Journal of Animal Research: v.7 n.3, p. 549-551. June 2017

DOI: 10.5958/2277-940X.2017.00081.X

# Genetic Screening for Bovine Citrullinaemia in Holstein Friesian Cattle and its Crosses

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**Received:** 30 March, 2017 **Revised**: 10 April, 2017 **Accepted:** 11 April, 2017

**ABSTRACT**

Fifty Holstein Friesian (H.F) cattle were screened for Bovine Citrullinaemia (BC) using PCR-RFLP. Blood samples were collected from jugular vein in 2 ml capacity vaccutainers ($K_2$EDTA) and the DNA was isolated by using whole blood extraction kit. The PCR was performed for amplification of polymorphic region of ASS gene (185 bp) on bovine chromosome 11. The PCR products were digested with *Ava II* endonuclease enzyme. The normal allele in unaffected cattle produced two fragments of 103 bp and 82 bp. No animal was found carrier for ASS gene. The genotype frequency of normal individuals and the gene frequency of normal allele were found to be one.

**Keywords:** Bovine Citrullinaemia (BC), holstein friesian cattle, ASS gene, PCR-RFLP

Genetic disorders cause physical or functional anomalies by producing negative impact on vitality. Bovine citrullinaemia (BC) is a Holstein-specific autosomal recessive genetic disorder (Meydan *et al*., 2010). With the wide use of artificial insemination (AI) and international trading of semen and breeding bulls, the genetic diseases have already been spread to a large population, as animal carriers of the diseases look normal. In India, where Holstein Friesian (HF) bulls and their semen are extensively used for crossbreeding programmes with indigenous cattle, it has become necessary to screen all HF and HF crossbreds, especially AI bulls, to minimize the risk of spread of these diseases among future bulls or bull mothers (Patel *et al*., 2007). In BC the homozygous cattle dies during the first seven days of life (Robinson *et al*., 1993). The carriers of BC have been detected in USA (Robinson *et al.*, 1993) and Australia (Healy *et al*., 1991). There is insufficient information of BC determination and its incidence in Madhya Pradesh. Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) is the most reliable and inexpensive method for identification of BC. The investigation was therefore, undertaken with the objectives to standardize PCR-RFLP technique for the detection of BC in Holstein Friesian and its crosses, to find out the incidence of BC and to determine their gene and genotype frequency.

## MATERIALS AND METHODS

The experiment was designed to screen 25 males and 25 females Holstein Friesian crossbred cattle from Bull Mother Farm Bhadbhada, Bhopal, Dairy unit, College of Veterinary Science and A.H., Mhow and nearby villages. Blood samples were collected from jugular vein in 2 ml capacity vaccutainers ($K_2$ EDTA) and were stored at 4°C until further procedure. The DNA was isolated from the blood samples by using whole blood extraction kit (Merk Genei, Mumbai catalogue number (cat#) 612102300011730). The quality of DNA was checked and quantization was done by double beam Spectrophotometer or thermo scientific NanoDrop 1000 and 2% agarose gel electrophoresis. The DNA was stored at -20ºC.

The PCR was performed using oligonucleotide sequences consisting of 29 base pair (F: 5’ GGCCA

GGGACCGTGTTCATTGAGGACATC 3' and R: 5' TTCCTGGGACCCCGTGAGA CACATACTTG 3') as reported by Meydan *et al*. (2010). Amplification reactions of the DNA samples extracted from the blood were prepared in a final volume of 25 µl comprised of 12.5 µl of 2X PCR Master Mix with final concentration of 1X (Merck Genei cat# 610602200031730), 7.5 µl of Deionised Water, 1.0 µl of Forward Primer (10 pmoles), 1.0 µl of Reverse Primer (10 pmoles) and 3.0 µl of Genomic DNA (30 ng/ µl) in PCR tube. The cycling conditions that were set with initial denaturation at 94ºC for 3 minutes, 30 cycles each of denaturation, annealing and extension at 94ºC for 30 seconds, 58ºC for 30 seconds and 72ºC for 30 seconds, respectively and final extension at 72ºC for 10 minutes. The PCR amplification was determined by 2% agarose gel electrophoresis. The gel was visualized under UV transilluminsator (Bio- Rad) for gene amplification.

The PCR products of 185 bp (5 µl) were digested with restriction enzyme *Ava II* (ILS, Gurgaon Cat#R0149S) (1 µl), the manufacturer's 10 X restriction buffer (3 µl) and water (21 µl) in the final reaction volume of 30 µl. The reaction mixture was centrifuged for few seconds for uniform mixing and then incubated at 37ºC for 1 hour. The polymorphism was checked by 3% agarose gel electrophoresis. The gene frequencies, genotype frequencies and incidence were calculated by standard formulas. All the procedures have been conducted in accordance with the guidelines laid down by the Institutional Ethics Committee.

## RESULTS AND DISCUSSION

The DNA extracted was found to be within the acceptable purity ratio of 1.6-1.9. The primers used in the study successfully amplified the DNA fragments of 185 bp for BC in all the 50 animals. Previous studies adopted more or less similar protocol and primers for the amplification of ASS gene (Padeeri *et al.,* 1999; Patel *et al.,* 2006; Meydan *et al.,* 2010). The size of amplification product was same for all the animals. The amplified DNA fragment of 185 bp is depicted in Fig. 1.

The restriction endonuclease *Ava II* known for their high potency in detecting polymorphism was used to digest the PCR product in the present study as earlier used by Padeeri *et al.* (1999), Patel *et al.* (2006) and Meydan *et al.* (2010). The amplified PCR product of 185 bp for the ASS locus upon digestion with the *Ava II* restriction enzyme yielded 103 bp and 82 bp for normal animals shown in Fig. 2.

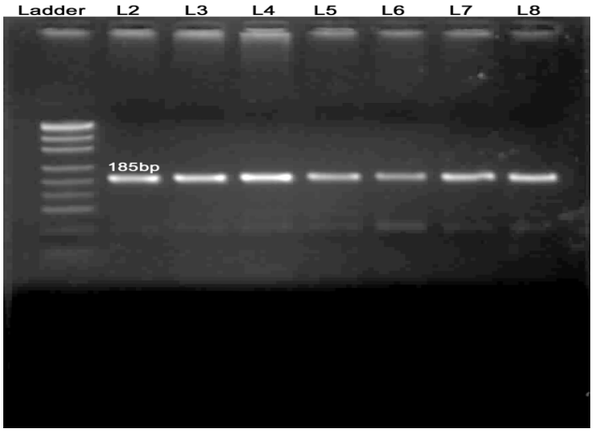


**Fig. 1:** Electrophoregram (2% Agarose Gel) reveling amplified DNA by PCR using BC specific primers. L1: Molecular ladder pUC19/ mspI. L2, L3, L4, L5, L6, L7 and L8: amplified DNA of 186 bp for ASS gene for B.C.

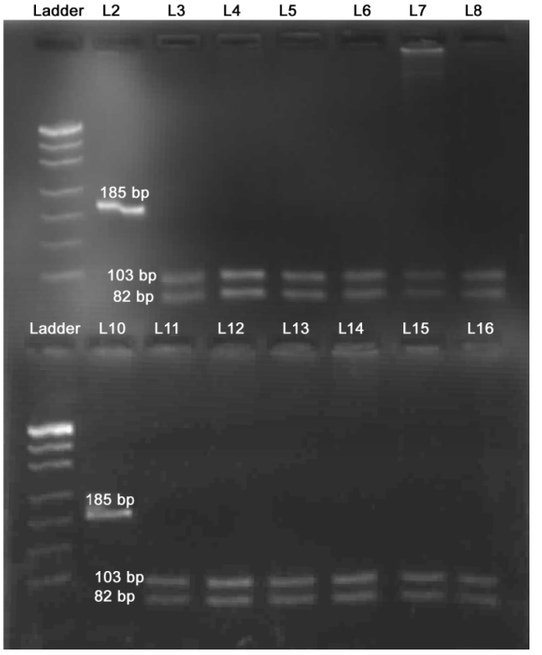


**Fig. 2:** Electrophoregram (3% Agarose Gel) of *Ava II* digested PC R product of 185 bp generated by amplification of genomic DNA using citrullinemia specific primers. L1 and L9: Molecular ladder (pUC19/mspI). L2 and L10 shows undigested PCR products. L3, L4, L5, L6, L7, L8, L11, L12, L13, L14, L15 and L16: Two bands of 103 bp and 82 bp of B.C free animals.

This is in agreement with findings of Padeeri *et al.* (1999) and Patel, *et al.* (2006) in Indian cattle. On the other hand, Robinson *et al.* (1993) and Viana *et al.* (1998) used different primers and found 99 bp and 78 bp in normal homozygotes, 177, 99 and 78 bp in heterozygote and 177 bp in recessive homozygote.

In the present study no carriers were found for BC. Thus the genotype frequency of normal individuals and the gene frequency of normal allele was one. The results obtained in this study also correspond to earlier reports (Grupe, *et al.,* 1996, Nassiry *et al.,* 2005, Patel *et al*., 2006; Oner *et al.,* 2010). By contrast, carriers of BC have been detected in USA (Robinson *et al.*, 1993) and Australia (Healy *et al*., 1991). This might be due to the differences in the use of affected breeding lines between different regions. Animals homozygous for BC allele dies during earlier part of their life, therefore, possibility of spread in the population is much less.

## CONCLUSION

The study demonstrates the genotype frequency and the gene frequency of normal allele to be one. Out of fifty animals screened, no animal was found to be heterozygous for BC in the Holstein population. PCR-RFLP analysis was found to be a strong and reliable method for identification of BC. The study demonstrates a need for further examination of more Holstein cattle in Madhya Pradesh, preferably by testing the breeding sires to avoid unrecognized spread of BC (if any).

## ACKNOWLEDGEMENTS

Authors are thankful to the Dean, College of Veterinary Science and A.H, Mhow (MP) for providing necessary facilities. The authors also expresses cordial thanks to the Dr. D.N. Rank, Department of Animal Genetics and Breeding, AAU, Anand (Guj.) and Managing Director, Livestock and Poultry Corporation, Bhopal (M.P) for the co-operation rendered during the study.

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