

Genotyping of the Holstein-Friesian crossbred cattle for *CD18* gene using PCR-RFLP

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Abstract

Aim: The present study was undertaken in Holstein-Friesian (HF) crossbred cattle with the objective to find out genotype of HF crossbred cattle for Bovine Leucocyte Adhesion Deficiency (BLAD) by using PCR-RFLP.

Materials and Methods: 50 blood samples were collected from HF crossbred cattle and subjected to PCR. The amplified PCR products were digested using *Taq* I restriction enzyme at 65 °C overnight. After restriction digestion, the final PCR products were electrophoresed on 2.5 % agarose gel.

Results: All the 50 animals under present investigation were found to be normal as the amplified PCR product upon digestion with *Taq* I restriction enzyme, revealed two bands of 313 bp and 54 bp for normal animals.

Conclusions: In the present investigation *D128G* carrier frequency was found to be 0 %. However, recent reports suggest that the mutant gene has already been observed in the HF crossbred cattle population of India, which makes it necessary to screen the animals to avoid the risk of spreading BLAD in the breeding cattle population.

Key words: bovine leucocyte adhesion deficiency, *CD18*, genotyping, Holstein-Friesian, PCR-RFLP.

Introduction

Bovine Leucocyte Adhesion Deficiency (BLAD) is a Holstein-Friesian (HF) specific autosomal recessively inherited disorder characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis, periodontitis, loss of teeth, delayed wound healing, persistent neutrophilia and death at an early age [1]. BLAD is a disease characterized by a reduced expression of the adhesion molecules on neutrophils, called β_2 integrins (a complex of the *CD11/CD18* family of proteins that are structurally and functionally related to glycoproteins).

The disease is caused by a mutation, which replaces adenine at nucleotide position 383 with guanine, resulting in an amino acid change (aspartic acid to glycine; *D128G*). The mutation ultimately leads to a *CD18* protein with impaired function. The carrier frequency of BLAD among US Holstein cattle was found to be 15% among breeding bulls and 8% among cows [2]. Use of breeding HF bulls imported from the USA in many countries lead to a high incidence of BLAD carriers in their native/crossbred cattle population including India [3-8].

The present study was therefore undertaken to describe the BLAD carrier prevalence, the geographical distribution and the related gene frequency among the HF crossbred cattle of Maharashtra state of India.

Materials and Methods

Ethical approval: The experimental design and plan of the present study was duly approved by the Institution Animal Ethics Committee of Bombay Veterinary College, Parel, Mumbai, Maharashtra.

Sample collection: Experimental material for the present study comprised 50 blood samples of HF crossbred cattle, maintained with the farmers of Shingawe village of Manchar in Pune district of Maharashtra.

***Cd18* genotype analysis by DNA polymerase chain reaction (DNA-PCR) test:** The genomic DNA was isolated from blood samples using QIAamp Kits (QIAGEN, USA) following manufacturer's instructions. The quantity and quality of DNA was checked by spectrophotometer (ND-2000, Thermo, USA) and agarose gel electrophoresis, respectively. We targeted a 367 bp fragment of fifth exon of *CD18* gene comprising BLAD locus [2]. Oligo primers (F 5'-AGG TCA GGC AGT TGC CTT CAA -3' and R 5'-GGG GAG CAC CGT CTT GTC CAC-3') as described by [9] specific to BLAD locus custom synthesized at Sigma were utilized in the present study. PCR mixture contained 1X PCR Buffer, 10 mM dNTPs, 5 pmoles each of Forward and Reverse primers, 2.0 mM MgCl₂, 1U *Taq* DNA Polymerase and 100 ng Template DNA. The PCR reaction included the following steps: Initial denaturation at 94°C for 2 minutes followed by 30 cycles of 30 seconds at 94°C, 30 sec at 63.8°C, 30 sec at 72°C and final extension for 7 min at 72°C. The amplified

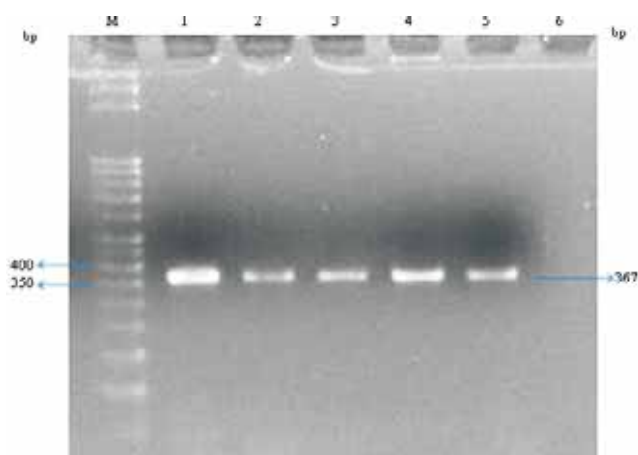


Figure-1: Agarose gel electrophoresis of the PCR amplified fragment of *CD18* gene. Lane M: 50 bp DNA ladder, Lanes 1 to 5: PCR product specific to a fragment of 5th exon of *CD18* gene (367 bp) Lane 6: negative control

PCR products were digested using *Taq* I restriction enzyme at 65 °C overnight. After restriction digestion, the final PCR products were electrophoresed on 2.5 % agarose gel.

Results

The present study was carried out to genotype HF crossbred cattle for BLAD, an autosomal recessive genetic disorder using PCR-RFLP. The DNA samples with an OD ratio of 1.8 to 2.0 (OD_{260:280}), appeared as a single compact fluorescent band free from shearing and contamination on agarose gel electrophoresis were subjected to PCR. For the PCR amplification, a suitable annealing temperature was tested from a range of 55–65 °C in the mastercycler gradient, consistent results were obtained at 63.8 °C. PCR amplification revealed 367 bp fragment of the 5th exon of *CD18* gene (Figure-1). The amplified PCR product upon digestion with *Taq* I restriction enzyme, revealed two bands of 313 bp and 54 bp for normal animals (Figure-2). Our results are in accordance with the findings of [6, 7, 10]. Three bands *viz.*, 367 bp, 313 bp and 54 bp as observed by [6, 7] expected for heterozygous/carrier animals, were not observed in the present investigation.

The carrier frequency for the *D128G* allele among Holstein cattle in the United States was found to be 23% [2], 21.47% in Denmark [8], 13.5% in Germany [11], 2.88% in Argentina [12], 1% in Pakistan [13], 2.8% in Brazil [14], 3.33% in Iran [15], 4 % in Turkey [16], 0.16% in China [17] and 1.2 % in Macedonia [18]. Recent reports of BLAD carriers have been reported in Antalya region of Turkey where the carrier frequency was found to be 2 % [19]. One of the investigations from Brazil has reported the BLAD carrier frequency of 0.77 % in the Girolando cattle [20]. In India, the heterozygote frequency was found to be 1.33%, 4.76%, 7.69%, 3.64%, and 3.23%, in Holstein cows by previous studies [5, 6, 7, 10, 21], respectively under various investigations.

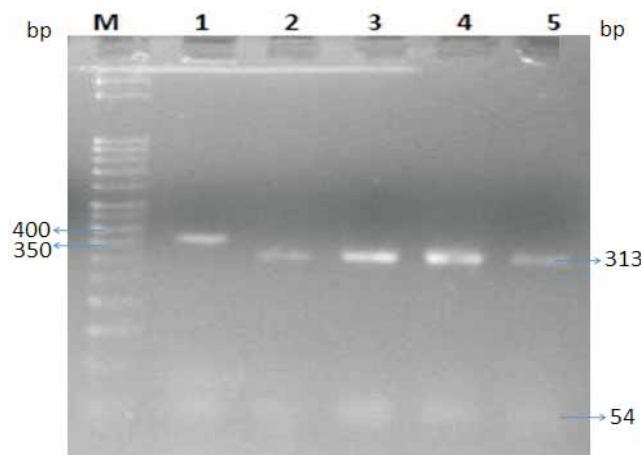


Figure-2: PCR-RFLP of *CD18* gene fragment by using *Taq* I restriction enzyme. Lane M: 50 bp DNA ladder, Lane 1: PCR product of 367 bp, Lanes 2 to 5: RE digestion product of 313 and 54 bp for normal animals.

Discussion

The development of artificial insemination (AI) has facilitated production of large number of progenies from a superior sire. The importation of selected HF bulls or their semen to use on the native cattle population is a routine practice worldwide. These practices have increased the milk production in the HF crossbred cattle and also enhanced the genetic relatedness among the individuals. The selection pressure within a breed and AI programmes are major factors to spread undesirable genetic disorders like BLAD. Lack of efficient methods for identifying autosomal recessive genetic disorders and use of carrier bulls lead to the high levels of BLAD allele in various countries. The BLAD affected calves were reported as descendants of common ancestral heterozygous sires [8].

Since the mutant gene has already been observed in the HF crossbred cattle population in India [5, 6, 7, 10, 21], these populations need regular screening to avoid the risk of spreading BLAD in the breeding cattle population [21]. As per the CMU (Central Monitoring Unit) norms 2010, it is mandatory to screen breeding bulls for BLAD, Citrullinaemia and DUMPS. As a first step we have screened 50 HF crossbred bulls. Although we could not get any carrier, the probability of carrier/heterozygous bulls could not be ruled out if a large number of HF crossbred population is tested.

Conclusion

In the present study, 50 blood samples collected from the HF crossbred cattle maintained with the farmers were screened for BLAD. The PCR amplified 367 bp product upon digestion revealed two bands of 313 and 54 bp respectively, specific for normal animals. In the present investigation *D128G* carrier frequency was found to be 0 %. However, a large number of crossbred cattle need to be screened to make realistic estimates of BLAD allele frequency in the Indian crossbred cattle population and to avoid unintentional transmission of recessive genetic disorders.

Author's contributions

ASK and AYD: Substantially contributed to design and plan of the study. ASK and AYD: Drafted the manuscript, analysed and interpreted the results. ASK, AYD, UDU, MPS and VDP: Revised manuscript for important intellectual content. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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