# POLYMERASE CHAIN REACTION BASED DIAGNOSIS OF *LEPTOSPIROSIS* IN BOVINE ABORTION BY DETECTION OF *LIPL*32 GENE

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**Abstract**

*Polymerase chain reaction based detection of etiology of abortion has gained much importance in medical and veterinary sector for appropriate, foolproof, efficient and timely diagnosis.* LipL32 *is one of the outer membrane proteins that could be relied upon for diagnosis of leptospirosis. Standard protocol for diagnosis of leptospirosis by detection of lipl*32 *gene by PCR has been developed in this study. The DNA extracted from the foetal stomach contents of bovine abortion, using DNA isolation kit was used as the template for the PCR. Primers were procured for the amplicon size 757bp. The PCR technique was standardised based on five abortion cases positive for Leptospira. Polymerase chain reaction was found successful in detecting 0.0004 ng of DNA*

**Key words:** *Leptospira, lipl32 gene*, bovine abortion, PCR

Bacterial infection is one of the important reasons for abortion in animals and human being. The common bacteria may vary between *Brucella, Leptospira, Chlamydophila, E.coli, Salmonella, Listeria* and *Campylobacter* in bovine abortion (Renukaradhya *et al*., 2002; Tramuta *et al*., 2011). *Leptospira* is one of the important causes for abortion in bovines. Several methods have been devised for the diagnosis of leptospirosis in animals like dark field microscopy, ELISA, Micro-agglutination test etc. Polymerase chain reaction based detection is of much importance in diagnosis of most of the infectious agents in medical and veterinary sector. LipL32 is a specific outer membrane protein present in *Leptospira* (Flannery *et al*., 2001). Presence of LipL32 protein could be made as a marker for the presence of the organism. So the gene responsible for the LipL32 (*ie., lipl*32 gene) could act as template for the PCR. In the clinical samples, concentration of the etiological agent may vary depending on various reasons. This study concentrated also on the minimum sensitive concentration of DNA that could be detectable by PCR of *lipl32* gene.

## Materials and Methods

Abortion materials from bovines formed the samples in this study. The stomach contents of aborted foetuses were the source for the DNA of the suspected etiological agent. Using the Qiagen DNA isolation kit, the samples were processed and the DNA extracted. The DNA concentration was estimated by Nanodrop2000c (Thermoscientific). The *lipl*32 gene, which was found unique in pathogenic

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*Leptospira* was the gene selected for the PCR primers. Published primers for *lipl*32 gene were procured with an amplification product size 757bp (Cheema *et al.,* 2007).

**Primers: F- 5' -** CGC GCT GCA GTT ACT TAG TCG CGT CAG AAG – **3'**

**R- 5' –** CGA ACC GTC TGG TGG CTT TCG CAG CTG GCG C - **3'**

The PCR technique was standardized based on five *Leptospira* positive abortion cases showing positive results in dark field microscopy of the sera of aborted cattle. The PCR reaction was performed with 25µl of total volume (Table 1 and 2).

Five samples which were found positive for *Leptospira* by *lipl32* PCR, were subjected to DNA estimation using Nanodrop2000c (Thermo Scientific). The concentration of each of the five sample was adjusted to 8ng/ µl. One in ten serial dilution of all the samples were prepared (up to 10,00,000 times dilution). The diluted samples were also subjected to DNA estimation by Nanodrop. All the serially diluted DNA samples were mixed with master mix (Table 1). Twenty five microlitre volume of each PCR mix were subjected to PCR as per the protocol (Table 2). The amplified products were run in 1.5% low EEO electrophoretic agarose gel (HiMedia) in 1X Tris Borate EDTA buffer (SRL) with 0.5 µg/ml ethidium bromide at 5V/ cm for half an hour. The maximum dilution at which the sample gave detectable results, were noted. The average of the concentration of diluted samples which gave detectable bands was considered as the minimum sensitive DNA concentration that could be appreciated by PCR, which was visualized using Gel doc system (Bio-rad)

**Table 1.** Components of PCR mixture

| Reagents | Quantity |
|---|---|
| 10X PCR buffer (with 15 mM $MgCl_2$) | 2.5 µl |
| Forward primer (25 pmol/µl) | 1 µl |
| Reverse primer (25 pmol/µl) | 1 µl |
| Taq Polymerase (3U/µl) | 0.33 µl |
| dNTP (2.5 mM each/µl) | 1 µl |
| DNA template | 5 µl |
| Nuclease free water | 14.17 µl |
| **Total** | **25 µl** |

**Table 2.** PCR Protocol for *lipl*32 gene

| Process | Temperature & time | Cycles |
|---|---|---|
| Initial denaturation | 95° C 7 minutes | 1 |
| Denaturation | 95° C 45 seconds | 35 cycles |
| Annealing | 60° C 30 seconds | |
| Extension | 72° C 45 seconds | |
| Final extension | 72° C 5 minutes | 1 |

**Results and Discussion**

On Nanodrop estimation of DNA, it was found that the concentration of DNA present in the sample ranged from 8 to 85ng/ µl (Table 3). Such minute concentration of DNA could be detected after PCR by horizontal agarose gel electrophoresis. This was found to be in accordance with the results obtained by Cheema *et al.* (2007)

The above PCR protocol for the detection of *lipl*32 gene of *Leptospira* was successful resulting in detectable quantity of the product with amplicon size of 757bp (Fig. 1)

It was found that the PCR of *lipl*32 gene was sensitive for detection of *Leptospira*. Thus the protocol in the present study could be effectively utilized for the detection of leptospirosis from affected foetal stomach content.

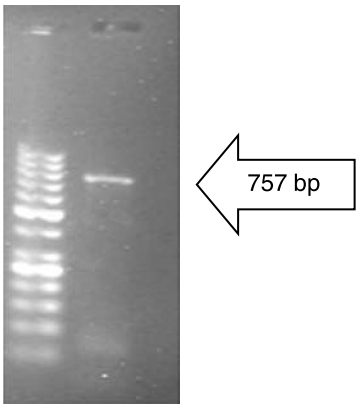
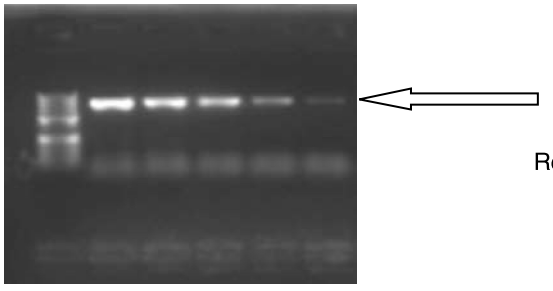



**Fig. 1.**
The image of the band showing the amplicon in 1.5% agarose gel
Lane 1 - 50 bp ladder.
Lane 2 – Amplicon size of 757bp

For finding out the sensitivity of *lipl32* based PCR, the concentration of the isolated DNA of all the five positive cases was adjusted to 8ng/µl, which was very well detectable by PCR in agarose gel electrophoresis with ethydium bromide. Ten fold serial dilutions of the DNA utilized for PCR detection of *lipl32* gene were depicted in the electrophoresis run (Fig.2 & 3). A faint band could be appreciated in the 8$^{th}$ lane (Fig. 3). Five microlitres of DNA concentration 0.00008ng/ µl, when used for PCR could be detected. Further dilutions could not reveal bands in subsequent lanes. Thus, it was found that the normal PCR of *lipl32* gene is sensitive for detection up to 0.0004 ng of extracted DNA in the sample indicating that PCR was one of the most sensitive methods for the detection of leptospirosis from abortion samples that could detect even one in thousand nanogram dilution of DNA as reported by Cheema *et al*. (2007), who suggested that PCR of *lipl32* gene was the sensitive and specific method for detection of leptospirosis.

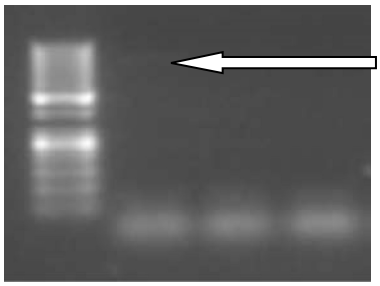
**Table 3.** Concentration of DNA in samples, extracted by DNA isolation kit

| Sample No. | Concentration of DNA, ng/ µl |
|---|---|
| 1 | 26 |
| 2 | 38 |
| 3 | 8 |
| 4 | 72 |
| 5 | 85 |



Lane: 1 2 3 4 5 6

Lane: 1 & 7 - 50bp marker
Lane 2 – 8 ng
Lane 3 – 0.8 ng
Lane 4 - 0.08 ng
Lane 5 – 0.008 ng
Lane 6 – 0.0008 ng



Lane: 7 8 9 10

**Fig. 2 and 3.** Amplified *lipl*32 gene of *Leptospira* in serially diluted sample

Lane 8 - 0.00008 ng
Lane 9 – 0.000008 ng
Lane: 10 - master mix alone (without DNA template).

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