Incidence of bovine leukocyte adhesion deficiency carrier in breeding sires of West Bengal


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Abstract: Bovine leukocyte adhesion deficiency (BLAD) is a genetically transmitted disease and inherited as an autosomal recessive defect. Keeping this in view the present study was conducted to estimate incidence of carrier animals in a herd of breeding bulls for mutation in Integrin α2 gene, reared at Frozen Semen Bull Station (FSBS), Harirnghata, Nadia, West Bengal, India. A total of 80 bulls of different age group were randomly selected from different cattle breeds (Sahiwal, Gir) to screen presence of BLAD syndrome in breeding sires. Genomic DNA was isolated from blood of the selected bulls. PCR parameters were standardized to obtain 570 bp amplicon. This amplified PCR product (amplicon) of ITGB2 gene was digested using TaqI (5’T”!CGA3’) restriction enzyme for screening of mutation in BLAD allele. TaqI PCR-RFLP revealed no such mutation thus indicating the absence of bovine leukocyte adhesion deficiency (BuLAD) allele in animals under study.

Key words: BLAD, ITGB2, PCR-RFLP, TaqI

Introduction
Bovine Leukocyte Adhesion Deficiency (BLAD) is an autosomal lethal recessive disorder. In BLAD affected animals expression of ITGB2 gene is greatly reduced, effecting regular leukocyte function. Heterodimeric α2 integrin are adhesion molecules intercede the movement of neutrophiles across membranes to annihilate foreign bodies [Kehrli et al., 1992 and Poli et al., 1996]. Thus animal with homozygous BLAD allele is affected by recurrent infections with diarrhea, pneumonia, delayed wound healing coupled with higher mortality and hampering production of the herd [Kehrli et al., 1990]. The only way to avoid transmission and economic losses due to BLAD is early detection of carriers in herds maintained at Frozen Semen Bull Stations, employed for the purpose of artificial insemination. It has been established that in BLAD affected cattle the codon at 382nd to 384th nucleotide position; GAC (aspartic acid: D) changes to GGC (glycine: G) due to mutation at the position A’!G at 383rd position in the cDNA of the CD18 gene (D128G) [Gerardi et al., 1996; Jorgensen et al., 1993; Rutten et al., 1996; Shuster et al., 1992]. Differences between healthy and affected calves by means of PCR-RFLP method using restriction endonuclease TaqI or HaeIII can be ascertained in early ages [Shuster et al., 1992]. The present study was conducted to screen for the presence of BLAD allele in breeding sires from different indigenous breeds (Sahiwal, Gir) maintained at Frozen Semen Bull Station (FSBS), Harirnghata, Nadia, West Bengal, India.

Material and Methods
Animals surveyed and blood collection: For this study a total of 80 breeding bulls maintained at Frozen Semen Bull Station (FSBS), Harirnghata, Nadia, West Bengal, India were chosen randomly from different cattle breeds (Sahiwal, n=41; Gir, n=39). Approximately 5 ml of venous blood was collected aseptically from the jugular vein in BD® Vacutainer (Becton, Dickinson and Company, 162 USA) containing EDTA as anticoagulant and transported to the lab on ice.

PCR amplification of ITGB2 gene segment:
Nucleic Acid isolation: DNA isolation was carried out as per standard phenol-chloroform procedure [Sambrook and Russel, 2001]. The isolated DNA was checked for purity and quantity using Nanodrop Spectrophotometer (ND-1000, Thermo Fisher Scientific, USA) and the integrity was assessed by running in 0.8% agarose gel. The isolated genomic DNA was stored at -20°C until further use.

PCR amplification: PCR amplification was performed in thin walled 0.2ml PCR tubes to amplify a 570 bp fragment of the ITGB2 gene. 25 ll reaction mixture was optimized as approximately 50-100 ng of genomic DNA, 2.5 lI 10X PCR buffer, 2 mM MgCl2, 1l of 10mM dNTPs, 10 lM of forward and reverse primers (enumerated in...
Table 1), 1U of Taq DNA Polymerase (Thermo Scientific, USA) and the residual volume was made up with Nuclease Free water (NFW). The PCR amplification was performed in a thermocycler (iCycler®, Bio-Rad, USA) and the cycling parameters have been enumerated in Table 1 (Sharma et al., 2009). For all the PCR runs, a non-template PCR was performed to rule out chances of contamination. The PCR amplified products were resolved on 2.5% w/v agarose gel in Tris acetate EDTA (TAE) buffer (1X). The agarose gel stained with ethidium bromide was visualized under UV light in a gel documentation system (G: Box, Syngene, UK).

Restriction digestion of amplicon: The amplified PCR products (amplicon) of ITGB2 gene were digested with TaqI \( (5'\text{T}^{'!}C\text{GA}3') \) restriction enzymes at 37°C for 3 hour [Sharma et al., 2009]. The digested restriction fragments were resolved in 3% w/v agarose gel stained with ethidium bromide in TAE buffer (1X) for 3–5 hr at 50 V and visualized under UV light in a gel documentation system (G: Box, Syngene, UK).

Results and Discussion
A 570 bp fragment comprised of exon 5 (173 bp), intron 5 (159 bp), and exon 6 (238 bp) of the ITGB2 gene was amplified (Fig.1). All the breeding sires revealed a monomorphic pattern of 429, 87, and 54 bp bands, when 570 bp amplicons of ITGB2 gene were digested with TaqI restriction enzyme (Fig.2). These monomorphic patterns were due to existence of two TaqI \( (5'\text{T}^{'!}C\text{GA}3') \) sites at 54th and 483rd position, whereas the codon at 382nd to 384th nucleotide position changes (GAC>GGC) due to mutation in the middle A to G at 383rd position in BLAD affected cattle. Thus first restriction frame of TaqI \( (T\#C\text{GA}) \), that occurred at 54th position in our amplicon, would have abolished which should have caused BLAD syndrome. In this situation, only two fragments of 483 and 87 bp and four fragments of 483, 429, 87 and 54 bp, would have appeared in case of BLAD homozygote and heterozygote respectively. Hence, pattern indicates lack of BLAD allele in the corresponding amplified region of ITGB2 gene.

**Table-1:** Table showing primer code, location of the primers within the ITGB2 gene, primer sequences amplicon size, cycling parameters for amplification

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Location within bovine genome</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
<th>Cycling parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLAD-11F</td>
<td>Exon 5, Intron 5</td>
<td>AGGTCAAGCCATTGCGTCAA</td>
<td>570 bp</td>
<td>94°C-3 min</td>
<td>Sharma et al., 2009</td>
</tr>
<tr>
<td>BLAD-11R</td>
<td>Exon 6</td>
<td>CACGCGGCCACCTG</td>
<td></td>
<td>94°C-45 sec, 61°C-40 sec, 72°C-45 sec, 72°C-5 min</td>
<td></td>
</tr>
</tbody>
</table>
In India an utmost portion of its cattle population is non descriptive and herd average of milk yield is far below than most of the developed country. Though, through successful implementation of grading up, the country has improved most of its non descriptive cattle population with the aid of artificial insemination [Mondal et al., 2016]. In this course intensive exploitation of elite sires enhances the risk of spreading of genetically transmitted disorders [Citek et al., 2004]. The present study was conducted one such lethal inherited disorder Bovine Leukocyte Adhesion Deficiency, in breeding sires from different indigenous cattle breeds (Sahiwal, Gir) maintained at Frozen Semen Bull Station (FSBS), Haringhata, Nadia, West Bengal, India.

Earlier extensive studies have been carried out to estimate frequency of BLAD allele in different parts of the world and carrier frequency was estimated 13.4% in Denmark [Jorgensen et al., 1993], 3.5% in Argentina [Poli et al., 1996], 10.4% in Hungary [Janosa et al., 1999], 5.7% in Brazil [Ribeiro et al., 2000], 8.1% in Japan [Nagahata, 2004], 3.3% in Iran [Nasreen et al., 2005], 2.5% in Romania [Vatasescu-Balcan et al., 2007] and 1% in Pakistan [Nasreen et al., 2009] in Holstein animal and it’s crosses respectively. In India, frequency of BLAD carrier animals has been estimated 1.33% [Muraleedharan et al., 1999], 3.23% [Patel et al., 2006], 21.82% [Kumar, 2009], 7.31% [Mahdi et al., 2010] and 3.64% [Yathish et al., 2010] in HF and HF Crossbred sires in earlier studies. But, till date frequency of carrier animals in Bos indicus cattle has been estimated nil, which is very much accordance with this study.

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References