

Research Article

Prevalence and serological correlation of leptospirosis among pet dogs and their owners in central Tamilnadu, India

Meera J^{1,2}, Prabhusaran N^{2*}, Vallab Ganesh Bharadwaj B²,
Chitra Rajalakshmi P², Uma A² and Natarajaseenivasan K³

Article Info

¹Department of Microbiology, PSP Medical College Hospital and Research Institute (Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai), Oragadam, Chennai, India

²Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre (Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai), Tiruchirappalli, India

³Director, ICMR-Regional Medical Research Centre, Dibrugarh, Assam, India

Corresponding Author:

Dr. N. Prabhusaran,

Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre, Tiruchirappalli, India;

Received: 07/02/2026

Accepted: 14/03/2026

Published: 20/03/2026

DOI: 10.5281/zenodo.19128168

Publisher's Note: IJABR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: ©2026 by the author(s). Licensee IJABR Press, India. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution- Share Alike (CC BY - SA) license.

Abstract

Leptospirosis is an anthroponozoonotic disease of global distribution. It is a contagious disease of animals and accidentally infects humans. This study hypothesized that seroprevalence of leptospirosis among dogs and pet owners increased and correlated. The present study aimed to determine the culture of leptospire and seroprevalence of leptospiral specific antibodies in pet dogs and its owners. A prospective cross-sectional observational study was undertaken to identify the presence of leptospiral antibodies in 54 pet owners (37 subjects whose dogs were not vaccinated and 17 controls whose dogs were unvaccinated), in central Tamilnadu. Seventy dogs of age 2 months to 15 years were included in the study to screen for the presence of leptospire and its specific antibodies, among them 53 were non-vaccinated as case and 17 as control - vaccinated. The EMJH culturing followed by direct dark field microscopy, Dinger's ring observation and CAAT showed 12 pet dogs and one pet owner positive. No culture positive observed among control groups. The seroprevalence by ELISA was possible with 20 (19 subjects and one control) and 18 (16 subjects and 2 controls) among pet dogs and pet owners respectively. MAT positivity was not observed among control dogs (vaccinated), but two of the control pet owners reactive, whereas 24 pet dogs and its consequent 12 pet owners showed positive to MAT. The serovars Javanica (66.7%) dominated followed by Pomona (50%) and Grippotyphosa (42.7%) among pet dogs, whereas the serovars Javanica (50%) followed by Canicola (41.7%) and Icterohaemorrhagiae (33.3%) found among pet owners. The observation of this study contribute to understand the seroprevalence of leptospirosis in the study area and apprehend the prominence of continued surveillance and awareness.

Keywords: Leptospirosis, pet dogs, pet owners, occupational risk groups.

Introduction

Leptospirosis, a bacterial infectious disease caused by the cork screw shaped spirochete called *Leptospira interrogans*. It is an emerging and re-emerging public health issue globally, that affect both animals and humans. Humans get accidental transmission by sapronosis and infected animals [1,2]. The inter-human transmission is rare, but urine of the infected patients may act as a source of transmission [3].

Seasonal flooding, water stagnation after rain and mixing of rain water and drainage are the subsistence environment for the infected animals thereby leptospires disseminated and exposed to humans. In same way, swimming in the pool may infect humans, as the animals are largely exposed to that open water [4,5], the biggest question mark – Is there any leptospiral transmission if people urinating in the swimming pools or in any water ways? According to the literature, eventhough, the urination of humans in swimming pool is not directly involving in the transmission of leptospires, but indirectly the urine chemicals (uric acid) mixes with chlorine that create dangerous chemicals like cyanogen chlorine and trichloramine [6] that affects heart, lungs and central nervous system. The uric acid and its related byproducts enhance the growth of leptospires [7] and the survival rate increased thereby the exposed humans get infection [8,9,10].

Among various infectious microbes causing acute undifferentiated febrile illness (AUFI), *Leptospira* is considered as one of the major pathogens that cannot be differentiated and in many situations, the clinical manifestations mimic like other tropical infections [1,11]. Thus clinicians misdiagnosed, under-reported and still neglected this from their pyrexia gallery. In the fever clinics, a standard proforma of excluding various infectious etiology and to diagnose specific infections like dengue, leptospirosis have to be maintained. Understanding such infectious AUFI is limited due to lack of smart and rapid

laboratory diagnostic capacity [12] and non-classification ability of the patients clinically is a continuous clinical challenge for clinicians [13].

Animals acquired leptospires from contaminated environment and are predominantly puddle borne, thereby the pets and other domestic animals transmit such infection to humans. Leptospirosis is considered as a serious infection that affect dogs, cats and other domestic animals including cow, goat, sheep, horse, pig etc. Understanding the transmission, symptoms and prevention can keep the animals and animal handlers safe from such infection [14]. Being a pet parent, providing healthy and safe environment for pets is the prime thing than to allow the pets in house.

The severity of leptospirosis is mainly determined by the serotypes which infects, and can range from asymptomatic to multiorgan involvement of vital functioning [15]. Interestingly, no serogroup specific clinical presentations occur. High levels of morbidity observed among pediatric [16], geriatric [17], untreated [18] and patients with comorbid conditions [19]. Thus, this study aimed to determine the culture of leptospires and seroprevalence of leptospiral specific antibodies in pet dogs and several risk groups including canine pet owners, farmers, butchers and laboratory workers.

Materials and Methods

This study included areas in and around Tiruchirappalli, Tamilnadu, INDIA, which consist of more than 35% of the population of agricultural background. The study was performed at a tertiary care teaching hospital where the samples were collected from private veterinary clinics (dogs and its owners). All the subjects and samples were included according to the random sampling strategy. A prospective cross-sectional observational study was done with 54 pet owners (37 cases and who have close contact with pet animals, duration of contact in years, history of contact with carcasses and with animal excreta, and 17 as control).

Seventeen pet owners were taken as control who were defined as dog exposures and those dogs are vaccinated. A total of 70 dogs of age 2 months to 15 years were included in the study to screen for the presence of leptospire and its specific antibodies. The number of dogs and the number of pet owners were not matched in this study due to unwillingness of 16 pet owners. Thus 70 dogs (53 unvaccinated as subjects and 17 vaccinated as control) and 37 canine pet owners were included in this study. Seventeen dogs which received regular vaccination and which were not allowed to mingle with other dogs were also included as control. Severe injured and older dogs were excluded. The pet owners who were having the pets for only 3 months were excluded.

This study was approved by Institutional Ethical Committee (IEC) to include human subjects (Ref: 211/TSRMMCH&RC/ME-1/2022-IEC No. 056 dated 14.03.2022) and for animals, permission was obtained from International Animal Rescue, Trichy. Informed consent in a vernacular language was obtained from all study subjects and from pet owners for inclusion of their pets in the study. Blood samples of approximately 5ml were collected for culture and serology. A field side inoculation was performed on the leptospiral selective media aseptically and serum was separated. The urine samples were collected in a sterile container and transported to laboratory for culturing and direct dark field microscopy (DFM). After appropriate time of incubation, the presence of leptospire were observed under high power direct dark field microscope (DDFM). The prompt and referred observation showed typical leptospire with hooks at both ends and vigorous translational and rotational motility. The dark

Table 1: Age wise distribution of pet dogs (n= 70)

Breeds	<1 year	1-5 years	6-10 years	10-15 years	Total
Labrador	-	11	-	-	11 (15.7)
Doberman	-	8	1	1	10 (14.3)
German Shepherd	1	-	4	-	5 (7.1)
Pug, Spitz, Dash	1	2	-	-	3 (4.3)

field microscopy and dinger ring positive tubes were aseptically maintained at room temperature for serogroup confirmation by Cross agglutination absorption test (CAAT).

Two serological tests were included in the study Genus specific Enzyme Linked Immuno Sorbant Assay [*Leptospira* IgM ELISA commercial kit was used (Panbio, Standard diagnostics, Republic of Korea, Lot No. 261006)] and Serovar specific Microscopic Agglutination Test (MAT) using panel of live leptospiral serovars including Australis, Autumnalis, Canicola, Grippotyphosa, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes, Sejroe and Semaranga.

Initially the results of ELISA and MAT were compared and then compared with culture results. The identified serogroups from MAT and CAAT (culture) were compared and evaluated. By this test results, the sensitivity, stability, precision and correlation of each method was done. All the data were entered in MS-Excel and assessed using basic descriptive statistics. The numerical data were calculated as high, moderate and low and further described in percentages.

Results

A total of 70 pet dogs included in this study; out of that 17 were taken as controls which were vaccinated regularly. The agewise distribution of the pet dog varieties were depicted in table 1. The owners of the pet dogs also included in this study and were screened for leptospiral imprints. Out of 70 pet owners, 54 gave consent for serological screening for leptospirosis; among them 42 were males and 12 were females, and their contact periods with pets were impregnated in table 2.

Country dog	-	14	2	2	18 (25.7)
Cross dog	-	3	3	-	6 (8.6)
Control group	2	10	3	2	17 (24.3)
Total	4 (5.7)	48 (68.6)	13 (18.6)	5 (7.1)	70

(Values in parenthesis represent percentages)

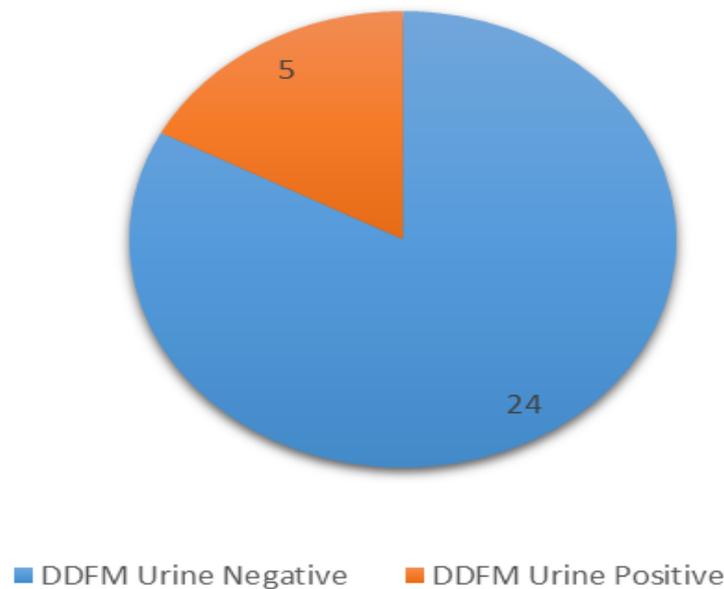
Table 2: Duration of contact in pet owners

Duration of contact	< 1 years	1-5 years	> 5 years
Total number of Pet owners (n=54)	3 (5.5)	42 (77.8)	9 (16.7)
Number of pet owners as cases (n=37)	2 (5.4)	31 (83.8)	4 (10.8)
Number of pet owners as controls (n=17)	1 (5.9)	11 (64.7)	5 (29.4)

(Values in parenthesis represent percentages)

DDFM revealed few to moderate number of highly motile leptospire from the urine of the pet owners; but they are clinically stable and no symptoms observed. Collection of urine from the pet dogs were not possible. Among the 37 pet owners, 24 were possible to collect the urine and 5 (20.8%) samples showed positive to DDFM. No urine samples were subjected for culturing and CAAT determination (Figure 1).

Figure 1: Direct Dark field microscopy of urine samples



Among the 53 pet dogs and 37 pet owners, 12 and one showed positive to EMJH semisolid culturing respectively, where mild (thread) to thick Dinger's ring were observed. The preliminary DFM revealed leptospire that are moderate to high motile and the CAAT analysis also confirmed the same. Among the 13 isolates, Javanica (8/ 61.5%) dominated, followed by Canicola (3/ 23.1%), Pomona (1/ 7.7%) and Icterohaemorrhagiae (1/ 7.7%). The one pet owner positivity is correlated with their pet dog with the serovar Javanica. All control pet dogs and pet owners showed negative to EMJH culturing.

Figure 2: Overview of EMJH semisolid culturing

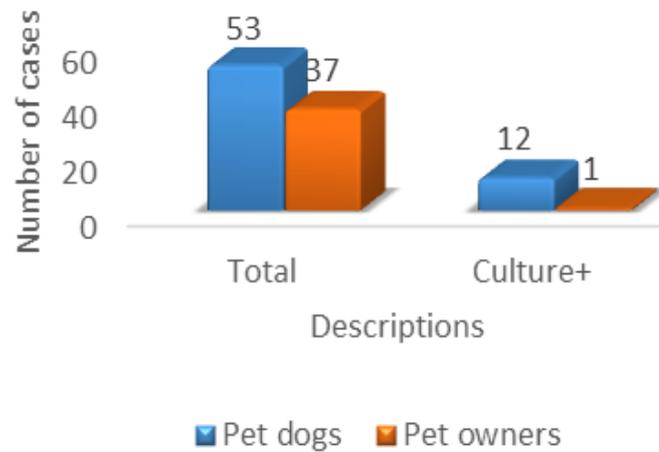
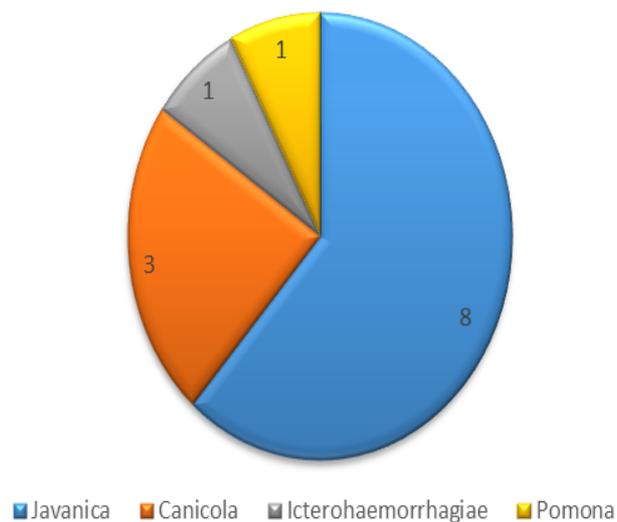


Figure 3: Culture positivity and serovar determination

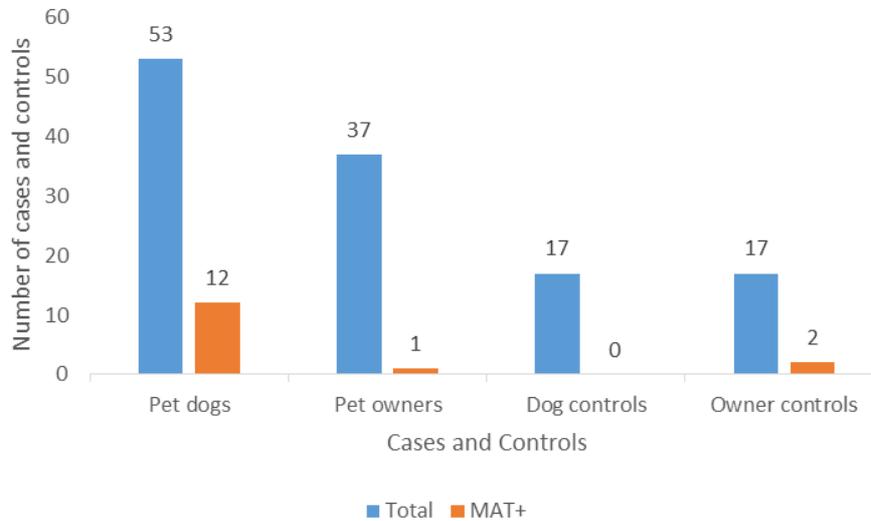


Serological determination

Out of 37 sera samples of pet owners subjected for IgM ELISA, 16 were positive. Out of 17 samples from healthy individuals included as controls, 2 were positive for anti leptospiral antibodies by ELISA. All pet owners and pet dogs were subjected for Microscopic Agglutination Test (MAT) and further both the results were compared. Among pet dogs, ELISA showed positive to 19 samples (35.8%) who are not vaccinated and one control dog reactive to ELISA.

Among the MAT positivity using live leptospiral antigens, it was identified the serological evidence among 12 pet owners (32.4%), 24 pet dogs (45.3%) and two healthy controls (6.7%) (Figure 4). Out of them, the serovar determination was matched with 8 pet dogs with their respective owners indicated that the dog is the confirmed source for their infections.

Figure 4: MAT positivity among cases and controls



The serovar specificity of the subjects and control included in this study also have some impact to understand the transmission and predominance. Among the MAT positive pet dogs (n=24), the serovar Javanica dominating with 16 samples (66.7%), followed by Pomona with 12 (50%) etc. There are poly-serovar involvement in most cases. The detailed serovar distributions were depicted in table 3.

Out of 37 pet owners, 12 showed positive to MAT thereby Javanica dominating with 6 samples (50%) followed by Canicola with 5 samples (41.7%); here also poly serovar involvement observed and recorded in table 3. No control pet dogs have imprints of leptospiral serovars; as all are vaccinated. Among human controls (owners of the control pet dogs), two showed (6.7%) positive to MAT where one recorded as polyserovar (Table 3).

Table 3: MAT seropositivity

No	Population	Serovar determined	Number of samples	Highest titre
1	Pet dogs N=53 MAT+=24	Javanica	16 (66.7%)	1:2560
		Pomona	12 (50%)	1:640
		Grippotyphosa	10 (42.7%)	1:160
		Australis	9 (37.5%)	1:1280
		Canicola	8 (33.3%)	1:2560
		Icterohaemorrhagiae	4 (16.7%)	1:160
2	Pet owners N=37 MAT+=12	Javanica	6 (50%)	1:2560
		Canicola	5 (41.7%)	1:320
		Icterohaemorrhagiae	4 (33.3%)	1:640
		Australis	3 (25%)	1:160
		Grippotyphosa	2 (16.7%)	1:320
3	Pet dog controls N=17	No MAT positive	0	-
4	Pet owner control N=17 MAT+=2	Pomona	2 (100%)	1:320
		Australis	1 (50%)	1:80
		Autumnalis	1 (50%)	1:160
		Canicola	1 (50%)	1:80

In the diagnostic methods of leptospirosis, ELISA is considered as the prediction of positivity against genus level leptospire, but MAT is the WHO reference test which confirms the leptospiral infection by using a battery of live leptospiral antigens (WHO laboratory manual, 2007). The sensitivity and specificity of ELISA and MAT were well assessed by standardizing the ELISA reader at 460nm and inclusion of reactive titre of 1:80 and above in MAT respectively. Further, the positive reactivity results were well analyzed among individual risk groups and the percentages of seropositivity towards serological investigations were tabulated (Table 4).

Table 4: Comparative analysis of ELISA and MAT in human subjects

Groups	Total No.	No. of reactivity	
		ELISA	MAT
Pet dogs	53	19 (35.8)	24 (45.3)
Pet dog controls	17	1 (5.9)	0
Pet owners	37	16 (43.2)	12 (32.4)
Pet owner controls	17	2 (11.8)	2 (11.8)
Total	124	18 (14.5)	38 (30.6)

(Values in parenthesis represent percentages)

The MAT results of pet owners and their respective pet dogs were compared and depicted in the Table 5. Out of them, MAT results were matched among five pet dogs and their respective pet owners. Predominantly, the titre value was observed as high among pet dogs, compared to pet owners. In one case, the MAT titer value serovar Grippotyphosa is high in pet owners than pet dog. Further, the high titre values are not much matched, but dogs are having high titer than their owners. The serovars Australis, Canicola, Grippotyphosa and Javanica are found common among the dogs and their owners indicated the possible of exposure of the pet owners to dogs.

Table 5: Comparison of MAT results of pet owners and their pets

Reference	Serovars	High titre	Reference	Serovars	High titre
PO 1	Australis Grippotyphosa	1:160 1:320	PD 1	Australis Grippotyphosa Pomona	1:320 1:160 1:640
PO 3	Australis Canicola	1:80 1:320	PD 3	Australis Canicola Pomona Javanica Grippotyphosa	1:160 1:1280 1:320 1:640 1:160
PO 5	Javanica Canicola	1:1280 1:160	PD 5	Javanica Canicola	1:2560 1:1280
PO 14	Javanica	1:160	PD 14	Grippotyphosa Pomona Javanica	1:160 1:640 1:2560
PO 17	Australis	1:80	PD 17	Javanica Australis	1:320 1:320

[PO – Pet owners; PD – pet dogs]

Discussion

One of the bacterial spirochetal infections called leptospirosis caused varied clinical symptoms among humans and animals [17]. Even though this disease is common in tropical and sub-tropical regions, due to poor sanitation, water stagnation, high temperature, the infection observed in animals and animal contacting humans. Leptospiral diagnosis is important to save lives of pet dogs and prevent the spread of infections; late diagnosis is often foremost reason for death [11].

Generally the dogs which are young (pups less than 12 weeks), geriatric dogs (lesser immunity), dogs in rural areas (living together with infected dogs), small breed from urban environment (due to medium intensity), inadequate and non-vaccinated dogs (unawareness) are very prone to leptospirosis [18,19]. In this study also, the dogs whose age between 1 and 5 are more infected than other groups. The pet dogs which are protected in housing environment also get infected if they have contact with contaminated environments. The spirochetes that are lively contaminated in soil and water, become more viable due to climate and other optimal conditions. Interestingly, biofilm formation in renal tubules of the animals enhance the ability of persistence in the environment [20,21].

Among gender, male dogs get more infected in this study (78.4%) and the same type of increased trend of male dogs infection were observed in other studies also with 69% [22] and 33% [23]. Male dogs were at suggestively having higher risk of leptospirosis than female; also the dogs' age between 4 and 7 were at greater risk than others [24]. Controversially, 88.4% and 55.5% of female dogs were reported for seropositivity to leptospirosis than males [25,26].

All pet owners of the pet dogs were invited for the study, and all are interested to participate and ready to learn about various infections spread through dogs, but only 52.8% were consented for providing blood samples for determining

seroprevalence. Among pet owners, males are dominated in this study with 78.4%, whereas in other studies females dominated with 89 and 74% in Los Angeles and Japan respectively [11,27].

All breed and aged dogs are at high risk of leptospirosis. Handling such pets in the restricted places provide effective infection control than allow them to roam and contact with other dogs. The serological evaluation of leptospiral antibodies in pet dogs and its owners was accomplished for the first time in India, but it was done in other countries like Tanzania [28], Brazil [29] and Malaysia [30].

In this study, from one dog, the direct dark field microscopy of urine sample showed positive to leptospire, while serology showed negative, due to recent and active infection. Eventhough leptospire demonstrated from urine; but no IgG antibody have been developed due to the requirement of two weeks generation time. IgM based ELISA may be convenient, which detects IgM antibodies. IgM antibodies appear earlier and initially against infection, that is part of body's early immune response [26].

There is no specific study or data available regarding the contact period of dogs and their owners for any infections. Touching or handling such pet dogs by the owners is not an issue for leptospirosis but exposure to the urine and other body fluids cause infections. Compared to other pets, dogs like to associate directly to dirty backyards, stagnant water and in drainage water if high temperature found [27,29]. Thus, this study identified that there is no connection between the years/ period of exposure of the owners to the pet dogs and leptospirosis infection. Notwithstanding the direct contact with infected dogs, the rate of human infection and infectious risk have been moderately lesser and the concurrent positive serology of the pet owners and pet dogs.

There is a reasonable variations in leptospiral serology among human and dog and this may reflect dissimilar infection configurations based on host species. Interestingly, pathogenic

leptospire cause acute disease in humans by accidental infection without involvement of renal carrier, whereas dogs presenting diverse disease grades of acute or chronic and colonization in the renal tubules leads to long-term shedding and act as reservoir to infect others [37,38].

In this study, while interview with pet owners, they are not having much awareness about the spread of leptospirosis through their pets' urine. They do not realize of harboring pathogens by their pets. Many pet owners purchase or collect pet animals for their companionship and for their children. Few of them take the pets from illegal markets or from domestic environment are not subjected for health checkups, may harbor and shed wide range of pathogens including leptospire. In common, microbiological assessment are not done for such pet animals that are considered as clinically healthy but difficult to determine the illness and further potential sources of infection to humans [34].

This zoonotic infection remains a public health importance and has becoming a life threat that escalating more prevalence mainly due to climate change, potential habitat and global warming. Antibiotic fail effectiveness when the leptospire infiltrates vital organs like liver, spleen and kidneys, and cause multiorgan dysfunction (MOD) to multiorgan failure (MOF). Thus, further development of next generation drugs and vaccines required to overcome leptospirosis in animals and humans.

Conclusion

If pet dogs are found infected with leptospirosis, they are allowed to excrete in a single and separate place where to dispose the wastes and clean it immediately with disinfectants. This symptomatic shedding of urine cause infection to pet owners while they are in contact with shedding urine which carries leptospire [35]. Pet dogs in the residence required special attention as described as follows,

- a. Effective vaccines are available for pet dogs against leptospirosis infection and control urinary shedding of leptospire [39], but does not defend against all leptospiral strains and serovars, also not having long lasting immunity [40]. No effective vaccine available for humans, thus personal hygiene and special care to be taken for the pet handlers to get rid from the infection, required multi-antigenic vaccines could trigger better protection.
- b. Preventing contamination from the environment, avoid exposing to drainages and stagnant water also to other dogs.
- c. Cleaning their shelters using mild disinfectants and regular disposal of the litters
- d. Frequent health checkups to analyze the presence of infectious etiology including leptospirosis
- e. Pet animals are not allowed inside the residence and train them to stay in their restricted housings
- f. Dogs hunt rats and rodents that have a high potential of acquiring leptospirosis

The health status of the pet dogs are to be monitored periodically and also exhibit any clinical sign if the house residents are immunocompromized, pregnant women, geriatric and pediatric age groups, and individuals with chronic diseases

Authors' contributions:

Meera J: Investigation, writing, initial edition

Prabhusaran N: Conceptualization, data curation, methodology, review writing, final drafting

Vallab Ganesh Bharadwaj B: Data curation, methodology and secondary drafting

Chitra Rajalakshmi P: Co-supervision, data management and analysis, initial editing

Uma A: Supervision, data curation and final editing

Natarajaseenivasan K: Conceptualization, methodology and expert input

Acknowledgement: None stated.

Conflict of Interest: The authors declare that there are no conflicts of interest

Funding: This work received no specific grant from any funding agency

Declaration of Non-Use of AI: The authors confirm that no artificial intelligence tools were used in this study.

References

1. Baby, K.K, et al., (2024). Leptospirosis in India: insights on circulating serovars, research lacunae and proposed strategies to control through one health approach. *One Health Outlook.* 6, 11.
2. Shanti, C, et al., (2023). Epidemiological status of leptospirosis in India. *Journal of Pure and Applied Microbiology.* 17, 1968-1977.
3. Haake, D.A, et al., (2015). Leptospirosis in humans. *Current Topics in Microbiology and Immunology.* 387, 65-97.
4. Jackson, L.A, et al., (1993). Outbreak of leptospirosis associated with swimming. *Pediatric Infectious Disease Journal.* 12, 48-54.
5. Forster, K, et al., (2013). Characterization of a virulent *Leptospira interrogans* strain isolated from an abandoned swimming pool. *Brazilian Journal of Microbiology.* 44, 165-170.
6. Tsamba, L, et al. (2019). Analysis of chlorination by-products in swimming pool water by membrane introduction mass spectrometry - Influence of water physicochemical parameters. *Rapid Communication in Mass Spectrometry.* 33, 710-718.
7. Kadis, S, et al., (1974). Urea utilization by *Leptospira*. *Infection and Immunity.* 10, 793-801.
8. Jackson, L.A, et al., (1993). Outbreak of leptospirosis associated with swimming. *Pediatric Infectious Diseases Journal.* 12, 48-54.
9. Jittimance, J, et al., (2019). Prevalence and control of leptospirosis in people and surveillance of the pathogenic *Leptospira* in rats and in surface water found at villages. *Journal of Infections and Public Health.* 12, 705-711.
10. Gajjala, A, et al., (2020). Donot drink the pool water: a case of severe leptospirosis. *American Journal of Research in Critical Care Medicine.* 201, A6962.
11. Parra, B.E.L, et al., (2023). Fatal acute undifferentiated febrile illness among clinically suspected leptospirosis cases in Colombia, 2016-2019. *PLoS Neglected Tropical Diseases.* 17, e0011683.
12. Budihal, S.V, et al., (2014). Leptospirosis diagnosis: competency of various laboratory tests. *Journal of Clinical and Diagnostic Research.* 8, 199-202.
13. William, F.W, (2020). Fever of Unknown Origin: Review, Recent Advances, and Lingering Dogma. *Open Forum Infectious Diseases.* 7, ofaa132.
14. Nakashiro, H, et al., (2024). Leptospirosis transmitted from a pet dog. *BMJ Case Reports.* 17, e261369.
15. Brown, K, et al., (2008). Leptospirosis in the family dog: a public health perspective. *Canadian Medical Association Journal.* 178, 399-401.
16. Doneray, H, et al., (2023). Pediatric leptospirosis: a case report and review of literature. *Eurasian Journal of Medicine.* 55, 150-156.
17. Daher, E.F, et al., (2019). Leptospirosis in the elderly: the role of age as a predictor of poor outcomes in hospitalized patients. *Pathology and Global Health.* 113, 117-123.
18. Taylor, A.J, et al., (2015). A systematic review of the mortality from untreated leptospirosis. *PLoS Neglected Tropical Diseases.* 9, e0003866.

19. Gancheva, G, (2022). Prognostic value of early clinical diagnosis of leptospirosis. *Journal of IMAB*. 28, 4450-4455.
20. Rajapakse, S, et al., (2022). Leptospirosis: clinical aspects. *Clinical Medicine (Northfield)*. 22, 14-17.
21. Yanagihara, Y, et al., (2022). *Leptospira* is an environmental bacterium that grows in waterlogged soil. *Microbiology Spectrum*. 10, e0215721.
22. Sykes, J.E, (2023). Updated ACVIM consensus statement on leptospirosis in dogs. *Journal of Veterinary Internal Medicine*. 37, 1966-1982.
23. Santos, A.A.N, et al., (2021). *Leptospira interrogans* biofilm formation in *Rattus norvegicus* (Norway rats) natural reservoirs. *PLoS Neglected Tropical Diseases*. 15, e0009736.
24. Meganathan, Y, et al., (2022). Biofilm formation and social interaction of *Leptospira* in natural and artificial environments. *Research in Microbiology*. 173, 103981.
25. Erni, S, (2022). Canine leptospirosis identification on small animal practice. *Journal of Kedok Hewan*. 16, 1-5.
26. Sathiyamoorthy, A, et al., (2017). Seroprevalence of canine leptospirosis in Namakkal, Tamilnadu by Microscopic agglutination test. *Journal of Cell and Tissue Research*. 17, 5991-5996.
27. Ward, M.P, (2002). Prevalence of and risk factors for leptospirosis among dogs in the United States and Canada: 677 cases (1970-1998). *Journal of American Veterinary Medical Association*. 220, 53-58.
28. Hernandez, R.C.V, et al., (2017). Prevalence and risk factors associated with serovars of *Leptospira* in dogs from Culiacan, Sinaloa. *Veterinaria Mexico OA*. 4.
29. Dhruv, D, et al., (2020). Detection and prevalence of canine leptospirosis from Navsari District of South Gujarat, India. *Microbial Research Journal International*. 30, 103-110.
30. Villanueva, S, et al., (2025). Evaluating a response to a canine leptospirosis outbreak in dogs using an owner survey. *Veterinary Sciences*. 12, 119.
31. Said, K, et al., (2018). Owner awareness and knowledge of canine leptospirosis as a zoonotic disease in Morogoro, Tanzania. *Tanzania Journal of Health Research*. 20: 1-8.
32. Benitez, A.N, et al., (2021). Spatial and simultaneous seroprevalence of anti-*Leptospira* antibodies in owners and their domiciled dogs in a major city of Southern Brazil. *Frontiers in Veterinary Sciences*. 7, 580400.
33. Lea, J.S.X, et al., (2025). Leptospirosis in humans and animals in Malaysia: A review from 1976 to 2023. *Veterinary World*. 18, 673-685.
34. Benitez, A, et al., (2017). Spatial and simultaneous representative seroprevalence of anti-*Toxoplasma gondii* antibodies in owners and their domiciled dogs in a major city of Southern Brazil. *PLoS ONE*. 12, e0180906.
35. Levett, P.N, et al., (2021). Two methods for rapid serological diagnosis of acute leptospirosis. *Clinical and Vaccine Immunology*. 8, 2-5.
36. Ko, A.I, et al., (2009). *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. *Nature Reviews Microbiology*. 7, 736-747.
37. Bertelloni, F, et al., (2025). Leptospirosis in unconventional mammal pets. *Veterinary Sciences*. 12, 285.
38. Vyn, C.M, et al., (2024). Canine leptospirosis: A One Health approach for improved surveillance, prevention, and interdisciplinary collaboration. *Canadian Veterinary Journal*. 65, 609-612.
39. Stephania, B.E, et al., (2022). Efficacy of commercially available vaccines against

canine leptospirosis: a systematic review and meta-analysis. *Vaccine*. 40, 1722-1740.

40. Isabela, R.A, et al., (2023). Human leptospirosis: in search for a better vaccine. *Scandinavian Journal of Immunology*. 98, e13316.