

## A STUDY ON CASE PREVALENCE OF CANINE LEPTOSPIROSIS IN CHENNAI USING MICROSCOPIC AGGLUTINATION TEST

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### ABSTRACT

*Leptospirosis is a globally re-emerging zoonotic disease caused by pathogenic species of the genus Leptospira affecting humans and animals. Dogs serve as important reservoirs and sentinels, playing a significant role in disease transmission to humans. The present study was conducted for the serodiagnosis of canine leptospirosis in Chennai between December 2024 to June 2025 using the gold standard test - Microscopic Agglutination Test (MAT) and to identify the predominant circulating serovars of Leptospira spp. The present study revealed 47% seropositivity with Canicola (31.91%) and Australis (27.65%) being the predominant serovars among the 100 sera samples screened. Laboratory confirmation remains essential for accurate diagnosis and epidemiological assessment of leptospirosis, and this study highlights MAT as a vital tool for surveillance, disease control, and reduction of zoonotic risk of leptospirosis.*

**Keywords:** Dogs, Leptospirosis, MAT, seropositivity, serovars

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### INTRODUCTION

Zoonotic diseases that involve both humans and animals can be transmitted either by domestic pets or wild animals as they act as reservoir of zoonotic pathogen (Ghasemzadeh and Namazi, 2015). Leptospirosis is a neglected emerging zoonotic disease caused by pathogenic species of the genus *Leptospira* that affects humans and animals characterized by febrile illness, multiple organ failure and death

(Bharti *et al.*, 2003). Dogs act as maintenance host for some serovars of *Leptospira* spp. that transmit infection to other animals and humans through direct and/or indirect contact with urine or contaminated water and soil (Levett, 2001). The worldwide annual incidence of leptospirosis were 1.03 million cases and 58900 deaths (Zeng, 2023). The incidence of leptospirosis is significantly higher in tropical countries than temperate regions as the humid and warm environment paves way for longer survival of *Leptospira* species. India's tropical monsoon climate provides an ideal ecological niche for survival and transmission of leptospires due to seasonal flooding and heavy rainfall. The gold standard test for diagnosing leptospirosis is Microscopic Agglutination Test (MAT) (WOAH, 2021) which detects anti-leptospiral antibodies in the blood within 1-2 week from the onset of clinical signs and identification of prevalent serovars in that particular geographical region could aid in understanding the disease epidemiology (Ahmed *et al.*, 2009). Herein, we present the findings of a study that was carried out to detect the presence of leptospiral antibodies in clinical cases of dogs suspected for leptospirosis using MAT to understand the burden of positive cases that might pose a major public health concern.

## MATERIALS AND METHODS

### Study area and sample collection:

A study was conducted for serodiagnosis of leptospirosis using MAT in clinically suspected dogs (n=100; male=52, female=48) for the disease in and around Chennai region of Tamil Nadu state. Blood/

Serum samples (n=100) were collected randomly from suspected dogs (<10years of age) with no recent history of vaccination and clinical signs of anorexia, lethargy, vomiting, diarrhoea, polyuria, polydypsia, jaundice, hematuria, and bleeding tendencies which were presented to the Teaching Veterinary Hospital, (TVH), Madras Veterinary College, Chennai-7 (12°55'N to 13°10'N and 80°0'E to 80°25'E) during December 2024 to June 2025. Samples from dogs brought to Zoonoses Research laboratory, CAHS, TANUVAS, Madhavaram Milk Colony, Chennai-51 that were suspected for leptospirosis based on history given by the pet owners were also included. Using serum vacutainer tubes with clot activator, 3-4 ml of blood was drawn aseptically using sterile syringe and needle by venepuncture. The serum was separated by centrifuging at a speed of 700 x g (1500 rpm) for 15 min and were subjected to MAT for detection of leptospiral antibodies (Bojiraj *et al.*, 2017 and Senthilkumar *et al.*, 2022).

### Microscopic Agglutination Test (MAT):

The serum samples were subjected to MAT for the serodiagnosis of leptospirosis. An antigen panel of twelve serovars (approximately 2×10<sup>8</sup> leptospires per ml) mentioned in Table. 1 of reference strains maintained in Zoonoses Research Laboratory, Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University was used in this study. Briefly, serum samples were subjected to 1:50 dilution in phosphate buffer saline (PBS) from which 30µL of diluted serum samples and an equal volume of leptospiral

antigen, were added to each well in a microtitre plate, to make the final serum dilution as 1:100. The microtitre plates were incubated at 37°C for 2 hours and then the serum-antigen mixtures were examined under a dark field microscope. The presence of agglutination and/or reduction of 50% free cells in comparison with the respective negative control was defined as positive at >1:100 dilution.

## RESULTS AND DISCUSSION

A total of 100 serum samples were screened for leptospirosis with panel of twelve serogroups of leptospire (Table. 1) by microscopic agglutination test. MAT titre of >1:100 was considered as positive and 47 samples were seroreactive to one or more serogroups of leptospira. The seropositivity rate among dogs were 47% in and around Chennai in Tamil Nadu. Among that the serovars prevalent in dogs were Canicola (31.91%), Australis (27.65%), Autumnalis (19.14%), Tarassovi (12.76%), Pyrogenes (10.63%), Hardjo (8.51%) and other serovars were less than 5%. The agglutination reaction for positive cases were shown in Figure.1

The widely used and gold standard serological test MAT is used to confirm the leptospirosis and determine the disease epidemiology. As MAT titre of 1:100 is considered to be positive (WOAH, 2021) seropositivity rate among dogs in the present study were 47% in and around Chennai in Tamil Nadu of which serovar Canicola (31.91%) and Australis (27.65%)

were highly predominant. The results were in accordance with seroprevalence study conducted in dogs in and around Chennai (62.5%) as reported by Vijayanand *et al.* (2009). Similarly, Senthil *et al.* (2013) reported 57% reported around 62.5% seropositivity among dogs in Namakkal district. Sarangi *et al.* (2024) reported seropositivity rate of 61.54% of which Australis (58.3%) was found to be highly predominant serogroup. The seropositivity rate reported by Bojiraj *et al.* (2017) was 47.2% around Tamil Nadu which could be due to inclusion of samples throughout the state. The moderate seropositivity of 28.40% in and around Namakkal district reported by Sathiyamoorthy *et al.* (2017) could be due to sampling from non-endemic region for Leptospirosis. The seropositivity rate may vary in different geographical areas and agro-climatic zones with different micro and macro climatic and ecological conditions prevailing. As Chennai is an endemic region for leptospirosis (Choudhary *et al.*, 2023), higher level of seropositivity in dogs have been reported in this study. Senthilkumar *et al.* (2023) conducted a seroepidemiologic study of canine leptospirosis from the year 2017 to 2021 and found that the predominant serogroup was Australis (47.5%), followed by Autumnalis (33.5%), Canicola (24.2%), Pomona (13.4%), Tarassovi (11.2%), and Grippotyphosa (9.4%). From the year 2023 to 2024, the unvaccinated strains including Australis, Autumnalis and Ballum were highly prevalent among dogs in Chennai, which were consistently reported across multiple MAT-based studies (Prabhavathy *et al.*, 2024; Sarangi *et al.*, 2024) suggesting

a diverse range of circulating serovars. The present study revealed the emergence of Canicola and Australis serovars among dogs indicating variation from the previous studies which could be due to various factors as described by Choudhary *et al.* (2023).

In the present study, out of 100 blood samples of dogs, the seroprevalence rate were 35% and 22% among male and female dogs, respectively. The increased positivity of 35% among the male dogs (chi-square values being significant at  $P < 0.05$ ), were similar to the findings reported by Reagan *et al.* (2022) and Bale *et al.* (2021) which could be due to straying behaviour of male dogs. Senthil *et al.* (2013) and Hemant *et al.* (2024) reported that there was no significant difference of seroprevalence between male and female dogs. The seroprevalence rate among different age group of dogs in this study were 15% for more than 6 years of age, 22% between 3-6 years of age, 16% between 1-3 years of age, and 4% for less than 1 year of age. The increased positivity of 22% among the age group of 3-6 years were similar to the findings by Kumar *et al.* (2009) and Hemant *et al.* (2024). The prevalence rate among dogs in Chennai reported by Sarangi *et al.* (2024) were 66.67% and 54.55% among male and female dogs, respectively with 35.7% among young, 77.42% in adult and 57.57% in older dogs. The low seroprevalence in the present study compared to Sarangi *et al.* (2024) could be due to environmental

exposure levels, sampling type and sample size included in the study. The transmission of leptospirosis peaks during rainy and post-monsoon months (July–October) due to high moisture and flooding, which supports the survival of leptospires (Bharti *et al.*, 2003; Vijayachari *et al.*, 2008). In contrast, the present study includes samples collected during December 2024 to June 2025 which is typically a cold and dry season of the year, leading to reduced environmental survival of leptospires and therefore lower MAT positivity noticed in clinical cases suspected for leptospirosis.

## CONCLUSION

MAT remains an indispensable tool for seroepidemiological studies despite its limitations, such as the requirement for live cultures and technical expertise. Identification of predominant serovars provides valuable information for formulating region-specific vaccines and implementing effective zoonosis control strategies. The present study confirms the circulation of leptospiral infection among dogs, posing a potential zoonotic threat. Routine surveillance using MAT is essential for early detection, appropriate treatment and prevention, thereby reducing the zoonotic risk of canine leptospirosis. Strengthening public awareness and vaccination programs on leptospirosis will aid in reducing disease burden.

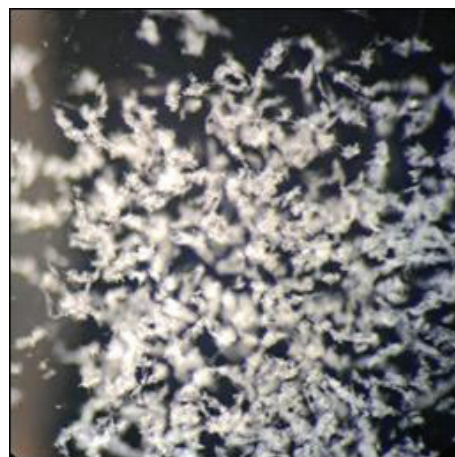
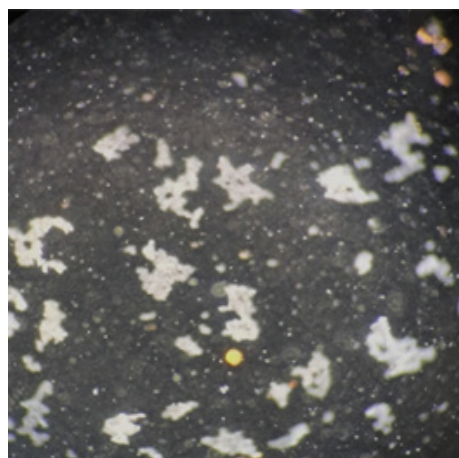
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**Table.1. Reference strains of *Leptospira* used in the study**

S. No	Species	Serogroup	Serovar	Strain
1.	<i>L. interrogans</i>	Australis	Australis	Ballico
2.	<i>L. interrogans</i>	Autumnalis	Rachmati	Rachmati
3.	<i>L. borgpetersenii</i>	Ballum	Ballum	Mus 127
4.	<i>L. interrogans</i>	Canicola	Canicola	Hond Utrecht IV
5.	<i>L. kirschneri</i>	Grippotyphosa	Grippotyphosa	Moskva V
6.	<i>L. interrogans</i>	Hebdomadis	Hebdomadis	Hebdomadis
7.	<i>L. interrogans</i>	Icterohaemorrhagiae	Icterohaemorrhagiae	RGA
8.	<i>L. borgpetersenii</i>	Javanica	Poi	Poi
9.	<i>L. interrogans</i>	Pomona	Pomona	Pomona
10.	<i>L. interrogans</i>	Pyrogenes	Pyrogenes	Salinem
11.	<i>L. interrogans</i>	Sejore	Hardjo	Hardjoprajitno
12.	<i>L. borgpetersenii</i>	Tarassovi	Tarassovi	Perepelitsiu



**Fig.1. Positive MAT result showing agglutination reaction between antigen and antibody**

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