Genetic Screening of Deficiency of Uridine Monophosphate Synthase in Holstein Freisian Crossbred Cattle

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Abstract

Fifty Holstein Friesian (H.F) crossbred cattle were screened for Deficiency of Uridine Monophosphate Synthase (DUMPS) using PCR-RFLP. Blood samples were collected from jugular veins in 2 ml capacity vacutainers containing 2 mg/ml (K₂ EDTA). DNA was isolated from the blood samples by using whole blood extraction kit. PCR was performed for amplification of polymorphic region of Uridine Monophosphate Synthase (UMPS) gene (108 bp) on bovine chromosome 1. The PCR products of 108 bp were digested with AvaI endonuclease enzyme. The normal allele in unaffected cattle produced three fragments of 53 bp, 36 bp and 19 bp. No animal was found carrier for UMPS gene. The genotype frequency of normal individuals and the gene frequency of normal allele were found to be one.

Keywords : Deficiency of Uridine Monophosphate Synthase (DUMPS), Holstein Friesian crossbred cattle, UMPS gene, AvaI, PCR-RFLP

Introduction

Genetic disorders cause physical or functional anomalies by producing negative impact on vitality. Deficiency of Uridine Monophosphate Synthase (DUMPS) is the autosomal recessive genetic disease which is Holstein-specific (Meydan et al., 2010). With the wide use of artificial insemination (AI) and international trading of semen and breeding bulls, this genetic disease have already been spread in 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). DUMPS is caused by single point mutation (C → T) at codon 405 within exon 5. The Uridine Monophosphate Synthase (UMPS) gene was mapped to the bovine chromosome one (Robinson et al., 1983 and Citek et al., 2006).

DUMPS cause early embryonic mortality during implantation in the uterus (Schwenger et al., 1994). The mutant alleles were identified in study carried out in U.S.A (Shanks and Robinson, 1989), and Argentina (Kumar et al., 2010). There is insufficient information of DUMPS 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 DUMPS.
The investigation was therefore undertaken with the objectives to standardize PCR-RFLP technique for the detection of DUMPS in Holstein Friesian crossbred cattle to find out the incidence of DUMPS 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 and Dairy unit, College of Veterinary Science and A.H., Mhow and nearby villages. Blood samples were collected from jugular veins in 2 ml capacity vacutainers containing 2 mg/ml K<sub>2</sub>EDTA. The collected blood samples were brought to the laboratory on ice with proper care and stored at 4°C until further analysis. 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 quantification was done by Nano Drop 1000 (Thermo Scientific) and 0.8% agarose gel electrophoresis. The DNA was stored at -20°C. Polymerase chain reaction (PCR) was used to amplify one region of UMPS gene for DUMPS for each sample as described by Meydan et al. (2010). The sequences of oligonucleotide primers employed in PCR assay were

F: 5' GCAGATGGCCTAGAACATTCTG 3' and R: 5' GCTTCTAACTGAACTCCTCGAGT 3'

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 (Merk Genei cat# 61060200031730), 7.5 µl of deionised Water, 1.0 µl of Forward Primer (10 p moles), 1.0 µl of Reverse Primer (10 p moles) and 3.0 µl of Genomic DNA (30 ng/µl) in PCR tube. The cycling conditions were set with initial denaturation at 94°C for 5 minutes followed by 40 cycles each of denaturation at 94°C for 60 seconds, annealing at 58°C for 60 seconds, extension at 72°C for 90 seconds and final extension at 72°C for 5 minutes.

PCR amplification was determined by 2% agarose gel electrophoresis. The gel was visualized under UV trans illuminator (Bio- Rad) for gene amplification. The restriction digestion of 108 bp with restriction enzymes Ava I (ILS, Gurgaon Cat# R0149S) was used for DUMPS. Five µl PCR products were digested with 1 µl restriction enzymes, 3 µl manufacturer’s 10 X restriction buffer and 21 µl nuclease free water (Molecular Biology water, Sigma) in the final reaction volume of 30 µl. The reaction mixture was spinned for few seconds for uniform mixing and then incubated at 37°C for 1 hour. The polymorphism was checked by 3% agarose gel electrophoresis. The gene frequencies, genotype frequencies and incidence were calculated by standard formulas.

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 108 bp for UMPS gene. Previous studies adopted more or less similar protocol and primers for the amplification of UMPS gene. The 108 bp fragment of cattle was amplified in all the 50 animals. The size of amplification product was same for all the animals. The amplified DNA fragment of 108 bp is depicted in Figure 01.

PCR-RFLP technique was applied to detect genetic variation in the amplified region of UMPS gene. The restriction endonuclease Ava I 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 108 bp for the UMP synthase gene locus on digestion with AvaI yielded 3 bands of 53 bp, 36 bp and 19 bp for normal animals (Figure 02). This is in agreement with findings of Padeeri et al. (1999), Patel et al. (2006) and Meydan et al. (2010) in Holstein cattle. In present study no carriers were detected as DUMPS carriers should yield four fragments of 89 bp, 53 bp, 36 bp and 19 bp and mutants should yield undigested band of 108 bp as reported by Meydan et al. (2010). The results obtained in this study also correspond to findings of Patel et al. (2006) in Indian Holstein population. Carriers for DUMPS were also not detected in Polish and Turkish Holstein cattle (Kaminski et al., 2005, Meydan et al., 2010 and Oner et al., 2010). On the contrary the frequency of the mutant allele for DUMPS has been estimated
at 1-2% in US Holstein cattle, 0.96% in Argentinian Holstein bulls and 0.11% in Argentinian Holstein cows during the 1990s (Kumar et al., 2010). Two HF carriers were found among 314 AI bulls in Hungary (Fesus et al., 1999). Lin et al. (2001) also reported two carriers out of 1468 HF animals screened for DUMPS in Taiwan. The differences between studies reflect differences in the use of affected breeding lines between different regions. It is possible that with the mounting selection pressure, the international gene pool may diminish, and consequently the risk of dissemination of genetic defect will increase. It is therefore recommended to screen breeding bulls for their breed-specific genetic diseases before they are inducted in artificial insemination programmes, to minimize the risk.
Conclusion

The study demonstrates the gene and genotype frequency of normal animals to be one. Out of fifty animals screened, no animal was found to be heterozygous for DUMPS in the Holstein population. PCR-RFLP analysis was found to be a strong and reliable method for identification of DUMPS. 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 DUMPS (if found).

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