Status of genetic disorders among indigenous and crossbred breeding bulls in India

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In the present investigation, screening of genetic disorders, viz., Bovine leukocyte adhesion deficiency (BLAD), Deficiency of uridine monophosphate synthase (DUMPS), Bovine citrullinemia (BC) and Factor XI deficiency (FXID), was carried out in 438 bulls of exotic and indigenous breeds, viz., Holstein Friesian, Jersey, Khillar, Deoni and Malnad Gidda, using PCR based techniques. Of all bulls screened, two Holstein Friesian bulls were found to be the carrier for BLAD. None of the bulls screened was found to be the carriers of DUMPS, BC and FXID. Though the frequency of unfavorable allele was very low or nil, considering the economic and lethal impact of the diseases, it is recommended to screen the bulls intended for breeding purpose for genetic disorders to minimize the spread of defective alleles in the population.

Keywords: BC, BLAD, DUMPS, FXID, genetic disorder, PCR-RFLP.

The known genetic disorders, viz., Bovine leukocyte adhesion deficiency (BLAD), Deficiency of uridine monophosphate synthase (DUMPS), Bovine citrullinemia (BC) and Factor XI deficiency (FXID), in breeding bulls are of economical importance in bovine breeding. Unrecognized dissemination of specific genes, especially through artificial insemination, is of greater concern in cattle breeding. So the present study was carried out with the objective to screen and identify the carriers for BLAD, DUMPS, BC and FXID in bulls.

A total of 438 cattle bulls of different breeds, viz., 177 Holstein Friesian, 139 Jersey, 30 Khillar, 40 Deoni and 52 Malnad Gidda, were utilized for the study. A volume of 10 mL blood sample was collected aseptically by jugular vein-puncture using vacutainer tubes containing EDTA and was stored at 4°C till further use. Within 24 h of collection of blood, genomic DNA was isolated by high salt method. The genotypes for BLAD, DUMPS and BC were identified by using PCR-RFLP technique. Primers, annealing temperature, PCR product sizes and restriction enzymes used for identification of BLAD, DUMPS, BC and FXID are presented in Table 1. Genotypes were determined using agarose gel electrophoresis (1.5%). A volume of 10 μL of PCR products for BLAD, DUMPS, BC and FXID genotypes were digested with specific enzyme. The digested products were separated on 3% agarose gel and analyzed by visualizing the gels under Gel doc system (Bio-Rad, USA).

The primers used in the experiment (Table 1) successfully amplified the DNA fragments of 136, 282, 198 and 320 bp for BLAD, DUMPS, BC and FXID, respectively. The PCR products for BLAD, DUMPS and BC were digested with TaqI, AvaI and AvaII restriction enzymes, respectively. After digestion of the PCR products, the normal allele in unaffected BLAD cattle produced two fragments of 108 and 28 bp, while BLAD carriers’ exhibited three fragments of 136, 108 and 28 bp (Fig. 1A), and affected animal showed one band of 136 bp. The amplified product of uridine monophosphate synthase upon digestion by AvaI, yield two bands of 213 and 69 bp for normal animals (Fig. 1B). Carrier animals produce three bands of 282, 213 and 69 bp, and DUMPS affected animals produce single band of 282 bp. None of the bulls screened were either affected or carrier for BC. In case of FXI gene, the PCR product from the unaffected animals exhibited a single fragment of 244 bp (both copies of gene-homozygous genotype); whereas in heterozygous (carrier) condition, one copy of gene would be normal and another copy would be...
of 320 bp because of 76 bp insertion (mutation), and affected animal would produce a fragment of 320 bp. All the screened bulls were found to be negative for FXID (Fig. 1D).

Among the 438 bulls screened, two Holstein Friesian animals were found to be carriers (heterozygote) for BLAD (Fig. 1A). The PCR products of those bulls found to be carrier for BLAD were custom sequenced using the automated ABI DNA Sequencer from Amnion Biosciences Pvt. Ltd., Bangalore, India to confirm the sequences. Sequence data were analyzed using BioEdit software. This disease is caused by a mutation, which replaces adenine at 383th position with guanine that changes amino acid aspartic acid to glycine. The mutation ultimately leads to a wrong protein (CD18) that is impaired in function. Among the screened animals, none of the animals was found either carrier or affected with DUMPS, BC and FXID.

Table 1 — Primers8,9,18, PCR product size, annealing temperature and restriction enzymes (RE) used for identification of BLAD, DUMPS, BC and FXID

<table>
<thead>
<tr>
<th>Genetic disorder</th>
<th>Primer sequence (5’→3’)</th>
<th>PCR product size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLAD</td>
<td>F: CCTTCCGGAGGGCAAGGGCT R: CTCGGTGATGCCATTGAGGGC</td>
<td>136</td>
<td>57</td>
<td>TaqI</td>
</tr>
<tr>
<td>DUMPS</td>
<td>F: AGGGTCTTATGGAACAGGT R: GGCTTACCTCTTGCTTAACTG</td>
<td>282</td>
<td>54</td>
<td>AvaI</td>
</tr>
<tr>
<td>BC</td>
<td>F: GGCCAGGGACCGTGTTAGTGAGGACATC R: TTCTGGGACCCCGTGACACATTTG</td>
<td>198</td>
<td>57</td>
<td>AvaII</td>
</tr>
<tr>
<td>FXID</td>
<td>F: CCCACTGGCTAGGAATCGTT R: CAAGGCAATGTCATATCCAC</td>
<td>244</td>
<td>55</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1 (A-D) — Illustration of BLAD, DUMPS, BC and FXID genotypes on agarose gels: A. BLAD genotype (Lane 12), [Carrier for BLAD with 3 bands of 136, 108 & 28 bp (not visible in gel)]; B. DUMPS [Normal homozygote animal showing two fragments of 213 & 69 bp]; C. Bovine citrullinemia [Lanes 1 to 12: Normal homozygote showing two fragments, 109 & 89 bp]; D. FXID [Lanes 1 to 10 showing product size 244 bp, normal homozygote genotype].
The spread of genetic disorders in cattle in recent years is caused by the extensive use of few elite sires, which were latent heterozygous carriers. Similar results of very low incidence of BLAD were observed in earlier studies carried out for Holstein population. Among Holstein Friesian and Holstein Friesian crossbred population, the percentage of BLAD carriers was estimated as 3.23%. In the present investigation, none of the males was either carrier or affected with DUMPS, BC and FXID. The results obtained in our study are in agreement with the earlier reports of very low or lack of incidence of genetic disorders among cattle population in Poland, India, Turkey, and Iran. Similar results were obtained for BC and FXID. Contrary to this, presence of FXID carriers have been reported from USA, Japan, Turkey, and Iran. Among Indian cattle, BC carrier bull belonging to Holstein breed were identified, while one Holstein cattle identified as carrier for DUMPS, BC and FXID. With the wide use of artificial insemination and international trading of semen and breeding bulls, if proper screening is not done, there is possibility of spreading of recessive genetic diseases as carrier animals of the disease look normal. Monitoring of all these diseases in young sires is recommended, especially in Holstein Friesian and its crossbreds, to avoid unrecognized dissemination of such defective genes. The results of the present study also suggest that the PCR and PCR-RFLP technique can be employed as an efficient and routine technique for screening of various genetic disorders for identification of carrier and/or affected animals.

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References