Full Length Article

SCREENING OF DOGS FOR LEPTOSPIROSIS IN CAUVERY DELTA REGION OF TAMIL NADU

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ABSTRACT

Leptospirosis is a zoonotic disease caused by pathogenic species of Leptospira interrogans. Dogs can act as maintenance host for some serovars and transmit to other animals and humans through direct and/or indirect contact with urine or contaminated water and soil. A study was conducted to identify the presence of Leptospira and assessment of seroprevalence in apparently healthy dogs in Cauvery delta region (Thanjavur, Thiruvarur and Nagapattinam districts) of Tamil Nadu. Serum (n=50) and urine (n=50) samples were collected randomly from 50 dogs with history of non vaccination to leptospirosis. Dark field microscopic (DFM) examination revealed the presence of Leptospira in 12.0 per cent of serum and 24.0 per cent of urine samples. Serological screening was done by microscopic agglutination test (MAT) using a battery of 12 Leptospira serovars (representing the serogroups of Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Tarassovi), which revealed 56.0 per cent seropositivity to either one or several serovars, which was Icterohaemorrhagiae (46.4 per cent), Grippotyphosa (32.1 per cent), Australis (32.1 per cent), Canicola (25.0 per cent) and Autumnalis (17.9 per cent) with highly significant difference ($p < 0.05$) in seropositivity between serovars. The predominant serovar detected in dogs in this study was Icterohaemorrhagiae followed by Grippotyphosa. It is concluded that presence of leptospirosis in dogs in Cauvery delta regions of Tamil Nadu poses the risk of transmission to animals and humans and dogs can be used as sentinel animals for identification of leptospirosis.

Keywords: *Leptospira*, Dogs, Seroprevalence, Cauvery delta region, Tamil Nadu

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INTRODUCTION

Leptospirosis is a globally important anthropozoonotic disease of animals and humans caused by pathogenic species *Leptospira interrogans.* Transmission of leptospirosis to humans and animals is mainly by contact with urine of rodents, domestic and wild animal reservoirs (Balakrishnan *et al.,* 2006). Numerous mammalian species act as maintenance hosts (natural carriers) including feral, farm and pet animals (Levett, 2001; Balakrishnan and Manicavasaka Dinakaran, 2014). Dogs may act as reservoirs and may succumb to severe illness similar to humans (Larson *et al.,* 2017). Humans act as incidental hosts (Haake and Levett, 2015; Bonhomme and Werts, 2022).

Antigen detection by dark field microscopy (DFM), antibody detection by microscopic agglutination test (MAT) and molecular techniques (PCR) are the widely accepted techniques in diagnosis of leptospirosis (Balakrishnan *et al.,* 2006; WOAH, 2021; Senthilkumar *et al.,* 2022). Seroprevalence of leptospirosis in dogs was 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) and 47.2 per cent (Bojiraj *et al.,* 2017). Prabhavathy and Cecilia Joseph (2018) 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 (Senthil *et al.,* 2013) and seroprevalence in dogs was found to be 14.0 per cent (Sathiyamoorthy *et al.,* 2018).

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). 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. There are scanty reports of prevalence of *Leptospira* serovars in many districts and no reports in dogs in Cauvery Delta regions of Tamil Nadu. The present study was carried out to know seropositivity of leptospirosis in dogs in Cauvery Delta region of Tamil Nadu.

MATERIALS AND METHODS

Study area and sample collection

A study was conducted for the serological screening for leptospirosis in apparently healthy dogs (n=50) to know the *Leptospira* serovar positivity in Cauvery delta regions (Thanjavur [n=20], Thiruvarur [n=15] and Nagapattinam [n=15] districts) of Tamil Nadu. Serum (n=50) and urine (n=50) samples were collected randomly from 50 dogs with no history of vaccination to leptospirosis. Samples were collected from dogs brought to Veterinary Clinical Complex, Veterinary College and Research Institute (VC&RI), Orathanadu and Veterinary Dispensaries 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. Mid-stream urine samples (50 ml from each animal) were collected aseptically from dogs by urinary catheterization in a sterile Uricol urine collection container and also in sterile containers containing 10 ml of phosphate-buffered saline as a stabiliser. Urine samples were centrifuged at 8,000 rpm for 20 min and the supernatant was discarded carefully without disturbing the pellet. The pellet was used for dark-field microscopic (DFM) examination for identification of Leptopsiral organisms (Bojiraj *et al.,* 2017; Senthilkumar *et al.,* 2022).

Identification of Leptospira by dark field microscopic (DFM) examination

Initial screening of serum and urine samples was carried out by DFM at once after collection or preferably within one hour after collection. A volume of 10 µl sample was placed on a clean, grease-free glass slide and covered with a coverslip. The wet mount was examined for 100 microscopic fields under a dark field microscope at 100X oil immersion for the identification of spirochaetes like structure (Chandrasekaran and Gomathi, 2004; WOAH, 2021; Senthilkumar *et al.,* 2022).

Serological screening by microscopic agglutination test (MAT)

Serological screening for leptospirosis was done by microscopic agglutination test (MAT) using a battery of 12 *Leptospira* serovars representing the serogroups of Australis, Autumnalis, Ballum, Canicola, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Javanica, Pomona, Pyrogenes and Tarassovi (Senthilkumar *et al.,* 2022) obtained from Zoonoses Research Laboratory, Tamil Nadu Veterinary and Animal Sciences University, Chennai and maintained in EMJH medium at Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Orathanadu. The concentration of growth of *Leptospira* serovars in EMJH liquid leptospiral medium at 30±2°C was assessed using the Helber bacteria counting chamber (0.02 mm) and estimating the number of leptospires 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 $2x10^8$ leptospires per ml of culture were used as antigen for MAT (Faine *et al*., 1999; WOAH, 2021). 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). Briefly, 1:50 dilution of the sera were prepared and then the diluted sera (30µl) were mixed with pooled antigen (30 µl) in 96 well U-bottom microtitre plate and kept for incubation at 37ºC 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.

Statistical analysis

The per cent positivity (Smith, 2005) and Chi-square test for comparing the statistical significance between different parameters such as seropositivity and serovars positivity of leptospirosis in dogs between breeds, sexes, age groups, management aspects and spatial distributions using IBM SPSS statistics for windows (Version 20.0, 2011) were followed in this study.

RESULTS AND DISCUSSION

Presence of spirochaete-like organisms suggestive of *Leptospira* was detected by DFM in six serum samples (12.0 per cent) and 12 urine samples (24.0 per cent), in which all serum positive dogs were also positive in urine samples. Chandrasekaran and Gomathi (2004) recommended that DFM can serve as a standard screening test for early and rapid diagnosis of leptospirosis and Monte *et al.* (2012) used blood and urine samples from dogs for detection of *Leptospira.* Similarly, Towheed *et al.* (2019) reported that 44.4 per cent dog urine samples were positive and Desai *et al.* (2020) reported that 39.39 per cent urine samples were positive by DFM. The lower percentage of *Leptospira* detection in urine samples in this study might depend on level of renal carriage and secretory pattern of *Leptospira* through urine of dogs. The amount of infection may also vary in different geographical areas, which depends on prevailing epidemiological risk factors associated with dog population.

The seropositivity with one or several serovars of pathogenic *Leptospira* was observed in 28 dogs (56.0 per cent) (Table 1). It concurs with the findings of Vijayanand *et al.* (2009) who reported 62.5 per cent seropositivity in Chennai and stated that seropositivity of leptospirosis is comparatively higher in endemic areas. Soman *et al.* (2014) reported 36.36 per cent seropositivity in canine sera from Kerala and Bojiraj *et al.* (2017) who reported 47.2 per cent of seropositivity in dogs. But, the amount of seropositivity in this study is in contrast with the findings of Patil *et al.* (2014) who reported 17.5 per cent seroprevalence in canine species in Mumbai; Sathiyamoorthy *et al.* (2018) who reported that 14.00 per cent positivity to pathogenic serovars of *Leptospira interrogans* from infected dogs suspected for leptospirosis and apparently healthy dogs in and around Namakkal (Tamil Nadu); Sophia Inbaraj *et al.* (2019) reported 10 per cent seropositivity in clinically suspected dogs from various parts of the country and Bale *et al.* (2021) reported 28.0 per cent seroprevalance of leptospirosis in dogs. It is reported that, detection of pathogenic *Leptospira* serovars in dogs represents a potential infection, environmental contamination and risk of spread of leptospirosis to people (Piredda *et al.,* 2021).

The amount of seropositivity may vary in different geographical areas and agro-climatic zones with different micro and macro climatic and ecological conditions prevailing. The agricultural activity in Cauvery delta regions is paddy cultivation followed by sugarcane cultivation and these agricultural fields might be highly inhabited by rodent population. So, water bodies and environment may have chance of being heavily contaminated by the urine of rodents. This might be the reason for higher level of seropositivity in dogs in this study.

Age-wise, one young (<1 year) (16.7 per cent) and 27 adult (>1 year) (61.4 per cent) dogs were seropositive. Significant difference (p <0.05) was noticed between young and adult dogs (Table 1). The finding of this study is in accordance with the findings of Kumar *et al.* (2009), who reported that highest seropositivity was observed in 3-5 years age group and lowest in less than 1 year of age. But, in contrast with the findings of Senthil *et al.* (2013) who reported higher prevalence in young dogs and lower prevalence in adult dogs that is 26.3 per cent in 1-2 years, 28.9 per cent in 2-3 years, 37.2 per cent in 3-4 years, 19.1 per cent in 4-5 years and 6.4 per cent in 5 years and above. Desai *et al.* (2020), who reported age-wise prevalence of 63.63 per cent in 1-≤3 years age group, 27.77 per cent in >3- ≤6 years age group and 51.85 per cent in >6- ≤12 years age group. The amount of seropositivity observed in adult dogs in this study is in accordance with Desai *et al.* (2020), but in contrast with the seroprevalence in young dogs. It concurs with the findings of Bale *et al.* (2021) who reported higher seroprevalance in adults when compared to young dogs. All age group of animals are equally susceptible to leptospirosis. But, the amount of infection may vary because of frequency of exposure to the contaminated environment. Young dogs in this study might not have been exposed more frequently to the causal factors when compared to adult dogs, hence low percentage of prevalence in young dogs is being reported.

Sex-wise, 16 male (66.7 per cent) and 12 female (46.1 per cent) dogs were seropositive in this study. No significant difference (p >0.05) was noticed between male and female dogs (Table 1). It agrees with the findings of Senthil *et al.* (2013) who reported 20.9 per cent in male and 21.0 per cent in female dogs with no significant difference between sexes. Similarly, Desai *et al.* (2020) who reported sex-wise prevalence of 42.10 per cent in male and 55.55 per cent female dogs. However, Kumar *et al.* (2009) who reported that male dogs were more seropositive (8.31 per cent) than females (5.41 per cent) and Bale *et al.* (2021) who reported higher seroprevalance in males as compared to females. Both male and female dogs are equally susceptible to leptospirosis. Hence, there is no significant difference in sex-wise seropositivity to *Leptospira* infection was observed in dogs in this study.

Breed-wise, 16 non-descript (66.7 per cent), five Labrador (41.7 per cent), one Pomeranian (100 per cent), three German shepherd (42.9 per cent), one Chippiparai (100 per cent) and two Dobermann (40.0 per cent) dog breeds were seropositive in this study. No significant difference was was (p >0.05) was noticed between breeds (Table 1). Kumar *et al.* (2009) documented the presence of different serovars of *Leptospira* in 17 dog breeds and reported more seropositives in large sized breed of dogs like Labrador, German shepherd and Doberman. He also reported that Spitz had maximum (26.31 per cent) seroprevalence among all breeds. Senthil *et al.* (2013) reported the seroprevalence of 17.0 per cent in small breeds (Pomeranian, Poodles, Pug, Dachshund and Spitz), 14.7 per cent in larger breeds (Labrador, Great Dane, Golden retriever, German shepherd, English mastiff and Terrier breeds) and 90.0 per cent in mongrel (stray dogs). It is suggested that, all dog breeds are equally susceptible to leptospirosis. Hence, no significant difference in seropositivity to *Leptospira* infection noticed between different dog breeds in this study.

Seropositivity to *Leptospira* was noticed in 7 owned dogs (35.0 per cent), 12 semi-owned dogs (60.0 per cent) and 9 stray dogs (90.0 per cent) in this study. Significant difference (p <0.05) was noticed between owned, semi-owned and stray dogs (Table 1). It concurs with the findings of Bale *et al.* (2021) who reported highest seroprevalance in non-descript breed. Higher seroprevalence of leptospirosis in stray dogs might be due to the nature of free roaming and more frequency of exposure to the contaminated environment.

In Cauvery Delta region of Tamil Nadu, 15 dogs (75.0 per cent) in Thanjavur district, eight dogs (53.3 per cent) in Thiruvarur district and five dogs (33.3 per cent) in Nagapattinam district were seropositive for leptospirosis in this study. No significant difference (p >0.05) was noticed between different geographical locations in the study areas (Table 2). Reports on canine leptospirosis in Cauvery Delta regions are not available. This is the primary report of seropositivity of leptospirosis in dogs in Cauvery Delta regions of Tamil Nadu. However, Kumar *et al.* (2009) who reported serum samples collected from Chennai, Tamil Nadu showed highest seroprevalence (15.21 per cent) with maximum number of serovars; Bojiraj *et al.* (2017) who reported 47.2 per cent of seropositivity in dogs in Chennai and Sathiyamoorthy *et al.* (2018) who reported 14.00 per cent seropositivity from dogs suspected for leptospirosis and apparently healthy in Namakkal.

Out of 28 seropositive dogs (titre of 1:100 by MAT was considered as seropositive for leptospirosis), serovars Icterohaemorrhagiae in 13 samples (46.4 per cent), Grippotyphosa in nine samples (32.1 per cent), Australis in nine samples (32.1 per cent), Canicola in seven samples (25.0 per cent) and Autumnalis in five samples (17.9 per cent) were seroreactive in this study (Fig 1). Multiple serovars of *Leptospira* were found in a single serum sample. Highly significant difference (p <0.05) was noticed between *Leptospira* serovars. The predominat serovar was Icterohaemorrhagiae, followed by Grippotyphosa, Australis and Canicola in this study. The least seroreactive serovar was Autumnalis. The findings of this study coincide with the findings of Senthil *et al.* (2013), who reported that Icterohaemorrhagiae, Canicola, Grippotyphosa and Autumnalis were seroreactive serovars of *Leptospira* to canine serum samples. However, it is in contrast with the findings of Kumar *et al.* (2009) who reported that the serovar Canicola was the predominant followed by lcterohaemorrhagiae, Australis, Autumnalis, Tarassovi and Ballum. Similarly, Soman *et al.* (2014) reported seropositivity with Australis and Pomona in canine sera from Kerala; Bojiraj *et al.* (2017) who documented that the serovars Australis and Autumnalis were most predominant and Sophia Inbaraj *et al.* (2019) who reported seropositivity with serovar Grippotyphosa in sera samples from clinically suspected dogs from various parts of the country. There is an urgent need to increase the awareness to public, practitioners and pet owners and follow preventive measures, because of the close contact between people and their pets can predispose the spread of *Leptospira* infection (Azocar-Aedo and Monti, 2022).

CONCLUSION

Leptospirosis is one of the endemic diseases in animals in India. It is an important zoonotic disease and often neglected in animals and humans because of lack of diagnostic facilities such as dark field microscopic examination (DFM) and microscopic agglutination test (MAT) for early diagnosis and timely treatment of clinically infected animals and humans. In this study, *Leptospira* was identified in serum (12.0 per cent) and urine (24.0 per cent) of apparently healthy dogs. Seropositivity was noticed with several pathogenic serovars of *Leptospira* such as Icterohaemorrhagiae (46.4 per cent), Grippotyphosa (32.1 per cent), Australis (32.1 per cent), Canicola (25.0 per cent) and Autumnalis (17.9 per cent). The predominant serovar detected in dogs in this study was Icterohaemorrhagiae followed by Grippotyphosa. It is concluded that dogs can act as maintenance host and potential source to spread leptospirosis to humans and animals. Environment, particularly water bodies and soil can be contaminated by urine of clinically or subclinically infected and carrier dogs with one or several pathogenic serovars of *Leptospira.* It poses the greatest risk of transmission of leptospirosis to other susceptible animals and humans in Cauvery delta region of Tamil Nadu.

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Table 1. Epidemiological parameters related to seropositivity of leptospirosis in dogs

Epidemiological parameters	No. of dogs	MAT positive		P value
		No.	%	
Age				
Young (<1 year)	6	1	16.7	0.038*
Adult (>1 year)	44	27	61.4	
Sex				
Male	24	16	66.7	0.144^{NS}
Female	26	12	46.1	
Breed				
Non descript	24	16	66.7	
Labrador	12	5	41.7	
Pomeranian	1	1	100	0.455^{NS}
German shepherd	7	3	42.9	
Chippiparai	1	1	100	
Dobermann	5	2	40.0	
Management				
Owned dogs	20	7	35.0	0.015*
Semi-owned dogs	20	12	60.0	
Stray dogs	10	9	90.0	
Total	**50**	**28**	**56.0**	

*Significant difference at $p < 0.05$; NS – Non Significant at $p > 0.05$

Table 2. Geographical distribution of seropositivity of leptospirosis in dogs in Cauvery Delta regions of Tamil Nadu

Cauvery Delta regions	No. of dogs	MAT positive	
		No.	%
Thanjavur district	20	15	75.0
Thiruvarur district	15	8	53.3
Nagapattinam district	15	5	33.3

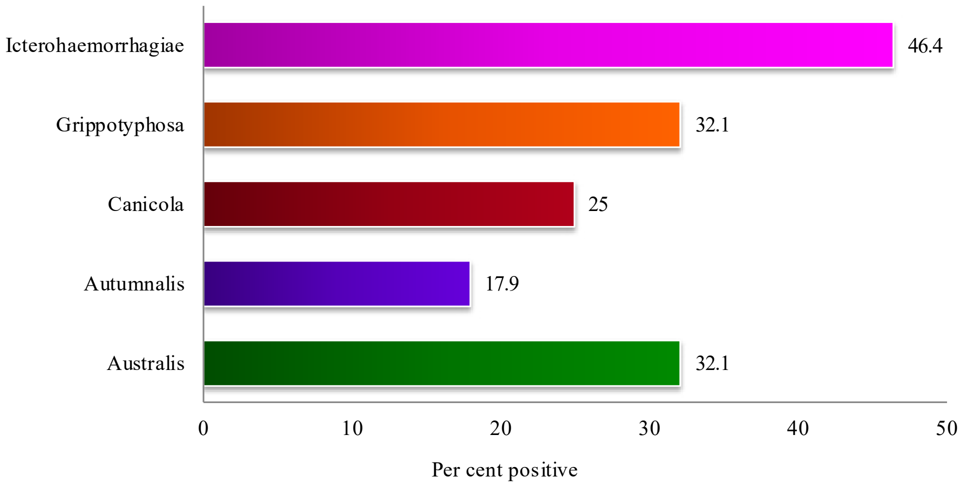

Fig. 1. Serovars of *Leptospira* spp. found among MAT positive dogs

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