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Prevalence of genetic diseases in Holstein crossbred (Frieswal) bulls in India

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Carrier animals of autosomal recessive disorders are most likely to transmit the mutant allele to the next generation. The same problem may assume dreadful significance if the carriers are bulls intended to be used in artificial breeding programmes. Hence it is necessary to screen all animals, especially AI bulls to minimize the risk of spreading these diseases to next generation. Of the many genetic diseases identified in cattle, bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS), bovine citrullinaemia (BC), and factor XI deficiency (FXID) exist in Holstein Friesian breeds. Frieswal cattle is one of the crossbred cattle of India with 5/8 Holstein Friesian (HF) and 3/8 Sahiwal blood. As exported HF semen was used for the development of this breed, it is vulnerable to the genetic diseases reported in the HF.

BLAD is an autosomal recessive hereditary disease characterized by recurrent infections due to the reduced expression of the heterodimeric β2 integrin adhesion molecules on leukocytes resulting in multiple defects in leukocyte function (Nagahata 2004). At DNA level, a missense mutation due to substitution of adenine to guanine nucleotide at 383rd position in the CD18 gene located in the bovine chromosome 1 results in deficiency of CD11/CD18 glycoprotein complex located on the surface of neutrophils and other granulocytes (Kehrli *et al.* 1990). Bovine citrullinemia is a metabolic disorder of impaired urea cycle caused by non-sense mutation due to the transition of cytosine into thymine at 86th codon of argininosuccinate synthetase enzyme coding gene. Another disorder, FXI deficiency leads to prolonged bleeding and associated health problems and is caused by an insertion of 76 bp within the exon 12 of this gene (Marron *et al.* 2004). DUMPS causes early embryonic mortality during implantation due to impaired UMP synthase enzyme. At molecular level, a non-sense mutation due to transition of C→T for UMPs at codon 405 within exon 5 mapped to the bovine chromosome 1 (Citek *et al.* 2006). The present study was conducted to assess the prevalence of bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS), bovine citrullinaemia (BC) and factor XI deficiency (FXID) in Frieswal (Sahiwal × HF) young bulls.

Table 1. Primers, annealing temperature, PCR product size and restriction enzymes (RE) used for identification of BLAD, bovine citrullinaemia, DUMPS and factor XI deficiency

Genetic disease	Primer sequence (5'→3')	Annealing temperature (°C)	PCR product size (bp)	Restriction enzyme	Reference
BLAD	F-GAATAGGCATCCTGCATCATATCCACCA R-CTTGGGGTTTCAGGGGAAGATGGAGTAG	65°C	357 bp	Taq1	Meydan *et al. (*2010)
BC	F-GGCCAGGGACCGTGTTCATTGAGGACATC R-TTCCTGGGACCCCGTGAGACACATACTTG	65°C	198 bp	Ava2	Grupe *et al*. (1996)
DUMPS	F-AGGGTCTTAGTGGAGCAGGT R-GGCTTACCTCCTGCTTCTAACTG	65°C	282 bp	Ava1	Designed
FACTOR XID	F-CCCACTGGCTAGGAATCGTT R-CAAGGCAATGTCATATCCAC	60°C	244	_	Marron *et al*. (2004)

Blood samplesfrom Frieswal young bulls (151), reared in the bull rearing unit of the Institute, were collected aseptically from the jugular vein of animals in EDTA

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containing tubes. Genomic DNA was extracted by conventional phenol-chloroform method as described by Sambrook *et al*. (1989) with minor modifications. Genotyping of BLAD, DUMPS and BC were done by PCR-RFLP method. The genotypes of FXID were detected by PCR method. The primers, annealing temperature, PCR product sizes and restriction enzymes (RE) used for identification of each genetic disorder are presented in Table 1.

Polymerase chain reaction (PCR) was carried out in a final reaction volume of 10µl. PCR cocktail consisting of 50 to 100 ng of genomic DNA, 200 µM of each dNTP, 5 picomoles of each primer, 1 unit of Taq DNA polymerase and Taq buffer having 1.5 mM $MgCl_2$ were used for each reaction. DNA was amplified with initial denaturation at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 30 sec, specific annealing temperature for 45 sec, extension at 72°C for 35 sec, with final extension 72°C for 10 min. Amplification was assessed by 2% agarose gel electrophoresis. An 8 µl volume of each PCR products of BLAD, DUMPS and BC genes were digested with Taq1, Ava1 and Ava2, respectively, in a final volume of 15 µl. The digestion cocktail contained 10 units of enzyme. The digested products were separated on 3% agarose gel and analyzed by gel doc system. Both mutant allele and wild allele of BLAD are sequenced using Automated DNA Sequencer in the both directions. Raw sequence data were edited using Chromas (Ver. 1.45, http://www.technelysium.com) and the variations were confirmed by manual inspection of chromatograms. The BLAST algorithm was used to analyze the mutation in the generated sequence against the sequences in the NCBI Gen bank databases.

To trace the inheritance, the bull pedigree for the carrier bull calves were analyzed and representative pedigree chart was prepared using PROGENY9 (www.progenygenetics.com).

Normal BLAD allele produced 2 fragments of 201 and 156 bp while BLAD carriers produced 3 fragments of 357, 201 and 156 bp (Fig. 1). Out of 151 young bulls screened, 7 were found heterozygous for BLAD allele. A carrier prevalence of 4.6% was noticed for BLAD. The allelic frequency of BLAD mutant was 0.023. Chromatograph analysis confirmed the presence of mutated allele (Fig. 2). The pedigree analysis (Fig. 3) of all the carrier animals traced that the imported semen of exotic HF Valeriant could be the source of BLAD in Frieswal herd. Incidence of BLAD was very high (23%) in an earlier report (Shuster *et al.* 1992). Due to regular screening, prevalence rate across the world is reducing. Recently, low incidences of BLAD were observed in Holstein populations in different parts of the world (Meydan *et al*. 2010, Li *et al.* 2011) and in India (Rajesh *et al.* 2007, Arpita *et al.* 2012, Patel *et al.* 2012). The prevalence of carrier and affected animals in Karan Fries (Holstein and Tharparkar cross) was 3.64 and 1.84%, respectively (Yathish *et al.* 2010).

None of the young bulls in the present study were either affected or carrier for BC, DUMPS and FXID (Fig. 1). Similar results were reported for bovine citrullinemia (Rajesh *et al*. 2006, Citek *et al.* 2006, Meydan *et al.* 2010), FXID (Mukhopadhyaya *et al.* 2006, Cyrus *et al.* 2011) and DUMPS (Rajesh *et al.* 2006, Rahimi *et al.* 2006, Meydan *et al.* 2010). Contrary to this, presence of FXID carriers were reported from USA (Marron *et al.* 2004), Japan (Ohba

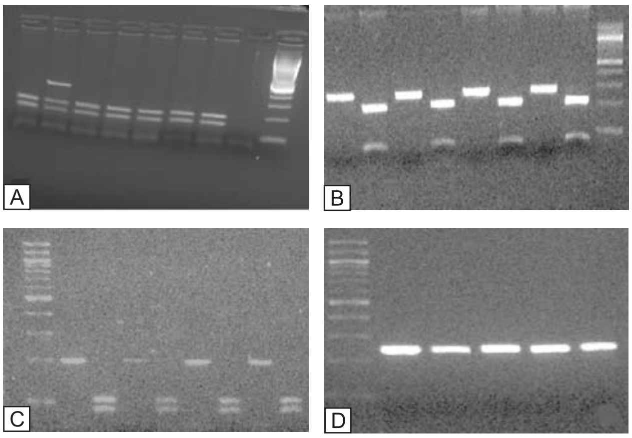

Fig. 1. Illustration of BLAD, DUMPS, BC and FXID genotypes on agarose gels. Lanes are numbered from left to right. Marker used is 100 bp. (A) BLAD genotypes; lane 2, digestion pattern for heterozygous animals with 3 bands of 357, 201 and 156 bp; lanes 1, 3, 4, 5, 6 and 7, normal homozygote pattern with 201 and 156 bp bands. (B) DUMPS genotypes; lanes 1, 3, 5, 7, undigested PCR products; and lane 2, 4, 6, 8 are digested products. All the digested products showed normal homozygote pattern of 213 and 69 bp. (C) Bovine citrullemia genotypes; lanes 2, 4, 6 and 8, undigested PCR products; lanes 3, 5, 7 and 9, digested products. All the digested products showed normal homozygote pattern of 109 and 89 bp. (D) FXIDgenotype; lanes 2– 6, PCR products showing the size of 244 bp normal of homozygote genotype.

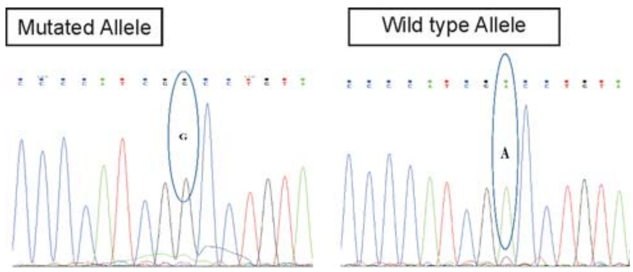

Fig. 2. Chromatograph analysis showed the presence of guanine in mutated allele instead of adenine in the wild type allele.

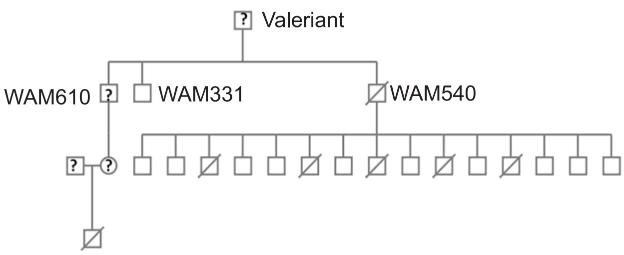

Fig. 3. Pedigree chart for the carrier bull calves identified.

et al. 2008, Ghanem and Nishibori 2009), Turkey (Meydan *et al.* 2010, Oner *et al.* 2010), India (Padeeri *et al.* 1999, Rajesh *et al.* 2007), Poland (Gurgul *et al.* 2009) and China (Li *et al*. 2011). DUMPS allele has been reported in America and Argentinian Holstein population in 1990s (Meydan *et al*. 2010).

The extensive use of few elite sires through AI makes it possible for the spread of genetic diseases. It is not possible to distinguish carrier animals and normal calves morphologically while affected cattle shows symptoms of recurrent infections and die early in life. Current molecular tools enables a rapid screening of breeding populations in order to eliminate the carriers from the population of potential breeding sires, thus decreasing the number of affected progeny. This study showed that PCR-RFLP analysis is a strong and reliable method for identification of BLAD, DUMPS and BC while simple PCR is enough for identifying FXID mutations.

Based on the results, the prevalence of recessive disorders of BLAD, DUMPS, bovine citrullinaemia and FXID seems low in India. It has to be taken with precaution as the actual number of clinical cases of BLAD, DUMPS, Bovine Citrullinaemia and FXID are unknown in India. As carriers were found for BLAD, sporadic cases may appear. Hence, the monitoring of all these diseases in young bulls should be continued.

SUMMARY

The present study was conducted to assess the prevalence of genetic diseases, viz. BLAD, DUMPS, BC and FXID in Frieswal (Sahiwal × HF) young bulls. We used the standard PCR-RFLP method to genotype animals for BLAD, DUMPS and BC while simple PCR was done to genotype FXID. The identified BLAD carriers were further verified by sequencing. A carrier prevalence of 4.6 % was noticed for BLAD in Frieswal young bulls while no carriers were identified for other genetic diseases. Pedigree analysis revealed that imported semen of Holstein Friesian bull Valeriant may be the source for the spread the BLAD in Indian Frieswal herd. The present study indicates the existence of BLAD carrier animals in Frieswal bull calves eventhough at a relatively low frequency. Hence it is recommended to screen young bulls at early stages for these defective genes in order to avoid spread within the population.

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