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Preliminary study on the status of feline leptospirosis in Kerala[#]

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Abstract

Leptospirosis is a disease of zoonotic importance in worldwide scenario and is caused by the infection of Leptospira species. The present study was undertaken to estimate the seropositivity of leptospirosis in cats of Kerala. A total of 200 serum samples were collected from apparently healthy cats from different parts of Kerala such as Thiruvananthapuram, Kollam, Alappuzha, Pathanamthitta, Kottayam, Thrissur, Ernakulam, Malappuram, Kozhikode, Wayanad, and Kannur. Among these samples, 12 samples (6 %) were found to be positive with the MAT titre above 1:20. The serovar identified in this study was serovar Djasiman, Autumnalis, Icterohaemorrhagiae, Australis, Grippotyphosa, and Hardjo. The highest prevalence in cat was for serovar Djasiman (50%) followed by serovar Autumnalis (25%). The majority of cats were crossbreds, males and in age groups between one and two years (58.33%). The majority of animals had both indoor with outdoor access, the presence of rodents and other animals in the household or surroundings.

Keywords: Cat, leptospirosis, seroprevalence, epidemiological study

Leptospirosis is a disease of zoonotic importance in the worldwide scenario and is caused by the infection of *Leptospira* species. It is reported in more than 150 species of mammals and identified to have more than 300 serovars (Picardeau, 2017). The spread of disease occurs as a result of contact with water contaminated with bacteria or contaminated urine. The transmission of disease in human beings occurs as a result of animal source or due to the recreational activities

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engaged in contaminated water (Murillo *et al.*, 2020). Rats are considered to be primary reservoirs for the infection and other mammals such as cattle, sheep, pigs, dogs and cats can also transmit the disease. To offer a rapid diagnosis in case of acute infection PCR can be used as a powerful tool meanwhile, MAT is recommended to identify infecting serovars of *Leptospira* species (Chandran *et al.*, 2019).

The seroprevalence of leptospirosis varies according to geographical regions from 4.8 to 35 percent (Murillo *et al.*, 2020; Spribler *et al.*, 2019; Rodriguez *et al.*, 2014; Mylonakis *et al.*, 2009). Although the infection in cats has been demonstrated and has the ability to develop antibodies, studies on feline leptospirosis are rare. Hence the study was undertaken to assess seroprevalence of feline leptospirosis in Kerala.

A total of 200 serum samples were collected randomly from apparently healthy cats from different parts of Kerala such as Thiruvananthapuram, Kollam, Alappuzha, Pathanamthitta, Kottavam, Thrissur, Ernakulam, Malappuram, Kozhikode, Wavanad. and Kannur during the period from December, 2021 to November, 2022. Epidemiological data such as age, sex, breed, housing environment, presence of other animals in the housing, and whether animals had access to rodents or not were also collected. The blood samples were collected in 5 ml clot activator vials for serum separation. Immediately after receipt, samples were centrifuged and sera samples were stored in -20°C.

Microscopic agglutination test was carried out using thirteen serovars as described by Faine *et al.*, (1999). The reference serovars maintained were serovar Australis, Autumnalis, Bataviae, Canicola, Djasiman, Grippotyphosa, Hardjo, Hebdomadis, Icterohaemorrhagiae, Pomona, Pyrogens, Tarasovi, and Javanica.

In the first step, 1:20 serum dilutions were prepared in PBS, 50 μ L of which was taken and mixed with 50 μ L of each of the four to six days old live leptospiral serovars separately. Antigen controls were set with 50 μ L PBS and 50 μ L of different live leptospiral serovars and the plates were incubated at 37°C for two

hours. After incubation, the result was read by examining a drop of serum-antigen mixture from each well under the 10X objective of DFM for agglutination of leptospires.

Samples positive at 1:20 dilution were again serially diluted up to 1:400. The combination at which 50 per cent or more leptospiral organisms were seen agglutinated was taken as positive. In the case of samples showing agglutination against more than one serovar, the reacting at the highest titre was presumed to be the infecting serovar. Sera samples show the same agglutination titres to more than one serovar were considered as mixed equals.

In the study, serum samples from apparently healthy 200 cats were collected from different parts of Kerala and among them, 12 showed seropositivity (6 per cent). This finding is in agreement with Murillo et al. (2020) who observed a seropositivity of 4.1 per cent in cats in Spain and most common serovar identified was serovar Cynopteri. Contrary to this finding, Lapointe et al. (2013) reported that 25 per cent of cats in Canada tested were seropositive by MAT and all the cats were positive for serovar Bratislava. Kakita et al. (2021) detected leptospiral antibodies in 16.6 per cent of cats tested in Japan and the predominant serovar was Javanica. Antibodies against Leptospira serovars Poi, Bratislava, Arborea, Ballum, Pomona and Lora were detected in 15.3 per cent of cats in Southern Italy. Among 12 samples two samples had mixed infection with two serovars and one sample had mixed infection with three serovars. Out of 12 samples, six samples (50 %) showed seropositivity to serovar Diasiman followed by serovar Autumnalis (25%) then followed by serovar Icterohaemorrhagiae (16.66%) and serovar Australis, Grippotyphosa and Hardjo each of 8.33 %. The major serovar observed was Diasiman followed by Autumnalis, Icterohaemorrhagiae. Least were Australis, Grippotyphosa and Hardjo.

In the present study, six per cent of apparently healthy cats, in Kerala, showed seropositivity for leptospirosis which is in agreement with previous studies where seroprevalence for leptospirosis in cats ranged from 4 to 33.3 per cent in different geographical

SI. No.	Age	Sex	Breed	Housing environment			Presence of other animal		Access to rodents		Serovar
				Indoor	Indoor with outdoor	Outdoor	Yes	No	Yes	No	
1	1.5 year	F	Crossbred	×	✓	×	✓	×	~	×	Djasiman
2	1 year	М	Persian	✓	×	×	✓	×	✓	×	Tarasowi
3	1year	М	Crossbred	×	×	✓	✓	×	 ✓ 	×	Djasiman
4	6 months	М	Crossbred	×	~	×	~	×	~	×	Djasiman and autumnalis
5	9 months	F	Crossbred	×	✓	×	✓	×	✓	×	Grippotyphosa
6	1 year	М	Persian	×	✓	×	✓	×	✓	×	Autumnalis
7	8 months	М	Crossbred	×	✓	×	✓	×	✓	×	Autumnalis
8	1 year	М	Crossbred	×	✓	×	×	✓	✓	×	Hardjo
9	10 months	F	Crossbred	×	×	✓	✓	×	 ✓ 	×	Djasiman
10	7 months	F	Crossbred	×	×	✓	✓	×	✓	×	Australis
11	2 years	М	Persian	~	×	×	~	×	~	×	lcteroheamorrhagiae, Djasiman, hardjo
12	2 years	F	Persian	~	×	×	~	×	~	×	lcterohaemorrhagiae, Djasiman

Table 1. Details of leptospira seropositive cats by MAT

areas (Murillo *et al.*, 2020; Sprißler *et al.*, 2019; Rodriguez *et al.*, 2014; Mylonakis *et al.*, 2009). Studies on seroprevalence study of leptospirosis in cats in Kerala are scarce. Divya *et al.* (2021) reported that one out of 11 cats tested by MAT was positive for the serovar Sejroe. One study regarding the seroprevalence of *Leptospira* spp. in India which was conducted in Salem district of Tamil Nadu by Natarajaseenivasan *et al.* (2002) reported that six out of nine cats had antibodies to leptospira tested by MAT and also showed high seroprevalence in rice mill workers.

In the present study, 50 per cent showed positive for *Leptospira* serovar Djasiman. This serovar is reported in Thailand by Spribler *et al.* (2019). According to several studies conducted commonly observed serovars were Australis, Autumnalis, and Canicola (Palerme *et al.*, 2019; Spribler *et al.*, 2019; Weis *et al.*, 2017; Chan *et al.*, 2014; Rodriguez *et al.*, 2014; Mylonakis *et al.*, 2009).

In the present study, 41.6 per cent of seropositive cats were under one year of age and 58.33 per cent were in age group between one and two years (Dorsch *et al.*, 2020). The present study showed that 41.6 per cent of seropositive cats were females and 58.33 per cent were males. In breed-wise classification, 66.66 per cent of seropositive cats were cross bred and the remaining were Persian cats. The housing condition of animals showed that 25 per cent of seropositive cats are reared exclusively indoor and 25 per cent of seropositive cats are reared exclusively outdoors and the majority (50 %) have both indoor and outdoor access (Rodriguez et al., 2014; Lehtla et al., 2022). When the presence of other animals or rodent status was considered, all seropositive animals, except one had the presence of at least one animal in the house or surroundings such as dogs, cats (Donato et al., 2022), and cattle (Ojeda et al., 2018). A study conducted in Chile by Azocar-Aedo et al. (2014) observed that contact of cats with livestock animals was significantly associated with the presence of *Leptospira* spp. antibodies and a study done on 90 asymptomatic cattle in the Alappuzha district revealed that 47 among them were seropositive which indicated that cattle can transmit the disease and can act as an asymptomatic carrier of the disease (Sreekutty et al., 2020).

Summary

The seroprevalence of feline leptospirosis observed in this study was 6 per cent and the highest prevalence was observed for the serovar Djasiman. Other serovars observed were serovar Autumnalis, Australis. Djasiman, Grippotyphosa, Icterohaemorrhagiae, and Hardjo. Majority of animals were males, crossbreds and of age between one and two years. The animals found seropositive had at least one animal in the household, access to the rodents, and were kept outdoor with indoor access.

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Conflict of interest

There are no conflicts of interest reported by the authors.

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