DIAGNOSIS OF BOVINE *LEPTOSPIROSIS* BY 16s rRNA BASED POLYMERASE CHAIN REACTION

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ABSTRACT

The present study was undertaken to diagnose acute case of bovine *leptospirosis* by PCR. A total of 115 serum samples collected from clinically suspected (33 cattle and 32 buffaloes) and apparently healthy (50) buffaloes were subjected to PCR. Among the clinically suspected animals, the PCR detected leptospiral DNA in 21 (32.31 per cent) samples. The clinical signs in these positive cases included abortion, repeat breeders, jaundice and haemorrhagic mastitis. Apparently healthy animals also gave positive results in PCR (6.00 per cent) which might be due to subclinical *leptospirosis.*

KEY WORDS: Bovines - *Leptospirosis* - Diagnosis - Polymerase Chain Reaction

INTRODUCTION

Leptospirosis is a zoonotic disease with worldwide distribution. Timely diagnosis is essential for prompt and specific treatment as early as possible to ensure a favorable clinical outcome. It is endemic in India and causes huge economic losses due to death of animals, decreased milk production, abortion, still birth and infertility (Thierman, 1984). The existence of this disease in India has been reported by many workers based on the serological studies by Microscopic agglutination test (Ramakrishna and Venkataraman, 1994; Srivastava and Kumar, 2003; Ramani Pushpa and Punya Kumari, 2005; Koteeswaran, 2006; Mariya, *et al.*, 2007 and Balakrishnan, 2014). Confirmation of the existence of the disease can be achieved by isolation of *leptospires* . Currently, there is no sensitive, specific, rapid and widely available diagnostic test for *leptospirosis* diagnosis. (Levett, 2001). Polymerase chain reaction (PCR) has been used to detect a large number of microorganisms, including those of clinical significance. It is a rapid, reliable and sensitive test for the diagnosis of *leptospirosis* (Van Eys *et al*., 1989). Different workers have reported the application of PCR for the diagnosis of *leptospirosis*. The first PCR assay to detect leptospirosis in cattle urine was developed in 1989 (Van Eys *et al*., 1989). The present study describes the diagnosis of *leptospirosis* in bovines by polymerase chain reaction.

MATERIALS AND METHODS

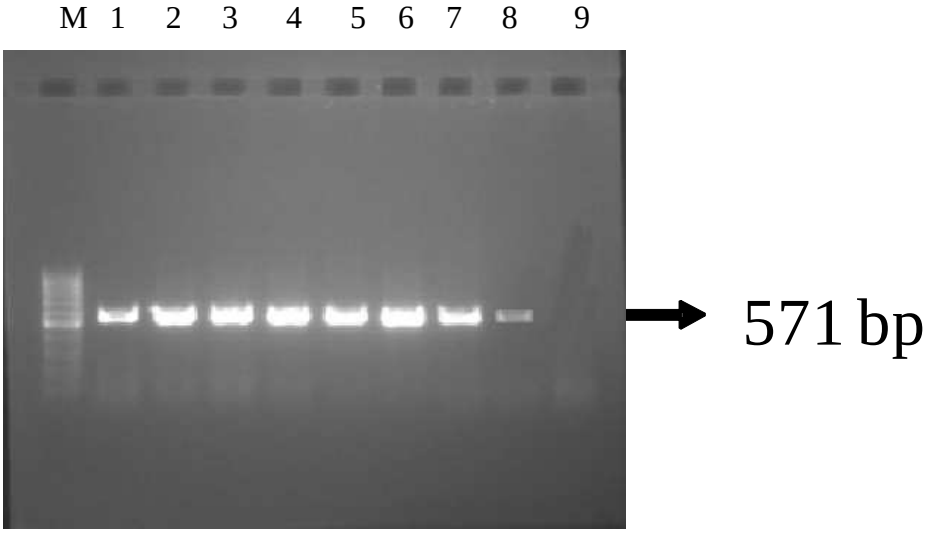
A total of 115 serum samples collected from clinically suspected (33 cattle and 32 buffaloes) and apparently healthy (50) buffaloes were subjected to PCR. Five ml blood of each clinically suspected and apparently healthy bovines were collected and allowed to clot at room temperature. Serum was separated by centrifugation at 2000 rpm for 20 minute and used in PCR. DNA was isolated as per the method of Boom *et al.* (1990). The PCR reaction was carried out as per the method described by Vitale *et al.* (2005). The primers which amplify 571bp fragment of 16S rRNA gene of pathogenic *Leptospira* were used. The sequence of the forward primer was E1: 5' – GGGAAAAATAAGCAGCGATGTG – 3' and the reverse primer was E2: ATTCCACTCCATGTCAAGCC-3'. The analysis of PCR product was carried out in 1.3 % agarose gel stained with ethidium bromide (0.5 µg / ml). 100 bp DNA ladder (Gene) and appropriate controls were incorporated to rule out false positive and false negative results. The gel was viewed under UV transillumination.

RESULTS AND DISCUSSION

Out of 33 cattle serum samples (cases of abortion 4, repeat breeding 6, jaundice 15 and haemorrhagic mastitis 8), only 14 cases were found positive for pathogenic *Leptospira* with an

amplicon of expected size 571bp by PCR using E1 and E2 published primers (Vitale *et al*., 2005). All the eight cattle suffered with haemorrhagic mastitis were found positive by PCR, whereas all the six repeat breeding cases were negative.

PCR results of bovine serum samples



M - Marker DNA (100 bp) 1-8 - Leptospira bovine serum samples

Among 32 buffaloes serum samples (abortion 6, repeat breeding 3, jaundice 10 and haemorrhagic mastitis 13), only seven buffaloes were found to be positive for *Leptosira* by PCR. All the buffaloes which suffered with abortion and repeat breeding were found negative by PCR, whereas seven animals with history of jaundice (3) and haemorrhagic mastitis (4) were found positive. Among 50 apparently healthy buffaloes three were found positive by PCR which was indicative of subclinical leptospirosis.

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