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Polymerase chain reaction in the diagnosis of leptospirosis in cattle

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Abstract
The PCR assays are a boost to new developments in diagnostics especially for Leptospirosis which is fastidious organism and other diagnosis techniques such as culture and MAT are time consuming. The present study aimed at an early diagnosis of disease to move forward with treatment, thus PCR proved to be an effective tool. A total of 400 cattle were screened under the study. The cattle belonged to the organized dairy farms of Jabalpur. Out of 400 cattle, 70 blood samples were collected with a history of repeat breeding, abortion, hemogalactia and mastitis. Polymerase chain reaction was performed for early diagnosis of disease under the study in serum samples of suspected cattle. 18 out of 70 (4.5%) samples were found positive for leptospirosis with G1/G2 primers.

Keywords: Chain reaction, diagnosis, leptospirosis, cattle

Introduction
Leptospirosis is a zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*, which belongs to the family *Leptospiraceae* and order *spirochaetales*. The pathogens include over 250 serologically defined types of serovars placed in 25 serogroups based on antigenic relationship (NCDC, 2015) [5]. The clinical presentation of leptospirosis is associated with a vast spectrum of signs, mild to severe disease with severe jaundice and multiple organ involvement. Acute systematic infection in adult dairy cattle is a cause of acute onset agalactia, called as milk drop syndrome (Ellis, 2015) [2]. In cattle, acute haemolytic syndrome of leptospirosis has been reported characterized by fever, icterus, anemia and hemoglobinuria including one or more additional findings of anorexia, apathy, dehydration, dyspnea, hyperpnea, bilirubinuria, mastitis and abortion in all affected cattle. Chronic infections with leptospirosis can result in abortions, stillbirths, and reduced fertility in cattle. Leptospirosis has been under-diagnosed due to non-specific symptoms, complex laboratory tests, fastidious nature of bacterium, and lack of diagnostic facilities. Bacteria are slow-growing and require a rich medium at a neutral pH, making it challenging to cultivate leptospires from natural sources (Zacarias *et al.*, 2008) [7]. Accurate and prompt diagnosis of leptospiral infection in bovine is difficult due to the limitation of current procedures; thus the present study describes the application of polymerase chain reaction (PCR) for detection of leptospiral deoxyribonucleic acid (DNA) in blood and urine samples suspected of bovine leptospirosis.

Material and Methods
- For the present study, 400 cattle were screened. The cattle belonged to the organized sector of Jabalpur. Out of 400 cattle 70 bio-samples were collected from cattle with a history of repeat breeding, abortion, hemogalactia, and mastitis.
- 05 ml of blood was collected aseptically from the jugular vein of properly restrained cattle in clot-activated vacutainers. They were kept in an upright position at room temperature for 2 hours for serum separation. The straw-colored serum was then poured into 1.5 ml sterile cryovials and aliquoted for future use. Samples were transported to the laboratory and stored at -20 °C till further use.
- DNA was extracted from blood samples collected in K3 EDTA vacutainers using DNeasy® Blood and Tissue kit for 50 reactions (QIAGEN, Germany).
- The extracted DNA samples from serum was subjected to PCR for molecular diagnosis. PCR was carried out as per the method of Gravekemp *et al.*, (1993) [3].
- G1/G2 primers were used for diagnosis of leptospirosis (Table 01) and the program was standardized (Table- 02).

Table 1: Details of primers used for PCR

S. No.	Primer used	Sequence	Expected amplicon size (bp)
1	G1 (F)	5' CTG AAT CGC TGT ATA AAA GT 3'	285 bp
2	G2 (R)	5' GGA AAA CAA ATG GTC GGA AG 3'	

Table 2: Standardized PCR programme

S. No.	Steps	Temperature (°C)	Time
1.	Initial denaturation	95 °C	10 min
2.	Denaturation	95 °C	1 min
	Annealing	53 °C	45sec
	Extension	72 °C	30 sec
3.	No. of cycles	35	-
4.	Final extension	72 °C	10 min

- Horizontal submarine agarose gel electrophoresis (1.5% w/v agarose gel was prepared in 1X TAE) was carried out to check the amplified products.

Results and Discussion

A total of 70 serum samples were collected out of which 18 (4.5%) samples were found positive for leptospirosis with G1/G2 primers (Table 03, Plate 01). The symptom wise distribution of leptospirosis revealed higher prevalence i.e. 32.35 per cent (11 out of 34 cattle) in the cases of abortion followed by 21.42 per cent (06 out of 28) in cases with the history of repeat breeding least i.e. 12.50 per cent (01 out of 08) in the cattle with mastitis (Table 04).

Table 3: Molecular diagnosis of leptospirosis in cattle

Screened	Suspected	Total Positive	Positive among screened cases (%)	Positive among suspected cases (%)
400	70	18	4.5	25.71

Table 4: Symptom wise distribution of leptospirosis in clinically ailing cattle

Symptom	Suspected	Positive	Percent positive (%)
Abortion	34	11	32.35
Repeat breeding	28	06	21.42
Haemogalactia / Mastitis	08	01	12.50

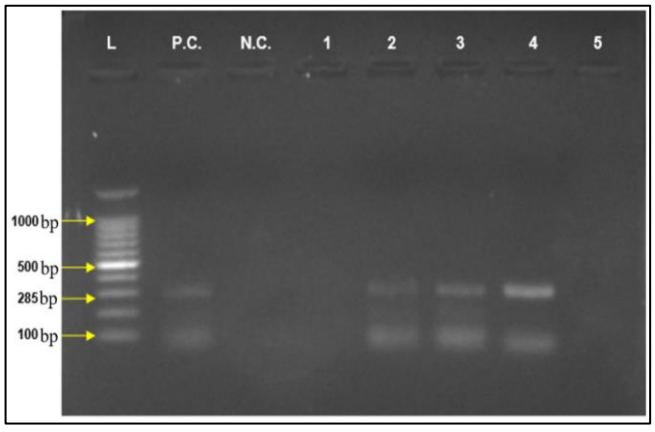

Plate 1: Amplified PCR product of genomic DNA isolated from samples of leptospirosis suspected cattle with G1/G2 primer (285 bp) in 1.5% agarose.

Lanes
L- 100 bp ladder
N.C. Negative control (without template)
01-05. Amplified PCR products suspected samples
02, 03 and 04 - PCR positive sample

The present study aimed at an early diagnosis of disease to move forward with treatment, thus PCR proved to be an effective tool. Leptospires can be isolated from blood during the first 10 days of illness. In past G1/G2 primers were used on human/cattle urine samples by a number of workers (Gravekamp *et al*., 1993; Jafari *et al*., 2011) [3, 4]. The findings of present study are similar to Patel *et al*. (2017) [6] who reported that 8.14% blood and 10.34% urine samples of cattle were found to be positive for leptospiral DNA. Similar finding pertaining to prevalence among clinically ailing cattle was reported by Chopra (2014) [1] who reported 56.66 present positivity in PCR in cattle using G1/G2 and Lipl32, Lipl 31 primers.

Conclusion

PCR is one of the most popular molecular tools used in disease diagnosis with specificity and the quickest possible time. Although the PCR assay are unable to identify serovars yet they are a tool for faster diagnosis of pathogenic leptospires in acute disease (first week) and carrier stage (chronic stage).

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