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Leptospirosis in dogs: A comprehensive review

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Abstract
Canine leptospirosis is a zoonotic bacterial disease with a worldwide distribution caused by motile spirochete belonging to the genus *Leptospira* involving rodents as the most important reservoir host. Generally, dogs get infected through direct contact with mucous membranes or broken skin with the urine of infected animals and indirect contact via contaminated soil or surface water. Infected animals exhibit various non-specific symptoms such as fever, vomiting, icterus, diarrhoea, haematuria, dyspnoea, uveitis, and abortions. Microscopic Agglutination Test (MAT) is the most acceptable diagnostic technique and is considered the gold standard test for the diagnosis of leptospirosis. The recommended antibiotic therapy for dogs showing clinical signs of leptospirosis is Penicillin or Tetracycline derivatives such as Ampicillin or Doxycycline. Accurate early diagnosis and treatment of affected dogs with a suitable therapeutic regimen coupled with periodical vaccination of healthy dogs with inactivated leptospiral vaccines are highly recommended for efficient control and prevention of canine leptospirosis.

Keywords: MAT, Leptospirosis, canine, doxycycline, jaundice, nephritis

1. Introduction
Leptospirosis is an infectious disease that affects both humans and dogs that is spread by zoonotic bacteria and has a global distribution. Worldwide, canines are affected with leptospirosis, sometimes known as Stuttgart's disease, which is brought on by gram-negative, strictly aerobic spirochetes of the genus *Leptospira*. According to studies by Ambily *et al.* (2013) [1] and Senthil *et al.* (2013) [2], the disease is endemic in southern Indian states like Tamil Nadu and Kerala and is commonly reported in tropical and subtropical areas of the world. Humans and other accidental hosts can become infected through reservoir hosts. Dogs typically become infected by direct contact with cutaneous abrasions, as well as through contaminated soil, vegetation, urine, food, and water (Desvars *et al.*, 2012) [3].
Depending on the serovar that is infecting the canine, the environment, and the host immunological response, leptospirosis in dogs typically causes sickness of different severity. Fever, anorexia, vomiting, lethargy, stomach discomfort, diarrhoea, jaundice, dehydration, stiffness, dyspnea, tachypnea, weight loss, uveitis, and abortions are the most often reported clinical symptoms in dogs.
Leptospirosis can be diagnosed using a history, clinical symptoms, lab results, serological tests, and molecular assays. For leptospiral diagnosis, the microscopic agglutination test (MAT) is regarded as the gold standard (Cumberland *et al.*, 1999) [4].
Penicillin or tetracycline derivatives such as Ampicillin, Penicillin G or Doxycycline are the recommended antibiotic therapies for dogs suspected of having leptospirosis (Sykes *et al.*, 2011) [5]. For efficient control and prevention of canine leptospirosis, which is a zoonotic illness, accurate early identification and treatment of sick dogs with appropriate therapeutic regimens along with periodic vaccination of healthy dogs with inactivated leptospiral vaccines are strongly recommended.

2. Epidemiology
2.1 Etiology
According to Bharati *et al.* (2003) [6], a motile spirochete from the genus *Leptospira* causes the disease known as leptospirosis in both humans and animals. Leptospires are elongated,

helically coiled, Gram-negative, extremely mobile bacteria. The organism's distinctive hooked ends can be used to distinguish it from other spirochaetes. *Leptospira* is a genus with two species: *L. interrogans sensu lato*, which includes all pathogenic strains, and *L. Biflexa*, which includes all saprophytic strains. More than 250 serovars belonging to these two species were categorized into antigenically related serogroups. Ten of these serogroups are crucial for canines and felines.

According to Sykes *et al.* (2011) [5], the most prevalent serovars of *Leptospira* that are typically recorded in dogs are Australis, Autumnalis, Canicola, Grippothyphosa, Hardjo, Icterohaemorrhagiae, Pomona, Saxkoebing, and Sejroe. *Leptospira interrogans* and *Leptospira Kirschner* are the main pathogens that affect dogs (Sykes *et al.*, 2011) [5]. In Brazil, *Leptospira Noguchi* was discovered in a sick dog (Silva *et al.*, 2009) [7].

2.2 Host and Reservoirs

Leptospirosis is mostly transmitted by definitive or reservoir hosts as well as accidental hosts, both of which are mammals. Dogs serve as both the reservoir and maintenance host for the serovar Canicola and may also accidentally harbour other serovars that could cause clinical illness (Burr *et al.*, 2009) [8]. For other serovars, rodents, skunks, raccoons, farm animals, and deer serve as reservoir hosts and regularly excrete the organisms in their urine.

2.3 Transmission

Most frequently, contact with contaminated urine results in the transmission of leptospirosis. For months to years, asymptomatic reservoir hosts may expel the organism in their urine. Direct contact between diseased animal urine and a host's injured skin or mucous membranes also results in infection. Inoculation of bite wounds, venereal or placental transfer, and consumption of tainted raw meat are other means of transmission (Meeyam *et al.*, 2006) [9]. The more common indirect transmission method involves exposing susceptible humans or animals to contaminated soil or surface water.

2.4 Risk Factors

Season, age, gender, contact with rodents, and access to outside water are some of the risk factors associated with leptospirosis. Epidemics of leptospirosis in both animals and people have been connected to prolonged periods of rain or flooding. A temperature range of 0 °C to 25 °C is ideal for organism survival. As freezing drastically reduces an organism's chance of surviving outside of its host, infection patterns in colder areas are probably seasonal (Goldstein, 2010) [10]. Young dogs (under 3 years) and older dogs (between 6 and 12 years) are more likely to contract leptospirosis. It might be because dogs in these age groups have compromised health states (Desai *et al.*, 2020) [11]. Moreover, young dogs are more active than older dogs, thereby higher chance that they will come into touch with contaminated urine.

According to reports, there is a higher risk for males, herding dogs, hounds, working dogs, and mixed breeds (Ward *et al.*, 2002) [12]. The increased prevalence of leptospirosis in intact males may be related to their propensity for wandering, which raises the risk of infection exposure. Sex, age, and breed were not found to be risk factors for acute leptospirosis (Lee *et al.*, 2013) [13].

Dogs living in proximity to outdoor water, those swim or drink from outdoor water sources are to be at greater risk of infection (Ghneim *et al.*, 2007) [14]. Rodents act as reservoir hosts as they carry and excrete the organisms in their urine for extended periods. Hence, contact with rodents increases the risk of leptospirosis. Dogs kept as pets are at risk of contracting *Leptospira* from stray animals. Stray dogs can become infected in several ways, including by direct contact with other animal species, practice of scavenging waste, food seeking, consumption of stagnant water, smelling other animals' urine, and licking female genitalia (Nandini, 2014) [15]. Dogs with outdoor activities or that consume raw meat were found to have a higher risk of leptospirosis (Meeyam *et al.*, 2006) [9].

3. Prevalence

India is recognized for having an endemic ailment called leptospirosis. According to Himani *et al.* (2013) [16], West Bengal, Orissa, Kerala, Tamil Nadu, Karnataka, Maharashtra, Gujarat, and the Andaman Islands consistently report the majority of leptospirosis outbreaks in India. Because of the availability of plenty of standing water and swampy settings, the incidence of canine leptospirosis is increased during the rainy season. Female dogs and dogs between the ages of 1-3 were shown to be more sensitive (Desai *et al.*, 2020) [11].

4. Pathogenesis

For the first 10 days following infection, *Leptospira* organisms are most abundant in blood. Subsequently, higher concentrations of organisms were found in the urine. Normally, the incubation period lasts 7 days, however, it can also be 2 to 30 days long. The number of organisms present, the host's age, and susceptibility all affect the clinical disease's severity. *Leptospira* organisms enter the host through the vaginal tract, conjunctiva, mucous membrane, and skin abrasions. Chemotaxis pathways are necessary for attachment and transmembrane passages (Mohammed *et al.*, 2011) [17]. Leptospires enter the body and quickly spread to the liver, spleen, kidneys, eyes, central nervous system, and urogenital tract, among other locations. The degree of internal organ damage varies and may be influenced by the host's sensitivity as well as the organism's virulence (Greene *et al.*, 2012) [18].

Invading leptospires enter the vascular compartment and cause endothelial injury, vasculitis, and hemorrhagic syndrome. The action of unidentified leptospiral poisons or toxic cellular components causes symptoms and lesions, once the number of leptospires in the blood and tissues reaches a critical level. Typically, these organisms prefer to proliferate in the renal tubular epithelial cells after entering the body, leading to tubular injury and renal insufficiency (Mohammed *et al.*, 2011) [17].

As organisms colonize and decimate renal tubular epithelial cells in the convoluted tubules of the kidney, renal failure develops in the subacute stage of leptospirosis. This results in acute renal insufficiency by impairing renal blood perfusion due to parenchymal swelling and thereby causing decreased glomerular filtration rate. Hypoxia secondary to renal ischemia may be the primary alteration producing nephropathy in leptospirosis patients. Hence, when therapy is initiated early in the course of infection, renal function may quickly improve. In severe situations, dehydration, significant bleeding, or increased capillary permeability related to vasculitis may result in hypovolemia and hypotension.

These organisms may also enter the portal circulation, producing hepatic involvement and hepatic cell necrosis and fibrosis, which together result in hepatic insufficiency.

Leptospira can also cause meningitis and uveitis by entering the CNS and the eye (Binder *et al.*, 1998) [19]. *Leptospira* involvement in the heart and lungs is a rare occurrence.

5. Clinical Signs

Fever, inappetence, vomiting, abdominal pain, diarrhoea, polyuria, polydipsia, myalgia, jaundice, epistaxis, and haematuria are some of the clinical symptoms of acute leptospirosis (Kohn *et al.*, 2010) [20].

Acute renal failure with oliguric, anuric, or polyuric symptoms can result from renal involvement in leptospirosis. Acute renal failure in dogs can manifest as polyuria, polydipsia, dehydration, vomiting, diarrhoea, inappetence, lethargic behaviour, or abdominal pain.

According to Greene *et al*. (2012) [18], hepatic involvement can range from a moderate increase in liver enzyme with or without hyperbilirubinaemia to severe liver failure with symptoms such as icterus, ascites, and hepatic encephalopathy. Respiratory signs such as tachypnoea or mild to severe dyspnoea may occur following acute respiratory distress syndrome or leptospiral pulmonary haemorrhage syndrome (LPHS). Dogs with LPHS develop multifocal intra-alveolar haemorrhages, which can be rapidly progressive and lead to massive haemoptysis and respiratory failure (Kohn *et al.*, 2010) [20].

Canine leptospirosis has been linked to hematemesis, hematochezia, hemoptysis, melena, epistaxis, petechial haemorrhages, and conjunctivitis (Minke *et al.*, 2009) [21].

Leptospirosis in dogs has been associated with cardiac symptoms in some cases. Leptospirosis may have caused myocardial injury in some dogs based on electrocardiographic abnormalities including ventricular tachyarrhythmias and elevated serum troponin concentrations (Mastrorilli *et al.*, 2007) [22]. Ocular symptoms include epiphora, mucopurulent discharge, diminished pupillary reflexes, conjunctivitis, pan-uveitis, scleral infection, aqueous flare, hyphaemia, papilloedema, retinal detachment, and retinal haemorrhages (Townsend *et al.*, 2006) [23]. Serovar Bratislava and Buenos Aires (serogroup Djasiman) infection in a dog was linked to abortion and infertility (Ellis, 1986 and Rossetti *et al.*, 2005) [24, 25].

6. Diagnosis

6.1 Direct identification of leptospires

Bacterial Culture: Ellinghausen-McCullough-Johnson-Harris (EMJH) medium is the most often used media. Fresh tissue, blood, or urine can be used as biological samples for cultures.

Darkfield Microscopy (DFM): Darkfield microscopy can be used to directly see leptospires in the blood and urine of affected dogs. However, several factors, such as the brief duration of leptospiremia or prior antimicrobial medication therapy, contribute to the limited sensitivity of DFM.

Tissue Biopsy: Giemsa or silver stains can be used to detect leptospires in tissue sections such as kidneys, and immunohistochemistry and immunofluorescence techniques can be used to provide a conclusive diagnosis (Goldstein, 2010) [10].

6.2 Polymerase chain reaction

Targeting the LipL32/hap1 gene, which is unique to pathogenic *Leptospira* spp., several PCR tests have been devised for the diagnosis of canine leptospirosis (Harkin *et al.*, 2003) [26].

6.3 Serological tests

Microscopic agglutination test: The "gold standard" test for acute leptospirosis is the microscopic agglutination test (MAT), which is the most commonly utilized test (Levett, 2001) [27]. By combining several dilutions of patient serum with live or dead, standardized leptospires, MAT analyses the serum to identify the agglutinating antibodies. Leptospires clump together when anti-leptospiral antibodies are present, and these clumps can be seen using dark-field microscopy.

Leptospirosis agglutination reactions are positive when the titre is 1:100 or more (OIE, 2021) [28]. Due to the typical delay in the development of serum antibodies, infected dogs may even be antibody negative during the acute stage of the disease. However, non-infected dogs which received bivalent or quadrivalent whole-cell anti-leptospiral vaccinations could have post-vaccinal titres of 1:6400 or higher to both vaccine and non-vaccinal serovars (Martin *et al.*, 2014) [29]. A fourfold rise in MAT or a convalescent titre of at least 800 to one or multiple serovars in an initial antibody-negative dog is highly suggestive of leptospirosis.

Enzyme-Linked Immunosorbent Assays: To boost specificity, ELISAs have been developed to identify antibodies against both the entire bacterial cell as well as specific recombinant antigenic proteins on the bacterial surface.

Latex Agglutination Test: For the quick, low-cost, and easy identification of Leptospira-specific antibodies in serum or plasma samples, the latex agglutination test (LAT) is used. The antibodies are found using the recombinant LipL32 antigen. Depending on the degree of agglutination and the length of time it took for agglutination to develop, the serum samples are rated as positive on a scale from +1 to +4. If no agglutination was shown within 5 minutes, the serum samples are deemed negative. When compared to the microscopic agglutination test, the latex agglutination test's specificity and sensitivity were 96% and 98.07%, respectively (Dey *et al.*, 2007) [30].

Point-of-care diagnostic assays: To identify canine Leptospira-specific antibodies, point-of-care (cage-side) lateral-flow serologic assays have just been commercially available.

A point-of-care ELISA called SNAP® Lepto (IDEXX Laboratories, Westbrook, ME, USA) finds antibodies against LipL32, a plentiful membrane protein of *Leptospira* (Curtis *et al.*, 2015) [31].

A lateral flow test called the WITNESS® Lepto (Zoetis, Parsippany-Troy Hills, NJ, USA) uses whole-cell extracts from *L. Kirschner* serovar Grippotyphosa and *L. interrogans* serovar Bratislava to find IgM antibodies. Only IgM is detected by WITNESS® Lepto, which simplifies the analysis and prevents cross-reactivity with IgG antibodies from dogs that have previously had a *Leptospira* vaccination. In comparison to the MAT, the trustworthy test WITNESS® Lepto has a higher rate of sensitivity and specificity for the diagnosis of acute leptospirosis (Kodjo *et al.*, 2016) [32].

Leptocheck®-WB kit is a fast, qualitative sandwich immunoassay for the detection of *Leptospira*-specific IgM antibodies in serum/plasma or whole blood (Zephyr Biomedicals, Tulip Diagnostics Pvt Ltd.). For the

serodiagnosis of recent leptospirosis, it is helpful. The specificity and sensitivity of Leptocheck® - WB were 95% and 95.7%, respectively when compared to the commercial ELISA test (Mahendra, 2021) [33].

6.4 Haemato-biochemical findings

Leucocytosis of mild to moderate severity and thrombocytopenia are frequent CBC abnormalities. Disseminated intravascular coagulation (DIC) characterized by extensive platelet consumption causes moderate to substantial thrombocytopenia (Karen, 2010) [34]. Azotemia, elevated blood liver enzyme activity, electrolyte abnormalities, and a little elevation in serum bilirubin concentrations are among the most frequent serum biochemical results. Leptospirosis-induced inhibition of the Na+ -K+ -ATPase can result in potassium wasting and hypokalaemia, which can both be caused by renal and/or gastrointestinal losses (Nandini, 2014 and Burth *et al.*, 1997) [15, 35].

6.5 Urine analysis

Proteinuria, glucosuria, bilirubinuria, haematuria, pyuria, and cylindruria are frequently seen in the urine (Mastrorilli *et al.*, 2007 and Kohn *et al.*, 2010) [22, 20].

6.6 Diagnostic imaging

Radiographic alterations indicative of leptospiral pulmonary haemorrhagic syndrome (LPHS), range from a mild interstitial pattern to a mild to severe reticulo-nodular pulmonary pattern with focal alveolar infiltrates (Baumann and Fluckiger, 2001) [36].

The most common abdominal sonographic findings related to kidneys include indistinct corticomedullary distinction, hyperechoic cortex, medullary band (Hyperechoic medulla), pyelectasia, and hyperechoic medullary rim sign (Chandrasekaran *et al.*, 2011) [37]. Hepatic changes, including hypoechoic parenchyma, hepatomegaly, and evidence of biliary sludge can also be found (Sonet *et al.*, 2018) [38].

7. Treatment

Leptospirosis is treated with antimicrobials and supportive care, with a particular emphasis on the treatment of the disease's renal or hepatic symptoms. Doxycycline or a Penicillin derivative can help with the primary objective of ending bacteremia and sterilizing the urine (Goldstein, 2010) [10]. The medication of choice for eliminating the bacterium from tissue is Doxycycline. Given that dogs with leptospirosis frequently exhibit gastrointestinal symptoms like vomiting, oral Doxycycline is not well tolerated by them, so initial therapy with an intravenous Penicillin derivative (such as Penicillin G, Ampicillin, or Amoxicillin) is frequently advised to end bacteremia until Doxycycline can be used. Dogs with leptospirosis need to be treated with Doxycycline 5 mg/kg every 12 hours or 10 mg/kg every 24 hours by mouth for a total of 14 days. Dogs with gastrointestinal signs should be treated initially with an intravenous 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) (Schuller *et al.*, 2015) [39].

7.1 Supportive therapy

The level of supportive treatment depends on the dog's overall health, the severity of the infection, the kidney condition, and the degree of dehydration. Intravenous crystalloid treatments to correct the electrolyte imbalances caused as a result of diarrhea and vomiting. If oliguria or anuria develops, vigorous fluid diuresis with isotonic fluids and osmotic diuretics (such as mannitol or 10% dextrose solutions) with or without furosemide should be tried. Depending on the clinical signs, antiemetics, and stomach protectants (such as H2-receptor blockers, and sucralfate) may be used. For at least 6 to 12 months after treatment, all healed patients must be continuously monitored for problems related to chronic renal failure, such as hypertension, gastrointestinal ulcers, vomiting, hypokalaemia, and Hyperphosphatemia. The extent of renal damage after treatment may play a key role in determining the long-term prognosis of affected dogs (Sessions and Greene, 2004) [40].

8. Prevention and Control

8.1 Control of infection in animals

Isolation of infected animals: The affected animals should be quarantined right away for at least two weeks, and the area, as well as the equipment, should be completely cleaned and disinfected. Bleach and disposable paper towels are used to clean the cages of patients with leptospirosis that has been suspected or confirmed, as well as any urine contamination outside the cage. The majority of cleaning products, such as bleach, povidone-iodine, and chlorhexidine, are typically quite good at preventing leptospirosis (Sessions and Greene, 2004) [40].

Use of protective clothing: When handling the dog, gloves ought to be used. When cleaning a cage or emptying a urinary collecting system, safety goggles and a face mask should be worn (Sykes *et al.*, 2011) [5].

8.2 Control of sources of infection

Rodent control: The control of the rodent population by chemical (zinc phosphide) means is effective.

Carriers: The urine of animals such as pigs, cattle, and dogs should be examined as they shed the organisms for long-term following recovery. Such urinary shedders should be kept in isolation and treated if possible.

8.3 Control of transmission

Drinking water should be microbiologically safe because leptospires can thrive in contaminated water. The water can be cleaned with potassium permanganate. Farmers, veterinarians, doctors, and other professionals who work directly with animals should be educated on leptospirosis, especially about diagnostic procedures, modes of transmission, and appropriate control measures (Srivastava, 2008) [41]. Decreased access to potential infection sources, such as outside water supplies, and reduced exposure to wildlife through fences are two further protective methods.

8.4 Vaccination

The commercial canine leptospirosis vaccinations available in India are bivalent vaccines comprising antigens from *Leptospira interrogans* (sensu lato) serogroups Icterohaemorrhagiae and Canicola. The Pomona, Grippotyphosa, Bataviae, Hardjo, and Bratislava serovars are not cross-protective. Tetravalent vaccine is advised to broaden the range of protection against *Leptospira interrogans* (sensu lato) serogroups Icterohaemorrhagiae, Grippotyphosa, Pomona, and Canicola. Irrespective of the breed, all dogs which are at risk should receive two initial vaccines spaced 2-4 weeks apart, followed by annual revaccination.

Leptospirosis vaccine administration must be closely watched for anaphylactic reactions including facial oedema, pruritus, hypotension, or dyspnea, especially in some breeds like Dachshunds and Pugs (Sessions and Greene, 2004) [40].

9. Zoonosis/public health considerations

One of the most important zoonotic diseases in India is leptospirosis. Sewage pipelines, sewage that overflows during heavy rain, and flooded roadways are examples of ecological zones where rats create an excellent habitat for the transmission of leptospirosis. Additionally, exposure to river or stream water as a result of outdoor pursuits like canoeing, rafting, fishing, or swimming might result in the spread of the infection. Leptospiral infection may be brought on by exposure to agricultural wet fields in rural locations that may have been contaminated with rat or farm animal urine. According to Himani *et al.* (2013) [16], farmers who work in rice fields run a high risk of contracting the so-called rice field workers' disease.

Along with farmers, employees of slaughterhouses, animal carers, and researchers who work with animals, veterinarians are thought to be at a higher risk. Several writers have proposed that leptospirosis can be transmitted from dogs to people (Barmettler *et al.*, 2011) [42]. Human leptospirosis can be deadly or asymptomatic and is frequently mistaken for a viral disease. The symptoms of mild leptospirosis in people include fever, myalgia, headache, anorexia, nausea, vomiting, and abdominal pain. Weil's syndrome, also known as icteric leptospirosis, is characterized by fever, jaundice, and azotemia (Langston and Heuter, 2003) [43].

10. Conclusion

Leptospirosis is a significant zoonotic disease that affects a variety of animal hosts worldwide but is particularly prevalent in tropical nations like India. In the coastal states of India, including Andaman and Nicobar Island, Tamil Nadu, Karnataka, Andhra Pradesh, and portions of Kerala, it is regarded as an endemic disease. Clinical signs of canine leptospirosis include hepatorenal failure, leptospiral pulmonary hemorrhagic syndrome (LPHS), uveitis, diarrhoea in the absence of infection (DIC), and abortions. Microscopic agglutination test (MAT) is the gold standard test for diagnosis. For dogs exhibiting clinical leptospirosis symptoms, penicillin or tetracycline derivatives like ampicillin or doxycycline are advised as antibiotic treatments. For effective control and prevention of canine leptospirosis, accurate early diagnosis, treatment with a suitable therapeutic regimen for diseased dogs, and routine vaccination of healthy dogs with inactivated leptospiral vaccines are strongly advised.

11. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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