

GENOTYPING OF THE HF CROSSBRED CATTLE FOR UMPS GENE USING PCR-RFLP

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Abstract: The present study was undertaken with the objective to identify the genotype of HF crossbred cattle for Deficiency of Uridine- 5'- Monophosphate Synthase (DUMPS) by using PCR-RFLP in HF crossbred cattle. The blood samples were collected from HF crossbred cattle from Tathawade cattle farm, Pune. The DNA isolated from the blood samples and subjected to PCR. The amplified PCR product of *UMPS* gene was digested using restriction enzyme *Ava* I at 37°c for overnight. After restriction digestion, the final PCR product was electrophoresed on 2 per cent agarose gel. Under the present investigation all animals were found to be normal. The amplified PCR product of 108 bp upon digestion by *Ava* I to detect the point mutation in a gene coding *UMPS*, yielded three bands of 53, 36 and 19 bp, respectively for normal animals. In the present study the all animals were wild for *UMPS* gene, the probability of carrier/ heterozygous bulls could not be ruled out if a large number of HF crossbred population is tested.

Keywords: DUMPS, PCR-RFLP.

Introduction

The deficiency of uridine monophosphate synthase is a hereditary recessive disorder in Holstein cattle causing early embryo mortality (Kaminski et al., 2005). In mammalian cells, uridine monophosphate synthase (UMPS) is the enzyme responsible for converting orotic acid to uridine monophosphate (UMP), which is an essential component of pyrimidine nucleotides. This enzyme actually has two enzymatic functions: orotic phosphoribosyl transferase (OPRTase) and orotidine monophosphate decarboxylase (OMPDCase), corresponding to the last two steps in pyrimidine synthesis (Patel et al., 2006). The mutation (C_T) in a gene for UMPS at codon 405 from exon 5 (Patel et al., 2006) of bovine chromosome 1 leads to a premature stop codon, which subsequently produces a functionally impaired enzyme (Schwenger et al., 1993). Carriers are phenotypically normal, but have only half the normal activity of uridine monophosphate synthase. Also, during lactation, carriers excrete an elevated level of orotic acid in milk and urine (Robinson and Shanks 1990). Embryos

homozygous for DUMPS do not survive to birth and usually die early in gestation. The embryos appear to be aborted or reabsorbed approximately 40 days after conception, leading to repeated breeding problems (Robinson et al., 1993; Lee et al., 2002).

Inactivation of this enzyme is caused by an autosomal recessive heredity mutation, which occurs in the gene of *UMPS*. The mutation (C \pm T) leads to the loss of the restriction site of *Ava* I site in codon 405 of the gene. This disorder is named as Deficiency of Uridine Monophosphate Synthase (DUMPS) in the Holstein cattle and characterized by lowered blood activity of enzyme *UMPS* (Schwenger, et al., 1993). In the world, DUMPS was spread through the descendents of an elite bull Skokie Sensation Ned born in 1957. DUMPS leads to embryonic death in early stage of pregnancy. Embryos homozygous for DUMPS die early in gestation at approximately 40 days of post fertilization. The embryos are often reabsorbed during first two months of gestation, leading to more services and longer than normal calving intervals causing serious reproductive problems in dairy herds. Heterozygous carrier shows a decrease of almost 50 % *UMPS* activity in kidney, spleen, muscles and mammary glands. It was evident that several cows produced five to ten times higher concentrations of orotic acids in their milk. A carrier to normal animal mating results in 50 % carriers. Several investigations were carried out in different countries. No carrier animals were found among the Holstein population in Poland, Iran and Turkey, but the mutant allele was detected in the studies carried out in U.S.A. and Argentina.

Materials and Methods

Total 40 blood samples were collected from the Holstein Friesian (HF) crossbred cattle maintained at Tathawade cattle farm, district Pune of Maharashtra State. Samples were collected aseptically from jugular vein using Vacutainers containing EDTA. The genomic DNA isolated from all blood samples by Genomic DNA Mini Kit. The quantification and purity of DNA were checked by UV spectrophotometry. The DNA samples with OD₂₆₀/OD₂₈₀ ratio ranging between 1.7 and 2.0 were subjected to further analysis.

For quality check, gel electrophoresis was carried out in 0.8 per cent agarose gel at 90 V for 30 minutes. To identify the FXID, the amplification reaction prepared in a final volume of 25 μ l. It contain 1X PCR buffer 2.5 μ l, dNTP 2.0 μ l, MgCl₂ 1.5 μ l, Forward primer 0.5 μ l (5'- GCA AAT GGC TGA AGA ACA TTC TG -3), Reverse primer 0.5 μ l (5'- GCT TCT AAC TGA ACT CCT CGA GT- 3') Taq DNA Polymerase 0.2 μ l, Template DNA 1.0 μ l (~100 ng), Nuclease free water 16.8 μ l. 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, annealing of

primers at 55°C for 45 seconds and extension for 30 seconds at 72°C, followed by final extension for 7 minutes at 72°C. The PCR products were resolved by electrophoresis on 2 per cent agarose gel followed by staining with Ethidium bromide in 1X TAE buffer. Amplified PCR products were confirmed by agarose gel electrophoresis (2.5 per cent). The amplified PCR products of expected size 108 bp were analyzed on 25 per cent agarose gel. The PCR products were digested overnight by using *Ava* I restriction enzyme at 37°C. The digested products were visualized on 12 per cent polyacrylamide gel and documented on Gel documentation system.

Results and Discussion

The present study was undertaken for DUMPS locus to genotype HF crossbred cattle by using PCR-RFLP. The DNA samples with an OD ratio of 1.8 to 2.0 (OD₂₆₀:₂₈₀) were subjected for PCR. For the PCR amplification, a suitable annealing temperature was tested from a range of 55-65°C in the Mastercycler gradient and consistent results were obtained at 55°C for DUMPS. PCR amplification revealed 108 bp fragment of UMPS gene (Fig.1).

The amplified 108 bp product upon digestion by *Ava* I to detect point mutation in a gene coding uridine monophosphate synthase, yielded three bands of 53, 36 and 19 bp, respectively for normal animals but 19 bp band is too short so it is not visible in RFLP. None of the animal showed four bands viz., 89 bp, 53 bp, 36 bp and 19 bp. So no animal was found to be carrier under present study. No carrier animals were found among Holstein population in Poland (Kaminski, et al., 2005), Iran (Rahimi, et al., 2006) and Turkey (Meydan, et al., 2010), but the mutant allele was detected in studies carried out in USA (Shanks, et al., 1987) Argentina (Poli, et al., 1996) and Taiwan (Lin, et al., 2010). Representative image of PCR-RFLP product of UMPS gene is presented in Fig.2. Since the mutant gene has already been observed in the HF crossbred cattle population of USA, Argentina and Taiwan and wide use of exotic semen in Indian cattle population during the last 5 decades, we cannot rule out the possibility of DUMPS in Indian HF crossbred cattle population in the future. So, as a precautionary measure it becomes essential to carry out further screening of animals to rule out any possibility of carriers in the local population.

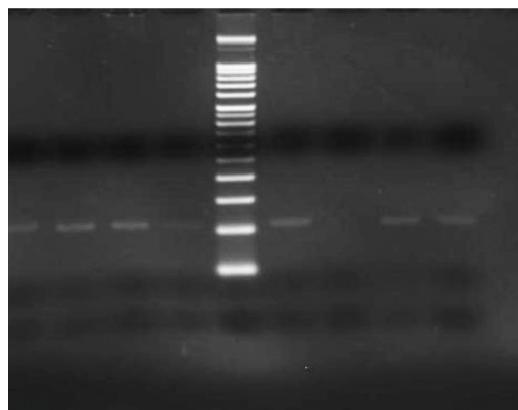


Fig.1: Dumps PCR size 108bp and 100 bp ladder

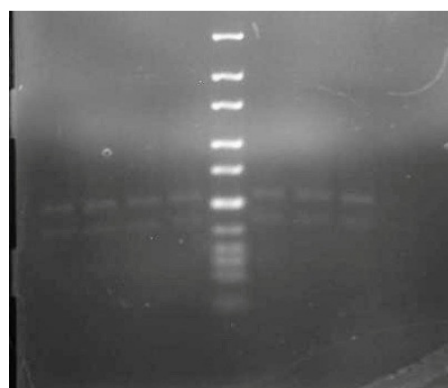


Fig. 2: Dumps RFLP of size 53, 36 and 19 bp, (19 bp is not visible)
Marker Ultra low range

Conclusion

All 40 animals genotyped for the DUMPS under present investigation were wild type and no heterozygous or homozygous recessive genotypes were found. It indicates that there are no carriers of DUMPS in the Indian HF crossbred cattle. However, in the years to come, it becomes essential to screen large number of crossbred cattle to avoid spread of such recessive genetic disorders.

References

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