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A retrospective analysis (2021-2024) of leptospirosis among suspected samples in different animal species from Tamil Nadu

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Abstract

Leptospirosis is a neglected emerging zoonotic disease occurring worldwide but most commonly noticed in tropical and subtropical countries. The present study was conducted to identify the presence of *Leptospira* and assessment of sero-prevalence in apparently clinically suspected livestock, small ruminants, companion and wild animals in Tamil Nadu. In this study, totally 2783 serum samples were collected during 2021 - 2024 of which 2649 from various companion animals like cattle, dogs, cats, sheep, goat, horse etc., and 134 serum samples from wild animals (lion, tiger, wolf and elephant). Serum samples from companion animals and wild animals were tested for Leptospirosis using MAT and the positive case rate of leptospirosis were, out of 1178 dog serum samples, 636 (53.9%) were positive, from 85 cat samples 20 (23.5%) were positive. Out of 1080 cattle serum samples, 296 (27.4%) were positive, out of 81 samples, 41 (50.61%) were positive in sheep and goats and out of 225 horse samples, 83 (36.88%) were positive for MAT. In wild animals, out of 134 serum samples 60 (44.77%) were positive for MAT test. The present study provides an updated overview of the *Leptospira* seroprevalence among domestic animals and wild animals in Tamil Nadu during 2021-2024, emphasizing the persistence and diversity of circulating serogroups in the region.

Keywords: Animal leptospirosis, microscopic agglutination test, sero-prevalance

1. Introduction

Leptospirosis is a worldwide zoonotic disease that affects humans, domestic and wild animals (Genovez, 2016) [16]. It is caused by members of pathogenic *Leptospira* spp. which comprises 66 different species that include more than 300 serovars (Caimi and Ruybal, 2020) [6]. It affects more than 160 mammalian species, such as dogs, cattle and humans; despite that, it is considered a neglected disease due to the lack of epidemiological data on the disease occurrence (Karpagam and Ganesh, 2020, Harren *et al.*, 2022) [13, 14]. Leptospirosis is most common in tropical and subtropical areas with high rainfall. Leptospirosis is becoming an increasingly significant public health problem, particularly in tropical developing countries. The disease is found mainly in places where humans come into contact with the urine of infected animals or a urine polluted environment (Padma Kumari *et al.*, 2016) [18]. Transmission of leptospirosis to humans and animals is mainly by contact with urine of rodents, domestic and wild animal reservoirs (Balakrishnan *et al.*, 2006) [3]. Numerous mammalian species act as maintenance hosts (natural carriers) including feral, farm and pet animals (Levett, 2001; Balakrishnan and Manicavasaka Dinakaran, 2014) [1, 2]. Dogs may act as reservoirs and may succumb to severe illness similar to humans (Larson *et al.*, 2017) [16]. Humans act as incidental hosts (Haake and Levett, 2015; Bonhomme and Werts, 2022) [5, 12]. It is estimated that wild animals are the source of >70% of all emerging infections (Chomel and Arzt, 2013) [7], as there is a close phylogenetic relationship between humans and other primates, creating a high potential for pathogen exchange. Humans are most commonly infected through occupational, recreational or domestic contact with the urine of carrier animals, either directly or through contaminated water or soil. Recent advances in diagnostic

approaches have led to a growing awareness of the broad distribution and diversity of leptospiral pathogens both in infection and in the environment (Costa *et al.*, 2015) ^[9]. Antigen detection by dark field microscopy (DFM), antibody detection by Microscopic Agglutination Test (MAT) and molecular techniques such as Polymerase Chain Reaction (PCR) are the widely accepted techniques in diagnosis of leptospirosis (WOAH, 2021; Senthilkumar *et al.*, 2022) ^[22]. Prevalence of leptospirosis is more among humans and animals with occupational exposure or in the area of agricultural activities. It is inevitable that indirect exposure by the human population to the environment contaminated with urine of cattle, sheep, goats, dogs, pigs, rodents and wild animals which are infected clinically and sub clinically by leptospirosis (Balakrishnan and Manicavasaka Dinakaran, 2014) ^[2]. Dogs generally contract infection through direct contact, soil, food and water bodies contaminated with urine of domestic animals, rodents and humans. In developing countries including India, leptospirosis can be considered as one of the emerging public health problem. The present study was carried out to determine the sero-prevalance of

leptospirosis in livestock, companion animals and wild animals in Tamil Nadu.

2. Materials and Methods

2.1 Study area and sample collection

A study was conducted for the serological screening for leptospirosis in livestock, companion animals (n=2649) and wild animals (n=134) to analyse the *Leptospira* serovar positivity in Tamil Nadu state. Serum samples were collected randomly from livestock, companion animals and wild animals with no history of vaccination to leptospirosis (Table 1). Blood/serum samples were collected randomly from animals with clinical signs of anorexia, lethargy, vomiting, diarrhoea, polyuria, polydipsia, jaundice, hematuria etc which were presented to the Zoonoses Research laboratory, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai in the study area. Using serum vacutainer tubes with clot activator, 3-4 ml of blood was drawn aseptically. After allowing the blood to clot, the serum was separated by centrifuging at 2,000 rpm for 15 min and then the serum samples were used for serological study (Senthilkumar *et al.*, 2022) ^[22].

Table 1: Serum samples from companion animals, livestock and wild animals

S. No	Species	No. of serum samples received during 2021-2024
1.	Dog	1178
2.	Cat	85
3.	Cattle	1080
4.	Sheep and Goat	81
5.	Horses	225
6.	Wild Animals	134
Total		2783

2.2 Serological screening by microscopic agglutination test

Serological screening for leptospirosis was done by MAT using a panel of 12 *Leptospira* serovars (Table 2) representing the serogroups of Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Tarassovi (Senthilkumar *et al.*, 2022) ^[22] obtained from Zoonoses Research Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai and maintained in EMJH medium. The concentration of growth of *Leptospira* serovars in EMJH liquid leptospiral medium at 30±2 °C was assessed using the Petroff-Hauser chamber (0.02 mm) and estimating the number of leptospire per field by dark field microscopy. The growth of the live cultures at least 4 days old, but not more than 10 days and densities of approximately 2x10⁸ leptospire per ml of culture were used as antigen for MAT (Faine *et al.*, 1999; WOAH,

2021) ^[10]. Around 500 µl of culture from each of these 12 *Leptospira* serovars was dispensed into a sterile 10 ml screw-capped tube, which was then utilized for further processing for MAT. All the serum samples were subjected to MAT (WOAH, 2021; Senthilkumar *et al.*, 2022) ^[22]. Briefly, 1:50 dilution of the sera was prepared and then the diluted sera (30µl) were mixed with pooled antigen (30 µl) in 96well U-bottom microtitre plate and kept for incubation at 37 °C for 2 hrs. Antigen control was kept by diluting 30 µl of pooled antigen in 30 µl PBS. After incubation, 10 µl of serum sample was placed on a clean glass slide alongside the respective antigen control. The slide was then examined under DFM (20X). The serum that showed 50% agglutination, leaving 50% free cells compared with a control culture was considered as positive (Titre of 1:100 and above) for leptospirosis (Figure 1).

Table 2: *Leptospira* reference strains used for the microscopic agglutination test

S. No.	Species	Serogroup	Serovar	Strain
1.	<i>L. interrogans</i>	Australis	australis	Ballico
2.	<i>L. interrogans</i>	Autumnalis	bangkinang	Bangkinang I
3.	<i>L. borgpetersenii</i>	Ballum	ballum	Mus 127
4.	<i>L. interrogans</i>	Canicola	canicola	HUterecht IV
5.	<i>L. kirschneri</i>	Grippotyphosa	grippotyphosa	Moskva V
6.	<i>L. interrogans</i>	Hardjo	hardjo	Hardjopraj
7.	<i>L. interrogans</i>	Hebdomadis	hebdomadis	Hebdomadis
8.	<i>L. interrogans</i>	Icterohaemorrhagiae	icterohaemorrhagiae	RGA
9.	<i>L. borgpetersenii</i>	Javanica	poi	Poi
10.	<i>L. interrogans</i>	Pomona	pomona	Pomona
11.	<i>L. interrogans</i>	Pyrogenes	pyrogenes	Salinem
12.	<i>L. borgpetersenii</i>	Tarassovi	tarassovi	Perepelicin

3. Result and Discussion

Leptospirosis is a zoonotic disease greatly influenced by ecological factors like rainfall, temperature and humidity. A one health approach to the epidemiology, management,

surveillance and control of leptospirosis relies on accessible and early diagnostics that can be applied to companion animals, livestock and wild animals.

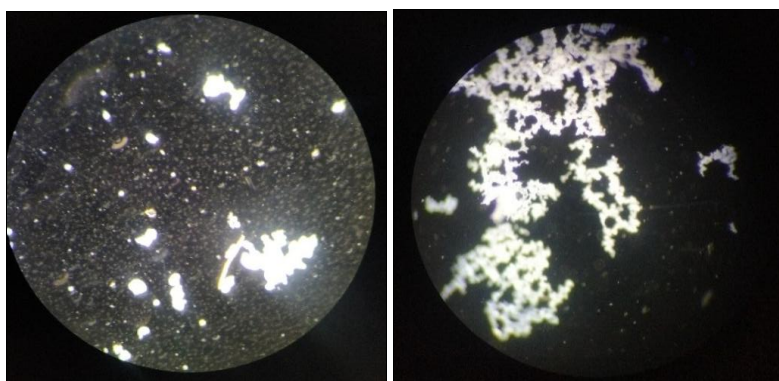


Fig 1: Agglutination pattern observed in the positive leptospirosis sample

In our study, totally 2783 serum samples were collected during 2021 - 2024 of which 2649 from various companion animals like cattle, dogs, cats, sheep, goat, horse etc., and 134 serum samples from wild animals (lion, tiger, wolf and elephant). Serum samples from companion animals and wild animals were tested for Leptospirosis using MAT and the positive case rate of leptospirosis were, out of 1178 dog serum samples, 636 (53.9%) were positive, from 85 cat samples, 20 (23.5%) were positive. Out of 1080 cattle serum samples, 296 (27.4%) were positive, out of 81 samples, 41 (50.61%) were positive in sheep and goats and out of 225 horse samples, 83 (36.88%) were positive for MAT. In wild animals, out of 134 serum samples 60 (44.77%) were positive for MAT test. In the year 2021, dog showed higher sero-positivity rate of 30.90%, while in the year 2022, 70% sero-positivity found in horse, whereas the sheep and goat showed sero-positivity of 88% in 2023 and in the year 2024, 63% of sero-positivity observed in dog (Fig 2 - 5). MAT test results in wild animals showed sero-positivity rate of 26% in the year 2022 and 40% sero-positivity in the year 2023 with MAT test. However, no positive results during the 2021 and 2024 in wild animals (Fig 6). The details of species-wise and year-wise distribution of *leptospira* serovars were presented in Table 3. In dogs, out of 1,178 canine sera tested, 636 (54.0%) were positive by MAT. The annual positivity ranged from 30.9% (2021) to 74.1% (2023). The Australis serogroup was consistently predominant across all years, followed by Autumnalis, Ballum, Canicola, and Pyrogenes in varying proportions. In 2022 and 2023, multiple serovars were recorded with nearly similar frequencies, indicating mixed exposure patterns in the canine population. In cattle, among 1,080 bovine samples, 296 (27.4%) were found positive. The positivity was highest in 2022 (34.5%) and lowest in 2021 (15.9%). The predominant serovars detected were javanica, hardjo, ballum, australis, and autumnalis, showing a wide serovar distribution. Also, hardjo and ballum were notably dominant during 2022, suggesting active circulation of these serovars in cattle herds. In horses, of 225 equine sera, 83 (36.9%) were positive. The predominant serovars included autumnalis, australis, ballum, and javanica during 2023 and 2024. In sheep and goats, 81 ovine and caprine samples were tested, with 41 (50.6%) being positive. The major serogroups identified were Australis, Pomona, Hardjo, Hebdomadis, and Pyrogenes, with distinct variation between years. Notably, in 2023, dual predominance of Hebdomadis and Pyrogenes was

observed. In cats, out of 85 feline samples examined, 20 (23.5%) were positive, all detected in 2024. The most common serogroups were Australis (45%), Grippotyphosa (25%), and Ballum (20%), indicating possible emerging exposure in cats during the study period. Across all species and years, Australis emerged as the predominant serogroup, indicating its widespread circulation and persistence in the region. Autumnalis and Ballum were the next most common serogroups, detected in multiple species, followed by Canicola, Hardjo, Javanica, and Pyrogenes, which showed species-specific occurrences. In cattle, Hardjo and Ballum were more prevalent, reflecting host adaptation, while Autumnalis and Australis dominated in dogs and horses. Among sheep and goats, mixed serogroups involving Australis, Pomona, and Hebdomadis were noted, whereas feline infections were primarily associated with Australis, Grippotyphosa, and Ballum. The results highlight the continued endemicity of leptospirosis with multi-serovar exposure among domestic animals during the study period.

The previous study of Choudhary *et al.*, 2023^[8] revealed that in animals, the highest prevalence of leptospirosis was found to be in buffalo (45.75%), followed by rodents (40%), dogs (26%), cows (24.26%), goats (19.42%), and pigs (15.8%) which was in accordance with our findings. Senthil Kumar *et al.*, 2023 screened 1466 serum samples, from which 528 (36.0%) samples were seroreactive to *Leptospira* on the MAT, a standard serological test referred by OIE which is correlating with this present study. Arumugam *et al.*, 2011^[1] study showed higher prevalence in Chennai (35.4%), followed by Thiruvallur (9.6%), Thiruvannamalai (5.5%), Thiruvavur (4.3%) and Kanniyakumari (3.9%). Seroprevalence of leptospirosis in animals and wild animals were reported in different areas of Tamil Nadu. Seroprevalence of leptospirosis in canines in Chennai was found to be 15.21 per cent (Kumar *et al.*, 2009)^[15] and 47.2 per cent (Bojiraj *et al.*, 2017)^[4]. Prabhavathy and Cecilia Joseph (2018)^[19] who reported that 95.0 per cent in dogs, out of which 52.63 per cent samples were positive to one serovar and 47.36 per cent samples were positive to several serovars. Leptospirosis in dogs was reported in Namakkal by Sathiyamoorthy *et al.*, 2017 showing an overall prevalence of canine leptospirosis at 28.40%. Serovar prevalence indicated that grippotyphosa was the predominant serovar for about 35.21% followed by 28.62% of autumnalis, 22.53% of australis, 16.90% of pomona, 15.49% of canicola, 12.67% of

icterohaemorrhagiae, 12.67% of pyrogenes and 1.40% of tarassovi. Out of 71 MAT positive serum samples, 18 (25.35%) were cross-reacted with more than one serovars. Chennai always has a higher prevalence than other parts of the state which was correlated with our present findings. Leptospirosis occurs in Chennai throughout the year. The data in this study shows that the cases are highly prevalent during monsoon (August - January) and increased after heavy rainfall and floods which implies that Leptospirosis is highly influenced by climate and environment. A notably high positivity in dogs (54%) and small ruminants (50.6%) suggests that these species act as important sentinel hosts reflecting environmental exposure to *Leptospira*. Australis emerged as the most predominant serogroup across all animal species and years, indicating its wide circulation in Tamil Nadu. Autumnalis, Ballum, and Hardjo were the other frequently detected serogroups, showing host adaptation tendencies. Year-wise variation, with a peak seropositivity in 2023, may be attributed to seasonal rainfall and flooding events that favour *Leptospira* persistence and transmission through stagnant water. The relatively lower positivity in cats compared to other species could be due to their solitary behaviour and limited environmental exposure and based on the limited available data. The detection of multiple

serogroups in the same host species also points to co-exposure or co-infection scenarios, reflecting complex transmission dynamics involving both maintenance and incidental hosts. These findings underscore the zoonotic risk associated with domestic animals, particularly dogs and cattle, which frequently interact with humans. Regular serological surveillance and control measures such as vaccination, rodent management and improved farm sanitation are essential to mitigate infection risk. Furthermore, integrating One Health approaches involving veterinary, public health and environmental sectors would enhance the understanding and management of leptospirosis transmission in endemic areas. The study confirms the continued endemicity of leptospirosis among domestic animals in Tamil Nadu, with Australis as the dominant serogroup. Continuous serological surveillance and preventive measures are essential to monitor emerging serogroups and reduce zoonotic transmission risks. The present study provides an updated overview of the *Leptospira* seroprevalence among domestic animals in Tamil Nadu during 2021-2024, emphasizing the persistence and diversity of circulating serogroups in the region. The overall seropositivity of 40.6% indicates a sustained endemic status of leptospirosis mainly among the livestock and companion animals.

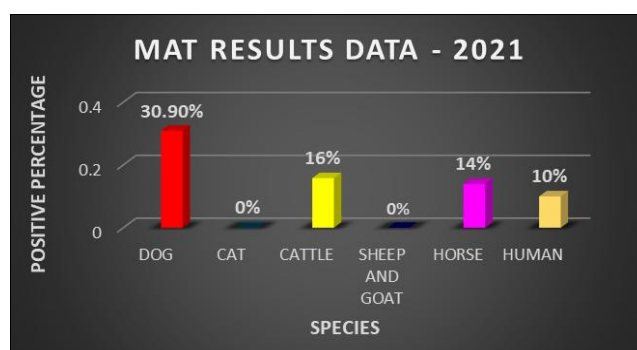


Fig 2: MAT results data for the year 2021 in which dog has highest positivity rate of 30.90%

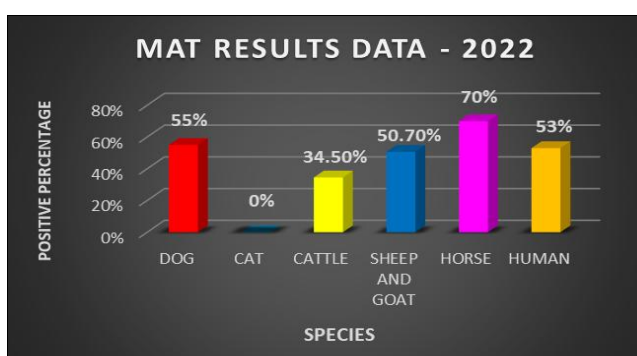


Fig 3: MAT result for the year 2022 in which horse has highest positive percentage 70% for MAT test

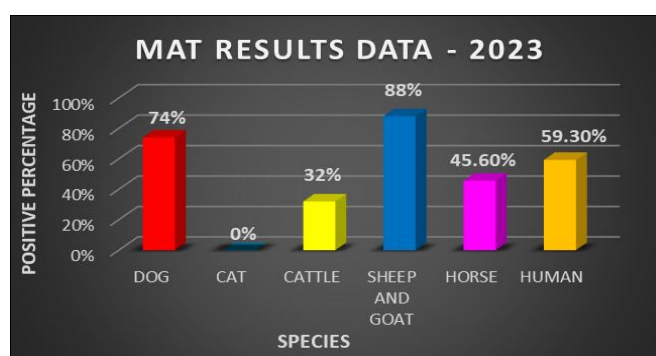


Fig 4: MAT result for the year 2023 in which sheep and goat has highest positive percentage 88% for MAT test

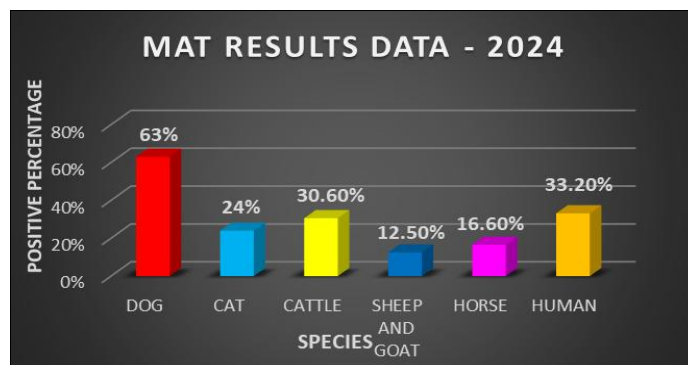


Fig 5: MAT result for the year 2024 in which dog has highest positive percentage 63% for MAT test

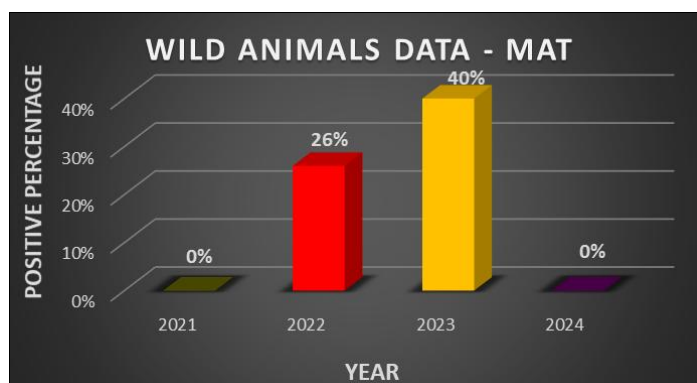


Fig 6: MAT test data of Wild animals in which the positivity rate was 26% in the year 2022 and 40% in the year 2023. No positive results in 2021 and 2024

Table 3: Data of samples received and MAT positive samples with predominant serovars

Species	year	No of samples received	No of samples positive for MAT	Predominant serogroups with positive percentages
Dog	2021	346	107	Australis (39%), Autumnalis (23.3%), Canicola (22.4%)
	2022	269	147	Australis (59%), Autumnalis (22.4%), Ballum (20.4%), Canicola (20.4%), Pomona (18.4%), Pyrogenes (18.4%)
	2023	251	186	Australis (51.6%), Autumnalis (28.5%), Ballum (30.1%), Pyrogenes (29%), Canicola (23.1%)
	2024	312	196	Australis (50.5%), Ballum (26.5%), Autumnalis (23.5%), Canicola (22.4%)
	Total	1178	636	
Cattle	2021	372	59	Javanica (30.5%), Australis (24%), Autumnalis (24%), Hebdomadis (22%), Icterohaemorrhagiae (22%)
	2022	510	176	Ballum (36.3%), Hardjo (36.3%), Grippytyphosa (22.1%), Javanica (17%), Autumnalis (14.7%).
	2023	22	7	Australis (85.7%), Javanica (71.4%)
	2024	176	54	Autumnalis (22.2%), Ballum (18.5%), Hebdomadis (14.8%), Pyrogenes (14.8%)
	Total	1080	296	
Horse	2021	56	8	Autumnalis (75%), Icterohaemorrhagiae (25%)
	2022	20	14	Ballum (50%), Autumnalis (43%)
	2023	125	57	Australis (36.8%), Autumnalis (29.8%), Javanica (24.5%), Ballum (19.3%), Canicola (15.8%)
	2024	24	4	Australis (50%), Javanica (25%), Pyrogenes (25%)
	Total	225	83	
Sheep and Goat	2021	0	0	-
	2022	65	33	Australis (36.3%), Pomona (24.2%), Autumnalis (18.1%), Hardjo (18.1%)
	2023	8	7	Hebdomadis (71.5%), Pyrogenes (71.5%)
	2024	8	1	Australis (100%)
	Total	81	41	
Cat	2021	0	0	-
	2022	0	0	-
	2023	1	0	-
	2024	84	20	Australis (45%), Grippytyphosa (25%), Ballum (20%)
	Total	85	20	

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