S.L. Vet. J. 71(2)
Clinical Communication
DOI: https://doi.org/10.4038/slvj.v71i2.93

CLINICAL PRESENTATION, DIAGNOSIS, AND TREATMENT OF LEPTOSPIROSIS IN FOUR OWNED DOGS FROM SRI LANKA

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SUMMARY: Leptospirosis is a zoonotic disease of global importance. This clinical communication reports the clinical presentation, diagnosis, treatment, and outcomes of leptospirosis in four owned dogs presented to the Veterinary Teaching Hospital, University of Peradeniya, within a period of four months. Clinical signs included anorexia, lethargy, and depression, with abdominal and lumbar pain on palpation. Only one dog had been properly vaccinated and dewormed. Common haematological and laboratory findings included anaemia, thrombocytopenia and elevated blood urea nitrogen (BUN). Diagnosis was confirmed using the microscopic agglutination test (MAT) in two dogs and nested polymerase chain reaction (PCR) targeting the *fla*B gene in the other two. Initial therapy consisted of fluid administration and antibiotics. Despite treatments, three dogs died within two to seven days, while one dog survived.

BACKGROUND

Leptospirosis is a zoonotic disease caused by a thin, filamentous, aerobic spirochete bacterium of the genus *Leptospira* (Ananda *et al*., 2008). It affects humans and a wide range of animals, with high levels of morbidity and mortality (Kalin *et al*., 1999; Santos *et al*., 2021). This disease has a major impact on people in both urban and rural environments (Bharti *et al*., 2003), particularly in tropical regions like South and Southeast Asia, which exhibit higher incidence rates compared to temperate regions (Nakashiro *et al*., 2024). Globally, more than 300 serovars of *Leptospira* have been identified, with most pathogenic serovars in dogs including *L. canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. pomona*, and *L. bratislava* (Kalin *et al.*, 1999*;* Khan *et al.*, 2019; Ko *et al.*, 2009). The acute forms of canine leptospirosis are most commonly associated with *L. canicola* and *L. icterohaemorrhagiae* serogroups (André-Fontaine, 2006). In Sri Lanka, thirteen different strains of leptospires from five species have been isolated in humans, with ten serogroups reported (Warnasekara and Agampodi, 2017). Dogs serve as a natural reservoir for *L. canicola* serogroups, while rodents, particularly rats, act as reservoirs for *L. icterohaemorrhagiae* serogroups (Burriel, 2015). Leptospirosis is infectious in moist habitats and spread directly through the urine of infected animals or indirectly via contaminated environments (André-Fontaine, 2006). In dogs, the disease typically affects adults aged 1 to 6 years, with an incubation period ranging from 3 to 20 days (Khan *et al*., 2019).

Clinical presentation in dogs varies by disease stage: per-acute, acute, sub-acute, and chronic (Langston and Heuter, 2003). The severity depends on the virulence of the organism and host susceptibility (Khan *et al*., 2019; Tansakul *et al*., 2024). Common clinical signs include anorexia, lethargy, vomiting, fever, weight loss, polydipsia, polyuria, diarrhoea, abdominal/lumbar pain, icterus, myalgia, renomegaly, petechiae, severe haemorrhage and thrombocytopenia (Geisen *et al*., 2007; Khan *et al*., 2019). Acute or icteric forms often involve hemoglobinuria, pulmonary haemorrhage, meningitis, renal failure, systemic collapse, and jaundice (Burriel, 2015; J. Warnasekara *et al*., 2019). Liver damage associated with leptospirosis is indicated by elevated levels of serum enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Among these, ALP typically shows a more pronounced increase compared to ALT, highlighting cholestatic hepatopathy. This condition arises from the spirochetes disrupting the intercellular junctions within hepatocytes (Khan *et al*., 2019).

The diagnosis of leptospirosis combines history, physical, and laboratory findings with confirmatory testing. Serological detection methods, such as the *Leptospira* microscopic agglutination test (L-MAT),

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are considered the gold standard for acute diagnosis when paired serum samples are available (Warnasekara and Agampodi, 2017). However, molecular methods such as polymerase chain reaction (PCR) have emerged as critical tools for early and accurate detection (Santos *et al*., 2021). PCR identifies pathogenic *Leptospira* DNA directly from blood, urine, or tissues, targeting specific genes like flaB or lipL32 (Hilbe *et al*., 2024). Nested PCR and quantitative PCR (qPCR) are particularly sensitive, allowing detection even at low bacterial loads (Alinaitwe *et al*., 2017; Miotto *et al*., 2018).

Patients with suspected or confirmed leptospirosis should receive a combination of antimicrobial and supportive care adjusted to each patient according to the severity of clinical signs and affected organ systems. The recommended antibiotics are IV penicillin derivatives (Ampicillin, Penicillin G) or oral doxycycline (Bharti *et al*., 2003). Other antibiotics such as cephalosporin (Cefotaxime, Ceftriaxone), macrolides (Azithromycin), tetracycline have been investigated for use in people with leptospirosis. However, their use in dogs has not been fully investigated and therefore they are not recommended as first-line therapies (Burriel, 2015).

CASE PRESENTATION

Four dogs were presented to the Veterinary Teaching Hospital with varying clinical signs. These included a 3-year-old, 15 kg neutered male German Shepherd (Dog 1), an 11-month-old, 25 kg intact female Labrador Retriever (Dog 2), a 10-year-old, 13 kg intact male crossbreed (Dog 3), and a 5-year-old intact male Pomeranian (Dog 4). Among them, only the Labrador Retriever had received proper vaccinations, including the DHL vaccine, administered less than one year ago. This vaccine contained the serogroups *Icterohaemorrhagiae* and *Canicola* (Nobivac®). Deworming was up to date only for the German Shepherd and the Labrador Retriever. The German Shepherd originated from a rural, agricultural area with a high incidence of leptospirosis in humans, in contrast to the other three dogs. Furthermore, the owner of the German Shepherd reported that the dog had ingested a rat one week prior to admission.

The clinical signs observed in all four dogs included anorexia (n=4), vomiting (n=4), and lethargy (n=4). Additional symptoms were reported, such as diarrhoea (n=2), haematochezia (n=1), and haematuria (n=2). Physical examination revealed hyperthermia (>39°C, n=2), hypothermia (<37°C, n=1), and varying degrees of dehydration (8%, n=2). Notable systemic findings included polydipsia (n=1), icterus (n=1), and severe abdominal pain (n=2). Specific abnormalities such as coffee-coloured urine with subsequent anuria (n=1), bounding pulse suggestive of hypovolemia, and tachypnoea with an elevated respiratory rate of 52 breaths per minute (n=1) were also observed. One dog died at initial examination before further analysis and treatment could be instituted.

Table 1. Summary of clinical signs and general clinical examination findings in the four dogs.

Parameter	Dog 1	Dog 2	Dog 3	Dog 4
History	anorexia, lethargy, vomiting, diarrhea, coffee-colored urine, later anuria	anorexia, vomiting, diarrhea, polydipsia, mild icterus	anorexia, hematochezia, hematuria, anuric for 2 days	reduced appetite, lethargy, hematuria, severe abdominal pain
Temperature (38.3°C - 39.2°C)	39.4°C,	37.5°C	36.7°C	39.4°C,
Heart rate (60 - 160 bpm)	120 bpm	84 bpm	120 bpm	-
Respiratory rate (10-30 breaths/min)	32	28	52	-
Mucous Membranes	pale	mildly icteric	Pale	pale
Dehydration	8%	not dehydrated	8%	mild
Pain on Palpation	abdominal	abdominal	lumbar region	abdominal
Other Findings	depressed, CRT <2 s	no gross clinical abnormalities	weak CRT >2 s	depressed, died shortly after presentation

LABORATORY AND OTHER INVESTIGATIONS

Routine Laboratory Tests

Blood samples for hematological and biochemical profiles (Tables 2, 3, and 4), as well as urine samples collected via catheterisation for urinalysis (Table 5), were analysed. Specific gravity (SG) values of urine were measured using dipstick and confirmed via refractometer.

Detection of Leptospira

Blood samples collected into EDTA tubes and urine samples collected into 15 ml sterile conical tubes collected from suspected dogs (1, 2 and 3) were submitted to Zoonotic Diseases Diagnostic and Research Laboratory, Department of Microbiology, Faculty of Medicine, University of Peradeniya. Only a blood sample could be collected from dog 4 and it was submitted to the same laboratory. Samples were processed within 2 hours of collection. Plasma was separated from blood samples through a two-step centrifugation process (3000 × g for 10 minutes, followed by 15,000 × g for 20 minutes), and sediments from urine samples were similarly processed. All processed samples were stored at -20°C until further analysis.

Qualitative and quantitative MAT were performed using plasma samples and a live *Leptospira* culture panel representing 13 serogroups *(Sejroe, Icterohaemorrhagiae, Canicola, Javanica, Shermani, Hebdomadis, Autumnalis, Panama, Grippotyphosa, Tarassovi, Bataviae, Semaranga,* and *Pyogenes*), with a cutoff titer of 1:400 for positivity. Additionally, DNA extraction from blood and urine sediments was performed using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Subsequently, nested PCR was carried out targeting the *fla*B gene of pathogenic leptospires (Koizumi *et al*., 2008). The PCR products were analysed by electrophoresis on a 2% agarose gel visualised under UV light. This assay included a positive control (DNA of *L. interrogans,* pathogenic), a negative control (DNA of *L. biflexa* serogroup *patoc,* non-pathogenic) and a blank containing only the reagents in each PCR run to ensure assay validity (Athapattu *et al*., 2020). In this study DNA quantification was not carried out.

Key Laboratory Findings

All dogs exhibited thrombocytopenia, anaemia, and leucocytosis to varying degrees (Table 2). Biochemical analyses revealed elevated blood urea nitrogen (BUN) and creatinine levels (could be done only in dog 4), indicating compromised renal function (Table 3). Urine specific gravity (SG) readings ranged from 1.031 to 1.005, reflecting variable renal concentrating ability across cases. Urinalysis showed proteinuria in three dogs, with severe proteinuria (300+) observed in one dog. Haematuria and pyuria were noted in some cases, alongside acidic urine pH in all four dogs (Table 4). MAT titres ranged from 1:400 (Dog 2) to 1:800 (Dog 1), with the *Canicola* and *Hardjo* serogroups identified as likely infecting serovars (Table 5). PCR testing confirmed leptospiral DNA in the blood samples of dogs 3 and 4, while dogs 1 and 2 tested negative for blood PCR despite positive MAT results. Urine PCR results were negative in all cases.

Table 2. Haematological parameters of four dogs

Dog	Days post-presentation	White Blood Cell ($\times 10^3/\mu L$)	Hematocrit (%)	Platelets ($\times 10^3/\mu L$)
1	–	31.11	19.8	62
2	1	5.18	36.4	35
	2	6.1	31.6	45
	3	16.82	30.5	15
	4	16.2	31.2	237
3	–	98.93	9.7	141
4	–	98.78	13.5	94

Table 3. Serum biochemistry parameters of four dogs

Dog	Days post presentation	BUN (10-28 mg/dl)	Creatinine (0.5-1.5 mg/dl)	ALT (21-102 mg/dl)	Icteric index <15-20 mg/dl
1	–	196	-	-	-
2	1	146.12	-	245.8	10
	2	148.41	-	261.4	25
	3	102.88	-	211.1	15
	4	24.67	-	24.76	-
3	–	32.56	-	-	-
4	–	77.0	4.4	269	-

Table 4. Urinalysis findings of four dogs on the day of presentation

Dog	Specific gravity	Blood /HPF	Protein	Leukocytes	PH
1	1.020	>100	300	-	5
2	1.020	3	100	-	6
3	1.020	>100	30	-	5
4	1.031	>100	100	30+	6

Table 5. Leptospirosis confirmatory diagnosis test results of four dogs

Dog	Samples	Test done	Result
1	Blood	MAT	Positive - *Hardjo* (1:800)
		PCR	Negative
	Urine	PCR	Negative
2	Blood	MAT	Positive - *Canicola* (1:400)
		PCR	Negative
	Urine	PCR	Negative
3	Blood	MAT	Negative
		PCR	Positive
	Urine	PCR	Negative
4	Blood	PCR	Positive

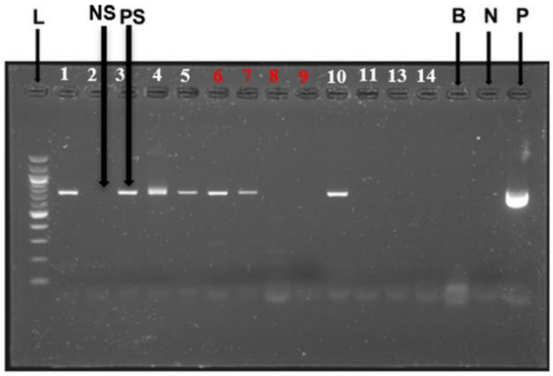

Figure 1: Agarose gel electrophoresis of PCR products for *Leptospira* detection from blood DNA

L:100bp DNA ladder, B: Blank (no template control), N: Negative Control (*Leptospira biflexa* serogroup patoc, non-pathogenic), P: (Positive control *Leptospira interrogans* serogroup MMC, pathogenic)
Lane 1-14: Canine blood DNA samples, Lane 6-9: Samples from the study dogs (Dog 4, Dog 3, Dog 2, and Dog 1, respectively)
Lane 1-5 and 10-14: Additional canine blood DNA samples (negative samples (NS) and positive samples (PS))

DIFFERENTIAL AND DEFINITIVE DIAGNOSIS

In all four cases, clinical evaluation and laboratory investigations revealed overlapping signs such as anorexia, lethargy, vomiting, and haematuria suggestive of renal and systemic infectious aetiologies. Acute renal failure was considered a key differential diagnosis in dogs 1, 3, and 4, based on clinical signs including anuria and dehydration, supported by elevated serum BUN levels. A urinary tract infection (UTI) was suspected in dog 4 due to the presence of bacteriuria and pyuria on urinalysis, but was ruled out in the other cases based on dipstick and urine sediment analysis.

Intoxication and gastroenteritis were initially suspected in dogs 1, 2, and 3, respectively, but were excluded following persistence of symptoms and normal faecal analysis. Dog 3 also presented with haematochezia and icterus, prompting evaluation for babesiosis, hepato-biliary disease, and immune-mediated haemolytic anaemia (IMHA), all of which were ruled out via specific diagnostic tests including blood smear, hepatic enzyme profiling, and ultrasound examination.

Positive MAT results confirmed leptospirosis in dogs 1 and 2, consistent with the clinical picture and laboratory data. In dogs 3 and 4, the diagnosis was confirmed by PCR detection of leptospiral DNA in blood samples, while negative PCR results in dogs 1 and 2 were likely due to timing of sample collection and disease progression. Notably, leptospiral DNA was not detected in urine samples from any case, suggesting intermittent shedding or limitations related to disease stage.

TREATMENT AND OUTCOME

In all cases, treatment was initiated with Lactated Ringer's solution (LRS) and normal saline (NS) to restore hydration and promote renal function. Fluids were administered intravenously (IV) and subcutaneously (SC) at a rate of 80 ml/h, accounting for maintenance needs, rehydration, and ongoing losses. Urine output was closely monitored throughout the treatment process.

In dog 1, since the animal was anuric, furosemide was administered in increasing doses (2 mg/kg bid IV, increased to 4 mg/kg bid IV after 30 minutes, and continued for 2 days). ciprofloxacin (10 mg/kg SID IV), acetylcysteine (10 mg/kg bid IV), omeprazole (1 mg/kg SID, IV), promethazine (0.2 mg/kg bid IV), vitamin B complex syrup (10ml bid PO), lactulose Syrup (0.5ml/kg bid PO), and lactulose enema (20 ml/kg, 3 parts lactulose to 7 parts water) per rectum as a retention enema (q12h) were also administered. Twenty-four hours later, the dog started urinating with improved water intake. Subsequent to the positive MAT results for leptospirosis, doxycycline (10 mg/kg SID, PO) was initiated and ciprofloxacin was discontinued. Irrespective of the treatment, the dog passed away within two days.

In dog 2, following a positive *Leptospira* diagnosis during the second visit, doxycycline (10 mg/kg bid PO) was added to the ongoing treatment, replacing clavulanate amoxicillin. Upon three days of presentation, the dog had shown a slight improvement in appetite and a reduction in vomiting, with normal vital parameters and mild icterus. Haematological and biochemical profiles revealed persistent thrombocytopenia and uraemia, with the icterus index elevated to 25 (Tables 2 and 4). During the subsequent follow-up visit three days later, the dog's appetite had fully returned, and icterus had significantly diminished. Haematological and biochemical results indicated normalisation of thrombocyte count and kidney function, although ALT levels were still elevated two-folds (Tables 3). Doxycycline was continued for an additional week, while the rest of the medications were discontinued. The patient made a full recovery after one week.

In dog 3, the treatment protocol included oral doxycycline (10 mg/kg SID, PO), erythropoietin (100 IU/kg), acetylcysteine (10 mg/kg bid IV), vitamin B complex (1 mL bid IV), omeprazole (1 mg/kg SID, IV), and ondansetron (0.5 mg/kg bid IV)

and for the dog 4, only initial hydration therapy could be administered before the deterioration of animal's condition. However, both dogs 3 and 4 also died on the following day and while on initial treatments, respectively.

DISCUSSION

The four cases presented shared a common history of anorexia, lethargy, vomiting, and diarrhoea, which reflect the systemic nature of leptospirosis and align with previous reports (Alinaitwe *et al*., 2017; Greenlee *et al*., 2005). Thrombocytopenia observed in all cases is a well-documented finding in leptospirosis, likely linked to leptospiraemia during acute infection (Kalin *et al*., 1999). Also, renal involvement was evident in all cases, as indicated by elevated BUN and creatinine levels, though not all cases progressed to renal failure. One dog retained normal concentration capacity, suggesting partial renal function preservation. Elevated ALT levels were observed in two dogs, but given the systemic nature of leptospirosis, this parameter may reflect inflammation or cellular stress rather than liver-specific damage.

The definitive diagnosis of leptospirosis was achieved using the MAT, which is regarded as the gold standard (Miller *et al*., 2011). However, a limitation of this study was the reliance on single plasma samples, with a cut-off value of 1:400 for vaccinated dogs or those exposed to other serogroups. While MAT is reliable in later stages of infection due to higher antibody titres, early-stage infections may yield false-negative results. The absence of paired samples in this study limits the ability to confirm acute infection via a fourfold rise in antibody titre. Molecular diagnostics such as PCR are more effective for early-stage detection within 10 days of exposure when leptospires are still circulating in the blood. After this period, organisms localise in immune-privileged sites such as renal tubules, which may lead to intermittent shedding in urine and potential false-negative PCR results (Bharti *et al*., 2003; Schuller *et al*., 2015).

Vaccination status played a crucial role in disease severity. Of the four dogs, only dog 2 was vaccinated with a bivalent vaccine (targeting *L. icterohaemorrhagiae* and *L. canicola*), about ten months prior to the infection, which likely contributed to the milder disease course and eventual recovery. These results show that even though the vaccination will reduce the severity of infection, it does not prevent the carrier state (André-Fontaine, 2006). According to World Small Animal Veterinary Association's vaccination guidelines, the immunity provided by the leptospirosis vaccine in dogs does not last beyond one year, necessitating annual boosters to maintain protection. While the vaccine is effective in reducing the risk of severe disease, it may not completely prevent infection. Dogs in high-risk environments, even with a proper vaccination history, may still become infected due to the variability in *Leptospira* serovars and the limited cross-protection between them.

Comparisons with cases reported in other countries reveal similarities in clinical presentation and laboratory findings, including thrombocytopenia and azotemia, regardless of vaccination status (Geisen *et al*., 2007; Miotto *et al*., 2018). Severe hepatic dysfunction is most commonly associated with the serovars *Icterohaemorrhagiae* and *Pomona*, whereas *Canicola* and *Grippotyphosa* are typically linked to minimal liver involvement (Langston and Heuter, 2003).

However, molecular diagnostics such as PCR are increasingly utilised in other countries to confirm leptospiral infections early, particularly in unvaccinated dogs. Studies in Europe and North America have reported higher incidence rates in unvaccinated dogs, similar to the findings here (Andrade-Silveira *et al*., 2024). However, vaccination rates also vary, with higher coverage in owned dogs in developed countries, potentially reducing disease severity and prevalence compared to Sri Lanka (André-Fontaine, 2006; Habuš *et al*., 2020; Sykes *et al*., 2011). In this study, DNA quantification was not performed as the samples were derived from clinical specimens, which often contain varying levels of DNA. Given the high sensitivity of nested PCR, even small amounts of target DNA can be amplified and detected making quantification less critical in this context. Due to practical constraints, additional samples which are not related to this study were incorporated into the gel electrophoresis to increase efficiency of gel electrophoresis and minimise resource usage.

In this study, doxycycline (10 mg/kg, PO, SID) was the antibiotic of choice, initiated promptly in one case and adjusted based on diagnostic confirmation in others. This approach aligns with current treatment guidelines (Gommeren and Daminet, 2017). Supportive care, including fluid therapy and symptomatic management, was critical in stabilising affected dogs, though mortality remained high in severe cases.

The findings from this report highlight the importance of timely diagnosis and intervention in canine leptospirosis. Enhanced diagnostic capabilities, comprehensive vaccination strategies, and public awareness of the zoonotic risks are crucial for improved disease management. Further, research into vaccine development and molecular epidemiology of *Leptospira* in endemic regions is necessary to mitigate its impact on canine and public health.

Clinical significance

Leptospirosis is an infectious disease which infects not only dogs, but other mammals. People can be infected with leptospirosis, causing potentially life-threatening illness making leptospirosis a particularly dangerous zoonotic disease. The efficacy of the current protocols of vaccination for leptospirosis is now being questioned. So far, various types of vaccines have been experimentally considered as good candidates for effectively preventing infection, or at least clinical disease. Until an effective vaccine for various animal species and safe for man is produced, prevention of losses is possible through antimicrobial treatment. Thus, awareness of veterinarians on the prevalence of this lethal disease among animals will enhance the possibility of early disease diagnosis which could result in decreased zoonotic potential. Taking necessary steps to make the public aware of the pets' role in acting as carriers would certainly be important.

Considering the number of reported cases of leptospirosis in companion animals within a short period of time in the year, it is of paramount importance for veterinarians, animal handlers, dog breeders, and dog owners to take the necessary precautions when dealing with animals with acute nephritis/renal failure until a confirmatory diagnosis is made to rule out leptospirosis. This case report highlights the importance of proper diagnosis of leptospirosis, together with a basic clinical diagnostic work-up.

ACKNOWLEDGMENTS

We acknowledge Dr. Sanduni Abeyratne, Dr. Dhanushka Hewapathirana, and Dr. Sewwandi Wijesooriya, who assisted in managing these patients, the support staff of the Veterinary Teaching Hospital, University of Peradeniya, and the owners of the dogs discussed in this article.

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