GENOTYPING OF HOLSTEIN FRIESIAN CROSSBRED CATTLE FOR BOVINE CITRULLINEMIA BY PCR-RFLP

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ABSTRACT

The present study was undertaken in 30 HF and HF crossbred cattle from the Holstein Friesian crossbreds of Nalawade cattle farm, Ambarnath and from Manchar village in Pune district with the objective to find out genotype for Bovine citrullinemia using PCR-RFLP. Citrullinemia is a rare inherited disorder caused by a deficiency or lack of the enzyme arginosuccinate synthetase (ASS) which plays important role in urea cycle, lack of this enzyme results in hyperammonemia. For this study, the isolation of DNA was done by phenol: chloroform method. The polymerase chain reaction (PCR) amplification of ASS gene was carried out which showed 185-bp DNA fragment. The PCR products were digested overnight by using Ava II restriction enzyme and it yielded two bands of 103 and 82 bp respectively, for normal animals. None of the animals showed three bands of 185, 103, and 82 bp, as reported in Bovine citrullinemia carriers, indicating all the HF and HF crossbred cattle were homozygous dominant genotype for ASS gene.

Key Words: HF, HF crossbreds, Citrullinemia, PCR- RFLP.

INTRODUCTION

Modern dairy cattle breeding increasingly involves programme based on international trade of semen from elite bulls with high genetic merit. With the widespread use of advanced reproductive technologies, including artificial insemination (AI) and multiple ovulation embryo transfer, individual bulls are able to quickly sire thousands of calves in many countries (Windsor and Agerholm, 2009). One of the most important issues concerned in animal breeding are genetic disorders as they have many negative influence on animal production. In cattle, inherited disorders are mostly caused by autosomal recessively inherited genes. The characteristic feature of autosomal recessive genes is that they are only expressed as a diseased phenotype if both alleles are present. Therefore, unrecognized dissemination of such defective genes is possible and autosomal recessively inherited disorders are of greater concern in cattle breeding than other disorders with dominant inheritance or recessive X-linked inheritance as these are easily recognized. Heterozygous individuals can be identified by different methods such as examination of progeny, analysis of enzyme activity in blood and genotyping of animals by genomic analysis. Recent developments within molecular genetics have made possible efficient and rapid identification of heterozygous animals by genomic analysis. Knowing the molecular basis of a defect, the direct detection of carriers is possible at the genetic level,

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thus preventing unintended breeding of the animal (Agerholm, 2007).

At present, there are identification records for several inherited bovine disorders such as Bovine leukocyte adhesion deficiency (BLAD), Deficiency of uridine monophosphate synthase (DUMPS), Complex vertebral malformation (CVM), Bovine citrullinaemia (BC) and Factor XI Deficiency (FXID) (Agerholm, 2007; Windsor and Agerholm, 2009). In cattle the autosomal recessive diseases are breed specific. Some of them are Holstein specific, which includes Bovine citrullinaemia (Harper et al., 1986). Through the wide use of artificial insemination (AI) and international trading of semen and breeding bulls, these genetic diseases have already been spread to a large population; as animals carrying 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 spreading these diseases among future bulls or bull mothers. The present study aims to investigate the occurrence of Bovine citrullineamia which is specific to HF and HF crossbreds (Patel et al., 2006). BC in Holsteins is an autosomal recessively inherited disease that was first described in the Australian Holstein population (Harper et al., 1986; Dennis et al., 1989; Robinson et al., 1993). This genetic disorder prevents the synthesis of argininosuccinate synthetase, the enzyme that catalyses the conversion of citrulline and aspartate to argininosuccinate at the consumption of ATP. Cattle affected by BC appear normal immediately after birth. However, by the 2nd day of life they become depressed and feed poorly, by the 3rd day, they are often seen aimlessly wandering about their enclosure or standing with their head pressed against a fence or wall. Between the 3rd and 5th day, the disease progresses rapidly. The calves appear to be blind and finally collapse. Homozygous calves die during the first 7 days of life (Harper et al., 1986). BC is caused by a transition of cytosine (CGA/arginine) into thymine (TGA/STOP codon) at codon 86 of the gene coding for argininosuccinate synthase leading to impaired urea cycle. The BC gene, Argininosuccinate synthetase (ASS) was mapped to the bovine chromosome 11 (Grupe et al., 1996; Patel et al., 2006). BC was disseminated throughout the Australian Holstein population following importation of semen from the US sire Linmack Kriss King (Healy et al., 1991; Healy, 1996). Identification of the molecular basis for genetic disorders enables a rapid screening of breeding populations in order to eliminate the carriers from the population of breeding sires, thus decreasing the number of affected progeny. The various studies proved that PCR-RFLP analysis is a strong and reliable method for identification of Bovine citrullinaemia. Considering the importance of inherited Bovine citrullinaemia disorder, the present study was undertaken in Holstein crossbreed cattle, to genotype HF crossbreds for Bovine citrullinaemia by PCR-RFLP technique.

MATERIALS AND METHODS

Total 30 blood samples were collected from the Holstein Friesian (HF) crossbred cattle maintained at Nalawade Cattle Farm, Ambarnath, Thane and adjacent villages of Munchar in Pune district of Maharashtra State. Samples were collected aseptically from jugular vein using Vaccutainers containing EDTA. The genomic DNA was isolated from all blood samples by phenol chloroform DNA extraction method (Sambrook et al., 1989) with slight modifications. The quantification and purity of DNA were checked by UV spectrophotometry. The DNA samples with OD260/OD280 ratio ranging between 1.8 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 to 60 minutes. To identify the ASS gene, the amplification reaction was prepared in a final volume of 25µl. It contained 1X PCR buffer 2.5µl, dNTP 0.5 µl, MgCl2 1.5µl, forward primer 1.0 µl (5' GGCCAGGGACCGTGTTCATTGAGGACATC 3'),

reverse primer 1.0 µl (5' TTCCTGGGACCCCGTGAGACACATACTTG 3'), Taq DNA Polymerase 0.2 µl, template DNA 2.0 µl (~60 ng), nuclease free water 16.3µl. The PCR reaction included the following steps: Initial denaturation at 94˚C for 3 minutes, Denaturation 94˚C for 30 sec, Annealing temperature 58˚C for 30 sec, repeat cycle for 35 times and final extension for 5 min at 72˚C. The PCR products were resolved by electrophoresis on 1 per cent agarose gel followed by staining with Ethidium bromide in 1X TAE buffer. Amplified PCR products were confirmed by agarose gel electrophoresis. Restriction digestion of the PCR products is done with the restriction enzyme Ava II. The PCR products from each tube were digested with restriction enzyme in the manufacturer's 10X assay buffer in the final reaction volume of 15 µl containing RE enzyme Ava II 1.5 µl, RE buffer 1.5 µl, PCR product 7.0 µl, and nuclease free water 5.0 µl. The reaction mixture was centrifuged for few seconds for uniform mixing and then incubated at 37˚C for overnight in the water bath.

RESULTS AND DISCUSSION

The isolated DNA by phenol chloroform method was checked for its quality and purity by using UV spectrophotometry. Most of the DNA samples were in the range of 1.8 to 2.0 (OD 260: 280). The genomic DNA samples appeared as single compact fluorescent band and without any shearing (Fig.1). For PCR, the annealing temperature was tested from 55-65˚C and consistent results were obtained at 58˚C. The PCR amplification yielded a 185bp product (Fig. 2). The amplified PCR product of 185 bp for Bovine Citrullinemia was digested with Ava II restriction enzyme. The RE digestion of PCR product revealed two bands of 103 bp and 82 bp (Fig. 3) for normal animals which were in accordance with Robinson et al., (1993) and Grupe et al., (1996). Li et al., (2011) reported 177bp PCR product in Chinese HF cattle with digestion in two bands of 98bp and 79bp fragments for normal animal and 177 bp, 98bp and 79bp fragments for the carrier animals. In the present study we observed only two bands of 103 bp and 82 bp in all the samples which indicate that all the animals are normal. The findings were in accordance with Robinson et al., (1993); Grupe et al., (1996) and Patel et al., (2006). All the animals under present investigation were homozygous dominant for ASS gene.

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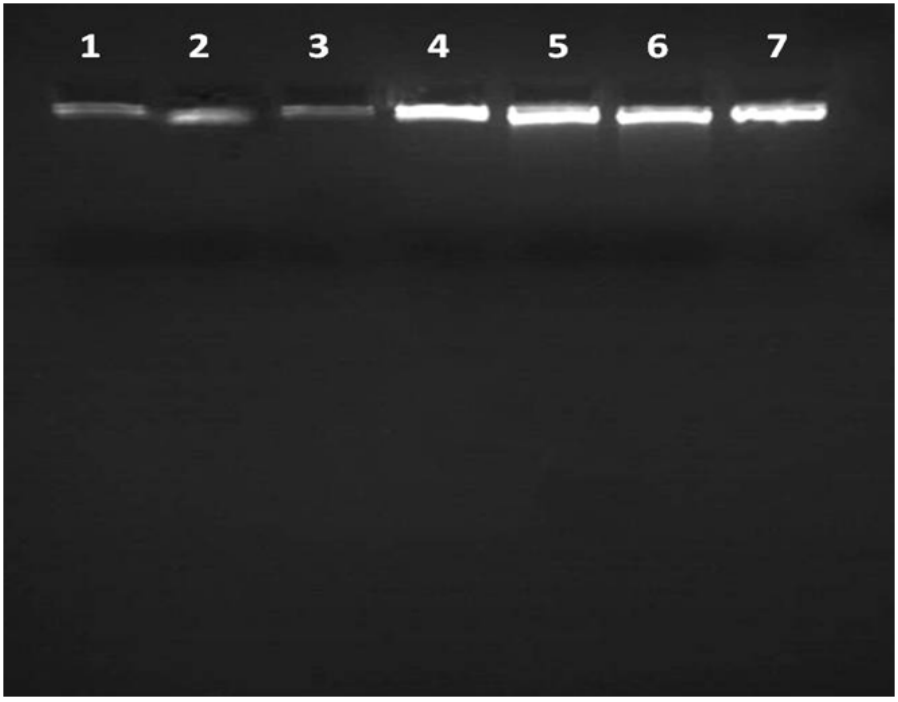

Fig.1

Genomic DNA from blood samples

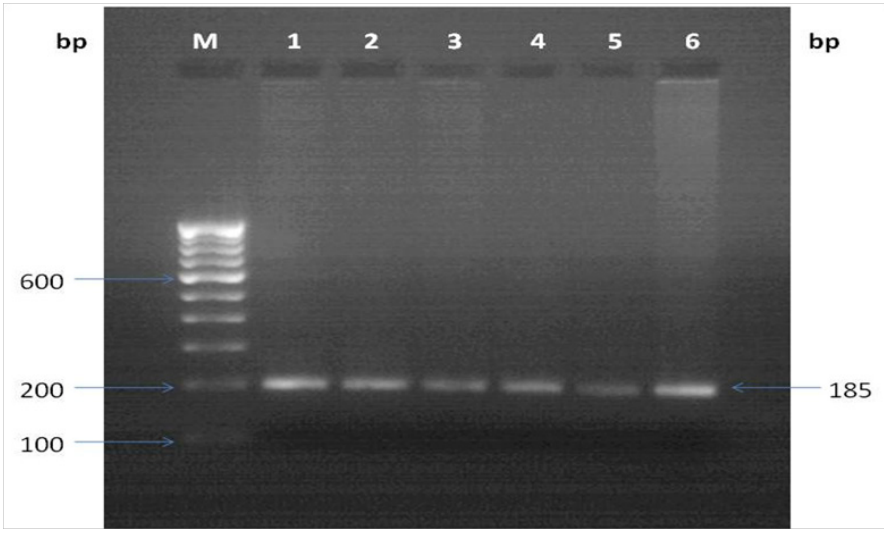

Fig.2: PCR product of ASS gene

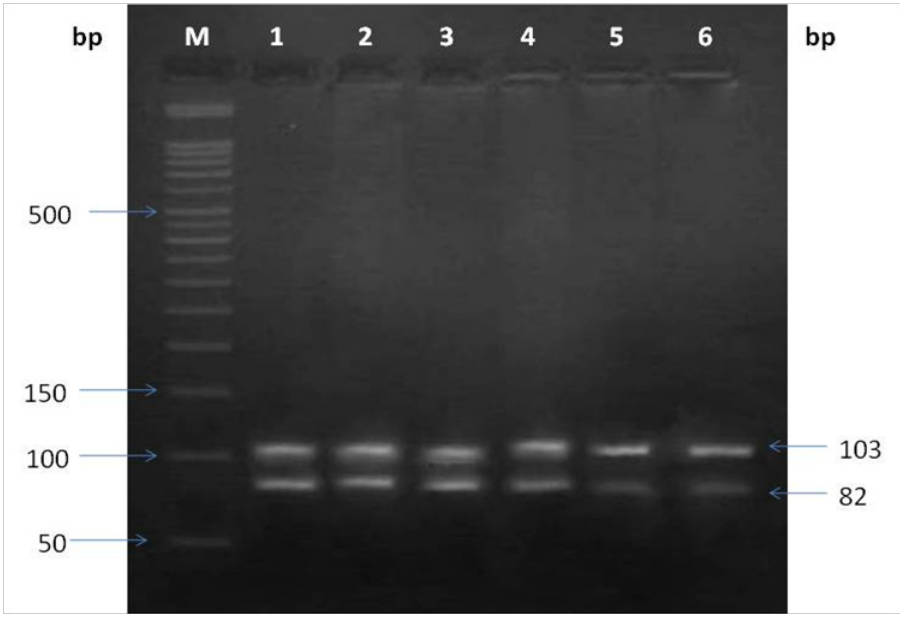

Fig. 3 Digested product of ASS gene