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Identification and isolation of *Leptospira* from bovine cases of reproductive disorders in Kerala, India

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Abstract
It is becoming more widely acknowledged that leptospirosis, a zoonosis of global importance, is a major but underdiagnosed cause of bovine reproductive failure. The purpose of this study was to look into the role of *Leptospira* in causing abortions, stillbirths and recurring breeding problems in cattle in Kerala. A combination of serological, molecular and cultural techniques were used to sample and assess a total of 20 animals. Five animals tested positive for *Leptospira* DNA by polymerase chain reaction (PCR) targeting the LipL32 gene, while two animals tested seropositive in microscopic agglutination test (MAT). *Leptospira* were successfully isolated from two samples (liver and blood). The results emphasise how crucial it is to use a variety of diagnostic techniques in order to accurately identify both active infections and carrier states.

Keywords: *Leptospira*, cattle, abortion, PCR, mat, isolation

Introduction
Leptospirosis is an infectious disease that is re-emerging and affects many types of domestic and wild animals. It is a serious threat to public health as it can spread from animals to people. Leptospirosis is a major cause of reproductive problems in cattle, such as infertility, abortion, stillbirth and weak calves, but it often goes unnoticed because due to non-specific symptoms and lack of awareness. The fact that the infection is chronic and that the organism likes to live in the kidneys and reproductive organs makes it hard for herds to get rid of the pathogen, even when there are no obvious clinical signs.
Bovine leptospirosis is still not diagnosed enough in many parts of India, including Kerala. Infected urine, water, feed, or fomites are the most common ways for the disease to spread. It is even harder to control because the organism is very resilient in the environment and carrier animals are common. The goal of the study was to find out the occurrence of *Leptospira* infection in cattle Kerala that have had problems with reproduction in the past by using a thorough diagnostic method.

Materials and Methods
The study included 20 cows that had clinical histories that suggested they had *Leptospira*l infection then or in the past. These included mid- to late-term abortions, stillbirths, and repeat breeding. Samples submitted to the Department of Veterinary Microbiology, i.e., blood and serum samples from 17 animals and urine samples from 3 animals were included. Also, samples of aborted foetal tissue, vaginal discharge, and post-mortem liver, kidney, and uterus were taken from cases where they were available.
Serological detection was performed using MAT, against 12 reference *Leptospira* serovars maintained at the Department of Veterinary Microbiology at a screening dilution of 1:200, following the protocol described by Faine *et al.* (1999) [1]. The samples were examined under a dark field microscope for agglutination.
Molecular detection using PCR assay targeting *LipL32* gene, which is specific to pathogenic *Leptospira* was performed. DNA was extracted using a Himedia Multisamples DNA extraction kit and PCR was performed using primers described by Amutha *et al.* (2007) [2].
For bacterial isolation, samples were inoculated into EMJH semi-solid medium and incubated at 28 to 30°C for up to 3 months. Cultures were monitored for Dinger's ring

formation and confirmed using dark field microscopy. Confirmatory identification of isolates was done using *LipL32* PCR.

Results

Out of the 20 cattle examined, two animals tested positive for *Leptospira* antibodies via MAT, showing reactivity to serovars Hebdomadis and Pomona. PCR targeting the *LipL32* gene revealed *Leptospira* DNA in five animals across blood, urine, and liver samples. *Leptospira* was successfully cultured in two cases (from blood and liver samples). Isolates showed typical morphology and were PCR confirmed. These findings underscore the value of multi-sample diagnostics over sole reliance on serum.

Animal no. (sample)	MAT	PCR	Isolation
1 (Blood, serum)	-	-	-
2 (Blood, serum)	-	-	-
3 (Blood, serum)	-	+ (blood)	-
4 (Blood, Serum)	-	+ (blood)	-
5 (Blood, serum)	-	-	-
6 (Blood, serum)	-	-	-
7 (Blood, serum)	+	-	-
8 (Blood, serum)	-	-	-
9 (Blood, serum)	+	-	-
10 (urine, aborted fetal tissue)	N. A	+ (urine)	-
11 (urine, vaginal discharge)	N. A	-	-
12 (Blood, serum, urine)	-	+ (blood, urine)	-
13 (Liver, kidney, uterus)	N. A	+ (liver)	+ (liver)
14 (Blood, serum)	-	-	+ (blood)
15 (Blood, serum)	-	-	-
16 (Blood, serum)	-	-	-
17 (Blood, serum)	-	-	-
18 (Blood, serum)	-	-	-
19 (Blood, serum)	-	-	-
20 (Blood, serum)	-	-	-

Discussion

Soman *et al.* (2014) [3] tested 100 bovine samples from central and northern Kerala using MAT and found a higher seropositivity rate of 47 per cent. Sreekutty *et al.* (2020) [4] reported an overall seropositivity of 52.2 per cent from 90 bovine serum samples collected from Alappuzha district. Divya (2021) [5] performed MAT on 29 bovine serum samples and identified a seropositivity of 20.68 per cent. Murigavelu *et al.* (2022) [6] examined 165 blood samples from slaughtered cattle in Thrissur district, revealing an overall seropositivity of 44.24 per cent.

Cheema *et al.* (2007) [7] collected 50 bovine serum samples across various states in India and found that four of the samples (eight per cent) tested positive in a lipl32 gene-specific real-time PCR. Senthilkumar *et al.* (2022) [8] identified *Leptospira* DNA in 9.2 per cent of urine samples from a total of 305 samples collected from cattle from Tamil Nadu. Patel *et al.* (2017) [9] detected positive amplicons in 9 out of 120 blood (7.5 per cent) and 33 out of 304 urine (10.85 per cent) samples from cattle, screened using lipl32 gene specific PCR in Gujarat. The findings demonstrate a strong link between *Leptospira*l infection and reproductive issues in cattle. Antibody detection using MAT detected only two positives, while PCR identified five, and cultures yielded two viable isolates. The discrepancy between MAT and PCR outcomes highlights the limitation of serological tests alone for confirmatory diagnosis of leptospirosis. The low detection rates could be ascribed to the intermittent shedding of leptospires in ruminants (WOAH, 2021) [10], with negative PCR results potentially reflecting a lack of detectable *Leptospira* excretion at the time of sampling.

In the present study, samples were collected from suspected cases of bovine leptospirosis as well as animals suffering from chronic reproductive disorders. This underlines the importance of repeated sampling over time to accurately determine carrier status, as suggested by Denipitiya *et al.* (2017) [11]. Furthermore, the comparative analysis by Nally *et al.* (2020) [12] found that no single assay was optimal, and there was a need for combining multiple diagnostic methods, such as real-time PCR alongside culture and FAT techniques, to enhance the accuracy of *Leptospira* detection in cattle. Moreover, the study reinforces the importance of considering the sample type *viz*., blood, urine or tissue, when diagnosing leptospirosis, as different sample types may yield varying detection rates in different species, with urine often showing higher positivity in cattle and pigs, due to the prolonged presence of leptospires in the urinary system. Additonally, while prior studies in cattle from Kerala employed a lower threshold of 1:50 for bovine samples (Sreekutty *et al.*, 2020; Murugavel *et al.*, 2022; Sriji *et al.*, 2022) [4, 6, 13], a higher cutoff titre of 1:200 was used in this study as majority of bovine samples were collected from animals with chronic reproductive disorders.

Isolation of *Leptospira* from internal organs affirms systemic infection and raises public health concerns. PCR-positive but culture-negative urine samples may indicate low bacterial load or intermittent shedding. These findings support previous reports by Ellis *et al.* (1982) [14].

Under the circumstances, comprehensive diagnostic protocol is essential in endemic areas like Kerala, where environmental factors favour transmission. Asymptomatic carriers may continue to spread the infection, underscoring the need for regular herd screening using all available tools.

Conclusion

This study confirmed the role of *Leptospira* in bovine reproductive disorders in Kerala. The presence of *Leptospira*l DNA and isolation of viable organisms

reinforces the need for a tiered diagnostic approach. Regular screening, vaccination, hygiene and biosecurity are critical in disease management and in reducing the zoonotic risk.

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References

1. Faine S, Adler B, Bolin C, Perolat P. *Leptospira* and leptospirosis. 2nd ed. Melbourne: Medisci Press; 1999. 272 p.
2. Amutha R, Chaudhury P, Garg AP, Vasan P, Cheema PS, Srivastava SK, *et al.* Cloning and sequence analysis of the gene encoding LipL32 of *Leptospira interrogans* serovar Sejroe. Vet Res Commun. 2007;31:513-519.
3. Soman M, Jayaprakasan V, Mini M. Epidemiological study on human and canine leptospirosis in Central and North Kerala. Vet World. 2014;7(1):10-14.
4. Sreekutty SS, Vrinda MK, Latha C, Sunil B, Ambily R. Seroprevalence of leptospirosis in cattle in Mannancherry panchayat of Alappuzha district. J Vet Anim Sci. 2020;51:115-118.
5. Divya D. Multi-Locus sequence typing for species/serovar identification of clinical isolates of *Leptospira* [master's thesis]. Pookode: Kerala Veterinary and Animal Sciences University; 2021. 94 p.
6. Murugavelu M, Vrinda MK, Latha C, Deepa J, Vinodkumar K. Seroprevalence of leptospirosis among slaughtered cattle in Thrissur, Kerala. J Vet Anim Sci. 2022;53:65-69.
7. Cheema PS, Srivastava SK, Amutha R, Singh S, Singh H, Sandey M, *et al.* Detection of pathogenic leptospires in animals by PCR based on lipL21 and lipL32 genes. Indian J Exp Biol. 2007;45:568-573.
8. Senthilkumar K, Ravikumar G, Parthiban ABR. Prevalence of urinary shedders and characterization of pathogenic *Leptospira* among cattle population in Tamil Nadu - Implications for control. Vet Ital. 2022;58(2):81-87.
9. Patel JM, Vihol PD, Prasad MC, Patel JH, Raval JK, Kalyani IH, *et al.* Polymerase chain reaction in the diagnosis of spontaneous leptospirosis in bovines. Int J Curr Microbiol Appl Sci. 2017;6(4):1723-1728.
10. World Organisation for Animal Health (WOAH). Leptospirosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris: WOAH; 2021. p. 324-335.
11. Denipitiya DTH, Chandrasekharan NV, Abeyewickreme W, Hartskeerl RA, Hapugoda MD, *et al.* Identification of cattle, buffaloes and rodents as reservoir animals of *Leptospira* in the District of Gampaha, Sri Lanka. BMC Res Notes. 2017;10:134-140.
12. Nally JE, Ahmed AA, Putz EJ, Palmquist DE, Goris MG, *et al.* Comparison of real-time PCR, bacteriologic culture and fluorescent antibody test for the detection of *Leptospira borgpetersenii* in urine of naturally infected cattle. Vet Sci. 2020;7(2):66-72.
13. Sriji AP, Vrinda KM, Latha C, Deepa J, Justin DK. Seasonal analysis of leptospirosis in apparently healthy dairy cattle of Pananchery panchayat in Thrissur district, Kerala. J Vet Anim Sci. 2022;53:657-662.
14. Ellis WA, O'Brien JJ, Neill SD, Ferguson HW, Hanna J. Bovine leptospirosis: microbiological and serological findings in aborted fetuses. Vet Rec. 1982;110:147-150.