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DETECTION OF GENETIC POLYMORPHISM IN CD18 GENE IN CATTLE BY PCR-RFLP

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BLAD is autosomal recessive genetic disease that affects Holstein breed world wide. It is a disease characterized by reduced expression of the adhesion molecules on neutrophils. The disease is caused by a mutation which replaces adenine at 383 with Guanine that changes amino acid, Aspartic acid to Glycine, leading to the expression of a wrong protein (CD18) that is impaired in function. Blood samples were collected from 42 HF and HF crossbred phenotypically normal bulls maintained at different sperm stations of Gujarat. PCR-RFLP was performed to detect point mutation in CD18, surface molecules of neutrophils. Results indicate that out of 42 bulls, 2 bulls appear to be carriers for BLAD. The condition is alarming and needs regular screening of HF and its crossbreds to avoid risk of spreading BLAD in breedable population of India.
Key words: BLAD, Autosomal recessive, CD18, nutrophils, PCR-RFLP

The autosomal recessive genetic diseases in cattle are breed specific and one of them is Bovine Leukocyte Adhesion Deficiency Syndrome (BLAD). It is a genetic disease that affects especially Holstein breed (Shuster et al., 1992). The defect was first identified in North American Holstein and was exported to other national Holstein. BLAD is a disease characterized by reduced expression of the adhesion molecules on neutrophils called β-integrins, a complex of CD11/CD18 family of proteins that are structurally and functionally related glycoproteins. These proteins help the neutrophils to migrate to the site of inflammation. 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). The carrier frequency of BLAD among US Holstein cattle once had reached to approximately 15% among active breeding bulls and 8% among cows. Besides, owing to the wide spread use of top breeding HF bulls imported from USA, many countries reported a high incidence of BLAD carriers in their black and white population (Pareek and Kaminiski, 1996; Lubieniecki et al., 1999).
The disease is caused by a mutation which replaces adenine at 383 with Guanine that change amino acid, Aspartic acid to Glycine. The mutation ultimately leads to a wrong protein (CD18) that is impaired in function (Shuster et al., 1992). However, continuous screening of young bulls before entering to Artificial insemination (AI) stations is reducing the incidence of BLAD carriers among HF animals. With the wide use of AI and international trading of semen and breeding bulls, this genetic disease can spread to a large population as carrier animals of the disease look normal. 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 this disease among future bulls and dams. Keeping the lethal effect of the diseases in dairy animals, the present study was undertaken to screen Holstein and its crossbreds to investigate occurrence of the disease in Indian dairy cattle.

MATERIALS & METHODS

CBlood samples were collected in heparinized blood collecting tubes from 42 HF and HF cross bred bulls belonging to sperm stations in Gujarat. The DNA was extracted by phenol-chloroform method as described by Sambrook et al., (1989). The quality and quantity of DNA were determined using agarose gel electrophoresis and UV spectrophotometry.
As described by Czarnik and Kaminski (1997), the 367 bp DNA fragment was amplified by Polymerase chain reaction (PCR), which was set by adding forward primer (5' AGG TCA GGC AGT TGC CTT CAA 3') and reverse primer (5' GGG GAG CAC CGT CTT GTC CAC 3'). The PCR mixture contained 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.8 pM each of forward and reverse primer, 0.5 Unit Taq DNA Polymerase, 100 ng genomic DNA and distilled water to make a final volume of 25 l. The PCR reaction included the following steps: predenaturation for 3 minutes at 94oC followed by 35 cycles of 30 seconds at 94oC, 30 seconds at 61oC, 30 seconds at 72oC and final extension for 10 minutes at 72oC for utilization of extra dNTPs in mixture.
The amplified PCR product was digested by using Taq I at 65C for overnight. The digested product was visualized on 2.5% agarose gel.

RESULTS AND DISCUSSION

In our investigation, out of 42 Holstein and Holstein Crossbreds bulls, two bulls were found heterozygous of BLAD (carriers), as shown in the figure, and rest were found to be homozygous normal. The percentage of recessive allele in the sample was calculated to be 4.76. The gene and genotype frequency of recessive allele was calculated 0.02 and 0.04762 in the 42 samples respectively. The size of PCR product was 367bp and it was subjected to RFLP analysis using Taq-1 restriction enzyme. In normal bulls, the PCR products yielded two fragments of 313bp and 54bp, whereas carrier (heterozygous) three fragments of 367bp, 313bp and 54bp.

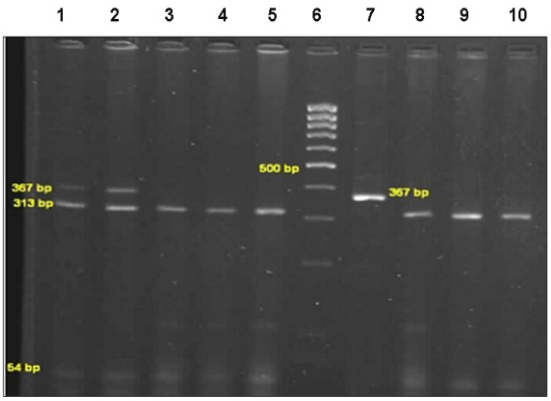

figure: Electrophoretic pattern indicates; Lane 1 & 2 show three bands of 367bp. 313bp and 54bp for heterozygous (carrier) for BLAD. Lane 3-5 & 8-10 show two bands of 313bp and 54bp for normal. Lane 6 indicates 100 bp DNA ladder. Lane 7 is PCR products of 367 bp

Development of artificial insemination enabled the advent of modern breeding practices worldwide. These practices involve importation of HF bulls or their semen, intense selection of bulls based on their daughters lactation performance and the widespread use of these few genetically superior bulls. During the last four decades, these practices have not only increased the milk production in HF cattle but also the within breed genetic relatedness among individuals, resulting expression of recessive genetic

diseases.

This has made the screening a mandatory practice for autosomal recessive disorders in farm-born HF and its crossbreds prior to their use for breeding programs. Though the initial incidence of BLAD is low, number of carriers could be substantially higher in coming days if the animals are not screened routinely for BLAD. As the selection pressure within a breed and AI programmes are major factors to spread of undesirable genetic disorders, a routine screening of bulls is required to reduce the recessive disorder in cattle population.

In India, Muraleedharan et al., (1999), Patel et al., (2006), Kumar (2009), Mahdi et al., (2010), and Yathish et al., (2010) have reported the carrier animal's frequency of 1.33%, 3.23%, 21.82%, 7.31%, and 3.64% in Holstein animals and its crosses respectively. However one recessive homozygous (affected) Karan Fries bull was observed by Yathish et al., (2010). The incidence of BLAD carriers among top sires was found to be 23 % in USA (Shuster et al., 1992), 10% in France (Tainturier et al., 1995), 13.5 % in Germany (Biochard 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). In our present investigation the frequency of carrier animals was found as 4.76%.

Routine and mandatory screening of HF animals contained the rapid spread of BLAD in several countries. Restricted breeding and long-term investigations have enabled a great reduction of this threat to the population. Hence, the routine screening of BLAD as well as other genetic disorders should be continued to reduce incidences in cattle population.

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