RAPID DIAGNOSIS OF BOVINE LEPTOSPIROSIS BY LipL32 BASED POLYMERASE CHAIN REACTION

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ABSTRACT

The present study was undertaken to diagnose acute cases of bovine leptospirosis by PCR. A total of 42 bovines serum samples suspected for leptospirosis were subjected to PCR to amplify LipL32 gene. The study revealed the amplicon size of 756 bp in 38 (90.47%) sera samples. The clinical signs in these positive cases included abortion, repeat breeders, jaundice and haemorrhagic mastitis. The present study indicated that the PCR which amplify the LipL32 is most sensitive, rapid and reliable method in diagnosing the leptospirosis in cattle. Since, LipL32 is conserved in all the pathogenic leptospires, it could be widely used for the diagnosis of leptospirosis in animals and human beings.

KEY WORDS : Leptospirosis – Diagnosis – PCR – LipL 32

INTRODUCTION

Leptospirosis is a spirochaetal disease and considered to be the most wide spread zoonosis in the world (WHO, 1999). It is endemic in India and causes huge economic losses due to death of animals, decreased milk production, abortion, still birth and infertility (Thiermann, 1982 and Balakrishnan *et al., 2014* ,2015). Timely diagnosis is essential for prompt and specific treatment as early as possible to ensure a favourable clinical outcome. Currently, there is no sensitive, specific, low cost, rapid and widely available diagnostic test for leptospirosis diagnosis. PCR based molecular techniques can be potentially rapid and sensitive means of diagnosis of leptospirosis. In the present study, LipL 32, an outer membrane protein gene which is conserved and expressed in all the pathogenic leptospires was targeted for the diagnosis of leptospirosis in animals. The present study was undertaken to diagnose acute case of bovine leptospirosis by PCR.

MATERIALS AND METHODS

5 ml of Blood was collected from clinically suspected bovines and allowed to clot at room temperature. Serum was separated by centrifugation at 2000 rpm for 20 min. and used in PCR. Deoxyribonucleic acid isolation was done as per the method of Boom *et al.* (1990) and Balakrishnan et *al.* (2014). The PCR reaction was carried out as per the method described by Haake et *al.* (2000). The primers which amplify 756 bp fragment of LipL32 gene of pathogenic *Leptospira* were used (Haake et *al.,* 2000). The sequence of the forward primer was LipL32 F: 5'-CTAAGTTCATACCGTGATTT-3' and the reverse primer was LipL32 R:5'-ATTACT-TAGTCGCGTCAGAA-3'). The PCR conditions consist of initial denaturation at 95°C for 10 minutes followed by 40 cycles of denaturation at 95°C for 40 seconds, annealing at 53°C for 30 seconds, extension at 72°C for 1 minute; and final extension at 72°C for 5 minutes. The analysis of PCR product was carried out in 1.5 % agarose gel stained with ethidium bromide (0.5 µg / ml). The gel was viewed under UV transillumination.

RESULTS AND DISCUSSION

A total of 42 serum samples collected from clinically suspected cattle were subjected to PCR using the primers of Haake et *al.* (2000). Out of 42 samples, 38(90.47%) samples were found positive for pathogenic *Leptospira as,* they yielded amplicon of expected size 756 bp. The present study indicated that the PCR which amplify the LipL32 is most sensitive, rapid and reliable method in diagnosing the leptospirosis in cattle. Since, LipL32 is conserved in all the pathogenic leptospires, it could be widely used for the diagnosis of leptospirosis in animals and human beings.

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