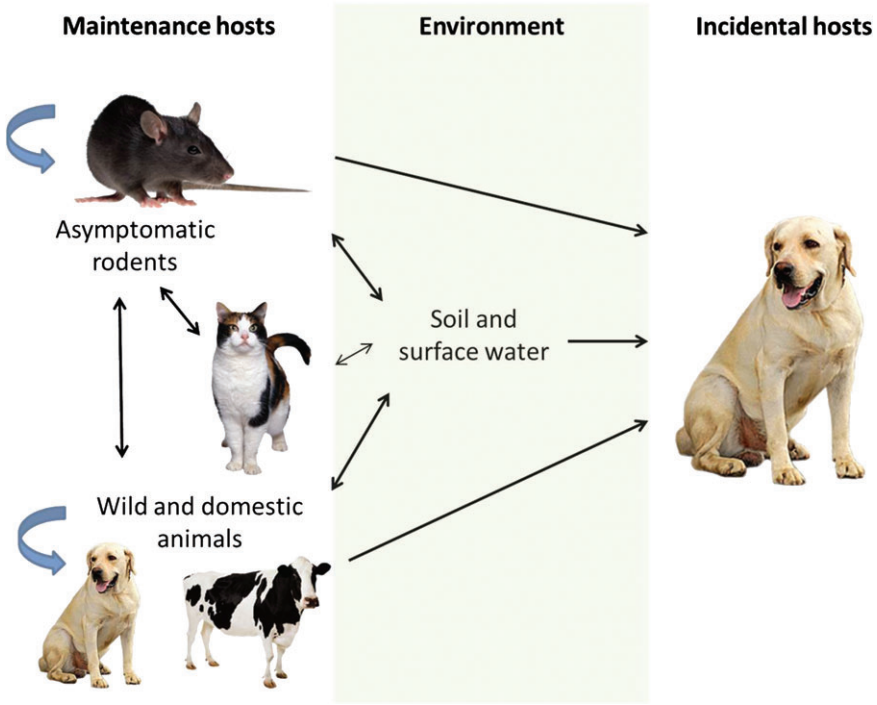

FIG 2. Transmission cycle of pathogenic *Leptospira* spp. Pathogenic leptospires are maintained in the environment by wild or domestic reservoir hosts. Incidental hosts become infected via either direct contact with reservoir hosts or contaminated soil and surface water. Cats are probably more likely to become infected via contact with prey due to their natural aversion to water. The role of dogs and cats as reservoir hosts requires further study

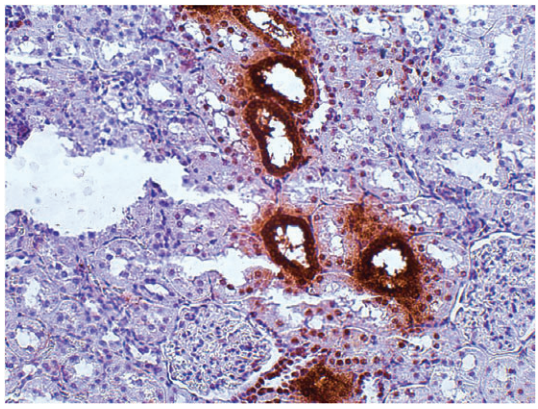

FIG 3. *Leptospira* in chronically infected renal tissue. Immunohistochemical staining for leptospiral outer membrane vesicles reveals entire organisms adhering to renal tubular cells. Leptospiral antigen is also present intracellularly in tubular epithelial cells and in the interstitium surrounding the affected tubules. Rat kidney (IHC; x200)

2014) (Fig 4). Consistent with the transmission cycle of leptospires, clinically affected dogs in the USA were more likely to be living in the proximity of outdoor water, swimming or drinking from outdoor water sources and having indirect exposure to wildlife (Ghneim *et al.* 2007). In a study from Italy, clinically healthy dogs living in kennels had a higher prevalence of antibodies to *Leptospira* spp. than dogs that were presented for veterinary check-ups (Scanziani *et al.* 2002). Similarly, dogs living in shelters had a slightly higher prevalence of urinary shedding of pathogenic leptospires compared with a general referral hospital dog population in Ireland (Rojas *et al.* 2010). This is likely due to crowding and potentially poor hygiene standards facilitating dog-to-dog transmission.

Analysis of risk factors for acute leptospirosis in dogs has yielded conflicting results and might be subjected to temporal changes (Lee *et al.* 2013). Males, herding dogs, hounds, working dogs and mixed breeds have been reported to be at an increased risk in the USA (Ward *et al.* 2002). In a cohort of dogs from Switzerland, puppies (<1 year) and male dogs were significantly over-represented compared with the general dog population ($P<0.001$) (Major *et al.* 2014). However, in other studies, sex, age or breed were not identified as risk factors for acute leptospirosis (Alton *et al.* 2009, Lee *et al.* 2013). In a recent study in the USA using the Veterinary Medical DataBase (VMDB), dogs weighing less than 6.8 kg (15 lbs) and, in particular, Yorkshire terriers had the highest hospital prevalence of leptospirosis between 2000 and 2009. This may be due to the fact that small breeds are suspected to have a higher risk for adverse effects following vaccination (Moore *et al.* 2005) and, therefore, are more likely not to be vaccinated. Alternatively, it could be speculated that this type of dog likely has a very close relationship with their owner and, therefore, is more likely to be presented to a veterinary hospital for treatment. **Based on the above findings, the panel recommends that practitioners should consider leptospirosis as a possible diagnosis regardless of the signalment of the patient.**

In cats, exposure to several serogroups has been identified, including Icterohaemorrhagiae, Canicola, Grippotyphosa, Pomona, Hardjo, Autumnalis, Ballum and Bratislava. The prevalence of

Table 3: Typical reservoir hosts of common leptospiral serovars (adapted from Bharti *et al.*, 2003).

Reservoir host	Host-adapted serovars
Pig	Pomona, Tarassovi
Cattle	Hardjo, Pomona
Horse	Bratislava
Dog	Canicola
Sheep	Hardjo
Rat	Icterohaemorrhagiae, Copenhageni
Mouse	Ballum, Arborea, Bim
Bat	Cynopteri, Wolffi

antileptospiral antibodies ranged between 0 and 48% (Larsson *et al.* 1984, Dickeson and Love 1993, Agunloye and Nash 1996, Mylonakis *et al.* 2005, André-Fontaine 2006, Markovich *et al.* 2012, Rodriguez *et al.* 2014). It has been suggested that cats are more likely to become infected by catching rodents harbouring leptospires rather than by contaminated water, due to their natural aversion to water (Shophet and Marshall 1980, Hartmann *et al.* 2013). No association has been found between the presence of antileptospiral serum antibodies and sex and/or breed. However, an association with age has been reported in several studies with older cats being more likely to have antileptospiral serum antibodies (Larsson *et al.* 1984, Mylonakis *et al.* 2005, Rodriguez *et al.* 2014). Antibody prevalence has been reported to be higher in outdoor cats, cats living in urban areas, cats that are known hunters and cats that live with another cat in the same household (Rodriguez *et al.* 2014). Several new studies have demonstrated that cats can shed leptospires in their urine and might, therefore, represent reservoir hosts of leptospires (Fenimore *et al.* 2012, Rodriguez *et al.* 2014).

PATHOGENIC MECHANISMS OF LEPTOSPIROSIS

After entering the host, pathogenic leptospires quickly establish a systemic infection via haematogenous spread. Unlike bloodstream infections with other Gram-negative bacteria, leptospires do not cause fulminant disease shortly after the onset of infection. This has been attributed to the low endotoxic potential of leptospiral lipopolysaccharide (Werts *et al.* 2001). During this initial phase, leptospires evade the host immune response by binding inhibitors of complement activation on their surface (Meri *et al.* 2005, Barbosa *et al.* 2009). Leptospiraemia continues until the host mounts an effective acquired immune response, which clears the organism from the bloodstream and most tissues. Thereafter, leptospires can persist in the immune-privileged sites, such as the eye and the renal tubules (Levett 2001).

Leptospirosis is a multi-systemic disease, affecting, in particular, the kidneys and the liver, but it also affects many other organs, such as the lungs, spleen, endothelial cells, uvea/retina, skeletal and heart muscles, meninges, pancreas and the genital tract. The exact mechanisms through which pathogenic leptospires cause organ dysfunction and tissue damage are not known and can vary among different organ systems. While vasculitis can be a feature in some cases of leptospirosis, most studies in humans and experimental animals do not support vasculitis as a constant primary event responsible for tissue damage (Medeiros Fda *et al.* 2010).

During the acute phase of leptospirosis, the predominant renal lesions are those of an acute interstitial nephritis, with tubular cell necrosis, apoptosis and regeneration (Nally *et al.* 2004, De Brito *et al.* 2006). However, glomerular abnormalities have been described in both dogs and experimental animals with leptospirosis, which indicate the structural and functional glomerular

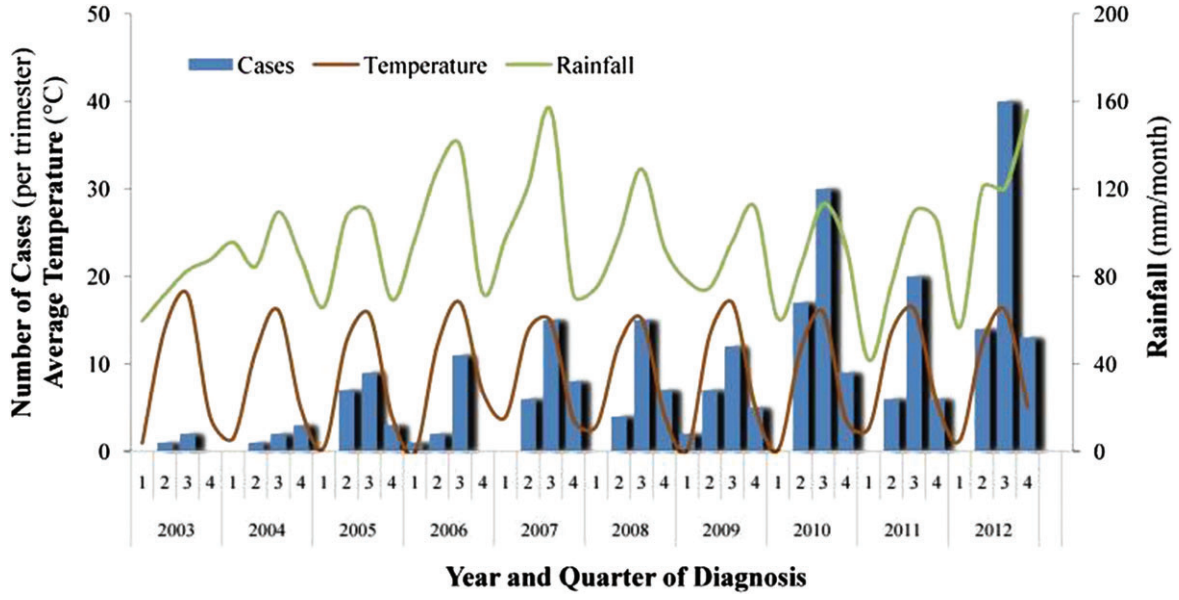

FIG 4. Distribution of 256 cases of leptospirosis by quarters for the 10 mainly affected cantons (2003–2012) and the corresponding temperature and rainfall curves (Major *et al.* 2014)

involvement (Mastrorilli *et al.* 2007, Schuller 2013). Tubular lesions are assumed to be due to direct effects of the organisms because renal lesions are generally associated with the presence of *Leptospira* (De Brito *et al.* 2006), and leptospiral outer membrane components have been shown to induce cell damage and inflammation in tubular epithelial cells in vitro (Yang *et al.* 2000). During this phase of infection, a clinically significant reduction in renal function is present in most, but not all, patients with leptospirosis (Levett 2001, Geisen *et al.* 2007).

The liver is another major organ damaged by leptospires. Histopathologically, a cholestatic hepatitis with complete or partial liver plate disruption, hepatocellular necrosis, binucleation of hepatocytes, periportal oedema with acute and chronic inflammatory cell infiltration and proliferation of Kupffer cells along the sinusoidal lining have been described (Nally *et al.* 2004; De Brito *et al.* 2006). Hyperbilirubinaemia was not correlated with hepatocellular necrosis in humans (Ramos-Morales *et al.* 1959). Hyperbilirubinaemia, however, coincided with the invasion of hepatic intercellular junctions by migrating leptospires and the subsequent disruption of bile canaliculi in experimentally infected hamsters (Ramos-Morales *et al.* 1959, Miyahara *et al.* 2014). In human patients, both icteric and non-icteric forms of leptospirosis have been described, the icteric form being considered more severe and rapidly progressive (Levett 2001). This may also be true in dogs. In a cohort of 254 dogs with acute leptospirosis, a serum bilirubin of at least 10 µmol/L (reference range 0.5–4.0 µmol/L) was strongly associated (OR 16.4; P<0.001) with a negative outcome (death or euthanasia) (Major *et al.* 2014).

LPHS is a severe manifestation of acute leptospirosis, which has been increasingly recognized in dogs and many other species in recent years (Kohn *et al.* 2010, Major *et al.* 2014). Histopathological lesions of LPHS lung tissue are similar across species and are characterized by various degrees of intra-alveolar haemorrhage in the absence of a marked inflammatory cell infiltrate or vasculitis (Nicodemo *et al.* 1997, De Brito *et al.* 1979, Nally *et al.* 2004) (Fig 5). Intra-alveolar oedema, fibrin and hyaline membranes, which are characteristic of disorders with diffuse alveolar damage such as acute respiratory distress syndrome (ARDS), can also be present, but are not a predominant feature (Nicodemo *et al.* 1997, Salkade *et al.* 2005, Croda *et al.* 2010, Klopfleisch *et al.* 2010). In contrast to liver and kidney, few leptospires are observed in the affected lung tissue in immunocompetent hosts and do not co-localize with the pulmonary lesions (Nally *et al.* 2004). The pathogenic mechanisms of LPHS are poorly understood. Several hypotheses, including systemic inflammatory, immune-mediated and direct leptospiral effects, are currently under investigation (Table 4). It is likely that the pathogenic mechanisms of LPHS are multi-factorial, with both host- and pathogen-related factors playing a role (Medeiros Fda *et al.* 2010).

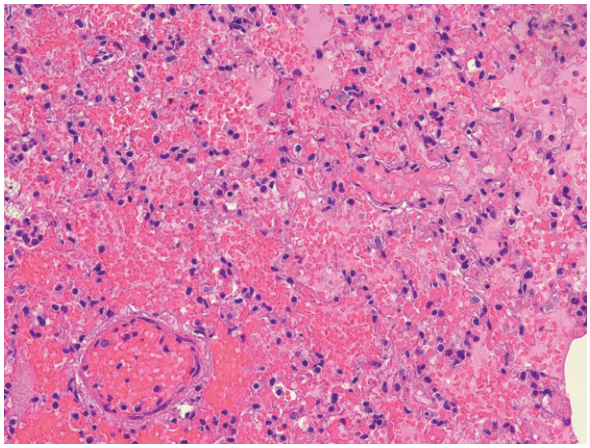

FIG 5. Lung tissue from a dog affected by LPHS. Extensive intra-alveolar haemorrhage is present in the absence of significant inflammatory cell infiltrates (H&E, x400)

It has been suggested that introduction of clones with enhanced virulence might be a contributing factor to the recent emergence of LPHS in humans (Ko *et al.* 2009). However, at present, available evidence to link specific leptospiral serovars with particular clinical manifestations in both humans and animals is weak (Triger 2004, Goldstein *et al.* 2006, Geisen *et al.* 2007, Medeiros Fda *et al.* 2010, Sykes *et al.* 2011). This may be partially due to the limitations of the current antibody tests, such as the MAT, to identify the infecting serogroup or serovar in acutely infected patients (Levett 2003, Miller *et al.* 2011).

Table 4. The leptospiral pulmonary haemorrhage syndrome (LPHS)

In recent years, LPHS has emerged as a severe form of leptospirosis in many species including humans and dogs. Patients with LPHS can develop fulminant pulmonary haemorrhage leading to high mortality. LPHS has been reported in cohorts of dogs from in Switzerland (Schweighauser *et al.* 2008; Major *et al.* 2014) and north eastern Germany (Kohn *et al.* 2010).
The pathogenic mechanisms of LPHS are poorly understood. It is likely that LPHS has a multi-factorial pathogenesis involving both host- and pathogen-related factors (Medeireios Fda *et al.* 2010).
It has been hypothesized that LPHS is caused by an increase in alveoar permeability due to the direct effects of pathogenic leptospires on host endothelial cells. Evidence from in vitro studies suggests that pathogenic leptospires bind to important endothelial adhesion molecules such as VE-Cadherin (Evangelista *et al.* 2014) and are able to induce changes in the expression of host proteins involved in cellular architecture and adhesion (Martinez-Lopez *et al.* 2010). While these mechanisms might primarily serve to facilitate tissue invasion by the pathogen, it is possible that they trigger a cascade of events culminating in LPHS.
Alternatively, it has been proposed that abnormal sodium transport by alveolar epithelial cells could be a cause of impaired pulmonary fluid handling, which could lead to lung injury. This hypothesis is based on a study documenting downregulation of the epithelial sodium channel and upregulation of the NaK_2Cl co-transporter NKCC1 in a hamster model of LPHS (Andrade *et al.* 2007).
However, there is also evidence to suggest that there is an involvement of the host immune response in the pathogenesis of LPHS. Deposition of antibody (IgG, IgM, IgA) and complement C3 has been documented in the alveolar basement membrane in an experimental guinea pig model (Nally *et al.* 2004) and in the alveolar surfaces and alveoar septae of naturally infected humans (Croda *et al.* 2010) in the absence of leptospiral antigen. Deposition of IgG and IgM was also present in lung tissues of naturally infected dogs with LPHS (Schuller 2013).

CLINICAL FINDINGS

Infection with pathogenic leptospires can lead to a wide range of clinical manifestations from subclinical to severe, and potentially lethal disease. The outcome of acute infection depends on the age and immune response of the host, and the virulence and inoculum size of the pathogen (Levett 2001). The incubation period until the development of clinical signs, such as fever, lethargy and inappetence, is approximately seven days in experimental studies, but can vary according to the immunocompetence of the host, infecting dose and serovar (Greenlee *et al.* 2005, Greenlee *et al.* 2004).

The most common clinical signs described in different case studies from Europe and the USA are listed in Table 5. Studies were included if the diagnosis of leptospirosis was based on positive polymerase chain reaction (PCR) results in blood or urine, high (≥1:800 in most studies) or increasing MAT titres and/or histopathological detection of leptospires by Levaditi silver staining.

The predominant clinical signs of acute leptospirosis relate to the presence of acute kidney injury (AKI) and liver impairment. In human patients with LPHS, respiratory signs can be the predominant initial clinical presentation (Trevejo *et al.* 1998), and this can very occasionally also be the case in dogs (Francey, unpublished data). In a recent study assessing the main organ manifestations (renal, hepatic, pulmonary, haemorrhagic) in 298 dogs with acute leptospirosis, 99.7% showed renal involvement, 35.4% hepatic involvement (as indicated by hepatic hyperbilirubinaemia), 68.8% pulmonary involvement and 18.4% showed signs consistent with disseminated intravascular coagulation (DIC). Although most dogs (43.6%) demonstrated involvement of two different systems, 24.5% had involvement of only one organ, 23.2% had involvement of three organ systems and 8.7% involved all the four organ systems (Major *et al.* 2014).

The clinical signs related to renal involvement include polydipsia and polyuria (PU/PD), which can develop with or without concurrent azotaemia, and can be a consequence of tubular dysfunction or an acquired vasopressin resistance of the inner medullary collection ducts (Magaldi *et al.* 1992). Leptospires can cause a specific hypokalaemic, non-oliguric form of acute renal failure due to the inhibition of the Na^+-K^+ ATPase (Seguro *et al.* 1990). Oliguric/anuric renal failure has been reported to develop in approximately 30% of dogs with acute leptospirosis (Major *et al.* 2014). Hepatic involvement can vary from mild liver enzyme elevations with or without hyperbilirubinaemia to severe liver failure with signs of hepatic encephalopathy (Greene 2012).

Fever can occur early in the course of disease and can be accompanied by pain, reluctance to move, weakness and a stiff gait (Poncelet *et al.* 1991, Kohn *et al.* 2010). Pain can be caused by myositis, meningitis and/or inflammation within other organs, such as the kidneys and the pancreas (Greene 2012).

Respiratory signs, such as tachypnoea and mild-to-severe dyspnoea, can occur in dogs with leptospirosis for many reasons, including pulmonary oedema due to overhydration, aspiration pneumonia, pain or acidosis; however, clinicians should also consider LPHS as a cause of dyspnoea in leptospirosis patients. Dogs with LPHS develop multi-focal intra-alveolar haemorrhage, which can be rapidly progressive and lead to massive haemoptysis and respiratory failure. LPHS is associated with mortality rates of up to 70%. Intra-alveolar haemorrhage can be detected even in dogs without overt respiratory signs (Kohn *et al.* 2010). Therefore, LPHS might be more common in dogs with leptospirosis than generally believed.

Pancreatitis is a described sequel to leptospirosis in human patients (Ranawaka *et al.* 2013). Pancreatitis can develop in dogs with acute leptospirosis and can explain the acute abdominal discomfort as well as anorexia and vomiting in dogs in which azotaemia and jaundice have resolved (Greene 2012).

Intestinal intussusception as a complication of acute leptospirosis, presumably associated with gastrointestinal inflammation and motility disorders (paralytic ileus), has been described in several case reports (Schweighauser 2009, Schulz *et al.* 2010).

Evidence of bleeding, such as haemoptysis, epistaxis, haematemesis, haematochezia, melaena, haematuria and petechiae, has been recognized in association with canine leptospirosis (Rentko *et al.* 1992, Birnbaum *et al.* 1998, Goldstein *et al.* 2006, Mastrorilli *et al.* 2007, Kohn *et al.* 2010). Disorders of the primary and/or secondary haemostasis play variable roles. It needs to be emphasized that animals with LPHS can show severe intra-alveolar haemorrhage in the absence of a systemic haemostatic disorder (Nally *et al.* 2004).

Cardiac manifestations have been described in humans and dogs with leptospirosis (Mastrorilli *et al.* 2007). Electrocardiographic abnormalities, such as ventricular tachyarrhythmias, and elevations of serum troponin concentrations in some dogs suggest myocardial damage (Mastrorilli *et al.* 2007). Myocarditis has been reported in humans who had died from leptospirosis (Shah *et al.* 2010).

In humans, neurologic involvement is a known complication of leptospirosis (de Souza *et al.* 2006). Aseptic meningitis has been described in up to 25% of humans with leptospirosis (Levett 2001), but there are no confirmed reports of meningitis/meningoencephalitis in association with canine leptospiral infections.

Uveitis is commonly recognized in humans and horses, and has been associated with persistence of leptospires in the vitreous humour, subsequent chronic inflammation and cross-reactivity of antileptospiral antibodies with intraocular antigens (Levett 2001, Brandes *et al.* 2007, Verma *et al.* 2008, Verma *et al.* 2012). In dogs with leptospirosis, different ophthalmological abnormalities, such as increased lacrimation, mucopurulent discharge, reduced pupillary reflexes, conjunctivitis, pan-uveitis, scleral injection, aqueous flare, hyphaema, papilloedema, retinal detachment and retinal haemorrhages, have been described (Keenan *et al.* 1978, Martins *et al.* 1998, Townsend *et al.* 2006).

Young dogs with leptospirosis have been reported to develop severe systemic or skin calcifications (Munday *et al.* 2005, Michel *et al.* 2011).

There are only a few reports of reproductive disorders in dogs in relation to leptospiral infection. Abortion and infertility were associated with serovar Bratislava infection in a dog (Ellis 1986). Serovar Buenos Aires (serogroup Djasiman) was isolated from an aborted foetus of an infected bitch (Rossetti *et al.* 2005).

Table 5. Clinical findings in dogs with leptospirosis

Reference	Rentko *et al.* 1992	Harkin *et al.* 1996	Birnbaum *et al.* 1998	Adin *et al.* 2000	Prescott *et al.* 2002	Goldstein *et al.* 2006	Steger-Lieb *et al.* 1999	Mastrorilli *et al.* 2007	Geisen *et al.* 2007	Gerlach *et al.* 2007	Kohn *et al.* 2010	Tangeman & Littman 2013
Country	**USA Massachusetts**	**USA New Jersey**	**USA New York**	**USA California**	**USA Ontario**	**USA New York**	**Switzerland**	**Italy**	**South Germany**	**North Germany**	**Northeast Germany**	**USA New Orleans**
Number of dogs	n=17	n=17	n=36	n=36	n=31	n=55	n=11	n=16	n=42	n=39	n=50	n=51
Anorexia % (n)	NR	NR	67 (24)	68 (24)	81 (25)	75 (41)	73 (8)	69 (11)	76 (32)	R	84 (42)	57 (29)
Vomiting % (n)	NR	NR	50 (18)	88 (33)	81 (25)	64 (35)	82 (9)	81 (13)	57 (24)	R	72 (36)	41 (21)
Lethargy % (n)	24 (4)	88 (15)	58 (21)	65 (23)	90 (28)	78 (43)	18 (2)	87,5 (14)	81 (42)	R	90 (45)	43 (22)
Abdominal pain % (n)	29 (5)	35 (6)	33 (12)	42 (15)	65 (20)	22 (12)	45 (5)	37,5 (6)	19 (8)	NR	36 (18)	NR
Diarrhoea % (n)	24 (4)	6 (1)	33 (12)	NR	NR	29 (16)	36 (4)	38 (6)	40 (17)	NR	50 (25)	12 (6)
Jaundice % (n)	NR	35 (6)	11 (4)	NR	29 (9)	13 (7)	36 (4)	17 (2)	45 (18)	R	10 (5)	NR
Dehydration %(n)	NR	NR	36 (13)	NR	52 (16)	26 (14)	27 (3)	NR	31 (13)	NR	6 (3)	NR
Stiffness /musculo-skeletal pain % (n)	12 (2)	17 (3)	25 (9)	23 (9)	35 (11)	NR	NR	44 (7)	NR	NR	8 (4)	2 (1)
Fever (rectal temp. ≥39.5°C) % (n)	6 (1)	6 (1)	11 (4)	15 (4)	13 (4)	9 (5)	18 (2)	19 (3)	36 (15)	R	8 (4)	NR
Hypothermia (rectal temp <38°C) % (n)	12 (2)	12 (2)	NR	22 (8)	NR	NR	36 (4)	38 (6)	17 (7)	NR	6 (3)	NR
Oliguria/ anuria % (n)	NR	NR	6 (2)	39(14)	NR	NR	18 (2)	44 (7)	NR	NR	20 (10)	4 (2)
Dyspnea/ tachypnea % (n)	6 (1)	NR	3 (1)	NR	35 (11)	NR	NR	44 (7)	NR	NR	38 (19)	2 (1)
Weakness % (n)	NR	NR	39 (14)	NR	NR	NR	18 (2)	NR	52 (21)	NR	NR	NR
PU/PD % (n)	NR	NR	50 (18)	NR	NR	31 (17)	27 (3)	NR	NR	NR	NR	NR
Weight loss % (n)	NR	NR	44 (16)	NR	NR	35 (19)	9 (1)	NR	17 (7)	NR	NR	NR

NR not reported, R reported, no numbers given

The role of leptospirosis as a cause of chronic kidney disease (CKD) in both cats and dogs requires further studies. Progression of tubulo-interstitial nephritis to tubular atrophy and renal fibrosis has been described in dogs infected with serovar Canicola (McIntyre 1952) and in rats infected with serovar Icterohaemorrhagiae (Sterling and Thiermann 1981). In a recent study, cats with kidney disease (acute and chronic) were more likely to have antibodies to *Leptospira* spp. and to shed pathogenic leptospires in their urine than cats without kidney disease (Rodriguez *et al.* 2014) which could support a link between leptospiral infection and kidney disease in cats.

Chronic hepatitis has been described in case reports in association with infection by serovars Grippotyphosa (Bishop *et al.* 1979) and Australis (Adamus *et al.* 1997). Amplification of leptospiral DNA from liver biopsies of dogs with chronic hepatitis was, however, unrewarding (Boomkens *et al.* 2005). At present, it is, therefore, not clear whether *Leptospira* spp. can be the causative agent of chronic hepatitis in dogs.

HAEMATOLOGY, CLINICAL BIOCHEMISTRY, URINALYSIS

Common haematological abnormalities are shown in Table 6. When first examined by a veterinarian, the majority of dogs present with a leucocytosis with WBC counts of up to 40×10^9/L. During the course of disease, leukaemoid reactions with WBC counts $>80\times10^9$/L have been reported (Kohn *et al.* 2010). In the leptospiraemic phase, a leucopenia can be encountered. Differential cell counts often reveal neutrophilia, sometimes with a left shift, lymphopenia and monocytosis.

Mild-to-severe thrombocytopenia is common in dogs with leptospirosis can raise the level of suspicion of leptospirosis in dogs with AKI. Low platelet counts can be caused by consumption due to activation, adhesion and aggregation to a stimulated vascular endothelium (Nicodemo *et al.* 1997), Kupffer cell phagocytosis (Yang *et al.* 2006), immune-mediated platelet destruction (Davenport *et al.* 1989, Kohn 2000) or splenic sequestration.

Approximately half of the dogs with leptospirosis present with anaemia, which is mostly mild to moderate. Causes of anaemia can be blood loss via the respiratory or the gastrointestinal tract and anaemia of inflammatory disease. Haemolysis due to the effect of leptospiral toxins on erythrocytic membranes appears to be rare in dogs compared with to cattle (Lee *et al.* 2000).

The most common biochemical abnormalities are shown in Table 7. Blood urea and creatinine concentrations are increased in the majority of dogs at presentation or during the course of disease. Hepatic injury as evidenced by increases in the activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and hyperbilirubinaemia almost exclusively occurs in conjunction with azotaemia (Goldstein *et al.* 2006, Geisen *et al.* 2007). Increases in the serum activity of ALP and total bilirubin are more frequent than increases in serum ALT activity (Table 7).

Electrolyte abnormalities, such as hypo- and hyperkalaemia, hyper- and hypophosphataemia, hyponatraemia and hypochloraemia, are known to be common in canine leptospirosis. They usually parallel the degree of renal and gastrointestinal dysfunction. Hypokalaemia can occur due to renal and/or gastrointestinal losses (Rentko *et al.* 1992, Goldstein *et al.* 2006), as well as potassium wasting due to the leptospiral-induced inhibition of the Na^+-K^+-ATPase (Seguro *et al.* 1990).

Increases of creatine kinase (and AST) activity and troponin I were reported in 44% and 69% of dogs with leptospirosis, which suggest skeletal and myocardial injury, respectively (Mastrorilli *et al.* 2007).

Increased activities of amylase and lipase can be caused by pancreatitis or enteritis, but can also reflect decreased renal excretion of these enzymes (Rentko *et al.* 1992, Mastrorilli *et al.* 2007).

Various abnormalities of haemostatic parameters have been reported in dogs with acute leptospirosis indicating that both hyper- and hypocoagulable states can occur (Mastrorilli *et al.* 2007, Francey *et al.* 2013). In one study 14% of dogs demonstrated thrombocytopenia together with prolongation of PT and aPTT leading to a suspicion of DIC (Kohn *et al.* 2010). Fibrinogen concentrations were found to be increased in 75% of dogs, consistent with an acute phase response (Mastrorilli *et al.* 2007). Other acute phase proteins such as C-reactive protein and haptoglobin were increased at admission in 100% and 94% of dogs, respectively, in one study (Mastrorilli *et al.* 2007).

Urinalysis reveals isosthenuria in the majority of dogs with leptospirosis, but hyposthenuria has also been described (Rentko *et al.* 1992, Adin and Cowgill 2000, Goldstein *et al.* 2006, Mastrorilli *et al.* 2007). Glucosuria secondary to acute tubular injury, haematuria, pyuria and granular casts can be present (Rentko *et al.* 1992, Birnbaum *et al.* 1998, Adin and Cowgill 2000, Mastrorilli *et al.* 2007, Kohn *et al.* 2010). Proteinuria is present in the majority of dogs. Urine protein electrophoresis revealed that both high molecular weight proteins consistent with glomerular damage and/or low molecular weight proteins consistent with a tubular origin can be present (Zaragoza *et al.* 2003, Mastrorilli *et al.* 2007).

The width of leptospires is below the resolution of light microscopy and thus, the organisms are not visible by routine urinary sediment examination.

DIAGNOSTIC IMAGING

Thorax

Radiographic changes indicative of leptospiral pulmonary haemorrhage syndrome (LPHS), typically initially appear in the caudodorsal parts of the lung fields; they are bilateral and non-lobar (Im *et al.* 1989). Lesions range from a mild interstitial pattern to a mild-to-severe reticulo-nodular pulmonary pattern with focal alveolar infiltrates (Baumann and Fluckiger 2001). A small amount of pleural effusion can be seen in some dogs. Radiographic abnormalities can be present in the absence of respiratory signs (Birnbaum *et al.* 1998, Baumann and Fluckiger 2001, Kohn *et al.* 2010). Thoracic radiography might underestimate the lesion type and the severity in dogs with leptospirosis as compared with thoracic CT (Gendron *et al.* 2014).

Thoracic CT findings in 10 dogs with LPHS have recently been described. While pulmonary lesions were distributed

Table 6. Selected haematological alterations in dogs with leptospirosis

Reference	Rentko *et al.* 1992	Harkin *et al.* 1996	Birnbaum *et al.* 1998	Adin *et al.* 2000	Prescott *et al.* 2002	Boutilier *et al.* 2003	Goldstein *et al.* 2006	Steger-Lieb *et al.* 1999	Mastrorilli *et al.* 2007	Geissen *et al.* 2007	Gerlach *et al.* 2007	Kohn *et al.* 2010	Tangeman & Littman 2013
Country	**USA Massachusetts**	**USA New Jersey**	**USA New York**	**USA California**	**USA Ontario**	**Canada Saskatchewan**	**USA New York**	**Switzerland**	**Italy**	**South Germany**	**North Germany**	**Northeast Germany**	**USA New Orleans**
Number of dogs	n=17	n=17	n=36	n=31	n=31	n=15	n=54	n=11	n=16	n=42	n=39	n=50	n=51
Anaemia % (n)	24 (4)	18 (3)	33 (12)	NR	45 (14)	NR	53 (29)	NR	38 (6)	45 (19)	(36)	50 (25) 68 (34)*	NR
Leucocytosis % (n)	47 (8)	53 (9)	31 (11)	55 (17)	58 (18)	27 (4)	37 (20)	81 (9)	63 (10)	81 (34)	74 (29)	68 (34) 80 (40)*	NR
Leucopenia % (n)	NR	NR	NR	NR	NR	7 (1)	NR	9 (1)	6 (1)	NR	NR	NR	NR
Neutrophilia % (n)	65 (11)	53 (9)	31 (11)	52 (16)	61 (19)	27 (4)	50 (27)	NR	63 (10)	65 (25)	NR	68 (30/44)	NR
Left shift % (n)	NR	NR	6 (2)	3 (1)	NR	NR	NR	81 (9)	NR	65 (25)	NR	25 (11/44)	NR
Monocytosis % (n)	29 (5)	NR	NR	NR	42 (13)	NR	NR	NR	NR	NR	NR	68 (30/44)	NR
Lymphopenia % (n)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	2 (1/44)	NR
Thrombocytopenia % (n)	56 (5/9)	24 (4)	14 (5)	55 (17)	35 (11)	NR	30 (13/44)	NR	7 (44)	53 (21/40)	28 (11)	58 (29) 74 (37)*	51 (26)

NR not reported
*During course of disease

Table 7. Selected biochemical alterations in dogs with leptospirosis

Reference	Rentko *et al.* 1992	Harkin *et al.* 1996	Birnbaum *et al.* 1998	Adin *et al.* 2000	Prescott *et al.* 2002	Boutillier *et al.* 2003	Goldstein *et al.* 2006	Steger-Lieb *et al.* 1999	Mastrorilli *et al.* 2007	Geissen *et al.* 2007	Gerlach *et al.* 2007	Kohn *et al.* 2010	Tangeman & Littmann 2013
Country	**USA Massachusetts**	**USA New Jersey**	**USA New York**	**USA California**	**USA Ontario**	**Canada Saskatchewan**	**USA New York**	**Switzerland**	**Italy**	**South Germany**	**North Germany**	**Northeast Germany**	**USA New Orleans**
Number of dogs	n=17	n=17	n=36	n=31	n=31	n=15	n=54	n=11	n=16	n=42	n=39	n=50	n=51
Increased creatinine % (n)	100 (17)	82 (14)	83 (30)	100 (36)	87(27)	80 (12)	93 (50)	55 (6)	100 (16)	57 (24)	72 (28)	88 (44) 92 (46)*	71 (36)
Increased urea % (n)	100 (17)	82 (14)	81 (29)	100 (36)	94 (29)	73 (11)	93 (50)	54 (6)	100 (16)	62 (26)	72 (28)	88 (44) 92 (46)*	75 (38)
Increased ALT % (n)	35 (6)	35 (6)	33 (12)	NR	26 (8)	33 (5)	32 (17)	55 (6)	69 (11)	74 (31)	28 (11)	78 (37/48)*	51 (26)
Increased AST % (n)	29 (5)	NR	39 (14)	NR	NR	NR	56 (30)	55 (6)	69 (11)	61 (22/36)	28 (11)	NR	47 (24)
Increased ALP % (n)	59 (10)	65 (11)	56 (20)	19 (7)	58 (18)	33 (5)	57 (31)	55 (6)	63 (10)	69 (29)	28 (11)	90 (42/47) 91 (43/47)*	59 (30)
Hyperbilirubinaemia % (n)	24 (4)	42 (7)	17 (6)	22 (8)	68 (21)	33 (5)	41 (22)	55 (6)	56 (9)	79 (34)	15 (6)	73(35/48) 83 (40/48)*	37 (19)
Hyperkalaemia % (n)	17 (3)	12 (2)	NR	NR	NR	NR	NR	NR	31 (5)	12 (5/41)	NR	NR	NR
Hypokalaemia % (n)	17 (3)	NR	NR	NR	NR	NR	41 (22)	NR	NR	NR	NR	NR	NR
Hyperphosphataemia % (n)	42 (7)	47 (8)	50 (18)	NR	NR	NR	78 (42)	55 (6)	94 (15)	45 (18/40)	NR	NR	55 (28)
Hypophosphataemia % (n)	12 (2)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hypochloraemia % (n)	12 (2)	NR	NR	NR	NR	NR	46 (25)	NR	25 (4)	NR	NR	NR	NR
Hyponatraemia % (n)	12 (2)	NR	NR	NR	NR	NR	17 (9)	NR	19 (3)	NR	NR	NR	NR
Hypoalbuminaemia % (n)	18 (3)	NR	NR	NR	NR	NR	35 (19)	64 (7)	75 (12)	NR	NR	NR	NR

NR not reported
*During course of disease

throughout all lung lobes, lesions were most pronounced in the caudodorsal lung fields. Pulmonary lesions included short-lived peribronchovascular thickening and bronchiolar dilatation, areas of consolidation and nodular lesions, which were predominately centrilobular. Pleural and mediastinal effusions were found in 3 and 2 out of 10 dogs, respectively. In this small cohort of dogs, the severity of the pulmonary lesions was not associated with survival to discharge (Gendron *et al.* 2014).

Abdomen

The most common abdominal sonographic examination findings relate to the kidneys and include cortical hyperechogenicity, renomegaly, mild pyelectasia, a medullary band of hyperechogenicity and a mild perirenal fluid accumulation (Forrest 1998).

Other findings on abdominal imaging include hepatomegaly, splenomegaly, evidence of ascites, enlargement and hypoechogenicity of the pancreas, thickening of the gastric and (rarely) intestinal wall and mild lymphadenomegaly (Rentko *et al.* 1992, Birnbaum *et al.* 1998, Adin and Cowgill 2000, Mastrorilli *et al.* 2007, Kohn *et al.* 2010).

CONFIRMATORY TESTING

As leptospirosis is a potential zoonosis, confirmation of a clinical suspicion in veterinary patients is important from a public health perspective. The clinical syndromes or conditions that should prompt a search for *Leptospira* infection are listed in Table 8. A positive culture of biological samples (blood, urine, tissue) is the definitive proof of infection, but culturing leptospires is difficult, requiring up to six months, and is not routinely available in Europe at present. Darkfield microscopy to identify entire leptospires in urine has poor sensitivity and specificity, and needs to be performed on fresh urine. The MAT to detect antileptospiral serum antibodies and PCR for detection of leptospiral DNA are currently the most useful diagnostic tools available for practicioners. Each of these tests has its strengths and limitations and their performance varies depending on a number of factors including the stage of the infection as well as prior antibiotic treatments as outlined below.

Serological tests

Microscopic agglutination test (MAT)

Despite the marked limitations, the MAT is the most widely used diagnostic test for acute leptospirosis. The MAT can also be used to document prior exposure to *Leptospira* spp. in dogs that are not suspected to have leptospirosis, but it does not provide any information about whether or not an animal is a carrier as antibody titres can be low in chronically infected animals (Arent *et al.* 2013).

The MAT is based on determining the ability of serial dilutions of patient serum to agglutinate live leptospiral serovars in vitro. MAT reactivity to a serovar suggests exposure to a serovar belonging to the corresponding serogroup (but not necessarily to the serovar tested) (Levett 2001). The panel of serovars tested should ideally be defined based on antibody prevalence data for the host species in the relevant geographic location, as failure to include the infecting serogroup can lead to false-negative results in infected animals. Based on the antibody prevalence data in Europe, serogroups Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Pyrogenes and Sejroe should at least be included in the test panel (Scanziani *et al.* 2002, André-Fontaine 2006, Geisen *et al.* 2007).

Quality control

MAT results are strongly dependent on the quality control in the laboratory with considerable interlaboratory variability (Miller *et al.* 2011). Practitioners are encouraged to submit diagnostic samples to laboratories that adhere to a proficiency scheme (Chappel *et al.* 2004). The International Leptospirosis Proficiency Testing Scheme, for example, is a collaborative project on behalf of the International Leptospirosis Society providing the participating laboratories with information about the quality of their MAT testing as an aid to improved performance.

MAT interpretation

The MAT has marked limitations with regard to sensitivity, specificity and repeatability, especially if single titres are interpreted (Miller *et al.* 2011, Fraune *et al.* 2013). Infected dogs

Table 8. Indications for confirmatory testing for leptospirosis

Clinical syndromes or conditions that should prompt a search for leptospirosis
- Acute kidney injury
- Isosthenuria associated with glucosuria without hyperglycaemia
- Acute hepatopathy ± jaundice
- Acute respiratory distress ± haemoptysis of unclear etiology with focal or generalized pulmonary reticulonodular interstitial pattern ± patchy alveolar consolidations

Clinical syndromes or conditions for which leptospirosis should be included as differential diagnosis
- Acute haemorrhagic gastroenteritis not due to parvoviral infection
- Acute febrile illness
- Uveitis, retinal bleeding

Additional features/laboratory abnormalities reinforcing a clinical suspicion of leptospirosis
- CBC abnormalities (thrombocytopenia, anaemia)
- Abnormal urine sediment (pyuria, haematuria, proteinuria, casts)
- Surface bleeding/coagulation abnormalities (rare)
- Ultrasonographic abnormalities (renomegaly, perirenal fluid accumulation, medullary band of increased echogenicity, mild pyelectasia)
- Epidemiologic clues (bathing or drinking in marshy areas or standing water, contact with wild rats)

can be antibody negative in the acute phase of the disease, due to the normal delay in the appearance of serum antibodies. On the other hand, non-infected dogs vaccinated with bivalent or quadrivalent whole cell antileptospiral vaccines can have post-vaccinal titres of 1:6400 or higher to both vaccinal and non-vaccinal serovars (Midence *et al.* 2012, Barr *et al.* 2005, Martin *et al.* 2014). Although the majority of vaccinated dogs have been shown to become antibody negative by week 15 postvaccination, vaccinal titres can persist for 12 months in a small percentage of dogs (Martin *et al.* 2014). The reactivity of antileptospiral antibodies with multiple serogroups often prevents the determination of the infecting serogroup. Moreover, the serogroup with the highest MAT titre can vary over time, indicating that the MAT does not reliably predict the infecting serogroup in acutely infected animals (Miller *et al.* 2011).

In a dog with a clinical suspicion of leptospirosis, the best way to confirm a recent infection using MAT is to test paired samples, collected one or two weeks apart (Miller *et al.* 2011, Fraune *et al.* 2013). Collection of a convalescent serum sample can be difficult in a clinical situation. Obtaining a serum sample for a follow-up titre at the time of discharge from the hospital could be a practical approach. A fourfold (two titre steps) or greater rise in MAT is highly suggestive of leptospirosis (for example, a titre of 200 rises to 800, corresponding to the fact that the serum is positive for two more consecutive dilutions) or when an initially antibody-negative dog exhibits a convalescent titre of at least 800 to one or multiple serovars. In a study of 42 dogs with a clinical suspicion of leptospirosis, the sensitivity of a single titre was 50% *versus* 100% for a paired antibody testing with a cut-off value of 1:800. With this cut-off, the specificity of a single titre was 100% *versus* 92% for paired antibody testing (Fraune *et al.* 2013). In a recent case series of 51 canine cases, paired antibody testing was necessary for diagnosis in 45% of the cases with a cut-off value of 1:1,600 in vaccinated dogs and 1:800 in non-vaccinated dogs (Tangeman and Littman 2013). Thus, the sensitivity of the MAT can be greatly improved when paired titres are interpreted.

For a dog with clinical signs consistent with leptospirosis and vaccinated with a bivalent vaccine against Canicola and Icterohaemorrhagiae, a single titre of at least 1:800 for one or more serogroup(s) has in the past generally been considered suggestive of leptospirosis (Fraune *et al.* 2013). A diagnostic algorithm for leptospirosis in dogs based on age, previous vaccination, kinetics of the agglutinating antibodies after infection or vaccination and the delay after onset of the disease was recently proposed (André-Fontaine 2013). **However, due to difficulties in the correct interpretation of a single MAT titre, the panel recommends interpretation of paired MAT titres in conjunction with the vaccinal history whenever possible.**

Enzyme-linked immunosorbent assay

Detection of antileptospiral IgM and/or IgG via ELISA is gaining popularity, as more patient-side assays are becoming commercially available. The performance of a rapid patient-side test detecting canine IgM against pathogenic leptospires was recently reported (Abdoel *et al.* 2011). A modified ELISA that detects canine IgG against serovars Icterohaemorrhagiae, Canicola, Pomona and Grippotyphosa in a semi-quantitative manner was recently licensed in Europe.

These assays provide a result within minutes, but suffer from the same limitations as those of the MAT with regard to the possible absence of antibodies in early infection or their presence due to recent vaccination. Re-testing of initially negative animals within a few days is advised. Further studies assessing the diagnostic performance of these ELISAs in well-characterised patient populations are needed. In the meantime, it is advised to use these tests in conjunction with paired MAT titres.

PCR

PCR assays for detection of leptospiral DNA in samples are offered by several European veterinary diagnostic laboratories. The PCR is a direct identification method and can be performed on blood, urine or tissue specimens.

Sensitivity and specificity of PCR

Several PCR assays for the diagnosis of canine leptospirosis have been described, targeting the *lipL32/hap1* gene, which is specific for pathogenic *Leptospira* spp. (Branger *et al.* 2005, Stoddard *et al.* 2009, Rojas *et al.* 2010), or *23S rDNA* (Harkin *et al.* 2003). Diagnostic performances of all PCR assays are not equivalent (Bourhy *et al.* 2011) and PCR assays validated for use in human clinical specimens, probably used by some veterinary diagnostic laboratories, might not perform similarly when applied to specimens from dogs (Bolin 2003). Unfortunately, diagnostic laboratories often do not report the target gene to the veterinary practitioner. Further studies are required to assess the sensitivity, specificity and positive and negative predictive values of different PCR assays in dogs.

Specimen of choice

Leptospires are generally found in blood for the first 10 days after infection and thereafter in urine (Greenlee *et al.* 2005), although this can vary depending on the immune response of the host and the infecting strain. In a study in dogs experimentally infected with *L. interrogans* serovar Canicola, both culture and *lipL32/hap1* PCR in blood were positive on day 4 and negative thereafter, whereas urine culture and *lipL32/hap1* PCR were negative on day 4 and positive on days 8, 19 and 26 (Branger *et al.* 2005). Findings in this untreated cohort reflect the classic concept of an initial leptospiraemic phase followed by urinary shedding. However, in naturally infected dogs, the exact time of infection is typically unknown. **The panel, therefore, recommends PCR testing of both blood and urine collected before antibiotic administration in each dog with a clinical suspicion of leptospirosis, regardless of the duration of the clinical signs.** Blood and urine specimens should be tested separately rather than being pooled, which potentially decreases the sensitivity through specimen dilution. After death, a clinical suspicion of leptospirosis can be confirmed by applying PCR to kidney tissue (Branger *et al.* 2005).

Preanalytic conditions

For blood testing, serum, plasma or whole blood collected in EDTA or heparinized tubes can be used. In the absence of

well-established general recommendations, clinicians are encouraged to follow the guidelines of their specific laboratory. For urine and tissue testing, storage in plain tubes is adequate. DNA in unprocessed blood is relatively stable (one week at +4°C for blood in EDTA). In one study, freezing of urine samples decreased the sensitivity of a *lipL32/hap1* PCR by more than 60% compared with fresh urine. Freezing of urine should, therefore, be avoided (Branger *et al.* 2005). DNA is less stable in unprocessed tissue and such samples should be sent to the laboratory at +4°C as soon as possible after collection.

Interpretation: status of infection in a clinically suspected animal

A positive PCR result indicates that leptospiral DNA is present in the sample. A positive PCR on blood together with consistent clinical signs is highly suggestive of acute leptospirosis. A positive PCR on urine indicates renal shedding, which can occur in both acutely infected animals and chronic renal carriers. Negative results on blood or urine do not rule out leptospirosis: leptospiraemia is transient (early stages of the disease) and urinary shedding is delayed after acute infection and can be intermittent. Negative results can also be due to recent antibiotic treatment. In a recent study, all of the 30 dogs with confirmed leptospirosis had negative PCR results on blood and urine most likely due to prior antibiotic treatment (Fraune *et al.* 2013).

Interpretation: infecting serovar

Routine diagnostic PCR provides no information on the infecting serovar. Recent methods of molecular typing such as Variable Number of Tandem Repeat (VNTR) and multi-locus sequence typing (MLST)) could offer interesting epidemiological perspectives (Salaun *et al.* 2006, Caimi *et al.* 2012) although they are presently not widely used in veterinary medicine. These methods require a relatively large amount of leptospiral DNA and so their direct application on clinical specimens without prior culture is often not possible.

Interpretation: carrier status

PCR on urine is the test of choice to detect renal carriers, which has been reported in 1.5% to 8% of dogs that are not suspected to have leptospirosis (Harkin *et al.* 2003b; Rojas *et al.* 2010; Llewellyn *et al.* 2013).

Complementarity of MAT and PCR

As long as there is lack of data on sensitivity, specificity and positive and negative predictive values of different PCR assays in dogs, the MAT remains the preferred confirmatory test for leptospirosis. PCR can be used in conjunction with MAT in patients with high vaccinal titres because previous vaccination does not lead to positive results by PCR (Midence *et al.* 2012). Considering the pathophysiology of leptospirosis, the PCR performed on blood in the first week after infection has the potential to be more sensitive and specific than a single MAT titre (Branger *et al.* 2005). Finally, the PCR performed on tissue can be more useful than MAT to detect chronic forms of leptospirosis (Adamus *et al.* 1997).

A direct comparison between the diagnostic accuracies of MAT and PCR in naturally infected dogs with suspected leptospirosis has not been performed. In a study of 33 dogs for which leptospirosis was a differential diagnosis, the PCR (blood and urine) and MAT results correlated well in 10 dogs that were strongly suspected to have leptospirosis, but markedly diverged in the group of 23 dogs for which a diagnosis of leptospirosis was only weakly or moderately suspected (Hugonnard *et al.* 2011). **Based on the current state of knowledge, the panel recommends that PCR results should always be interpreted cautiously and in conjunction with MAT results, taking into account the clinical context.**

TREATMENT OF LEPTOSPIROSIS

Effective treatment of canine leptospirosis consists of appropriate antimicrobial therapy and supportive care for the different organ systems involved. In light of the wide spectrum of possible organ manifestations, the therapeutic plan should be based on a thorough clinical and clinicopathological evaluation. Depending on the severity of the organ system dysfunction, therapeutic intervention should vary from simple monitoring to complex functional replacement, such as renal replacement therapies (RRTs) or mechanical ventilation. With the limited number of prospective clinical studies evaluating treatment of leptospirosis in humans and dogs, recommendations are mostly based on uncontrolled clinical observations. Appropriate clinical and laboratory monitoring of dogs treated for leptospirosis is, therefore, essential to avoid inappropriate therapeutic decisions.

Antimicrobial therapy

Although intuitive and recommended in most textbooks, the use of antibiotics for the treatment of human leptospirosis remains controversial. Many human patients with leptospirosis appear to recover with symptomatic therapy alone, even when not treated with antibiotics (Gulati and Gulati 2012). Two Cochrane systematic reviews failed to find sufficient evidence to provide clear guidelines for the use or the choice of antibiotics in affected individuals (Guidugli *et al.* 2000, Brett-Major and Coldren 2012). The 2012 review included four prospective randomized clinical trials comparing administration of intravenous (iv) penicillin with placebo in 403 humans, and it could not associate the use of antibiotics with improved survival or shorter hospitalization. This meta-analysis suggested a possible, but not a statistically significant, shorter duration of clinical illness in humans treated with antibiotics. With a limited number of available studies, a small number of patients and the high variability in the disease severity and manifestations, this meta-analysis had a low statistical power. On the other hand, the complex role of the immune response in the pathophysiology of leptospirosis is still largely unknown but, once triggered, immune-mediated mechanisms appear to induce some of the clinical manifestations, independently of the underlying bacterial infection itself (Minor and Mohan 2013). Despite this controversy, the World Health Organization clearly recommends antibiotic therapy in humans

with suspected leptospirosis, especially in the early stage of the disease (WHO 2003). **Even though data are sparser for dogs and difficult to extrapolate across species, the panel strongly recommends the use of appropriate antibiotics in dogs suspected to have leptospirosis, even before a definitive laboratory confirmation can be obtained.** The abundant evidence of severe clinical manifestations including death and the potential risk of zoonotic transmission justify this recommendation.

Leptospires are susceptible to a wide range of antibiotics. Antibiotics used in human and canine leptospirosis typically included iv penicillin derivatives or oral doxycycline, the latter being used to eliminate intra-renal persistence and long-term carriage in affected patients (Watt *et al.* 1988). The initial choice of antibiotic depends on whether the patient can tolerate oral doxycycline treatment. As dogs with leptospirosis commonly show gastrointestinal signs, such as vomiting, they usually do not tolerate oral doxycycline well, and initial therapy with an iv penicillin derivative (e.g. penicillin G, ampicillin, amoxicillin) is often recommended to terminate bacteraemia until doxycycline can be used. Human randomized clinical trials were not able to demonstrate any difference among the use of iv penicillin, iv cephalosporin, doxycycline or azithromycin on outcome (Brett-Major and Coldren 2012). First-generation cephalosporins have been shown to be effective in a hamster model of leptospirosis (Harris *et al.* 2011). The iv use of the third generation cephalosporins ceftriaxone and cefotaxime has gained popularity for the treatment of severe forms of leptospirosis in humans, where these antimicrobials have mostly replaced penicillin (Panaphut *et al.* 2003, Suputtamongkol *et al.* 2010). Fluoroquinolones have shown weaker efficacy than doxycycline in rodent models and are not recommended for treatment of dogs with leptospirosis (Truccolo *et al.* 2002).

One case report described a dog with persistent leptospiruria despite the sequential treatment with penicillin and doxycycline. The dog responded to therapy only when switched to streptomycin, possibly indicating a lack of sufficient drug penetration to the site of infection (Juvet *et al.* 2011).

In vitro susceptibility testing of pathogenic leptospires has been reported using clinical or wildlife isolates and it provides very useful information for decisions on antimicrobial strategies at the population level (Chakraborty *et al.* 2010, Harris *et al.* 2011). Such studies can unveil important information on the evolution of antimicrobial susceptibility under the pressure of commonly used antimicrobials. These tests are, however, of minimal use for routine individual clinical decisions given the difficulty in culturing leptospires.

Based on these data, the panel recommends that dogs with leptospirosis should be treated with 5 mg/kg q12h or 10 mg/kg q24h doxycycline for 14 days. Dogs with gastrointestinal signs initially should be treated with an iv penicillin derivative (e.g. 20–30 mg/kg q6–8h ampicillin, 25,000–40,000 U/kg q6–8h penicillin G or 20–30 mg/kg q6–8h amoxicillin). The dose should be adapted in dogs with decreased renal function. A safe and practical approach would be to double the administration interval in dogs with acute kidney injury (AKI) Grade 4 and higher creatinine level (>440 µmol/L). It is important that the dog receives a full 14-day course of oral doxycycline when the gastrointestinal signs are under control in order to eliminate renal colonization. Antimicrobial resistance of leptospires appears to be rare. In the case of persistence of clinical signs, other potential problems including preexisting chronic kidney disease or systemic bacterial infection with nosocomial pathogens should be considered.

Treatment of acute kidney injury (AKI)/acute renal failure

Treatment of dogs with AKI from leptospirosis generally follows the therapeutic recommendations for AKI from other aetiologies (Langston 2010). Correction of fluid, electrolyte and acid-base disorders with appropriate fluid therapy remains the mainstay of the treatment, together with the treatment of systemic hypertension and gastrointestinal complications, pain management and active nutritional support. Particular care should be taken to avoid iatrogenic fluid overload in animals with oliguria or anuria. After initial fluid resuscitation, if needed, fluid therapy should aim at maintaining physiological hydration status and intravascular volume. Fluid overload exacerbates dysfunction of organs such as the lung, gastrointestinal tract, pancreas and the brain. Furthermore, increased renal parenchymal pressure further decreases the already compromised renal perfusion and glomerular filtration rate.

Treatment of leptospirosis-associated AKI can sometimes result in an abrupt and profound polyuria with marked electrolyte wasting in the renal recovery phase. Dogs can, therefore, have rapidly changing fluid requirements, from half a maintenance rate (1 ml/kg/h) during anuria to more than 10x maintenance rates (>20 ml/kg/h) in the polyuric recovery phase. Fluid requirements, therefore, need to be monitored carefully through a closed urine collection system or regular determination of bodyweight (q4–6h) (Langston 2010). The high prevalence of pulmonary manifestations in dogs with leptospirosis in certain geographical areas further limits the tolerance to iatrogenic fluid excesses.

Treatment of dogs with gastrointestinal signs includes a combination of antiemetics and gastroprotectants. Intussusceptions should be considered in dogs with persistent vomiting before antiemetics are contemplated (Schweighauser 2009, Schulz *et al.* 2010). Phosphate binders or haemodialysis might be necessary to correct hyperphosphataemia in affected dogs.

Pain management is particularly important in the early phases of the disease when painful swelling of the kidneys, in addition to muscle, joint and gastrointestinal pain, can contribute markedly to the disease manifestations. Opioids, including buprenorphine or fentanyl, are usually recommended.

The use of enteral feeding tubes is strongly advocated in dogs with anorexia as they allow efficient and early nutritional support with minimal risk of complications (Langston 2010, Hinden *et al.* 2013). Total parenteral nutrition can be necessary in dogs with persistent vomiting.

While dogs with mild-to-moderate azotaemia do well with conservative treatment, renal replacement therapies (RRTs) are often necessary to bridge the time to recovery from renal failure in dogs with severe AKI (Langston 2010). Leptospirosis is con-

sidered one of the best indications for RRT in dogs, because of a favourable prognosis for renal recovery and a short duration of severe renal failure. A study including 36 dogs with leptospirosis reported more than 80% recovery in dogs with severe azotaemia undergoing RRT that had failed prior medical management (Adin and Cowgill 2000). Gradual renal recovery usually occurs after two to seven days of dialytic support. Although RRTs have no direct effect on renal recovery, they allow the full use of the recovery potential by restoring physiological fluid, electrolyte and acid–base balances, by providing the possibility of active nutritional support even in anuric animals and by restoring an acceptable quality of life during the critical phase of kidney failure (Fischer *et al.* 2004). Although the choice of the RRT modality is still a controversy in humans with AKI, both intermittent haemodialysis (IHD) and continuous RRT (CRRT) have been used successfully in dogs with leptospirosis and this choice is usually guided primarily by their respective availability rather than by theoretical arguments on modulation of inflammation. Specific treatment adaptations can be required for dogs with haemorrhagic syndromes that preclude conventional therapies with systemic heparinization (Francey and Schweighauser 2012).

Definitive indications for dialysis include oliguria or anuria with subsequent life-threatening hyperkalaemia or severe volume overload and advanced uraemia refractory to medical management. With the more widespread availability of RRTs in Europe, early initiation of dialysis appears to be indicated for dogs with leptospirosis, in analogy to humans where increased survival from leptospirosis and shorter duration of hospitalization were shown with early start of haemodialysis (Cerqueira *et al.* 2008). **The panel, therefore, recommends the use of RRTs for the severe renal form of canine leptospirosis. Early referral to facilities where RRTs are available is advised.**

Treatment of hepatopathy

Liver involvement can significantly contribute to the morbidity of the infection. As it manifests infrequently as severe liver failure with hepatoencephalopathy, hypoglycaemic seizures or ascites, its treatment is mostly supportive. The use of antioxidants and choleretics has not been assessed in dogs with leptospirosis. In most cases, animals will have significant improvement of their liver function by the time they can tolerate oral doxycycline, and thus require no dose reduction.

Treatment of leptospiral pulmonary haemorrhage syndrome (LPHS)

LPHS is a severe manifestation of leptospirosis and has become the main cause of death in affected areas (Schweighauser and Francey 2008a, Kohn *et al.* 2010). As the exact pathogenesis remains widely unexplained, the mainstay of the management is supportive. Systematic radiographic screening even in the absence of respiratory signs allows early precautionary measures to be adopted. These include minimization of manipulations and stress and avoidance of systemic hypertension, overhydration or hypervolaemia (Francey *et al.* 2013). Depending on the degree of pulmonary haemorrhage, dogs can require oxygen therapy and in severe cases, mechanical ventilation.

Treatment of active haemorrhage with desmopressin has yielded controversial results in humans (Pea *et al.* 2003, Niwattayakul *et al.* 2010) and it did not appear to improve outcome in dogs, at least when administered as ocular drops (Schweighauser and Francey 2008b). Plasma or whole blood transfusions are only indicated in dogs with associated systemic disorders of haemostasis, which is not the case in most dogs with LPHS (Francey *et al.* 2013).

Based on the hypothesis of an immune-mediated mechanism, immunomodulation has been investigated in affected humans with promising preliminary results. A combination of cyclophosphamide, pulse glucocorticoid therapy and therapeutic plasma exchanges to remove potentially auto-reactive antibodies improved survival (Trivedi *et al.* 2001, Meaudre *et al.* 2008, Trivedi *et al.* 2010, Taylor and Karamadoukis 2013). However, considering the complexity and the risk for complications, these therapies still need to be refined further before they can be recommended on a wide scale for affected dogs in clinical practice.

Treatment of haemostatic disorders

Haemostatic disorders in dogs with leptospirosis vary widely in severity and they are multi-factorial in origin. Hypocoagulable conditions from DIC, failure of coagulation factor synthesis, thrombocytopenia and thrombocytopathy compete with prothrombotic conditions associated with inflammation and renal disease (Francey *et al.* 2013). Thrombocytopenia is common in dogs with leptospirosis, but rarely necessitates specific therapy. The mainstay therapeutic options for DIC in dogs with leptospirosis are plasma transfusions (Bruchim *et al.* 2008, Ralph and Brainard 2012). Heparin is no longer recommended for treatment of DIC, unless the dogs are clearly hypercoagulable.

Treatment and prophylaxis for dogs living in the same household as infected dogs

The role of dogs and cats as reservoirs and potential sources of infection for other animals and humans is a subject of discussion (Jimenez-Coello *et al.* 2010, Hartmann *et al.* 2013). Concurrent infection of other dogs that reside in the same household can occur, probably following coincident infection from the same environmental source as they have usually a very similar risk of exposure.

The panel recommends 5 mg/kg q12h or 10 mg/kg po q24h doxycycline treatment for two weeks for the dogs living with dogs diagnosed with leptospirosis, while the treatment of cats living in the same household is currently not recommended.

Clinical follow up after recovery

Recovery of renal function can continue for several months after initial stabilization. This phase does not typically require hospitalization as long as the dogs can maintain adequate hydration and food intake. Some dogs with apparent full recovery and normalization of serum creatinine concentration can, however, have residual parenchymal damage and subsequently develop chronic kidney disease. A follow-up study of dogs with leptospirosis indicated that approximately 50% of the dogs surviving the acute phase of the disease displayed impairment of their renal function more than one year after hospital discharge (Kis *et al.* 2012). Long-term monitoring of renal function is, therefore, strongly recommended in these dogs.

The panel recommends that dogs with leptospirosis be re-examined no later than one week after hospital discharge and every one to three weeks thereafter until clinical stabilization. Further monitoring should be progressively extended to intervals of one, three and six months. Clinical assessment, including blood pressure measurement, as well as blood analysis (urea, creatinine, phosphate, electrolytes and albumin) and urinalysis, should be considered.

LEPTOSPIROSIS IN CATS

Cats can be infected with leptospires, but clinical signs are rarely described (Dickeson and Love 1993, Agunloye and Nash 1996). No significant difference in antibody prevalence between sick and healthy cats could be demonstrated in one study (Mylonakis *et al.* 2005). However, in another recent study, cats with kidney disease (acute and chronic) were more likely to have serum antibodies to *Leptospira* spp. and to shed pathogenic leptospires in their urine (Rodriguez *et al.* 2014). Urinary shedding of *Leptospira* spp. by healthy outdoor cats has also been demonstrated (Fenimore *et al.* 2012, Rodriguez *et al.* 2014).

Experimental infection of cats with serovar Pomona resulted in leptospiraemia and leptospiruria, as well as renal and hepatic lesions in the absence of clinical illness (Fessler and Morter 1964). In experimentally and naturally infected cats, interstitial nephritis is the most consistent histopathological finding reported (Fessler and Morter 1964, Rees 1964, Hemsley 1956, Arbour *et al.* 2012). In addition, a few studies of pet cats report an association between *Leptospira* spp. infection and clinical signs (Hemsley 1956, Fessler and Morter 1964, Rees 1964, Mason *et al.* 1972, Bryson and Ellis 1976, Agunloye and Nash 1996, Luciani 2004, Arbour *et al.* 2012). A case series of three cats with leptospirosis from the USA showed that all the three cats had renal failure, while liver disease was not present in these cats (Arbour *et al.* 2012). In one cat from the UK, leptospires were isolated from thoracic fluid, aqueous humour and kidneys, which, at necropsy, had widespread haemorrhages and straw-coloured fluid in the thoracic and peritoneal cavities (Bryson and Ellis 1976). In one study, a relationship was found between PU/PD and the presence of antibodies to *Leptospira* spp. (Luciani 2004).

In a recent study in captive wild felids in Brazil, 2 out of 57 animals had serum antibodies to *Leptospira* spp. indicating that wild felids can also be infected with *Leptospira* spp. (Ullmann *et al.* 2012).

The role of healthy cats as reservoir hosts and the role of leptospirosis as a clinical disease in cats might have been underestimated in the past and deserves further study.

LEPTOSPIROSIS PREVENTION

Vaccination

Before 1960, serovars Icterohaemorrhagiae and Canicola were thought to be responsible for most cases of canine leptospirosis. Since the introduction of a bivalent vaccine against serogroups Canicola and Icterohaemorrhagiae, infection with serovars that belong to these serogroups likely has become rare based on MAT antibody testing, and acute infections in dogs are now commonly caused by other serogroups, such as Grippotyphosa and Australis (Ellis 2010, Hennebelle *et al.* 2013).

The vaccines containing serovars of serogroups Canicola and Icterohaemorrhagiae induce serogroup-specific immunity, but only partial immunity to heterologous serogroups (Plesko and Lataste-Dorolle 1970, Adler and Faine 1978, Sonrier *et al.* 2000). Canine leptospirosis has been reported among European dogs after vaccination with bivalent Icterohaemorrhagiae and Canicola vaccines (Kohn *et al.* 2010). Thus, the current bivalent vaccines do not sufficiently cross-protect against serovars that are responsible for the majority of current infections in dogs. Quadrivalent vaccines that contain not only serogroups Canicola and Icterohaemorrhagiae but also Grippotyphosa and Pomona have been available in the USA since 2001. Recently, new vaccines containing serovars belonging to three (Icterohaemorrhagiae, Canicola and Grippotyphosa) or four (Icterohaemorrhagiae, Canicola, Grippotyphosa and Bratislava) serogroups (Klaasen *et al.* 2013) have become available in several European countries. However, more data are required to determine whether addition of these serovars will protect more dogs in Europe from leptospirosis than the available bivalent vaccines, as suggested by the limited data in the USA (Hennebelle *et al.* 2013). **Given the widespread recognition of leptospirosis in European dogs that have been vaccinated with bivalent vaccines, the use of quadrivalent vaccines is recommended in an attempt to increase the spectrum of protection.**

There is some debate as to whether vaccines containing *Leptospira* spp. antigens should be considered core or non-core. In fact, they should be classified as non-core vaccines as the term "core" implies that all dogs, independent of their lifestyle, need to be vaccinated. However, the number of dogs that never have access to wildlife, environmental water sources and potentially contaminated areas is probably very small. It should also be kept in mind that leptospirosis has been diagnosed in urban dogs with no apparent history of access to wildlife or water sources. Exposure to the urine of rodents or other wildlife that visit urban areas during the night might explain this phenomenon. All dogs "at risk" should be regularly vaccinated, as leptospirosis is a zoonotic disease and the disease in dogs can be severe and fatal if untreated.

After a basic vaccination with two applications three to four weeks apart, annual revaccination is recommended for all at-risk dogs, regardless of the breed. Vaccines have been shown to protect for at least 12 months (Klaasen *et al.* 2003). Although some veterinarians recommend more frequent vaccinations in dogs at a very high risk (e.g., hunting dogs in regions with high prevalence), the necessity to vaccinate more frequently than every 12 months has not been substantiated. At least in countries, where cold winter temperatures inactivate leptospires in the environment, annual revaccination should be performed in spring to assure best protection during the months with the highest occurrence of the infection.

Evidence to show the protective effect of currently available leptospirosis vaccines beyond 12 months is lacking. **Until more**

data become available, the panel recommends restarting a basic vaccination schedule with two doses administered three or four weeks apart in dogs that have not been revaccinated against leptospirosis for more than 18 months.

Concern has been raised regarding the development of anaphylactoid reactions in dogs after leptospirosis vaccination, especially in some small breed dogs, although such reactions can occur in any breed and small breed dogs are more susceptible to reactions with any vaccine (Moore *et al.* 2005). There is anecdotal evidence from veterinarians and industry that the prevalence of these reactions is decreasing, and now approximates the rate induced by vaccines for other pathogens. In a study on acute vaccine reactions in dogs in the USA utilizing a large database, vaccines that contained *Leptospira* spp. antigen were no more reactive than other vaccines for dogs (Moore *et al.* 2005).

The duration of immunity in dogs after natural infection is unclear, and it is unknown whether or not lifelong immunity results from natural infection. So far, there are no reports of reinfection of dogs with *Leptospira* spp. after successful treatment. However, dogs that have been infected once are at risk of ongoing exposure to the same environmental source, and, thus, should be optimally protected. The duration of immunity after natural infection is likely to be at least as long as that induced by vaccination; however, since dogs can also be exposed to infection with serovars from other serogroups, vaccination as soon as possible after clinical recovery is recommended.

Other preventive measures

Other methods of prevention include decreasing access to potential sources of infection, such as outdoor water sources, and minimizing exposure to wildlife through fencing and rodent control (Greene 2012).

In humans in endemic areas, doxycycline has been given at a low dose (200 mg per person once weekly) for prophylaxis with unclear benefit (Brett-Major and Coldren 2012). However, the widespread prophylactic use of antibiotics can select for resistant bacterial strains and is not recommended for dogs.

ZOONOTIC ASPECTS

Leptospirosis is a zoonotic disease. In humans, leptospirosis occurs after an incubation period of 2 to 20 days and is most often a mild, influenza-like illness. In a smaller percentage of humans, it is manifested by severe, multi-organ failure, with renal failure and hepatic damage with or without pulmonary haemorrhage. Abortion can occur during pregnancy (Levett 2001).

Humans are at increased risk of infection if they perform activities that involve animal contact, such as hunting wildlife species, abattoir work, dairy farming, veterinary practice and direct or indirect contact with wild rodents (Levett 2001, Baer *et al.* 2010). Recreational activities, such as swimming, canoeing, fishing, potholing and caving, are also associated with a significant risk of exposure due to the intense contact with water or soil (Monahan *et al.* 2009, Brockmann *et al.* 2010).

In developing countries, dogs are considered as reservoir hosts for *Leptospira interrogans* serovar Canicola and can represent a zoonotic risk to exposed humans (Brod *et al.* 2005, Maciel *et al.* 2008). The situation in industrialized countries is less clear. In one study from the USA, leptospiral DNA was amplified from the urine of 8% of the dogs included in the study using a conventional PCR assay (Harkin *et al.* 2003). However, in another study from the USA, none of the 100 dogs that were not suspected to have leptospirosis tested positive for leptospiral DNA in their urine using a real-time PCR (Foley, Sykes, unpublished). In Ireland, 37 (7%) of 525 dogs from local shelters and the University College Dublin Veterinary Hospital tested positive for the *lipL32* gene in their urine, a gene only found in pathogenic leptospires (Rojas *et al.* 2010). In a study from southern Germany, using the same PCR assay, 3 of 200 (1.5%) healthy dogs tested positive (Llewellyn *et al.* 2013). In order to better understand the role of dogs and cats as sources of human infection, more studies are required to determine the prevalence as well as the duration and magnitude of subclinical leptospiruria.

Generally, it is assumed that dogs that develop leptospirosis are incidental hosts for the infecting serovar and, as a result, shedding is likely to be brief when compared with that of reservoir hosts. In dogs that develop the disease, shedding might not commence until after the first week of illness. Shedding patterns can also vary geographically depending on the prevailing strains in a region. Dog-to-human transmission of leptospirosis has been suggested by several authors (Haunz and Cardy 1952, Barkin and Glosser 1973, Feigin *et al.* 1973). In a recent study, seropositivity to *Leptospira* serovars in veterinary staff working in a teaching hospital with a very high leptospirosis case load and in pet owners exposed to dogs with confirmed acute leptospirosis was uncommon (Barmettler *et al.* 2011). However, the exact risk of exposure of humans to infected dogs and cats is unknown.

It is generally assumed that leptospiruria ceases after the first few days of antibiotic treatment. However, PCR data from six human patients suggest that urinary shedding of leptospires is possible despite an appropriate antimicrobial therapy (Bal *et al.* 1994). In one case report, leptospires were observed using dark field microscopy in the urine of a dog after 10 days of treatment with penicillin and doxycycline (Juvet *et al.* 2011). The kinetics of urinary shedding of leptospires in dogs during treatment, therefore, deserves further study.

Generally, appropriate precautions should be taken when handling dogs suspected to have leptospirosis. Precautions recommended for veterinary hospitals dealing with canine patients with leptospirosis are outlined in Table 9.

Veterinarians should advise owners of dogs with suspected leptospirosis to promptly seek medical advice if the dogs become ill and to advise their own medical practitioner of their dog's illness. Pet owners should be referred to their medical practitioner for further advice about the disease in humans. Owners should be informed that their dog likely contracted leptospirosis through direct or indirect contact with wild or farm animals, which could represent an ongoing risk for human and companion animal infection.

Table 9. Recommendations for hygiene measures in Veterinary Hospitals

- Begin antimicrobial treatment of the patient with doxycycline as early as possible to interrupt shedding
- Use routine hospital disinfectants promptly and properly on surfaces that become contaminated with urine. Appropriate disinfectants include quaternary ammonium compounds, accelerated hydrogen peroxide solution, iodine-based disinfectants and dilute (1:32) bleach solutions.
- Place cage warning labels
- Minimize the movement of suspect dogs around the hospital
- If a urinary catheter is not in place, walk dogs outside to urinate frequently in an area that can be disinfected in order to minimize contamination of the hospital environment
- If urine output must be monitored, use an indwelling urinary catheter (as opposed to intermittent catheterization)
- Avoid contact between suspect dogs and pregnant or immunocompromised people
- Wash hands properly before and after handling affected dogs
- Wear gloves, a disposable gown, a mask and eye protection when handling soiled bedding or cleaning cages or runs
- Place soiled bedding in biohazard bags
- Inactivate urine with disinfectant (e.g. by diluting in 1:1 with 10% bleach solution)
- Treat all body fluids from affected dogs as medical waste
- Notify all personnel likely to have direct or indirect contact with a suspect patient of the risks. This includes laboratory personnel that handle body fluids or tissues.

Owners should be instructed to wash hands after handling their pet and to wear gloves when cleaning up urine spills until the course of antimicrobial drug therapy is completed. Routine household disinfectants should be used to clean urine spills, and dogs should be taken outside to urinate in a place that no other pets or humans are likely to have access.

FUTURE DIRECTIONS

Despite being an "old" disease, the understanding of the epidemiology, pathogenesis and optimal prevention and treatment strategies of leptospirosis in both humans and animals is limited. Future veterinary research should address the potential role of dogs and cats in the transmission cycle of *Leptospira* spp.; the pathogenic mechanisms of the more severe forms of leptospirosis, such as LPHS; and the development and continuous adaptation of vaccination strategies based on an improved understanding of the epidemiology of the disease in order to prevent clinical infection and urinary shedding in companion animals.

Leptospirosis is a zoonotic disease with similar clinical manifestations in most incidental hosts; therefore, findings in animal species have direct relevance to humans. Veterinarians, therefore, have an important role to play in advancing our knowledge with the goal to equally improve both human and animal lives.

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Conflicts of interest

The views expressed in this consensus statement are those of the authors. No conflicts of interest are disclosed.

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