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Detection of leptospirosis in the urine of cattle in North Karnataka, South India

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Abstract
The present study was conducted to detect the carrier status in Cattle. A total of 170 Cattle Urine samples were collected from Veterinary College Hospital with history of reproductive disorders, abortion, repeat breeding, and mastitis animals. All Urine samples were processed for extractions of DNA. Isolated DNA samples were amplified gene LipL32 of leptosira size 497bp of PCR. Out of 170 Urine samples 8 samples were found positive (4.70%) for *leptosira spp*. The result of present study showing evidence that there is a prevalence of leptospirosis infection in cattle. Carrier animals play a great role in maintenance and transmission of leptospirosis. PCR is an ideal test for detection of carriers.

Keywords: Leptospirosis, urine samples, LipL32 gene, PCR, cattle

Introduction
Leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira*. The organism affects many mammalian species, including humans. Animals may become in apparent carriers and shedding of leptospires, primarily in the urine, serves as a source of infection for other animals and humans [1].

In cattle, leptospirosis is an important cause of abortion, stillbirths, infertility, poor milk production and death, all of which cause an economic loss [2]. The clinical signs associated with bovine leptospirosis are variable and depend on the infecting serovar and the susceptibility of the animal. Clinically, bovine leptospirosis is difficult to diagnose because the signs are non-specific and easily confused with other diseases [3]. Traditionally, the reference method for diagnosis of leptospirosis is the microscopic agglutination test (MAT). However, this test has several drawbacks, including the requirement for a permanent stock of reference strain representing the appropriate serogroups, subjectivity involved in reading the results under dark-field microscopy, inability to differentiate titers of natural infection from vaccine titres and the failure to identify most chronic shedders.

Moreover, the assay is labour intensive and represents a biohazard to laboratory staff [4, 5]. Isolation of leptospires is time consuming, subject to contamination and may require 4–6 months [4].

A variety of molecular methods have been developed for the specific detection of pathogenic *Leptospira* spp. serovars in clinical samples. These include DNA Hybridization [6], in situ hybridisation and DNA probes, which have been used mainly for detection of leptospires in urine samples from animals infected experimentally with The polymerase chain reaction (PCR) also has been used to detect *Leptospira* spp. in urine samples from cattle experimentally infected with serovar *Hardjo bovis* [7].

A PCR to detect *Leptospira* spp. in the urine of naturally infected cattle using genus-specific primers has been reported by some workers [8, 9]. Recently, PCR with primers derived from the LipL32 sequence has been reported by Nassi and Co-worker [9].

The aim of the present study was to evaluate the use of PCR to detect leptospirosis in urine samples from cattle naturally infected with *Leptospira* spp. using primers based on a genomic region that encodes LipL32, a prominent lipoprotein expressed in the outer membrane of pathogenic *Leptospira* spp. Only [10]. Identify and correlate carrier status in animal leptospirosis.

Materials and Methods
Collection of urine samples
Total of 170 Clinical urine samples were collected from Veterinary College Hospital, Bidar

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With history of reproductive problems, repeat breeding, abortion and mastitis in cattle. Urine sample was collected aseptically from urinating cows. The urine sample was then transferred into 10ml collection tubes (Tarsons).

DNA extraction

All 170 urine samples were subjected to LipL32 sequence gene (497bp) to detect *Leptosira*. DNA extraction was carried out from Clinical urine samples using (QIAGEN, USA) Kit. As per the manufacturer's instructions. The extracted DNA was stored in aliquots at -20 ^{0}C.

Polymerase chain reaction (PCR)

PCR assay targeted to LipL32 (497 bp) was used for diagnosis of *Leptospirosis* as per Biofilm [11]. The nucleotide sequences of the forward and reverse primers are as follows for *LipL32* gene (497 bp).
Forward: 5' GAC GGT TTA GTC GAT GGA AAC 3' and
Reverse: 5' GGG AAA AGC AGA CCA ACA GA 3'
PCR amplification of *LipL32* (497 bp) fragment was setup in 25 µl reactions. The reaction mixture consisted of 4.0 µl (58 ng) of template DNA, 12.5 µl of 2x master mix which consist of 10x PCR buffer, 10 mM dNTP mix, and Taq DNA polymerase and 1.0 µl each (10 pmol) of the forward and the reverse primer.

The volume was made up to 25 µl by nuclease free water (6.5 µl). The cycling conditions were as follows; the first series of thermal cycling (per PCR) consisted of initial denaturation at94ºC for 2 minutes, followed by 30 cycles of denaturation 94ºC for 1 minute annealing at 50ºC for 1 minute and extension at 72 □C for 1 min.
Final extension was performed at 72□C for 10 min. The PCR products (5 µl) were loaded into the respective wells. Molecular weight marker (100 bp) and positive and negative controls were also run. The electrophoresis was carried out at 100 V for 45 min or until the tracking red dye migrated more than two third of the length of the gel tray. The gel was placed under UV trans-illuminator and the results were documented in a gel documentation system (Bio rad) to analyze and document the results.

Result

LipL32 (497 bp) fragment based PCR

Out of 170 clinical urine samples eight urine samples (4.70%) were positive LipL32 (497 bp) fragment based PCR of leptospirosis. The PCR amplicon size of 497bp was noted on agarose gel electrophoresis (Fig-1). The data were subjected to statistical analysis as method described by Snedecor and Cochran [12].
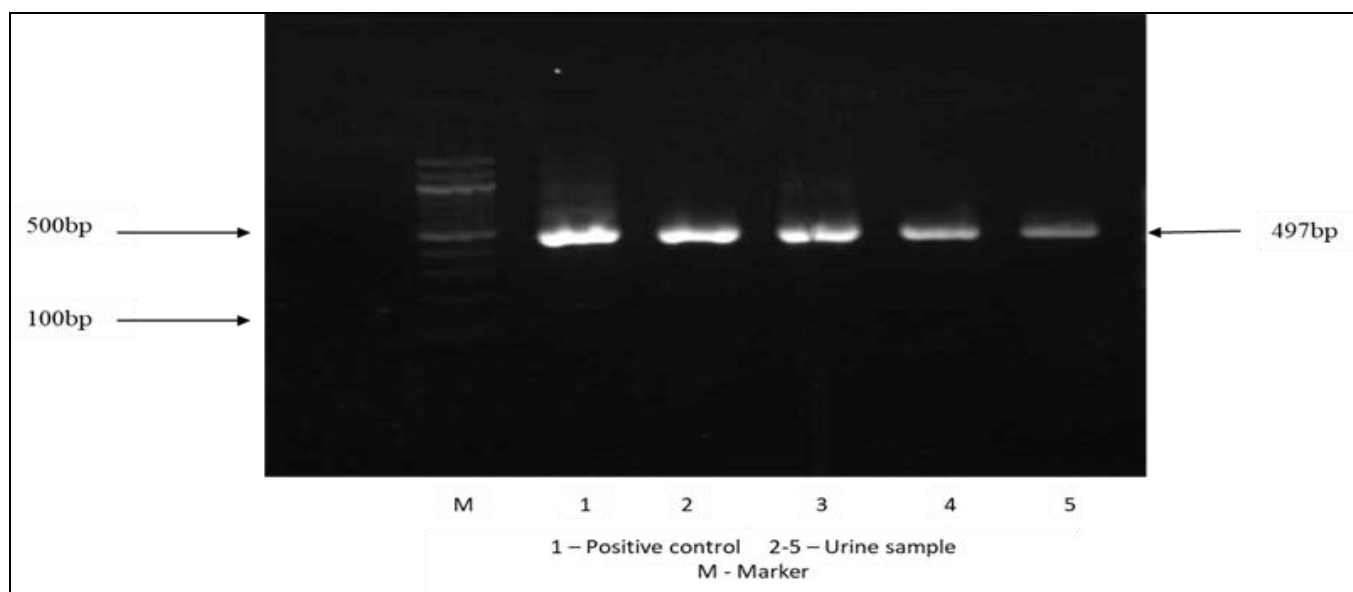

Fig 1: Detection of gene LipL32 of leptospira size 497bp by PCR in Cattle

Discussion

The DNA extraction from bovine urine was done by phenol: chloroform: is oamyl alcohol method as described by Krishnaveni, Sharma [13, 14] and the primers used in this study *LipL*32 was found effective in detection of pathogenic *leptospires*. The urine samples tested in this study was from reproductive disorder, mastitis history of early leptospiral infection.
The aim of this study was to determine whether PCR with primers derived from the *LipL*32 (Bal, Shi, Van) [15, 16, 17] could be used to directly detect pathogenic leptospires to overcome traditional diagnostic methods, such as leptospiral isolation and serology using the MAT. During the standardization of the method, we found that the *LipL* 32 internal primers could be used in a conventional PCR with just one round of amplification using DNA from reference serovars and clinical samples.

Conclusion

From the study it is concluded that PCR is an alternative method to detection of leptospires using the LipL32 coding region from Urine samples of cattle. Clinical cases like reproductive problems and mastitis can be significant for detection of leptospira by PCR. The present study showed 4.70% prevalence of leptospirosis in apparently healthy cattle. The PCR possesses advantages over more traditional methods. Hence, PCR using Lip32 gene is highly advantages for detection of leptospirosis in carrier status of cattle.

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