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SHORT COMMUNICATION

New Cases of Bovine Leukocyte Adhesion Deficiency (BLAD) Carriers in Indian Holstein Cattle

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ABSTRACT

Bovine Leukocyte Adhesion Deficiency (BLAD) is autosomal recessive genetic diseases that affects Holstein breed world wide. It is a disease characterized by reduced expression of the adhesion molecules on neutrophils. The disease caused by mutation which replaces adenine at 383 with guanine that change amino acid, aspartic acid to glycine, leading to wrong protein (CD18) that is impaired in function. Blood samples were collected from 126 Holstein phenotypically normal bulls maintained at different sperm stations in India. PCR-RFLP was performed to detect heterozygous (carrier). Results indicate that out of 120 bulls, 2 bulls (1.59%) appear to be carriers for BLAD. The gene and genotype frequency of recessive allele was estimated 0.008 and 0.016 in the 126 samples respectively. The condition is alarming and emphasizes regular screening of Holstein AI bulls and its crossbreds to avoid risk of spreading BLAD in breedable population of India.

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INTRODUCTION

Most of the autosomal recessive genetic diseases in cattle are breed specific and one of them is frequently occurring genetic disorder, Bovine Leukocyte Adhesion Deficiency Syndrome (BLAD) that affects especially Holstein breed (Shuster *et al*., 1992). The defect was first identified in North American Holstein and was exported to other countries. 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 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). BLAD carriers were among the most prominent bulls of the Holstein breed such as Osborndale Ivanhoe, Penstate Ivanhoe Star and Carlin-M-Ivanhoe Bell. Affected cattle with BLAD were linked to common ancestral sires that were carriers (Shuster *et al.,* 1992; Jorgensen *et al.,* 1993; Powell *et al.,* 1996). The carrier frequency of BLAD among US Holstein cattle once had reached to approximately 15% among active breeding bulls and 8% among cows (Shuster *et al.,* 1992). Besides, owing to the wide spread use of top breeding Holstein bulls imported from USA, many countries reported a high incidence of BLAD carriers in their black and white population; Denmark (Agerholm *et al.,* 1993), United Kingdom (Andrews *et al.,* 1996), Dutch (Bernadina *et al.,* 1993), Hungary (Fesus et al., 1999), Taiwan (Huang *et al.,* 2000), Japan (Nagahata *et al.,* 1997), Austria (Schilcher *et al.,* 1995), Brazil (Ribeiro *et al*., 2000), Iran (Noruzy *et al.,* 2005), China (Jianbin *et al.,* 2011), India (Muraleedharan *et al*., 1999, Patel *et al.,* 2007, Mahdi, 2008, Kumar, 2009, Patel *et al.,* 2011) etc. Holsteins with BLAD (affected) were also reported in many countries (Nagahata, 2004).

The molecular basis of BLAD is a single point, which replaces adenine to guanine at 383 of the CD18 gene that change amino acid, aspartic acid to glycine at amino acid 128 in the functional protein. The mutation ultimately leads to wrong protein (CD18) that is impaired in function (Shuster *et al.,* 1992).

Development of artificial insemination enabled the advent of modern breeding practices worldwide. These practices involve importation of Holstein bulls or their semen, intense selection of bulls based on their daughters lactation yield 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 Holstein and their crossbreds cattle but also the within breed genetic relatedness among individuals, resulting expression of recessive genetic diseases (Rusc and Kaminiski, 2007; Patel *et al.,* 2011).

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**.** 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 animals.

MATERIALS AND METHODS

Blood samples were collected in to the EDTA blood collecting tubes from 126 Holstein bulls stationed at sperm stations located at various part of country. 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 Kriegesmann *et al*. (1997), 343 bp DNA fragment was amplified by Polymerase chain reaction (PCR), which was set by adding forward primer (5'-CCTGCATCATATCCACCAG-3') and reverse primer (5'GTTTCAGGGGAAGATGGAG -3'). The PCR mix contained 1X PCR buffer, 1.5 mM $MgCl_2$, 10 mM dNTPs, 5 pM each of forward and reverse primer, 5 Unit *Taq* DNA Polymerase, 50ng genomic DNA and distilled water to make a final volume of 18µl. The PCR reaction included the following steps: predenaturation for 3 minutes at 94^{o}C followed by 35 cycles of 30 seconds at 94^{o}C, 30 seconds at 61^{o}C, 30 seconds at 72^{o}C and final extension for 10 minutes at 72^{o}C for utilization of extra dNTPs in mixture.

The amplified PCR product was digested by using *Taq*I at 65°C for overnight. The digested product was visualized on 2.5% agarose gel.

RESULTS AND DISCUSSION

In our investigation, out of 126 Holstein bulls, two bulls were found heterozygous of BLAD (carriers) and rest were homozygous (normal), as shown in the figure-1. The percentage of recessive allele in the sample was calculated to be 1.59. The gene and genotype frequency of recessive allele was calculated 0.008 and 0.016 in the 126 samples respectively. The size of PCR product was 343bp and it was subjected to RFLP analysis using *Taq*-1 restriction enzyme. In normal bulls, the PCR products yielded two fragments of 191bp and 152bp, whereas carrier (heterozygous) three fragments of 343bp, 191bp and 152bp.

In India, Muraleedharan *et al.* (1999), Patel *et al.* (2007), Kumar (2009), Mahdi et al. (2010), and Yathish *et al.* (2010), Patel *et al.* (2010) have reported the carrier animal's frequency of 1.33%, 3.23%, 21.82%, 7.31%, 3.64% and 4.76%, in Holstein animals and its crosses respectively. However one recessive homozygous (affected) Karan Fries bull was also 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 1.59%.

With the wide use of artificial insemination and international trading of semen and breeding bulls, these genetic diseases can spread to large population as animals' carrier 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 these diseases among future bulls and bull mothers. However, continuous screening of young bulls before entering in artificial insemination (AI) stations is reducing the incidence of BLAD carriers among HF animals.

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.

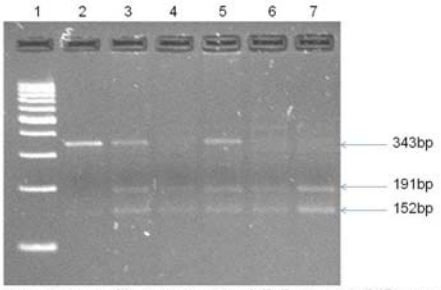

Fig-1: Electrophoretogram (2% agarose gel) of *Taq*I digested PCR product generated by amplification of genomic DNA using BLAD specific primers. Lane # 1: Gene Ruler 100bp DNA ladder (Thermo Scientific), lane # 2: PCR product of 343bp. Lane # 4,6 & 7: 191 and 152bp bands respectively of normal animals and lane # 3 & 5: 343, 191 and 152bp bands respectively of heterozygous animals

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