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SHORT COMMUNICATION

Polymorphism in Argininosuccinate Synthase Gene in Indian Holstein

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ABSTRACT

The present study investigated the occurrence of an autosomal recessive genetic disease, Bovine Citrullinaemia caused by mutation in Argininosuccinate Synthase (ASS) gene, in Indian Holstein cattle. The Polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP) analysis was performed on a group of 120 Holstein bulls to identify carrier (heterozygous) animals. Two out of 120 (1.67%) animals were found carrier for Bovine Citrullinaemia. The gene and genotype frequency of recessive allele was estimated 0.0083 and 0.0167 in the 120 samples respectively. It is possible that these could be descendant of LMKK bull as Holstein bulls and frozen semen doses were imported in past for crossbreeding programmes in India. It is therefore, strongly recommended to screen breeding bulls for their breed specific genetic diseases before they are inducted in Artificial Insemination programme, to minimize the risk in future Holstein breeding bulls.

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INTRODUCTION

Bovine Citrullinaemia is a genetic disease, which has only been reported in Holstein cattle (Fesus *et al*., 1999). It is urea cycle disorder in humans and animals. A deficiency of the urea cycle enzyme results in a lethal neurological disease in newly born calves. The urea cycle involves a series of biochemical steps in which nitrogen, a waste product of protein metabolism, is removed from the blood and converted to urea. Calves affected with the disease appear normal immediately after birth. However, by the second day of life they become depressed and feed poorly. By the third day, they are often seen aimlessly wandering about their enclosure or standing with their head pressed against a fence or wall. Between day 3 and 5 the disease progresses rapidly. The calves appear to be blind and then they collapse. Death usually occurs within 12 hours of onset of these clinical signs (Healy *et al*., 1990). The clinical signs of Citrullinaemia are believed to be as a consequence of accumulation of ammonia in the brain of the affected calves.

Bovine Citrullinaemia was reported in Australia in 1986 (Harper *et al*., 1986) and the mutation responsible traced to a North American sire named Greyview Crisscrossh, the semen of whose son Linmack Kriss King (LMKK) was used extensively in Australia (Healy *et al*., 1991). About 8% of bulls considered for Artificial Insemination in Australia have proven to be heterozygous for the defective gene. LMKK-derived semen has been used extensively in New Zealand. However, no carriers were found black & white cattle in Germany (Grupe *et al*., 1996), Korea (Lee *et al*., 2002), India (Patel *et al*., 2006), Czech Republic (Citek *et al*., 2006), Turkey (Oner *et al*., 2010) and Iran (Eydivandi *et al*., 2012). Many carriers were detected in Australia (Healy *et al.,* 1991), USA (Robinson *et al*., 1993), India (Muraleedharan *et al*., 1999), Hungary (Fesus *et al*., 1999), Taiwan (Lin *et al*., 2001), China (Mei *et al*., 2009; Li *et al*., 2011), etc.

It has been established that bovine Citrullinaemia is a consequence of a deficiency of Argininosuccinate synthetase (ASS), one of the enzymes of the urea cycle. The deficiency of ASS occurs when a calf inherits a copy of the mutant gene encoding for ASS from each parent. The mutation occurs almost in the center of a particular segment of ASS DNA. The mutation is caused by a transition of cytosine (CGA/ Arginine) to thymine (TGA/stop codon) at codon 86 within exon 5 in the gene coding for ASS leading to impaired enzyme, which cannot participate in urea cycle (Dennis *et al*., 1989). The gene is located on chromosome No.11 (BTA11).

MATERIALS AND METHODS

DNA was extracted from blood samples collected from 120 Holstein bulls of different part of the country, by phenol-chloroform method as described by Sambrook *et*

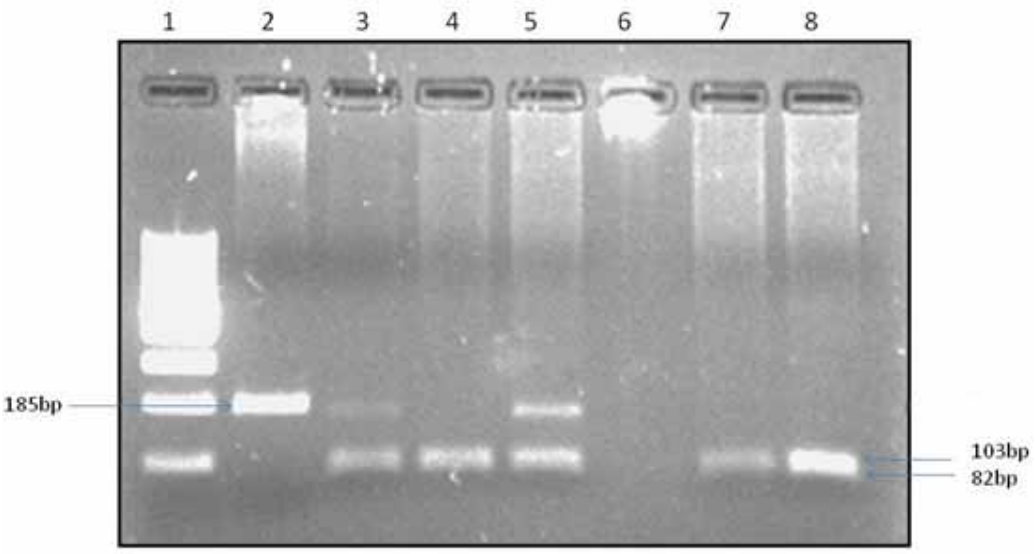

Fig. 1: Electrophoretogram (3% agarose gel) of *Ava* II digested PCR product generated by amplification of genomic DNA using Citrullinaemia specific primers. Lane # 1: Gene Ruler 100bp DNA ladder (Thermo Scientific), lane # 2: PCR product of 185bp. Lane # 4 7 & 8: 103 and 82bp bands respectively of normal animals and lane # 3 & 5: 185, 103 and 82bp bands respectively of heterozygous animals

al. (1989). The quality and quantity of DNA were determined using agarose gel electrophoresis and UV Spectrophotometry. For detection of mutation in a gene coding for Argininosuccinate Synthase, as described by Grupe *et al*. (1996), the 185 bp DNA fragment was amplified by PCR, which was set by adding sense primer (5' GGC CAG GGA CCG TGT TCA TTG AGG ACA TC 3') and antisense primer (5' TTC CTG GGA CCC CGT GAG ACA CAT ACT TG 3'). The PCR mix containing 1X PCR buffer, 0.2 mM dNTPs, 0.4 pM each of sense and antisense primer, 0.162 mM $MgCl_2$, 0.5 Unit *Taq* DNA Polymerase, 100 ng genomic DNA and finally added with sterilized distilled water to make a final volume of 25 µl. The PCR reaction included the following steps: Pre denaturation for 3 minutes at 94^{o}C followed by 40 cycles of 30 seconds at 94^{o}C, 30 seconds at 55^{o}C, and 30 seconds at 72^{o}C and final extension for 10 minutes at 72^{o}C for utilization of extra dNTPs in mixture.

The PCR product of 185 bp was seen on 1.5% agarose gel. The amplified or PCR products was digested by using *Ava* II (Restriction enzyme) and 1X reaction buffer at 37^{o}C for overnight. The digested product was visualized on 3% agarose gel.

RESULTS AND DISCUSSION

The amplified 185bp product upon digestion by *Ava* II, yielded two bands of 103 bp and 82 bp respectively for normal animals, and three bands of 185 bp, 103 bp and 82 bp in two Holstein bulls, indicating polymorphism in a gene coding for Argininosuccinate synthase (fig). Results indicate that out of 120 bulls, 2 bulls (1.67%) appear to be carriers for Citrullinaemia. The gene and genotype frequency of recessive allele was estimated to be 0.0083 and 0.0167 respectively. The occurrence of bovine Citrullinaemia was found high in Australia where this mutation is reportedly wide spread. Healy *et al.* (1991) reported that 50% of Australian national Holstein herds and 30% of breeding bulls in AI centres were descendants of Linmack Kriss King (LMKK), which was carrier for Citrullinaemia. In other countries like USA and Germany, the incidence of the Citrullinaemia is very low (Robinson *et al*., 1993; Grupe *et al*., 1996). In India, one case of Citrullinaemia was reported in a Holstein bull imported from Australia and that could be descendant of LMKK (Muraleedharan *et al*.., 1999). The bull was immediately culled from the sperm station to prevent spreading of mutant gene in Holstein and its crossbred populations. Later on, Patel *et al*., (2006) reported no carrier of Citrullinaemia in Holstein and their crosses in India. However, fresh cases of Citrullinaemia in 2 Holstein bulls is once again alarming and emphasizing to continue the screening of genetic disorders to prevent further replication of carriers in Holstein and their crossbred population in India. These carriers might be descendant of LMKK bull as Holstein bulls and frozen semen doses were imported in past for crossbreeding programmes in India.

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