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**Shyma VH**
Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Pookode, Kerala, India

**Bipin KC**
Associate Professor, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Pookode, Kerala, India

**Deepa PM**
Associate Professor, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Pookode, Kerala, India

**Tresamol PV**
Professor, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Mannuthy

**Ambily R**
Assistant Professor, Department of Veterinary Microbiology, CVAS, Mannuthy, Kerala, India

**Neethu P**
Research Assistant, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Pookode, Kerala, India

**Dinesh PT**
Assistant Professor, Department of Veterinary Surgery and Radiology, CVAS, Pookode, Kerala, India

**Corresponding Author:**
**Shyma VH**
Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, CVAS, Pookode, Kerala, India

# Diagnosis of leptospirosis in cattle with haemagalactia

**Shyma VH, Bipin KC, Deepa PM, Tresamol PV, Ambily R, Neethu P and Dinesh PT**

**Abstract**
Haemagalactia and cold mastitis is a classical clinical sign in cattle for leptospirosis. Samples *viz;* blood, serum, milk and urine was collected from 10 animals with haemagalactia. Samples were subjected to Microscopic agglutination test (MAT) and Polymerase chain reaction (PCR) to confirm the disease. Out of the sixteen animals included in the study, three were diagnosed with leptospirosis.

**Keywords:** Haemagalactia, leptospirosis, PCR, MAT

## Introduction
Leptospirosis in domestic animals is primarily characterized by fever, icterus, renal insufficiency and heightened mortality. Dogs are more susceptible to acute form of disease and resulting in renal damage. But the acute and severe form of leptospirosis is rare in ruminants. Clinical presentation of leptospirosis in livestock includes fever, jaundice, hematuria, haemagalactia and reproductive failure. Diagnosis of leptospirosis in livestock is difficult because of the low specificity, sensitivity and vague interpretation of various diagnostic tests due to the absence of specific clinical signs (Constable *et al.*, 2016) [4].
Polymerase chain reaction targeting *lipL32*, *lipL21*, *lipL41* is recommended for accurate diagnosis in livestock. Real-time PCR (qPCR) is faster than conventional PCR and extends widened sensitivity. But it is more expensive and often requires specialized machineries that may not be readily available.

## Materials and Methods
The study was conducted at the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Pookode during the period from January 2022 to November 2023. As per CCSEA guidelines, study involving clinical samples does not require the approval of Institutional Animal Ethics Committee and authors were permitted by animal owners for sampling.
A total of 16 milk samples were collected from cattle with haemagalactia from four northern and two central districts of Kerala. Whole blood and serum were also collected from these animals. Serum was subjected to microscopic agglutination test (MAT). The DNA was extracted from the milk and blood samples using Genomic DNA kit (Origin$^{TM}$), Kollam, Kerala.
Microscopic agglutination test:
Twelve serovars were maintained at Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Pookode during the tenure as reference strains which were procured from Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Mannuthy. The reference strains were *Leptospira interrogans* serovars Australis, Bataviae, Grippotyphosa, Ictero haemorrhagiae, Pyrogenes, Tarassovi, Hardjo, Pomona, Hebdomadis, Javanica, Autumnalis, Canicola. Microscopic agglutination test was carried out in the 16 serum samples using live Leptospira serovars as described by Faine *et al.* (1982) [5]. Serum samples having a titre of 1:100 and above were considered as positive as per Favero *et al.* (2017) [6].

**Polymerase chain reaction**

Deoxy ribonucleic acid (DNA) was extracted from16 milk and blood samples and template DNA from samples were subjected to PCR targeting *lipl32* gene as per Krishna *et al.* (2013) [7].

**Table 1:** Details of Primers

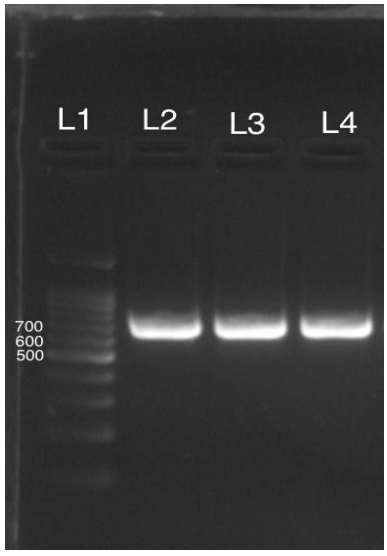
| Serial no: | Target gene | Primer sequence 5'-3' | Product size |
|---|---|---|---|
| 1. | *lipL 32* | Forward- 5'-CGC GCT GCA GTT ACT TAG TCG CGT CAG AAG-3'<br>Reverse – 5'-CGC GGT CGA CGC TTT CGG TGG TCT CTG CCA AGC-3' | 790 bp |

**Results and Discussion**

Among the sixteen suspected cases 3 animals showed seroreactivity in MAT with 1/100 or above titre. Two animals showed positive reaction to serovar Australis at 1/200 and 1/400 and one to Hardjo at 1/400. PCR of DNA extracted from these samples yielded positive result in two cases.

The 'rose milk' or 'haemagalactia' could be associated with intravascular haemolysis by the toxin haemolysin, damage to vascular endothelial cells in udder parenchyma by pathogenic serovars of leptospira. Similarly, Saravanan and Palanivel (2018) [9] reported haemagalactia due to leptospirosis in crossbred cows. They employed both MAT and PCR for confirmatory diagnosis. Tresamol *et al.* (2015) [10] reported haemagalactia in a leptospira positive cow. Hairgrove (2004) [11] reported flabby mastitis in 10 percent and reproductive wastage in 25 percent of a herd due to leptospirosis. *Lipl*32 is a gene conserved in pathogenic leptospires, so PCR targeting this gene is a reliable molecular technique for diagnosis. Cheema *et al.* (2007) [3] reported that eight percent of the samples found positive with PCR targeting *lipL32* gene in their study on livestock. Eventhough, Hardjo is considered as predominant serovar among cattle, here seroreactivity was obtained for serovar Australis in two cases. Ambily *et al.* (2013) [1] and Chandran *et al.* (2019) [2] reported Australis as the predominant serovar Ambily *et al.* (2013) [1], Chandran *et al.* (2019) [2] reported Australis as the predominant serovar among domestic animals of Kerala. Direct demonstration of leptospires from milk, urine and serum of affected animals are difficult and application of molecular techniques will aid in diagnosis of leptospirosis in cattle.

**Pic 1:** Blood tinged milk




L2- PC, L3, L4- samples

**Pic 2:** PCR. L1- ladder

**Conclusion**

As haemagalactia is a nonspecific clinical sign of leptospirosis, creating awareness among farmers about clinical signs, prevention and control of this zoonotic disease is very important.

**Acknowledgement**

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