

Occurrence of leptospirosis in dogs with renal and/or hepatic disease in Chennai, India

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Abstract

Leptospirosis is an emerging zoonotic bacterial infection affecting both humans and animals that usually exhibits multisystemic symptoms. However, the classical disease in dogs is characterized by renal disease or concurrent renal and hepatic diseases. This retrospective study was designed to identify the occurrence of leptospirosis in dogs presented with renal and/or hepatic illness in Chennai, India. A total of 78 dogs were selected, and details such as signalment, medical history, management as well as clinical observations were recorded. Serum samples collected from affected dogs were examined using the gold standard Microscopic agglutination test (MAT) with cut-off titer of $\geq 1:100$. Statistical analysis was performed using Pearson's chi-square tests for risk factor analysis. Of the total 78 dogs, 48 (61.54%) were seropositive for leptospirosis based on MAT having titres ranging from 1:100 to 1:1600. Among 12 regionally prevalent serogroups tested in MAT, Australis serogroup (n=28) was found to be highly prevalent, followed by Autumnalis (n=07), Ballum (n=03), Canicola (n=03), Javanica (n=03), Grippityphosa (n=02), Pomona (n=01) and Pyrogenes (n=01). Adult dogs (1 to 5 years) ($p<0.05$), dogs with history of rodent exposure ($p<0.05$) and dogs with outdoor activity ($p<0.01$) had a higher risk for leptospirosis, but no significant association was observed between sex, breed, vaccination status and occurrence of disease. This study showed around two in three dogs presented with renal and/or hepatic disease have the possibility of being affected with leptospirosis, which necessitates incorporation of regionally circulating serogroups in vaccines for protection against disease.

Keywords: Australis, Dogs, Leptospirosis, MAT, Renal and/or hepatic disease

Highlights

- MAT is the gold standard serological diagnostic test for leptospirosis.
- Forty eight dogs (61.54%) out of 78 dogs with renal and/or hepatic disease were seropositive for leptospirosis.
- Australis and Autumnalis serogroups were predominated.
- Adult dogs, rodent exposure and outdoor activity were identified as significant risk factors.

INTRODUCTION

Leptospirosis is an emerging zoonotic disease of many animal species and humans with global importance (Major *et al.*, 2014; Raj *et al.*, 2021). It is mostly predominant in temperate or tropical countries and endemic in areas affected by heavy rainfall and flooding (Pinto *et al.*, 2022). It is caused by gram-negative pathogenic spirochaete of Genus- *Leptospira*, which consists of 66 different species and includes more than 300 serovars (Schuller *et al.*, 2015; Caimi and Ruybal, 2020). The most common method of transmission to both animals and humans is by contact with soil and water contaminated by the urine of infected

animals. Monitoring leptospirosis in dogs as sentinels may also aid in estimating the risk of leptospirosis in humans (Koizumi *et al.*, 2013). A wide range of clinical symptoms, including anorexia, vomiting, depression, icterus, diarrhea and dehydration, may be present in infected dogs, or there may be mild and asymptomatic with a self-limiting febrile sickness (Rissi and Brown, 2014). The classical disease in dogs is characterized by renal disease or concurrent renal and hepatic disease (Mastrorilli *et al.*, 2007). In Tamil Nadu, the seroprevalence of leptospirosis in dogs has been recorded to be 15.21-37.80 per cent (Kumar *et al.*, 2009; LakshmiPriya *et al.*, 2012; Senthilkumar *et al.*, 2023),

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but mere literature is available on the prevalence of leptospirosis in dogs with renal and/or hepatic illness. The purpose of this study was to report the occurrence of leptospirosis in dogs diagnosed with renal and/or hepatic disease by utilizing Microscopic agglutination test (MAT) as a diagnostic method.

MATERIALS AND METHODS

Selection criteria and collection of samples: In the present study, dogs diagnosed with renal and/or hepatic disease presented to the Madras Veterinary College Teaching Hospital (MVCTH) and to the private clinics in Chennai from December 2022 to November 2023 were included. Before the collection of samples, pet owner's consent was obtained. The inclusion criteria of the selected dogs were: dogs showing clinical signs of renal and/or hepatic disease based on elevated serum biochemistry parameters such as blood urea nitrogen (BUN) >28 mg/dL, creatinine >1.7 mg/dL, alanine aminotransferase (ALT) >90 U/L and alkaline phosphatase (ALP) >100 U/L. The epidemiological parameters of each dog were recorded based on the owner's information, such as age, sex, breed, vaccination status and other risk factors (Table 1). The selected dogs were manually restrained for collection

Table 1. Epidemiological parameters of the dogs with renal and/or hepatic disease (n = 78)

Characteristics	No. of dogs (%)
Age	
Young (<12 months)	14 (17.95)
Adult (13-60 months)	31 (39.74)
Senior (61-120 months)	33 (42.31)
Sex	
Male	45 (57.69)
Female	33 (42.31)
Breed	
Small	17 (21.80)
Medium	43 (55.13)
Large	18 (23.07)
Vaccination status	
Vaccinated	20 (25.64)
Not vaccinated	58 (74.36)
Rodent exposure	
Exposed	38 (48.72)
Not exposed	40 (51.28)
Management	
Indoor	29 (37.18)
Outdoor	49 (62.82)
Area	
Urban	48 (61.54)
Suburban	30 (38.46)

of blood samples from cephalic or saphenous vein. Blood samples were collected using clot activator tubes, which helped in rapid formation of a clot, facilitating separation of serum and then centrifuged at 2500 rpm for 10 min. Sera were collected in a 1.5 mL microcentrifuge tube and stored at -20°C for further analysis.

Microscopic agglutination test: Microscopic agglutination test (MAT), which is considered as the gold standard serological test, was employed to detect anti-leptospiral antibodies as recommended by the World Organization for Animal Health (Anonymous, 2021). An antigen panel of 12 serogroups, namely Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Sejroe, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Tarassovi (approximately 2×10^8 leptospires/mL), with 4-5 days old culture maintained at Zoonoses Research Laboratory, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, was used in this study. Before performing the test, serum samples were thawed to room temperature and diluted to 1:50 in phosphate buffered saline (PBS) in a dilution plate. Then, an equal volume of diluted serum sample (50 μ L) and leptospiral antigen (50 μ L) were added to each well in a 96-well U-bottomed plate to make the final serum dilution of 1:100. The 96-well U-bottomed plate was incubated for 2 hours at 37°C, and then a dark field microscope (10x) was used to examine a drop (10 μ L) of serum-antigen mixtures on a clean, grease-free glass slide. The presence of antigen-antibody agglutinations and/or a reduction of 50% free cells were considered as positive at a dilution of 1:100. Highest titres were detected using two-fold serial dilutions up to 1:1600, and the last well with 50% agglutination was noted. The minimum cut-off titer for a positive MAT reaction was taken at a titer of $\geq 1:100$. For individual dogs, the serogroup with the highest MAT titer was recorded.

Data analysis: Statistical analysis was done using IBM SPSS Version 15.0. The total number of seropositive dogs was calculated based on age, sex, breed, vaccination status and other environmental risk factors. Then, Pearson's Chi-square test was employed (Abdul Rahman *et al.*, 2021) to measure the differences in proportions between generated categories, and a p-value of less than 0.05 and 0.01 were considered statistically significant and highly significant, respectively.

RESULTS

Among 78 dogs with renal and/or hepatic diseases selected for this retrospective study, 30 dogs (38.46%), 27 dogs (34.62%) and 21 dogs (26.92%) were found to be affected with only renal disease, only hepatic disease and both renal as well as hepatic diseases, respectively.

Seroreactivity by MAT: Of the 78 dogs, 48 (61.54%) were found seropositive for leptospirosis, confirmed by MAT having titres ranging from 1:100 to 1:1600. The titres of 1:100 and 1:800 were predominated, representing 33.33 per cent (16/48) and 29.17 per cent (14/48) of the positive animals, respectively, followed

by 1:1600 (14.58%, 7/48), 1:400 (12.50%, 6/48) and 1:200 (10.42%, 5/48) (Fig. 1). Among 12 regionally prevalent serogroups tested in MAT, Australis serogroup (58.3%) was found to be highly prevalent, followed by Autumnalis (14.6%), Ballum (6.3%), Canicola (6.3%), Javanica (6.3%), Grippotyphosa (4.2%), Pomona (2.1%) and Pyrogenes (2.1%) (Table 2). No seroreactivity was recorded against Sejroe, Hebdomadis, Icterohaemorrhagiae and Tarassovi serogroups. In nonvaccinated dogs, the most prevalent serogroups were Australis, Autumnalis, Ballum, and Canicola, whereas in vaccinated dogs, prevalent serogroups were Australis, Autumnalis and Javanica (Fig. 2). In

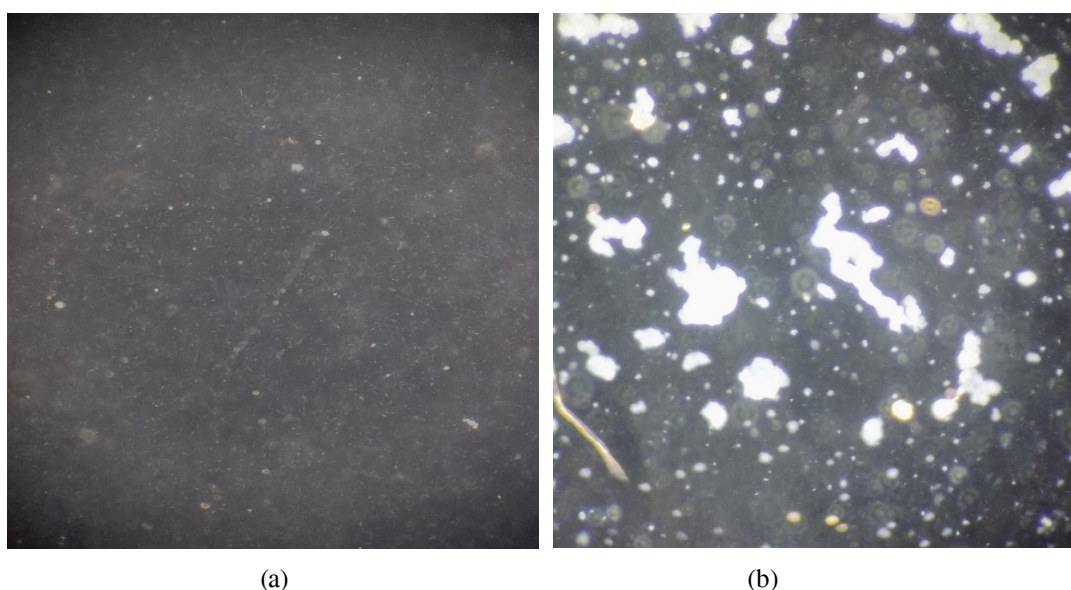


Plate 1. (a) *Leptospira* antigen control under DFM (10x); (b) Positive MAT reaction (1:100 dilution) indicated by antigen-antibody agglutinations under DFM (10x)

Table 2. Frequency of MAT reactions and titres of pathogenic *Leptospira* serogroups in 48 dogs with renal and/or hepatic disease from Chennai, India

Serogroups	Positive reaction (n)	Percentage (%)	Titres				
			1:100	1:200	1:400	1:800	1:1600
Australis	28	58.3	11	02	03	10	02
Autumnalis	07	14.6	02	01	02	02	-
Ballum	03	6.3	02	-	-	-	01
Canicola	03	6.3	-	02	01	-	-
Grippotyphosa	02	4.2	-	-	-	01	01
Sejroe	Nil	-	-	-	-	-	-
Hebdomadis	Nil	-	-	-	-	-	-
Icterohaemorrhagiae	Nil	-	-	-	-	-	-
Javanica	03	6.3	01	-	-	01	01
Pomona	01	2.1	-	-	-	-	01
Pyrogenes	01	2.1	-	-	-	-	01
Tarassovi	Nil	-	-	-	-	-	-
Total	48	100	16	05	06	14	07

Leptospirosis in dogs with renal and/or hepatic disease

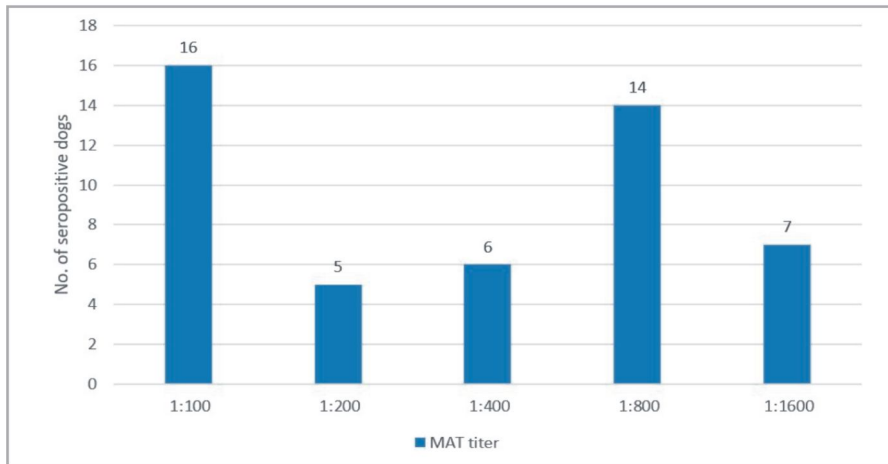


Fig. 1. Frequency of MAT titres from 48 seropositive dogs

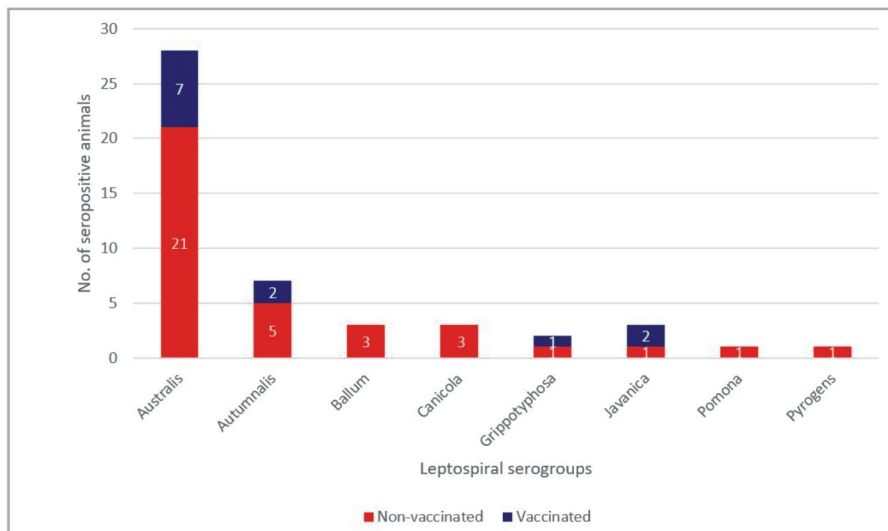


Fig. 2. Frequency of leptospiral serovars with highest titres detected from seropositive nonvaccinated dogs (n=36; red bar) and seropositive vaccinated dogs (n=12; blue bar)

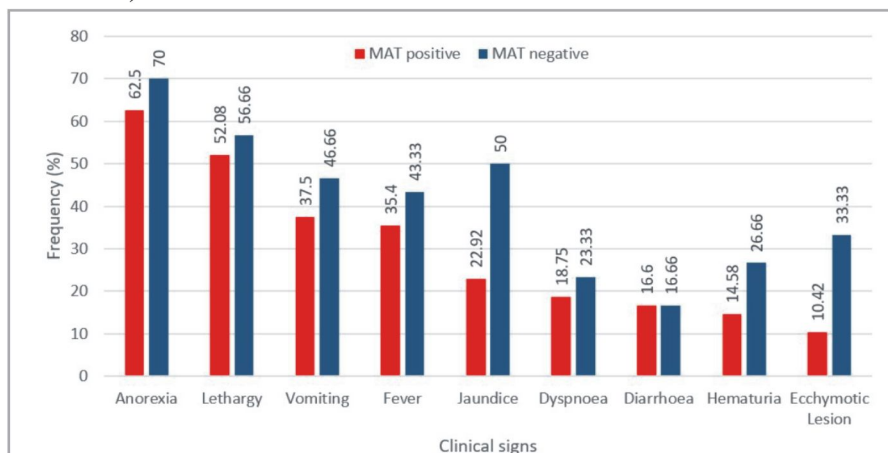


Fig. 3. Frequency (%) of clinical signs noticed in dogs diagnosed with renal and/or hepatic disease with leptospirosis (n=48; red bar) and without leptospirosis (n=30; blue bar)

Table 3. Frequency of clinical signs in dogs seropositive for leptospirosis (n=48)

Clinical signs	No. of dogs seropositive to leptospirosis (%)
Anorexia	30 (62.50)
Lethargy	25 (52.08)
Vomiting	18 (37.50)
Fever	17 (35.42)
Jaundice	11 (22.92)
Dyspnoea	09 (18.75)
Diarrhoea	08 (16.67)

leptospirosis positive dogs, most frequent clinical signs were anorexia followed by lethargy, vomiting, fever, jaundice, dyspnoea, diarrhea, hematuria and echymotic lesions (Table 3 and Fig. 3).

Risk factor analysis: Adult dogs between 1 to 5 years of age were found to be more affected with leptospirosis than young and senior dogs, indicating a significant association ($p < 0.05$) between age and occurrence of disease. Although male dogs (66.67%) were found to be more affected than female dogs (54.55%), there was no statistically significant ($p > 0.05$) association

between sex and occurrence of the disease. Medium sized breed dogs (66.44%) were found to be more seropositive for leptospirosis when compared to small and large breed dogs without any significant association ($p > 0.05$). There was no significant association observed between vaccination status and occurrence of the disease. Among environmental risk factors, dogs with a history of exposure to rodents (73.68%) and outdoor activity (77.58%) were significantly found to be seropositive for leptospirosis ($p < 0.05$ and $p < 0.01$, respectively) (Table- 4).

DISCUSSION

Microscopic agglutination test (MAT) is regarded as the standard serological test for diagnosis of leptospirosis (Anonymous, 2021). In this study, 48 dogs out of 78 dogs were detected as seropositive to leptospirosis using MAT, indicating a prevalence of 61.54 per cent which corroborated with the findings of Abhinay *et al.* (2012), Ambily *et al.* (2013), Paz *et al.* (2021) and Santos *et al.* (2021). In a study from Malaysia, Rahman *et al.* (2021) reported a prevalence of 42.7 per cent (53/124) in dogs with kidney and/or liver illness. Whereas other studies from Tamil Nadu

Table 4. Risk factor analysis with regard to occurrence of leptospirosis

Risk factors	No. of dogs	No. of dogs positive	Prevalence (%)	Pearson's Chi-square	p-Value
Age					
Young (<12 months)	14	05	35.71		
Adult (13-60 months)	31	24	77.42	7.47*	0.024
Senior (61-120 months)	33	19	57.57		
Sex					
Male	45	30	66.67	1.18 ^{NS}	0.277
Female	33	18	54.55		
Breed					
Small	17	10	58.82		
Medium	43	29	67.44	1.70 ^{NS}	0.428
Large	18	09	50.00		
Vaccination					
Vaccinated	20	12	60.00	0.03 ^{NS}	0.870
Not vaccinated	58	36	62.07		
Rodent exposure					
Exposed	38	28	73.68	4.62*	0.032
Not exposed	40	20	50.00		
Management					
Indoor	29	10	34.48	14.28**	0.000
Outdoor	49	38	77.55		
Area					
Urban	48	30	62.50	0.05 ^{NS}	0.825
Sub-urban	30	18	60.00		

($p < 0.05$, *Significant; $p < 0.01$, **Highly significant; S- not significant)

and other parts of India reported a prevalence ranging from 15.21 to 37.8 per cent (Kumar *et al.*, 2009; Lakshmi Priya *et al.*, 2012; Kumar *et al.*, 2013; Soman *et al.*, 2014; Behera *et al.*, 2021; Kanthala *et al.*, 2023; Senthilkumar *et al.*, 2023), which is comparatively lower than the present study owing to highly targeted population in our study.

MAT cut-off titer of $\geq 1:100$ was taken as positive (Anonymous, 2021) in the current study. Although it is advised that MAT results based on paired serum samples (collected 14 days apart) testing to identify four-fold rise in agglutinating antibodies is confirmatory for leptospirosis, previous studies stated an antibody titer of $\geq 1:100$ combined with consistent clinical signs were regarded as positive for leptospirosis (Chou *et al.*, 2008; Rahman *et al.*, 2021). However, in the current study, among 48 seropositive dogs, 14 dogs (29.17%) and 07 dogs (14.58%) were reactive at titres of 1:800 and 1:1600, respectively, with single serum sample testing, which was regarded as a confirmative diagnosis for leptospirosis (Paz *et al.*, 2021).

For MAT, twelve regionally prevalent serogroups were used in current study as employed in other studies from Tamil Nadu (Varadarajan *et al.*, 2015; Senthilkumar *et al.*, 2023) among which Australis serogroup was recorded as highest reactive serogroup, followed by Autumnalis, Ballum, Canicola, Javanica, Grippotyphosa, Pomona and Pyrogenes. This observation of two predominant serogroups, Australis and Autumnalis, was consistent with findings of other studies from South India (Ambily *et al.*, 2013; Sathiyamoorthy *et al.*, 2017; Abdullathief *et al.*, 2018; Senthilkumar *et al.*, 2023). Similarly, Schuller *et al.* (2015) and Delaude *et al.* (2017) also reported predominant serogroups as Australis and Autumnalis in European countries. Whereas, Kanthala *et al.* (2023) and Patil *et al.* (2014) reported a predominant serogroup as Pyrogenes in Gujarat and Mumbai, respectively, explaining the variation in local endemicity. In India, the extensive use of multivalent vaccines containing Icterohaemorrhagiae, Canicola, Grippotyphosa and Pomona might have replaced the circulating serogroups by antigenic shift, as reported by several other countries (Bertasio *et al.*, 2020).

Canine leptospirosis may be asymptomatic or cause a clinical syndrome of acute renal failure, hepatic illness, coagulation disorders, or a combination of syndromes (Stokes and Forrester, 2004; Andre-Fontaine, 2006). In the present study, the most frequent clinical signs in affected dogs were anorexia followed by lethargy, vomiting, fever, jaundice, dyspnoea, diarrhea, hematuria and ecchymotic lesions, which is

in agreement with Goldstein *et al.* (2006), Abdullathief *et al.* (2018) and Rahman *et al.* (2021). Clinically ill dogs with signs of acute renal failure and/or icterus should be considered as suspected cases of leptospirosis until a definitive diagnosis can be made (Van de Maele *et al.*, 2008) as among 48 affected dogs in the present study, 20 dogs (41.67%) had only kidney disease, followed by 15 dogs (31.25%) with both kidney as well as liver disease, and remaining 13 dogs (27.08%) had only liver disease.

Analysis of demographic risk factors contributing to the incidence of leptospirosis in particular region will be helpful for veterinarians in diagnosing and treating the dogs presenting with clinical signs and laboratory results suspicion of leptospirosis. In this study, adult dogs (1 to 5 years of age) were found to be significantly ($p < 0.05$) highly affected with leptospirosis than young and senior dogs, which is consistent with the findings of Stokes and Forrester (2004), Prabhavathy and Joseph, (2018) and Senthilkumar *et al.* (2023), whereas Abdul Rahman *et al.* (2021) did not observe any significant difference among different age groups in their study. There was no significant association observed between sex and occurrence of disease in the current study, which is in agreement with the findings of Spangler *et al.* (2020), Abdul Rahman *et al.* (2021) and Senthilkumar *et al.* (2023). Medium sized breed dogs (66.44%) were more seropositive for leptospirosis when compared to small and large breed dogs without any significant association ($p > 0.05$). The percentage of seropositive test results was higher for medium-sized breed dogs when compared to small and large breed dogs, which corroborated with the reports of Prabhavathy and Joseph (2018), but no identifiable significant association was observed, as reported by Stokes and Forrester (2004) and Spangler *et al.* (2020). Although some of the dog breeds may have increased outdoor activity, leptospiral organisms are unlikely to have particularly high predilection for any particular dog breed (Gautam *et al.*, 2010).

In the present study, there was no significant association observed between vaccination status and occurrence of the disease, as 75 per cent of the presented cases were not vaccinated. However, in both vaccinated and unvaccinated dogs, Australis and Autumnalis serogroups were predominated. The commercial tetravalent vaccine provides serogroup-specific immunity but does not provide protection against non-vaccinal serogroups, which necessitates the inclusion of regionally circulating serogroups for protection against the disease (Senthilkumar *et al.*, 2023).

A history of exposure to rodents ($p < 0.05$) and outdoor management ($p < 0.01$) were significant predisposing factors for leptospiral seropositivity in dogs diagnosed with renal and/or hepatic disease in the present study. Goh *et al.* (2019) also reported the presence of rodents as a significant predictor of dog seropositivity. Hence, proper history-taking during case presentation with consistent laboratory findings may facilitate the veterinarian to consider leptospirosis as one of the differential diagnoses.

The current study disclosed that around two in three dogs presented with renal and/or hepatic illness in Chennai have the possibility of being affected with leptospirosis. Therefore, veterinarians examining dogs with these ailments should suspect leptospirosis as a potential cause, especially in areas where the disease is endemic or during times of increased risk, such as after heavy rainfall or flooding, to ensure prompt and appropriate treatment, improving the chances of recovery of affected dogs.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SS: Presented PhD research work, involved in investigation, data generation, and preparing original draft; MVB: Major advisor, engaged

in conceptualization, supervision and final editing; MM: Involved in statistical analyses, methodology and editing; TVM: Methodology and interpretation; CS: Revised the manuscript; KM, TMAS: Guided SS for interpretation of MAT results. All the data has been collected and analyzed by the first author for her PhD research work.

Data availability statement: All data generated or analyzed during this study are included in this article. The corresponding author is willing to provide the raw data upon reasonable request.

Ethical statement: This study is not entitled to ethical approval as all the blood samples were collected from the suspected dogs for diagnostic purposes. Animals were treated in a humane non-invasive method, and all the procedures carried out in this study were in accordance with the ethical standards of the institution where the study was conducted.

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