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SHORT COMMUNICATION

First Case of Genetic Polymorphism in Uridine Monophosphate Synthase Gene in an Indian Holstein Bull

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ABSTRACT

The present study investigated the occurrence of an autosomal recessive genetic disease, bovine deficiency of uridine monophosphate synthase (DUMPS), in Indian Holstein cattle. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed on routine screening of 86 Holstein bulls station at various part of the country to identify carriers of the disease. One of the bulls was found carrier of DUMPS. It is therefore recommended to screen breeding bulls for their breed-specific genetic disorders before they are inducted in artificial insemination programmes, to minimize the risk of spreading.

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INTRODUCTION

Several genetic disorders in animals, controlled by recessive genes, are the cause of concern worldwide as animal carrier of the disease looks normal and may therefore be extensively used because of its breeding value. Hence, gene mutation or defective gene can easily spread to 50% of their progenies. In India, where HF animals are extensively used for crossbreeding programmes, it has become necessary to screen all HF and their crossbreds to minimize the risk of spreading these diseases among future bulls and bull mothers. Deficiency of Uridine Monophosphate Synthase (DUMPS) is one of such diseases especially in Holstein (Robinson *et al.,* 1993). In mammalian cells, the last step of pyrimidine nucleotide synthesis involves the conversion of orotate to uridine monophosphate (UMP) and is catalysed by UMP Synthase (Robinson *et al.,* 1983). The mutation (C→T) in a gene for UMP Synthase at codon 405 leads to a premature stop codon, which subsequently produces a functionally impaired enzyme (Schwenger *et al.,* 1993). The UMP Synthase gene is mapped on chromosome 1 (BTA1). Embryos homozygous for DUMPS do not survive to birth and usually die early in gestation, so no homozygous recessive animal was detected so far. 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). In the USA testing of reproductive bulls for DUMPS was started in 1988. It was established that all carriers are descendants of an elite bull Skokie Sensation Ned born in 1957.

Though the occurrence of DUMPS is very low as compared to other genetic diseases like BLAD, Citrullinaemia, FXI and CVM however, due to international trading of Holstein bulls and their frozen semen, many countries like Czech Republic (Citek *et al*., 2006), Poland (Kamiñski *et al*., 2005), India (Patel *et al*., 2006), Turkey (Oner *et al*., 2010), Pakistan (Rahimi *et al*., 2006), Romania (Vatasescu *et al*., 2006), Iran (Rezaee *et al*., 2009; Eydivandhi *et al*., 2011), etc. screened their Holstein cattle and found no carrier in the population. However, countries like China (Mei *et al*., 2009), Argentina (Poli *et al*., 1996), Japan (Ghanem *et al*., 2006) etc observed the presence of DUMPS carriers. Effects of carrier bulls in breeding programmes are deleterious, because if a bull is carrying one copy of the mutant gene (a heterozygote) and is mated with an unaffected cow, they will produce 50% heterozygous carriers in the population. If 2 heterozygous carriers are mated, then 25% of their offspring will be affected with the disease,

50% will be carriers, and only 25% will be normal. Considering the lethal effect of the diseases in dairy animals, genetic diseases are routinely screened in Indian dairy cattle (mainly bulls) so that the incidence of genetic disorders in dairy cattle can be reduced. This will also help in reducing economical losses to the organized farms or semen banks on rearing of carrier animals. This paper presents first case of heterozygous for DUMPS in an Indian Holstein bull found during routine screening.

MATERIALS AND METHODS

Blood samples were collected from 86 HF bulls stationed at various farms for the routine screening of chromosomal abnormalities and genetic disease diagnosis. DNA was extracted from blood cells by a standard phenol-chloroform method (Sambrook *et al*., 1989). The quality and quantity was estimated by using a spectrophotometer and agarose gel electrophoresis prior to PCR amplification. For detection of a possible mutation in a gene coding for UMP synthase, as described by Schwenger *et al*., (1993) with minor modifications, the 108-bp DNA fragment was amplified by PCR with sets of primers; forward (5'-GCA AAT GGC TGA AGA ACA TTC TG-3') and reverse (5'-GCT TCT AAC TGA ACT CCT CGA GT-3'). The amplification conditions include predenaturation at 94^{o}C for 3 min, followed by 35 cycles of 60 s for 94^{o}C, annealing of primers for 60 s at 60^{o}C, and extension for 60 s at 72^{o}C, followed by final extension at 72°C for 10 min. The PCR products of expected size were analysed on 2.5% agarose gel, stained with ethidium bromide and visualized under UV-transilluminator. The PCR products were next digested overnight by using *Ava*I restriction enzymes for DUMPS, in 1 × reaction buffer at 37^{o}C. The digested products were visualized on 2.5% agarose gel and documented on Gel Doc System.

RESULTS AND DISCUSSION

The amplified 108-bp product upon digestion by *Ava*I, to detect point mutation in a gene coding for uridine monophosphate synthase, yielded 3 bands of 53, 36 and 19 bp, respectively, in normal bulls, except one bull which digested product exhibited 4 bands of 89, 53, 36 and 19 bp (Fig 1), confirming presence of heterozygous for DUMPS in Indian Holstein which was validated by sequencing (ACCESSION No. JN039033).

The fewer cases were reported from a few countries except North America. In late 1987, the condition was declared an undesirable enzyme defect by the Holstein Association of America (HAA) and testing programmes were initiated, whereby heterozygotes were detected by half normal activity of erythrocyte UMP synthase (Robinson *et al*., 1993). From 1988 to 1991, 585 were identified as carriers out of 3461 animals screened for DUMPS. During the same period 1226 animals were tested in Europe with 414 shown to be carrier, higher percentage of carriers. Thus by 1992, over 1000 DUMPS carriers have been identified. Two carriers were found among 314 AI bulls, 682 bull mothers and 155 young bulls in Hungary (Fesus *et al*., 1999). Mutations in UMPS gene were also identified in 1.79% bulls and 0.96% cows in Argentina (Poli *et al*., 1996). Similarly two out of 1468 HF cattle were found carrier for DUMPS in Taiwan (Lin *et al*., 2001). However, DUMPS disorder in HF cattle is lethal during early embryonic period. On the basis of a modest number of animals examined, Jones *et al*., (1986) reported effects of age and sex on erythrocyte UMP activity; with newborn having 80% more than mature animals. With regards to sex males had 10% more activity than females. It is difficult to observe homozygous recessive genotype for DUMPS; however, Shanks *et al*., (1992) identified the homozygous recessive genotype for DUMPS in 35-days bovine embryos. Whereas no incidence of DUMPS carriers was observed in 2209 of Polish Holstein dairy herd (Kaminski *et al*., 2005). This was similar to the observation where Patel *et al*., (2006) screened 1250 including 976 cattle and 274 buffaloes in India, and found no DUMPS carrier. Though DUMPS carriers are reported less worldwide but it recommended continuing genetic screening of animals particularly Holstein and its crosses to avoid the risk of spreading such lethal disorders.

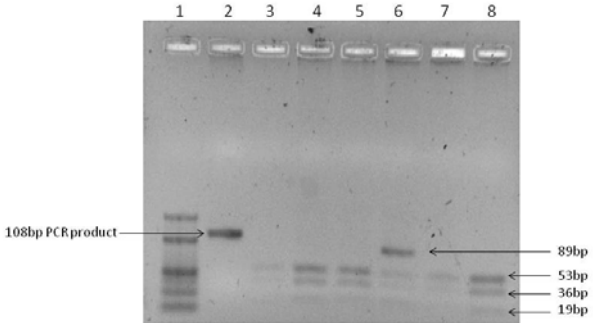

Fig. 1: Electrophoretogram of *Ava* I digested PCR product generated by amplification of genomic DNA using DUMPS specific primers. Lane # 1: O'Range Ruler™ 10bp DNA ladder (Fermentas), lane # 2: PCR product of 108bp. Lane # 3,4 5, 7 & 8: 53, 36 and 19bp bands respectively of normal animals and lane # 6: 89, 53, 36 and 19bp bands respectively of heterozygous animals.

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