Update on leptospirosis in dogs

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Leptospirosis is an important bacterial disease of dogs. Infection occurs through contact with infected urine. It additionally presents a public health concern due to its zoonotic nature. *Leptospira* can affect multiple organ systems, but the classical presentation is renal or hepatic dysfunction. However, other manifestations have since been recognised. Leptospirosis is perceived to, generally, be an acute illness but there are reports of chronic forms of disease. Disease can range from mild to severe, multi-organ failure or death.
One of the key issues with leptospirosis is the vast range of tests available for diagnosis. Timing and choice of test provides a continued challenge to clinicians.
For the past 50 years, a bivalent vaccine has been utilised for protection in canines. However, several years ago tri- and tetravalent vaccines were released to the veterinary market in response to emerging concerns to changing patterns of leptospirosis.

Which animals can be infected with leptospirosis?

Leptospirosis affects most mammalian species. Animals can be classified as maintenance hosts, eg. rodents, or incidental hosts, eg. dogs. Host-adapted leptospiral infections in maintenance hosts are typically asymptomatic with urinary shedding. This is in contrast with infection in incidental hosts usually resulting in clinical disease. An animal that is a maintenance host for one *leptospira* serovar can be an incidental host for many other serovars (Bharti *et al*, 2003; Ellis, 2010).
Rodents have a highly important role in maintenance and shedding of leptospires. There is variation between rodent species in their degree of renal carriage. This variation in renal carriage and distribution of rodent species around Europe is an important contributory factor to distribution of serogroups (André-Fontaine, 2006).
The significance of leptospirosis in cats, both as an asymptomatic reservoir and as a causative agent of kidney disease, is still being explored (Lapointe *et al*, 2013; Schuller *et al*, 2015).

What is the geographical distribution of leptospirosis in the UK and Ireland and which serovars are most important?

At present, there have been no large-scale surveillance studies undertaken in the UK or Ireland. Leptospirosis has not been a notifiable UK disease since 2010, so there is no mandatory reporting of cases in people (Forbes *et al*, 2012). Our knowledge of circulating serogroups and their prevalence is largely based on smaller studies. The serogroups most frequently identified in European studies are listed in Table 1 below.

Table 1. Serogroups most commonly identified in European studies (Ellis *et al*, 1983; Ellis, 2010; O'Grady *et al*, 2012; Schuller *et al*, 2015; Sykes *et al*, 2011).

Species	*Leptospira* serogroups
Dog	*L. interrogans* Canicola, Icterrohaemorrhagiae, Grippoytyphosa, Australis, Sejroe, Pomona
Livestock	*L. interrogans* Sejroe, Australis, Pomona
Equine	*L. interrogans* Australis, Copenhageni, Pomona

Leptospirosis is an endemic disease to Ireland and the UK (Ellis, 2010). It is likely that the serovars seen in Ireland will be similar to the UK because there are no export/import regulations. There have been several studies examining Irish dogs specifically. Rojas *et al*, (2010) found 7% of a subset of healthy-dog urine samples at the University College Dublin Veterinary Hospital contained pathogenic leptospires. Another Irish study identified 6% of dogs not suspected of leptospirosis having significantly elevated antibody titres to the following non-vaccinal serovars: Ballum, Bratislava, Mozdok, Altodouro and Hardjo (Schuller *et al*, 2015). A study on Irish beef suckler herds found sero-prevalence to Hardjo to be over 80% (O'Grady *et al*, 2012).

What is a serogroup or serovar?

One aspect of understanding leptospirosis that is complex is the ever-changing nomenclature of the bacteria, the key terms are defined in Table 2 below. The evolving classifications and grouping provides challenges for clinicians.

Table 2: Definitions of serovar, serogroup and genomospecies (Levett, 2001).

Serovar	Serogroup	Genomospecies
Based on differences in host antibody reactivity to lipopolysaccharide (LPS) structure. There are >250 serovars at present.	A group of antigenically related serovars that antibodies cross react and agglutinate to.	More recent organisation of leptospira genus on the basis of DNA similarities. There are currently >16 species

Serovar and sero-grouping are important from an epidemiological and diagnostics context as this system has been used since early research into leptospires in the 1900s. There is no correlation between serovar/serogroup

and genomospecies organisation (Levett, 2001). As applies to all pathogenic bacteria, viruses and parasites, the immune response in infected humans and animals as well as protection against re-infection are mainly serotype-specific. Therefore, *Leptospira* genomospecies are of minor importance to the epidemiology of leptospirosis.

How might leptospirosis present in a patient?

Infection occurs through contact with urine-contaminated water onto mucus membranes or broken skin. Once leptospires enter the blood stream, various organs can be affected. Renal and hepatic involvement is most common but additionally lungs, eyes and the reproductive tract can be affected (Delaude *et al*, 2017; Ellis, 2010; Schuller *et al*, 2015). Although leptospirosis is more commonly reported as an acute disease, it is important to highlight that it can be involved in chronic renal or hepatic disease (McCallum *et al*, 2018; Timoney *et al*, 1974). Table 3 summarises major clinical signs reported.

Table 3: Most common clinical signs in leptospirosis cases (Birnbaum *et al*, 1998; Geisen *et al*, 2007; Goldstein *et al*, 2006; Major *et al*, 2014; Mastrorilli *et al*, 2007; Rentko *et al*, 1992; Schuller *et al*, 2015).

Clinical sign	Prevalence (%)	Clinical sign	Prevalence (%)
Abdominal pain	19-65	Hypothermia	12-38
Anorexia	57-81	Jaundice	13-45
	67	Lethargy	24-90
Diarrhoea	6-50	PUPD	27-50
	33	Pyrexia	6-36
Dehydration	6-52	Vomiting	41-88
Dyspnoea	2-68		

There are also rarer reports of reproductive issues; such as abortion and infertility, and skin conditions, such as calcification (Munday *et al*, 2005). The role of leptospirosis in reproductive disease in livestock is well recognised but it has not yet been fully explored in dogs. Leptospires have been isolated from bitches with reproductive issues (André-Fontaine, 2006; Ellis, 2010; Graham & Taylor, 2012; Mori *et al*, 2017; Rossetti *et al*, 2005).

In recent years, studies have documented cases with severe respiratory tract involvement. This presentation is called leptospiral pulmonary haemorrhage syndrome (LPHS [Klopfleisch *et al*, 2010; Kohn *et al*, 2010; Major *et al*, 2014]). In one Swiss study, 68% of dogs diagnosed with leptospirosis had respiratory signs as part of their clinical presentation (Major *et al*, 2014). LPHS is recognised in human leptospirosis and has mortality rates associated with over 50% (Dolhnikoff *et al*, 2007). Although the pathogenesis is not well understood, it is thought that LPHS is associated with an immune-mediated response rather than a high bacterial load. Other bleeding disorders such as epistaxis, haematemesis and petechiae are seen with leptospirosis. This may be due to leptospires triggering vasculitis but the exact mechanisms have not been elucidated (Wagenaar *et al*, 2007).

What are common laboratory-test abnormalities in leptospirosis?

Laboratory tests will often reveal a range of non-specific abnormalities which might raise suspicion of leptospirosis and prompt confirmatory diagnosis. The most common abnormalities are listed in Table 4.

Table 4: Common diagnostic abnormalities in leptospirosis (Ellis, 2010; Goldstein *et al*, 2006; Kohn *et al*, 2010; Major *et al*, 2014; Musso & La Scola, 2013; Schuller *et al*, 2015; Troìa *et al*, 2018).

Investigations	Common abnormalities
Haematology	Left shift leucocytosis +/- preceding leucopaenia in early disease Thrombocytopaenia
Biochemistry	Elevated azotaemia Elevated liver enzyme activities Hyperbilirubinaemia
Coagulation profile	Prolonged PT and APTT
Urinalysis	Isosthenuria Proteinuria Pyuria Haematuria Hyaline or granular casts

When to test for leptospirosis?

Due to the wide range of clinical presentations, it is important to keep leptospirosis as a differential for the following presentations:

Other causes of acute kidney injury (AKI)

- Toxic, eg. non-steroidal drugs, ethylene glycol, aminoglycosides
- Infectious, eg. generalised sepsis, pyelonephritis, babesiosis
- Other, eg. urethral obstruction

Other causes of acute hepatitis

- Toxic, eg. NSAIDs, xylitol zinc, onion
- Infectious, eg. infectious canine hepatitis (CAV-1), bacterial infections

Other causes of coagulation disorders

- Toxic, eg. rodenticide
- Immune-mediated, eg. immune mediated haemolytic anaemia or thrombocytopaenia, disseminated intravascular coagulopathy (DIC)

Which diagnostic tests can be performed?

In order to confirm the diagnosis of leptospirosis, specific tests must be performed on blood or urine samples. There are numerous tests available and knowing when to use them is crucial to diagnosis. Tests are divided broadly into two categories:

- Serological tests that measure antibody response to leptospirosis infection (serological testing).
- Molecular tests that detect leptospiral DNA (molecular testing).

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Table 5 lists which types of tests fall into these categories.

Table 5: Serological and molecular tests to diagnose leptospirosis.

Serological	
Test	**Laboratories to send to**
MAT (microscopic agglutination test)	APHA (UK)
	AFBINI (Ireland)
ELISA (enzyme linked immunosorbent assay)	IDEXX laboratories
In house rapid ELISAs	IDEXX laboratories
	BioGal laboratories
Immunofluorescence Antibody assay	Bio-best laboratories
Molecular	
Test	**Laboratories to send to**
qPCR	IDEXX Laboratories Scanelis Laboratories Biobest Laboratories Langford Vets
Fluorescent *in situ* Hybridisation (FISH)	Langford Vets

What are the limitations with the currently available tests?

The MAT test is the current gold standard for leptospirosis diagnosis. Interpretation of MAT results can be challenging. The result given is an antibody titre (the highest dilution of sample where >50% of the sample has agglutinated). Examples of MAT test agglutination results can be seen in Figure 1.

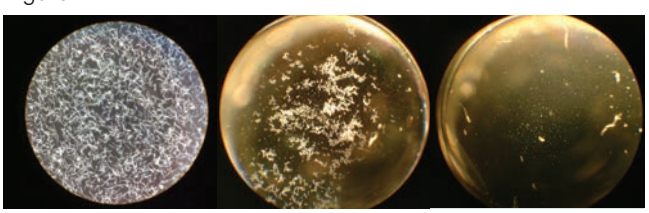

Figure 1: Agglutination results in a MAT test. The first well on the LHS a well of live leptospires prior to addition of patient sera. The central well shows agglutination of leptospires in a positive test result. The final well on the RHS shows no agglutination, a negative result. Images courtesy of George Souter, APHA.

There is variability of the cut off for a positive sample but veterinary medicine generally follows the same guidelines as Leptospirosis Burden Epidemiology Research Group (LBERG): ≥1:400 for a single sample and at least a four-fold increase between paired samples (Schuller *et al*, 2015). Paired sampling is highly recommended as it is impossible to differentiate between post-vaccine antibodies or active or previous infections from a single elevated antibody titre (Ellis, 2010; Schuller *et al*, 2015). Although vaccine response antibodies generally do not reach as high titres or remain elevated for as prolonged periods as post-infection antibodies, this is not consistently the case (Martin *et al*, 2014; Miller *et al*, 2011). High uptake of vaccination in UK dogs (between 65-95%, depending on the study) and the inability of the MAT test to distinguish between these antibodies is a key limitation of this test (Ball *et al*, 2014; Sánchez-Vizcaíno *et al*, 2018). A major drawback of serological testing is sampling too early in the disease course (before IgM antibody levels have risen) leading to a false negative diagnosis.

Although polymerase chain reaction (PCR) results are not challenging to interpret, there is the risk of false negative results depending on timing of antimicrobial therapy and, if it is a urine sample, intermittent shedding of leptospires. In case of sampling outside a hospital, storage and transport conditions and time until sample processing have an impact on PCR results due to possible disintegration of leptospiral DNA. If possible, samples for PCR should be taken prior to initiation of antibiotic therapy. Additionally, PCR results do not provide any information on infecting serogroup (Musso & La Scola, 2013).

Unfortunately, the majority of tests require external laboratory analysis, so it can take seven to 14 days to receive results. Due to this lag, modified ELISA patient-side tests, such as SNAP Lepto (IDEXX Laboratories) and Immunocomb (Biogal-Galed laboratories) can be useful for increasing confidence in a diagnosis of leptospirosis. However, both the European College of Veterinary Internal Medicine (ECVIM) consensus statement and test manufacturers recommend these tests be performed in conjunction with other serological and/or molecular tests. The ECVIM Consensus statement also recommends PCR testing be done in conjunction with MAT testing (Schuller *et al*, 2015).

Table 6: Provides a guide of timing of tests.

Duration of infection (days)	Disease stage	Test goal	Test
0-3	Leptospiraemic	Test for leptospires in blood	PCR on blood
3-5	Leptospiraemic	Test for leptospires in blood and/or antibodies	PCR on blood First MAT test Any ELISA test
7-10	Seroconversion Leptospiuric	Test for antibodies and/or test for leptospires in urine	PCR on urine First MAT test Any ELISA test
>10	Seroconversion +/- leptospiuric	Test for antibodies and/or test for leptospires in urine	Second MAT test PCR on urine

Table 6: Timing and choice of leptospirosis diagnostic tests
***leptospiraemic = leptospires in bloodstream**
***seroconverted = producing antibodies against leptospires**
***leptospiuric = leptospires in renal tubules**

How should leptospirosis be treated?

Treatment of leptospirosis requires antimicrobial and supportive therapy. The most common antimicrobials used are penicillin derivatives and doxycycline. The dosages are listed in Table 7. Penicillin and penicillin related antimicrobials are useful for treating the leptospiraemia but will not prevent leptospires colonising renal tubules. The use of doxycycline is required to eliminate persistent renal carriage. However, doxycycline is indicated only once the patient is eating and not vomiting, so more critically ill patients should begin therapy on intravenous penicillin.

Table 7: Antimicrobial options in leptospirosis infections.

Antimicrobial	Dose	Duration
Penicillin	25,000-40,000U/kg IV BID	Until gastrointestinal (GI) signs resolve
Amoxicillin-Clavulanic acid	20mg/kg IV q6-8h	Until GI signs resolve
Doxycycline	5mg/kg BID or 10mg/kg SID PO	14 days

In cases with severe acute kidney injury (AKI grade 4 or creatinine >440umol/L), the antimicrobial dosing interval should be increased (Schuller *et al*, 2015). Supportive therapy required will vary depending on which organ systems are affected. Patients are frequently managed with intravenous fluid therapy, antiemetics and analgesia.

Dogs with coagulation abnormalities may require plasma transfusions. Dogs with severe AKI (>grade 4) may be candidates for renal replacement therapy (RRT) at certain referral clinics (Schuller *et al*, 2015; Sykes *et al*, 2011). Dogs presenting with LPHS are treated with oxygen therapy (Sykes *et al*, 2011). Additionally, as a result of infection, animals may have developed chronic renal or hepatic insufficiency and need lifelong management of this. One study of dogs treated for AKI (of various aetiologies) found that 50% of dogs had permanent renal damage after discharge (Kis *et al*, 2012). Monitoring of biochemistry, particularly renal values and electrolytes, should be done every 24 hours while hospitalised. Additionally, careful monitoring of fluid ins and outs should be undertaken (Schuller *et al*, 2015; Sykes *et al*, 2011).

It is recommended to treat other dogs in the household with prophylactic antimicrobial therapy. Additionally, owners should be advised to disinfect thoroughly and avoid contact with contaminated urine while their dog is undergoing treatment (Sykes *et al*, 2011).

What factors might be associated with poorer outcomes?

Sykes *et al* (2011) reported survival rates of 80%. Major *et al* (2014) found that the presentation of hepatic dysfunction was most negatively associated with survival. This has been recognised in human studies where the presence of jaundice was associated with higher mortality than other presentations (Taylor *et al*, 2015).

Additionally, it is reported that some serogroups are associated with more severe disease than others. For example, André-Fontaine (2006) reported Australis and Sejroe serogroups being associated with milder or more chronic forms of leptospirosis than the Autumnalis and Grippotyphosa serogroups. Goldstein *et al* (2006) found dogs infected with the Pomona serogroup to have higher mortality rates. Although this relationship has not been examined formally in more robust veterinary studies, similar relationships between serogroup and disease severity have been acknowledged in human literature also. In a large human meta-analysis, patients infected with serovar Icterohaemorrhagiae had highest mortality when compared to all other serovars (Taylor *et al*, 2015).

How is leptospirosis prevented?

Vaccination is a cornerstone of preventing leptospirosis in dogs and also minimising the risk of urinary shedding of leptospires to humans (Klaasen *et al*, 2014). Leptospirosis vaccines contain inactivated whole-cell leptospires (Klaasen & Adler, 2015). Vaccination does not generate cross protection between serogroups. It is only protective against the serovars included in the formulation and those closely related to them. The duration of immunity from vaccine administration is approximately 12 months (Klaasen *et al*., 2014). Hence, these vaccines must be administered annually. Bivalent vaccines have been widely used since the 1960s and provide protection against the Canicola and Icterrohaemorrhagiae serogroups. In 2013, a broader version of the vaccine was released throughout Europe providing coverage against a further two serogroups, Grippotyphosa and Australis. These serogroups were included due to increased seroprevalence in continental Europe studies (Ellis, 2010; Klaasen & Adler, 2015; Klaasen *et al*, 2013; Renaud *et al*, 2013; Schuller *et al*, 2015). In the US, a tetravalent vaccine was also released but with the inclusion of Pomona rather than Australis serogroups. This reflects higher incidence of infections with the Pomona serogroup in the US than in Europe (Sykes *et al*, 2011).

Prior to the introduction of a bivalent vaccine in the 1960s, *L. interrogans* Canicola and *icterrohaemorrhagiae* were the most important serogroups associated with disease in dogs. Since then, leptospirosis has remained an important disease of dogs but the importance of the Canicola serogroup has diminished whilst identification of disease due to other serogroups has increased. Dogs are the only maintenance host of Canicola therefore high levels of vaccinated dogs have created a degree of herd immunity to this serogroup (André-Fontaine, 2006). There has been a lot of dog owner concern with respect to tetravalent vaccines and adverse reactions towards them. The Veterinary Medicines Directorate (UK) investigated this and found the incidence of adverse events with bivalent vaccines to be 0.015% (two in 10,000) and tetravalent vaccines 0.069% (seven in 10,000). According to this, leptospirosis vaccines fit into the category of 'rare' adverse effects (<10 in 10,000) (Veterinary Medicine Directorate, 2017). This incidence rate is similar to what has been reported for other core vaccines such as DHP (distemper, adenovirus and parvovirus).

Future perspectives

Leptospirosis remains an important pathogen to consider in a wide range of clinical presentations in dogs. Future work must focus on robust epidemiological studies of UK and Irish canines and improved diagnostics. Improved characterisation of clinical presentation in these canines will also improve clinician recognition of the disease. The importance of other species' (particularly livestock) role in spread of leptospirosis must also be explored.

REFERENCES

1. André-Fontaine, G. (2006). Canine leptospirosis-Do we have a problem? *Veterinary Microbiology*, 117(1), 19–24. https://doi.org/10.1016/j.vetmic.2006.04.005
2. Ball C, Dawson S, & Williams N. (2014). Leptospira cases and vaccination habits within UK vet-visiting dogs. *Veterinary Record*. https://doi.org/10.1136/vr.102085
3. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, Vinetz JM. (2003). Leptospirosis: a zoonotic disease of global importance. *The Lancet Infectious Diseases*, 3(12), 757-771. https://doi.org/10.1016/S1473-3099(03)00830-2
4. Birnbaum N, Barr SC, Center SA, Schermerhorn T, Randolph JF, & Simpson, KW. (1998). Naturally acquired leptospirosis in 36 dogs: Serological and clinicopathological features. *Journal of Small Animal Practice*, 39(5), 231-236. https://doi.org/10.1111/j.1748-5827.1998.tb03640
5. Delaude A, Rodriguez-Campos S, Dreyfus A, Counotte, MJ, Francey T, Schweighauser A, Schuller S. (2017). Canine leptospirosis in Switzerland - A prospective cross-sectional study examining seroprevalence, risk factors and urinary shedding of pathogenic leptospires. *Preventive Veterinary Medicine*, 141, 48-60 https://doi.org/10.1016/J.PREVETMED.2017.04.008
6. Directorate VM. (2017). Leptospira vaccination in dogs. Retrieved from https://www.gov.uk/government/news/leptospira-vaccination-in-dogs
7. Dolhnikoff M, Mauad T, Bethlem, EP, & Carvalho, CRR. (2007). Pathology and pathophysiology of pulmonary manifestations in leptospirosis. *Brazilian Journal of Infectious Diseases*, 11(1), 142–148. https://doi.org/10.1590/s1413-86702007000100029
8. Ellis WA. (2010). Control of canine leptospirosis in Europe: time for a change? *Veterinary Record*, 167(16), 602-605. https://doi.org/10.1136/vr.c4965
9. Ellis WA, O'Brien JJ, Cassels JA & Montgomery J. (1983). Leptospiral infection in horses in Northern Ireland: Serological and microbiological findings. *Equine Veterinary Journal*, 15(4), 317-320. https://doi.org/10.1111/j.2042-3306.1983.tb01809.x
10. Forbes AE, Zochowski WJ, Dubrey SW, & Sivaprakasam V. (2012). Leptospirosis and weil's disease in the UK. Qjm, 105(12), 1151-1162 https://doi.org/10.1093/qjmed/hcs145
11. Geisen V, Stengel C, Brem S, Müller W, Greene C & Hartmann K. (2007). Canine leptospirosis infections – Clinical signs and outcome with different suspected Leptospira serogroups (42 cases). *Journal of Small Animal Practice*. https://doi.org/10.1111/j.1748-5827.2007.00324.x
12. Goldstein RE, Lin RC, Langston CE, Scrivani PV, Erb HN, & Barr SC. (2006). Influence of infecting serogroup on clinical features of leptospirosis in dogs. *Journal of Veterinary Internal Medicine*. https://doi.org/10.1892/0891-6640(2006)20[489:IOISOC]2.0.CO;2
13. Graham EM & Taylor DJ. (2012). Bacterial Reproductive Pathogens of Cats and Dogs. *Veterinary Clinics of North America*: Small Animal Practice, 42(3), 561-582 https://doi.org/10.1016/j.cvsm.2012.01.013
14. Kis I, Schweighauser A, & Francey T. (2012). Long term outcome of dogs with acute kidney injury. In *Proceedings of the ACVIM Forum* (p. 798)
15. Klaasen, E., & B. Adler. (2015). Recent advances in canine leptospirosis: focus on vaccine development. *Veterinary Medicine: Research and Reports*, 245. https://doi.org/10.2147/vmrr.s59521
16. Klaasen HLBM, van der Veen M, Molkenboer MJCH, & Sutton, D. (2013). A novel tetravalent Leptospira bacterin protects against infection and shedding following challenge in dogs. *Veterinary Record*. https://doi.org/10.1136/vr.101100
17. Klaasen HLBM, van der Veen, M, Sutton D, & Molkenboer MJCH. (2014). A new tetravalent canine leptospirosis vaccine provides at least 12 months immunity against infection. *Veterinary Immunology and Immunopathology*, 158(1-2), 26-29. https://doi.org/10.1016/j.vetimm.2013.08.002
18. Klopfleisch R, Kohn B, Plog S, Weingart C, Nöckler K, Mayer-Scholl A, & Gruber AD. (2010). An emerging pulmonary haemorrhagic syndrome in dogs: similar to the human leptospiral pulmonary haemorrhagic syndrome? *Veterinary Medicine International*, 2010, 928541. https://doi.org/10.4061/2010/928541
19. Kohn B, Steinicke K, Arndt G, Gruber AD, *et al*. (2010). Pulmonary Abnormalities in Dogs with Leptospirosis. *Journal of Veterinary Internal Medicine*, 24(6), 1277-1282. https://doi.org/10.1111/j.1939-1676.2010.0585.x
20. Lapointe C, Plamondon I & Dunn M. (2013). Feline leptospirosis serosurvey from a Quebec referral hospital. *Canadian Veterinary Journal*, 54(5), 497-499.
21. Levett PN. (2001). Leptospirosis. *Clinical Microbiology Reviews*, 14(2), 296-326. https://doi.org/10.1128/CMR.14.2.296-326.2001
22. Major A, Schweighauser A, Francey T, Major A, *et al*. (2014). Increasing Incidence of Canine Leptospirosis in Switzerland. *International Journal of Environmental Research and Public Health*, 11(7), 7242-7260. https://doi.org/10.3390/ijerph110707242
23. Martin LER, Wiggans KT, Wennogl SA, Curtis K, *et al*. (2014). Vaccine-associated leptospira antibodies in client-owned dogs. *Journal of Veterinary Internal Medicine*. https://doi.org/10.1111/jvim.12337
24. Mastrorilli C, Dondi F, Agnoli C, Turba ME, *et al*. (2007). Clinicopathologic features and outcome predictors of Leptospira interrogans Australis serogroup infection in dogs: a retrospective study of 20 cases (2001-2004). *Journal of Veterinary Internal Medicine*, 21(1), 3-10
25. McCallum KE, Cogan T, Swales H, Warland JH, *et al*. (2018). Hepatic leptospiral infections in dogs without obvious renal involvement. *Journal of Veterinary Internal Medicine*, 33(1), 141–150. https://doi.org/10.1111/jvim.15340
26. Miller MD, Annis KM, Lappin MR, & Lunn KF. (2011). Variability in Results of the Microscopic Agglutination Test in Dogs with Clinical Leptospirosis and Dogs Vaccinated against Leptospirosis. *Animals*, 426-432.

27. Mori M, Bakinahe R, Vannoorenberghe P, Maris J, *et al*. (2017). Reproductive Disorders and Leptospirosis: A Case Study in a Mixed-Species Farm (Cattle and Swine). *Veterinary Sciences*, 4(4), 64. https://doi.org/10.3390/vetsci4040064
28. Munday JS, Bergen DJ, & Roe WD. (2005). Generalized calcinosis cutis associated with probable leptospirosis in a dog. *Veterinary Dermatology*, 16(6), 401-406. https://doi.org/10.1111/j.1365-3164.2005.00476.x
29. Musso D, & La Scola B. (2013). Laboratory diagnosis of leptospirosis: A challenge. *Journal of Microbiology, Immunology and Infection*. https://doi.org/10.1016/j.jmii.2013.03.001
30. O'Grady L, Ryan EG, Leonard N, Doherty ML, *et al*. (2012). Seroprevalence of Leptospira Hardjo in the Irish suckler cattle population. *Irish Veterinary Journal*, 65(1), 1-11. https://doi.org/10.1186/2046-0481-65-8
31. Renaud C, Andrews S, Djelouadji Z, Lecheval S, *et al*. (2013). Prevalence of the Leptospira serovars bratislava, grippotyphosa, mozdok and pomona in French dogs. *Veterinary Journal*. https://doi.org/10.1016/j.tvjl.2012.10.002
32. Rentko VT, Clark N, Ross LA & Schelling SH. (1992). Canine Leptospirosis: A Retrospective Study of 17 Cases. *Journal of Veterinary Internal Medicine*, 6(4), 235-244. https://doi.org/10.1111/j.1939-1676.1992.tb00345.x
33. Rojas P, Monahan AM, Schuller S, Miller IS, *et al*. (2010). Detection and quantification of leptospires in urine of dogs: A maintenance host for the zoonotic disease leptospirosis. *European Journal of Clinical Microbiology and Infectious Diseases*, 29(10), 1305-1309. https://doi.org/10.1007/s10096-010-0991-2
34. Rossetti CA, Liem M, Samartino LE & Hartskeerl RA. (2005). Buenos Aires, a new Leptospira serovar of serogroup Djasiman, isolated from an aborted dog fetus in Argentina. *Veterinary Microbiology*, 107(3-4), 241-248. https://doi.org/10.1016/j.vetmic.2005.01.015
35. Sánchez-Vizcaíno F, Muniesa A, Singleton DA, Jones PH, *et al*. (2018). Use of vaccines and factors associated with their uptake variability in dogs, cats and rabbits attending a large sentinel network of veterinary practices across Great Britain. *Epidemiology and Infection*. https://doi.org/10.1017/S0950268818000754
36. Schuller S, Arent ZJ, Gilmore C, Nally, J. (2015). Prevalence of antileptospiral serum antibodies in dogs in Ireland. *Veterinary Record*, 177(5), 126. https://doi.org/10.1136/vr.102916
37. Schuller S, Francey T, Hartmann K, Hugonnard M, *et al*. (2015). European consensus statement on leptospirosis in dogs and cats. *Journal of Small Animal Practice*. https://doi.org/10.1111/jsap.12328
38. Sykes JE, Hartmann K, Lunn KF, Moore GE, *et al*. (2011). 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *Journal of Veterinary Internal Medicine*, 25(1), 1-13. https://doi.org/10.1111/j.1939-1676.2010.0654.x
39. Taylor AJ, Paris DH & Newton PN. (2015). A systematic review of the mortality from untreated leptospirosis. *PLoS Neglected Tropical Diseases*, 9(6), 1-19. https://doi.org/10.1371/journal.pntd.0003866
40. Timoney JF, Sheahan BJ, Timoney PJ. (1974). Leptospira and Infectious Canine Hepatitis (ICH) virus antibodies and nephritis in Dublin dogs. The *Veterinary Record*, 316-319.
41. Troìa R, Balboni A, Zamagni S, Frigo S, *et al*. (2018). Prospective evaluation of rapid point-of-care tests for the diagnosis of acute leptospirosis in dogs. *Veterinary Journal*, 237, 37–42. https://doi.org/10.1016/j.tvjl.2018.05.010
42. Wagenaar JFP, Goris MGA, Sakundarno MS, Gasem MH, *et al*. (2007). What role do coagulation disorders play in the pathogenesis of leptospirosis? *Journal of Tropical Medicine and International Health*, 12(1), 111-122. https://doi.org/10.1111/j.1365-3156.2006.01792.x

READER QUESTIONS AND ANSWERS

1. WHAT IS THE GOLD STANDARD TEST FOR LEPTOSPIROSIS?
- **A.** PCR
- **B.** MAT
- **C.** SNAP Lepto
- **D.** Culture

2. WHAT ARE THE MOST COMMON PRESENTING CLINICAL SIGNS OF LEPTOSPIROSIS?
- **A.** Vomiting & diarrhoea
- **B.** PUPD
- **C.** Pyrexia
- D. Jaundice

3. WHICH OF THESE SEROGROUPS DOES TETRAVALENT VACCINATION IN EUROPE NOT INCLUDE?
- **A.** Pomona
- **B.** Canicola
- **C.** Icterrohaemorrhagiae
- **D.** Australis

4. WHICH ANTIBIOTIC IS USED TO PREVENT RENAL CARRIAGE OF LEPTOSPIROSIS?
- **A.** Penicillin
- **B.** Marbofloxacin
- **C.** Clindamycin
- **D.** Doxycycline

5. WHICH CLINICAL PRESENTATION APPEARS TO BE ASSOCIATED WITH HIGHEST MORTALITY?
- **A.** LPHS
- **B.** Neurological
- **C.** Hepatic dysfunction
- **D.** Bleeding disorder

ANSWERS: 1 B; 2 A; 3 A; 4 D; 5 C