

DIAGNOSIS OF LEPTOSPIRAL MASTITIS IN A COW BY POLYMERASE CHAIN REACTION

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Leptospiral mastitis is popularly known as flabby udder in cattle. It is caused by a number of serovars of *Leptospirae* especially serovar *hardjo*. Serological diagnosis of leptospirosis depends on microscopic agglutination test (MAT). Though MAT is considered as gold standard test (OIE, 2005), it is difficult to differentiate acute infections from chronic infections by MAT without paired serum sampling technique. Polymerase Chain Reaction (PCR) can be useful for a rapid diagnosis of leptospirosis particularly acute infections (Ramadass, *et al.*, 1997 and Gravekamp, *et al.*, 1993). The present study describes the diagnosis of leptospiral mastitis in a cow by polymerase chain reaction.

Blood (5 ml) was collected from a cow suffering from haemorrhagic mastitis and allowed to clot at room temperature. Serum was separated by centrifugation at 2000 rpm for 20 min. and used in PCR and MAT. The PCR assay was carried out as per the method of Vitale, *et al.* (2005) with slight modifications. One ml of serum was centrifuged at 14000 rpm for 30 min. to pellet the Leptospire. The pellet was washed thrice in PBS by centrifuging at 14000 rpm for 30 min and resuspended in 100µl sterile nuclease free water. This suspension was kept in a boiling water bath for 30 min. and used in PCR. Amplification of DNA was performed in 25 µl reaction mixture containing 12.5 µl of supermix (Genei), 1µl of each primer (Sigma) and 11.5 µl of extracted DNA. The reaction components were mixed and subjected to amplification in a thermal cycler. The thermal profile consisted of initial denaturation at 94 °C for 5 min. followed by 40

cycles of denaturation at 94 °C for 1 min., primer annealing at 60 °C for 1 min. and extension at 72 °C for 1 min. The final extension was carried out at 72 °C for 5 min.

The primers which amplify 571bp fragment of 16S rRNA gene of pathogenic leptospira were used (Vitale, *et al.*, 2005). The sequence of the forward primer was 5' – GGGAAAATAAGCAGCGATGTG – 3' and the reverse primer was 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.

A panel of 12 serovars (*australis*, *autumnalis*, *ballum*, *canicola*, *grippotyphosa*, *hardjo*, *hebdomadis*, *icterohaemorrhagiae*, *javanica*, *pomona*, *pyrogenes* and *tarassovi*) was used in MAT. A 5 day old culture of leptospire grown in Ellinghausen McCullough – Johnson – Harris medium containing 2×10^8 organisms / ml was used as antigen. MAT was performed as per the OIE (2005) by quantitative assay, in which the serum samples were serially two fold diluted from 1:50 to 1:51600. The serum showing 50 % reduction of free leptospire with or without agglutination with a titre of $e^{3.7} 100$ was considered as positive.

The cow examined in this study exhibited the clinical signs including pyrexia, anorexia and agalactia. The milk was pink to red in colour with blood clots. The udder was flabby without heat

and pain and all four quarters were equally affected which concur the observations of Durfee and Allen (1980). In recent years, PCR has been proved to be useful for the detection of leptospiral DNA in clinical samples like urine (Van Eys, *et al.*, 1989 and serum (Merien, *et al.*, 1992 and Gravekamp, *et al.*, 1993) The success of PCR depends on the method of DNA extraction. The addition of Guanidine thiocyanate, Cetyl trimethyl ammonium bromide, Phenol extraction to the specimen (Boom, *et al.*, 1997) is some of the options for DNA extraction. In the present study, simple boiling method was used to extract the leptospiral DNA which makes the PCR technique an even more rapid and cost effective technique, similar method with slight modifications was used to extract the leptospiral DNA from urine, serum and milk sight (Senthil Kumar, *et al.*, 2001). The presence of 571 bp amplicon was detected in the present study which confirms the amplification of 16s rRNA gene of pathogenic Leptospire (Vitale, *et al.*, 2005). The presence of antibodies against *L. hardjo* in the serum was detected by MAT and the antibody titre was found to be 1: 100. Since the MAT is unable to differentiate the active infection from carrier state, a combination of PCR and MAT will be helpful to diagnose a case of leptospiral mastitis. Cross reactions among the serovars could not be observed in this study.

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