

PCR based identification of bovine leukocyte adhesion deficiency syndrome (BLAD) carriers in Karan Fries bulls

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ABSTRACT

Bovine leukocyte adhesion deficiency syndrome (BLAD) is a lethal autosomal and recessive inherited disease in Holstein Frisian (HF) and its crosses, characterized by a missense mutation in CD18 gene at the position of 383. This gene is part of $\beta 2$ integrin gene, which is responsible for migration of leukocytes from blood vessels to the site of inflammation. BLAD affected animals exhibit a deficiency of $\beta 2$ integrin of leukocyte and so neutrophil functions are severely impaired. In this study 52 Karan Fries (KF) bulls were examined and 4 animals were found to be carriers for this disorder. Undesired allele frequency was estimated as 0.038. Although this syndrome has been reported from many countries this paper is the first documented report for Karan Fries bulls in India. This also calls for screening of other population of HF and its crosses in the country and castration of carrier bulls can be a solution to prevent further propagation of the recessive alleles of BLAD.

Key words: BLAD, CD18 gene, Karan Fries, Missense mutation

Bovine leukocyte adhesion deficiency (BLAD) is an autosomal recessive genetic disease that especially affects Holstein breed and its crosses (Shuster *et al.* 1992). The animal with BLAD is characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth, periodontitis, loss of teeth, delayed wound healing, persistent neutrophilia and death at an early age (Nagahata *et al.* 1987, Hagemoser *et al.* 1983). Most of the calves positive for BLAD also exhibit stunted growth and a persistent progressive neutrophilia. BLAD is a lethal, autosomal and recessive inherited disease (Healy 1996, Agerholm *et al.* 1993).

The determining gene CD18 is located on the Bta 1 and the disease is the result of a mutation, which changes guanine to adenine at the 383rd nucleotide (Kehrli *et al.* 1991). There is a deficiency in leukocyte surface glycoproteins known as Integrins. These proteins are responsible for the cell-cell interactions necessary for neutrophils to adhere to vascular endothelium, enter the tissues and destroy invading pathogens (Kishimoto *et al.* 1989). The integrin family includes

leukocyte function-associated antigen (LFA 1), macrophage antigen (Mac-1) and p150, 95. These 3 adhesion molecules are heterodimers, composed of a unique alpha subunit (CD11a, CD11b and CD11 c) and a common beta subunit (CD18) (Springer 1990). Since integrin expression requires intracellular association of both CD11 and CD18 subunits, CD18 defect prevents all integrin functions (Kishimoto *et al.* 1989). BLAD affected animals have deficiency in $\beta 2$ integrin of leukocyte and so neutrophil functions are severely impaired (Kehrli *et al.* 1990, Nagahata *et al.* 1995). BLAD animals demonstrate progressive neutrophilia and excessive leukocytes (100 000 leukocytes/ μ l) (Kehrli *et al.* 1994). Most calves with BLAD die before diagnosis, probably before 1 year of age. Some cows are able to survive for more than 2 years; however their reproductive and milk performance is poor. Two point mutations were identified in the gene that encodes bovine CD18 in Holstein cattle affected with BLAD (Shuster *et al.* 1992). One mutation replaces adenine with guanine at nucleotide 383, it is a missense mutation resulting in an aspartic acid to glycine substitution at amino acid 128 (D128 G) in the glycoprotein while the other replaces cytosine with thymine at nucleotide 775. The latter is a silent mutation and therefore causes no alteration in the amino acid sequence. In this study karan Fries bulls were screened for their BLAD status.

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MATERIALS AND METHODS

Blood (10 ml) was collected from 52 Karan Fries Bulls (Holstein Friesian $\times$ Tharparkar) maintained at the Institute. Genomic DNA was isolated from the blood by using DNA kit. The quality of the DNA was assessed by 0.6% submarine horizontal agarose gel electrophoresis. The PCR was performed using primers consisting of 21 bases each (5'-AGG TCA GGC AGT TGC CTT CAA -3' and 5'-GGG GAG CAC CGT CTT GTC CAC -3') as reported by Czarnik and Kaminski (1997). The PCR was performed in 25 μ l of final volume: 100 ng/ml sample DNA, 1 U *Taq* DNA polymerase. The PCR mixture was heated and kept at 95°C for 5 min, then revolved in 30 cycles, each cycle consisting of 30 sec of each of the temperature levels of 94, 61 and 72°C. After the last cycle the samples were kept at 72°C for 5 min and the PCR procedure was finished. After the PCR, a fragment of 367 bp length was amplified. The PCR products were migrated on 2% agarose gel. On the gel the band of 367 bp length was studied in UV light and the fragment was cut with *Taq* I restriction enzyme.

RESULTS AND DISCUSSION

The restriction enzyme *Taq* I digested the PCR product into 2 fragments of 54 and 313 bp in normal homozygous (TL/TL) animals. Heterozygous animals (BL/TL) revealed 3 fragments of 54, 313 and 367 bp (Fig. 1). On electrophoresis we observed that 4 animals out of 52 were heterozygous for BLAD and no homozygous recessive animal was found. The genotypic frequency of heterozygous carrier animals was estimated as 7.311% and undesired allele frequency was 0.038. In order to confirm the occurrence of CD 18 mutation, DNA samples of carrier animals were sequenced by use of automated DNA sequencer. The results justified the enzyme digestion for carrier animals (GenBank Accession Numbers: EU826000 and EU826001).

In India, the carrier animals' frequency were found 1.33% and 3.23% in Holstein cows by Muraleedharan *et al.* (1999) and Patel *et al.* (2007) respectively. The incidence of BLAD carriers among top sires was 23% in USA (Shuster *et al.* 1992), 10% in France (Tainturier *et al.* 1995), 13.5% in Germany (Boichard *et al.* 1995), 2.88% in Argentina (Poli *et al.* 1996), 16% in Japan (Nagahata *et al.* 1995), 2.8% in Brazil (Ribeiro *et al.* 2000) and 3.33% in Iran (Norouzy *et al.* 2005).

As imported HF bulls or their semen doses have been extensively used during the last five decades in India, mainly for crossbreeding programmes, it became mandatory to screen HF and its crossbreds for autosomal recessive disorders before they are used in breeding programmes. Although the initial incidence of BLAD is low, the actual number of carriers could be substantially higher in the future if the animals are not regularly screened for BLAD. The artificial insemination programme in dairy animals accelerates the spread of undesirable genetic disorders.

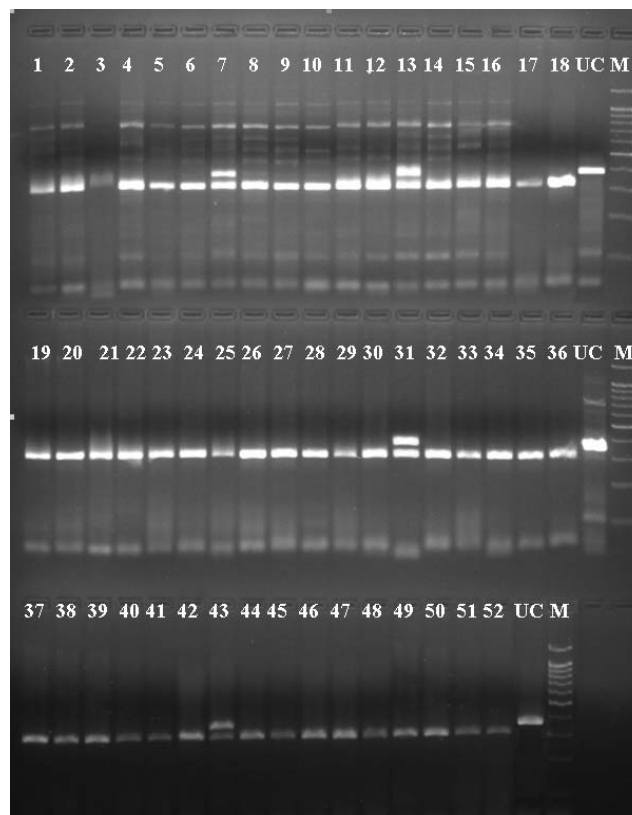


Fig 1. *Taq*I digestion of PCR Product on 2% Agarose Gel. UC: Un cut sample; M: 100 bp Marker; Wild type Samples: 54 bp, 313 bp.; Carrier samples: 54 bp, 313 bp and 367 bp. Lane: 7, 13, 31 and 43.

Therefore, caution is required for reducing the risk of dissemination of recessive defects resulting from increased selection pressure within the dairy industry, which is presently dominated by HF breed worldwide. The present findings and eradication of the BLAD carriers are of great significance in India for animal improvement programmes. This also calls for screening of other population of HF and its crosses in the country and castration can be a solution to prevent further propagation of the recessive alleles by KF carrier bulls.

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