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Seroprevalence of *Leptospira* spp. in cattle of Pandalam municipality - hotspot in Pathanamthitta district, Kerala#

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Abstract

Leptospirosis is one of the leading zoonotic tropical disease and has been endemic in India since the 20th century. The present study was undertaken to assess the occurrence of Leptospira spp. among cattle and their role in disease transmission in Pandalam municipality of Pathanamthitta district, which has been recognised as hotspot for human leptospirosis by the State Surveillance Unit, Directorate of Health Services (DHS), Kerala in the year 2023. A total of 101 apparently healthy dairy cattle were randomly selected, and blood and urine samples were collected from the same animal. Blood samples were analysed by Microscopic Agglutination Test (MAT) using a panel of 25 reference serovars, while urinary shedding was assessed by Polymerase Chain Reaction (PCR). An overall seropositivity of 63.36 per cent was observed in Pandalam municipality, with antibody titres ranging from 1:50 to 1:3200. Among the seropositive samples, 21.87 per cent samples showed titres of 1:400 and above. None of the cattle exhibited any overt clinical signs, however farmers reported history of various reproductive disorders among the cattle. Molecular detection of urine samples revealed 7.92 per cent positivity for the genus-specific 16S rRNA gene, while 0.99 per cent samples were positive for the lipL21 gene, indicating shedding of leptospires in urine. The study highlights the importance of identifying circulating serovars in carrier animals such as cattle, in which infection is usually asymptomatic and their role in the maintenance and transmission of the disease. These findings will aid in adopting proper interventions for effective prevention and control of leptospirosis in endemic regions such as Kerala.

Keywords: *Leptospirosis, microscopic agglutination test, cattle, Kerala, polymerase chain reaction*

Leptospirosis is one of the leading zoonotic causes of morbidity and mortality, accounting for an estimated one million cases and 60,000 deaths globally each year (Costa et al., 2015; CDC, 2025). Leptospirosis disproportionately impacts socioeconomically disadvantaged communities in tropical and subtropical regions worldwide (CDC, 2025). Transmission occurs via direct contact with infected urine or indirectly through contaminated soil or water. Human leptospirosis is a dead-end infection, as human to human transmission is virtually unknown. The higher transmission rates observed in low-socioeconomic communities are largely attributable to inadequate sanitation infrastructure.

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Leptospirosis has been endemic in India since the early 20th century, with majority of outbreaks reported from the coastal states (Gupta et al*.,* 2023; Himani et al*.,* 2013). According to DHS, Kerala, a total of 3552 confirmed human leptospirosis cases and 251 confirmed deaths due to leptospirosis were reported in 2024. Among the districts in Kerala, maximum number of hotspots for human leptospirosis was reported in Ernakulam, Alappuzha, Kottayam, Pathanamthitta and Thiruvananthapuram districts. Pathanamthitta was identified as a leptospirosis hotspot in humans by the State Surveillance Unit, DHS, due to the clustering of cases across multiple municipalities and several affected panchayats, indicating sustained environmental exposure. Pathanamthitta has been identified as a leptospirosis hotspot by the State Surveillance Unit, DHS, due to clustering of cases across two municipalities and 10 panchayats, indicating sustained environmental exposure, and in 2024 alone, the district reported 170 confirmed human cases with six deaths.

The carrier animals in India include rats, pigs, cattle, bandicoots and dogs. Cattle are the maintenance host and reservoir for *Leptospira interrogans* serovar Hardjo which is a significant etiology of bovine abortion and the most common human leptospiral infection. Furthermore, cattle serve as a natural host for several other Leptospira serovars where infections are typically asymptomatic, though chronic cases can result in various reproductive disorders. Chronically infected cattle intermittently excrete leptospires in their urine for periods spanning 28 - 40 weeks (Leonard et al*.,*1992; Monti et al*.,* 2023).

Effective control of leptospirosis mandates comprehensive surveillance to identify the circulating strains, geographic morbidity patterns, reservoir species, and at-risk populations. Intervention strategies are designed to disrupt critical links within the transmission cycle, primarily concentrating on improved environmental hygiene and animal immunization.

Thus, the present study aims to identify the seroprevalence of leptospirosis in cattle and leptospiral shedding in cattle urine. The understanding of animal and occupational drivers will facilitate effective preventive and control measures in the region.

Materials and methods

Sampling process

The present study was conducted to estimate the occurrence of leptospirosis in apparently healthy cattle in Pandalam municipality of Pathanamthitta district during the period of May 2025 to September 2025. A total of 101 apparently healthy dairy cattle above one year were randomly selected for the study, with two animals sampled from each household, and blood and urine samples were collected from the same animals. The sample size was calculated using Epi Info™ statistical software using a 95 per cent confidence interval (CI), and the precision was estimated at 10 per cent. The cattle population in Pandalam municipality was 2683 according to the 20th livestock census (2019). The prevalence of bovine leptospirosis was considered to be 44.24 per cent by Microscopic Agglutination Test (MAT), in Kerala (Murugavelu, 2021).

Blood samples were collected aseptically from the jugular vein by intravenous puncture using a sterile 18G disposable hypodermic needle. Four millilitres of blood was collected into clot activator vials for serum separation. The blood-filled vials were placed in a slightly inclined position and allowed to stand at room temperature for one to two hours to facilitate clot formation and separation of serum. Following clot formation, the serum was carefully harvested and transferred into sterile two millilitre Eppendorf tubes. The serum samples were stored at -20°C until further analysis by Microscopic Agglutination Test (MAT).

Urine samples were collected from the same dairy cattle by mid-stream collection and transferred into 100 mL sterile sample collection containers containing an equal volume of phosphate buffered saline (PBS) with a pH of 7.2. The urine samples were transported to the laboratory at atmospheric temperature and subsequently processed for DNA isolation and detection of *Leptospira* spp. by PCR.

Microscopic Agglutination Test (MAT)

All the serum samples were subjected to MAT using a panel of 25 standard strains of Leptospira representing different serovars and serogroups as shown in Table 1. The MAT was carried out as per World Organisation for Animal Health (WOAH, 2025) guidelines using live Leptospira organisms grown in liquid Ellinghausen McCullough Johns Harris (EMJH) culture medium at 30 ± 2°C and the culture were at least 4 days old, but not more than 10 days old.

The test was carried out in a 96 well 'U' bottom microtitre plate with serum dilutions ranging from 1:50 up to 1:12800. Initially all the wells of the microtitre plates were filled with 50 µL PBS. In first well of each row, 50 µL of serum sample diluted to 1:25 was added and serial two-fold dilution were then prepared across eight wells in each row, and 50 µL was discarded from the eighth well. A constant volume of 50 µL of a particular serovar with a density of 2 X 10^8 leptospires/ mL was added in each row and incubated at 37 ºC for two to four hours (Faine, 1982). All the final dilution mixtures were observed by placing 10 µL drop of reaction mixture on a clean, grease-free slide under Dark Field Micrscope (DFM) without a coverslip and the results were recorded. The highest titre showing 50 per cent agglutination or 50 per cent reduction in free leptospires compared to negative control was taken as end point titre (Fig 1.).

Polymerase chain reaction (PCR) of urine samples

The DNA isolation was done using HiPurA® Urine DNA Purification Kit (HiMedia, Mumbai), followed by PCR targeting the genus specific *16S* rRNA gene and the virulence genes *lipL21, lipL32, lipL41* and *loa22* genes. Primers used for PCR are listed in Table 2. Duplex PCR for amplification of the 16S rRNA and *lipL32* genes was standardised at an annealing temperature of 56.8 °C for 30 sec, as described by Murugavelu, (2021). Uniplex PCR for *lipL21* was standardised at an annealing temperature of 54 °C for 45 sec, following Archana et al. (2019). Duplex PCR for amplification of *lipL41* and *loa22* genes was standardised at an annealing temperature of 59.8 °C for 45 sec in the laboratory. After amplification, visualisation of PCR product was carried out after performing agarose gel (1.5 per cent agarose gel in Tris-acetate EDTA (TAE) electrophoresis buffer (1X) electrophoresis at 80V for 50 minutes. The DNA isolated from standard culture of *Leptospira* spp. was used as positive control.

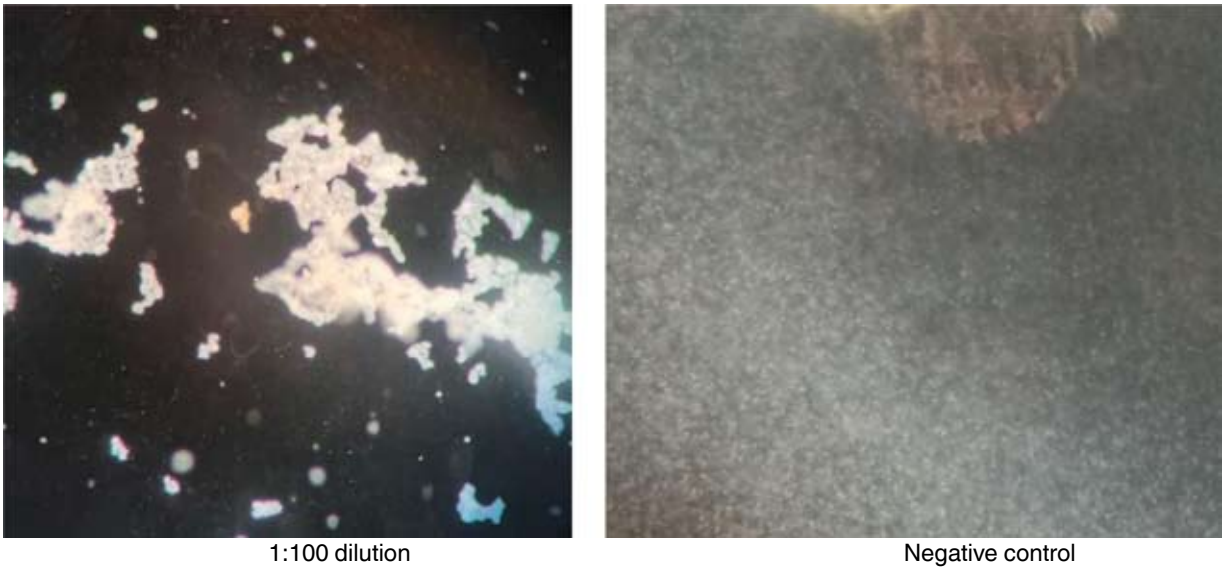
Results and discussion

Occurrence of Leptospira spp. in cattle by MAT

Out of the 101 sera samples collected from Pandalam municipality, 63.36 per cent (64/101) were seropositive. Of these, 49.50 per cent showed MAT titres between 1:50 and 1:200, indicating past exposure to *Leptospira* spp. in the study area, while 13.86 per cent exhibited titres of 1:400 and above, suggestive of active clinical infection. Similar results were obtained for Sreekutty et al. (2020) who reported 40 per cent seroprevalence among cattle of Alappuzha, Kerala. Contrary to the present study, Sriji (2022) reported a lower seropositivity of 23.53 per cent in the pre-monsoon and 32.35 per cent in the post-monsoon in the cattle of Thrissur, Kerala, indicating regional variation in the prevalence of leptospirosis. Such

Table 1. Serovars of Leptospira spp. used in MAT

Sl. No.	Serovar
1.	*Leptospira interrogans* serovar Australis Ballico
2.	*Leptospira interrogans* serovar Autumnalis Bankinang 1
3.	*Leptospira borgpetersenii* serovar Ballum Mus127
4.	*Leptospira interrogans* serovar Bankinang +1
5.	*Leptospira interrogans* serovar Bataviae Swart
6.	*Leptospira interrogans* serovar Canicola Hond Utrecht IV
7.	*Leptospira weilii* serovar Celledoni
8.	*Leptospira interrogans* serovar Djasiman
9.	*Leptospira kirschneri* serovar Grippotyphosa Moskva V
10.	*Leptospira interrogans* serovar Hardjoprajitno
11.	*Leptospira interrogans* serovar Hebdomadis
12.	*Leptospira fainei* serovar Hurstbridge BUT 6
13.	*Leptospira inerrogans* serovar Icterohaemorrhagiae RGA
14.	*Leptospira borgpetersenii* serovar Javanica
15.	*Leptospira inadai* serovar Kaup LT 64-68
16.	*Leptospira noguchii* serovar Louisiana LSU 1945
17.	*Leptospira alexanderi* serovar Manhao L-60
18.	*Leptospira borgpetersenii* serovar Mini Sari
19.	*Leptospira noguchii* serovar Panama CZ 214
20.	*Leptospira borgpetersenii* serovar Poi
21.	*Leptospira interrogans* serovar Pomona
22.	*Leptospira interrogans* serovar Pyrogenes Salinem
23.	*Leptospira weilii* serovar Sarmin
24.	*Leptospira santarosai* serovar Shermani 1342 K
25.	*Leptospira borgpetersenii* serovar Tarassovi Perepelitsin



1:100 dilution Negative control

Fig 1. Dark field microscopic view of Microscopic agglutination test (MAT)

Table 2. Primers used for the identification of Leptospira

Sl. No.	Primer	Sequence	Size (bp)	Reference
1.	16S rRNA F	5'GAACTGAGACACGGTCCAT3'	430 bp	Tansuphasiri et al. (2006)
	16S rRNA R	5'GCCTCAGCGTCAGTTTTAGG3'		
2.	*lipL32* F	5'ATCTCCGTTGCACTCTTTGC3'	474 bp	Tansuphasiri et al. (2006)
	lipL32 R	5'ACCATCATCATCATCGTCCA3'		
3.	*lipL41* F	5'CTGGTTTGAAAGTGGCAGGT3'	243 bp	Archana et al. (2019)
	lipL41 R	5'CCAAACCTTGTCCGAAAGAA3'		
4.	*lipL21* F	5'CGGGTCGACTCCAGTACTGACACAGGACAAA3'	507 bp	Archana et al. (2019)
	lipL21 R	5'CGGCTGCAGTTATTGTTTGGAAACCTCTTGA3'		
5.	*loa22* F	5'AGAGGAGAATTCAGCTCCTGAGC3'	525 bp	Roshini et al. (2023)
	loa22 R	5'TGGTGCCTGCAGCGCAAAACGGA 3'		

high seroreactivity indicates sustained circulation of leptospires within livestock populations and reflects the endemic nature of leptospirosis in Pathanamthitta district, Kerala.

The predominant serovars detected were Australis and Javanica (21.78 per cent each) followed by Hardjo (15.84 per cent), Pomona and Pyrogenes (14.85 per cent) and Autumnalis and Kaup (13.86 per cent each) as shown in Fig. 2. Serovar Bataviae was not detected in any of the samples. From the tested serum samples, 14 samples (13.86 per cent) were positive with highest antibody titre of 1:400 and above indicative of active infection. Multiple serovars were detected in the same sample, which may be attributed to cross-reactivity of antibodies, as cross-reactive antibody titers against non-infecting serovars can remain detectable even after the antibody titer against the infecting serovar declines below detectable levels (Mummah et al., 2024). Australis was the predominant serovar according to Bojiraj et al. (2017) in cattle of Tamil Nadu, while Hardjo was identified as predominant serovar in cattle by Divya et al. (2021) among cattle in Thrissur, Kerala. The high prevalence of Australis, Javanica, Pomona and Pyrogenes were reported in rodents by Shilpa et al. (2024) in Thrissur. Balamurugan et al. (2016) reported that in endemic states of India, ruminants play an important role as maintenance hosts for intermediate serovars such as Kaup. The reservoir host for serovar Kaup has been identified as bandicoot, indicating that bandicoots and other rodents may serve as a potential source of infection for cattle in the study area.

In the present study, 15.24 per cent cattle revealed a MAT titre of 1:50, which is indicative of past exposure to leptospirosis. Murugavelu et al. (2022) reported a seropositivity of 44.24 per cent in slaughtered

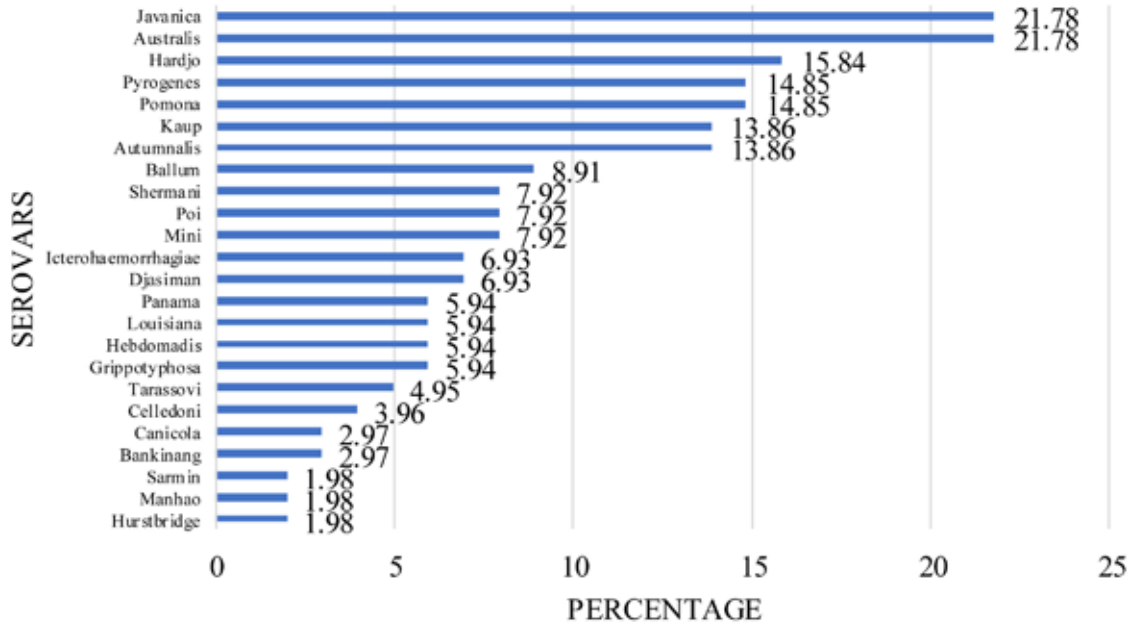


Fig 2. Serovars detected in dairy cattle based on MAT

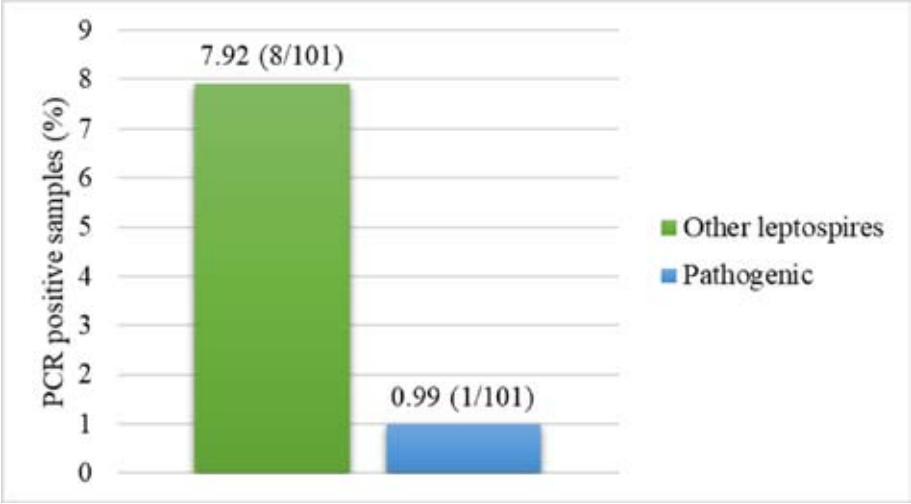


Fig 3. Presence of Leptospira spp. in cattle urine

cattle in Thrissur. This finding may be attributed to the selection of apparently healthy cattle in the present study. Shivakumar and Krishnakumar (2006) suggested that in endemic regions a MAT titre of 1:400 and above is indicative of clinical infection in cattle. In the present study, 13.86 per cent samples had a titre of 1:400 and above. However, none of the cattle exhibited overt clinical signs, although during survey farmers reported history of various reproductive disorders among the cattle. This was in accordance with study done in Brazil by Loebel et al. (2025), where antibody titres ranged from 1:200 to 1:800 in cattle, still showed no clinical signs of the disease. Uribe et al. (2025) reported a high seropositivity of 85 per cent in cattle of endemic areas of Chile but none of the seropositive animals exhibited clinical signs suggestive of leptospirosis.

Occurrence of Leptospira spp. in cattle urine by PCR

On subjecting urine samples to PCR targeting 16S rRNA gene, 7.92 per cent samples (8/101) were positive. Only 0.99 per cent samples (1/101) could detect *lipL21* gene were found from Pandalam municipality (Fig. 3). None of the samples revealed the presence of virulence genes *viz., lipL32, lipL41* and *loa22*. Results indicating low level renal carriage with limited shedding of pathogenic leptospires. These findings are in accordance with the study conducted by Nally et al. (2018) where positivity in urine was 7.20 per cent from beef cattle in the United States. The lower detection of leptospiral DNA in urine samples may be attributed to frequent antibiotic treatment in animals, which has been reported to reduce urinary shedding of leptospires (Adler, 2015). Hamond et al. (2014) reported that as shedding of leptospires in urine is intermittent, a single negative PCR result does not exclude infection. All urine-positive animals were also found to be seropositive by MAT, with antibody titres ranging from 1:50 to 1:800, indicating concurrent serological evidence of exposure among cattle shedding leptospires in urine.

Conclusion

The present study demonstrates a high occurrence of leptospirosis among apparently healthy dairy cattle in Pandalam municipality of Pathanamthitta district, confirming the endemic nature of *Leptospira* spp. infection in this recognised human leptospirosis hotspot area. The substantial seropositivity detected by MAT, together with the predominance of serovars commonly associated with rodent reservoirs, indicates sustained circulation of leptospires within cattle populations and strongly suggests an active role of rodents as a major source of infection for cattle. The presence of multiple serovars further reflects a complex transmission cycle involving livestock-rodent-environment interactions. The detection of elevated antibody titres in clinically normal cattle highlights their role as asymptomatic carriers, while farmer-reported reproductive disorders point to possible subclinical impacts on bovine health. Molecular detection of Leptospira in urine, confirms renal carriage and intermittent shedding, contributing to environmental contamination. Taken together, these findings underscore that rodents likely act as a critical link in maintaining infection in cattle and may also facilitate spillover to humans in endemic settings. Continuous surveillance of livestock and rodent populations, along with integrated control measures focusing on rodent control, environmental sanitation, and occupational risk reduction, is essential for effective prevention and control of leptospirosis in endemic regions such as Kerala.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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