Polymorphism in DRB3 exon 2 by PCR-RFLP and its association with mastitis in Murrah buffaloes

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Murrah buffaloes (n=25) were used to explore the genetic polymorphism in DRB3 exon 2 by PCR-RFLP and to find out the association with mastitis. The gDNA was isolated from whole blood samples as per standard protocol and a 304 bp PCR was amplified. When 304 bp product of exon 2 of DRB3 gene was digested with RsaI, it revealed 10 alleles (a, b, c, f, i, o, s & t) with 12 different genotypes (a/a, b/b, c/c, f/f, i/i, o/o, s/s, t/t, o/g, i/f, l/t, & i/s). HaeIII revealed 5 alleles (a, b, d, e & i) and 8 genotypes (a/a, e/e, d/d, b/b, a/e, a/b, a/d & b/i). On digestion with PstI, only 4 alleles (x, y, z & s) with 7 different genotypes (x/x, y/y, z/z, x/y, x/z, s/z & y/z) were observed. The following genotypes a/a, c/c, and f/f (RsaI); b/b, d/d, e/e, and b/f (HaeIII); x/x, y/y, x/y, and y/z (PstI) were observed only in healthy animals. These results revealed that the DRB3.2 gene was more polymorphic in the healthy animals than mastitis cases.

Keywords: BuLA-DRB3.2, PCR-RFLP, Murrah buffalo, mastitis

Since long time, scientists have given main emphasis on improvement of production traits with little or no attention to improve disease resistance traits. Major histocompatibility complex (MHC) of farm animals comprises a group of closely linked genes, most of which are polymorphic and play central role in the immune responsiveness and resistance/susceptibility to diseases that involve immune intervention. BoLA is classified into four regions namely class IIb, class Ila, class III and class I from centromere to telomere direction. The class IIa region has DQ and DR as major loci. There are at least three DRB genes i.e. DRB1, DRB2 and DRB3. Among these, DRB1 is a pseudogene, DRB2 is expressed at lower level and DRB3 gene is highly expressed and polymorphic. Class II region genes encode heterodimeric glycoproteins, which are composed of α and β chains, expressed on the surface of antigen-presenting cells. These molecules bind processed peptides of exogenous foreign antigens and present them to specific T helper cells. The extensive structural polymorphism of the class II molecules is considered to be responsible for differences among the individuals in the immune response to different infectious agents. The MHC of buffalo is called buffalo lymphocyte antigen (BuLA). Expression of MHC genes is thus an essential component of studies on immune response and resistance/susceptibility to diseases. Associations have been found between MHC and various diseases in farm animals. DRB3.2 alleles have been found to be associated with somatic cell count (SCC) and mastitis resistance in cattle. Mastitis is economically important disease of dairy cattle and buffaloes. If linkage disequilibrium is established between MHC alleles and mastitis, the marker can be used in marker assisted selection (MAS) for improving resistance to mastitis. These markers can be used to select the animals at young age before actual expression of the traits of interest. This will not only improve the selection intensity, but also decrease generation interval, increase accuracy of prediction leading to enhanced genetic progress per unit of time and cost of production. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) has been found useful for DRB3 typing in cattle and buffaloes. Since the MHC is known to control the progression of many infectious diseases, it is argued that developing markers for these loci may be helpful in identifying superior haplotypes for disease resistance provided that association between the trait and these markers can be established. Keeping the above facts in view, the present investigation was undertaken with the objective to study the polymorphism in exon2 of DRB3 gene in Murrah buffaloes using PCR-RFLP and to explore the association between DRB3.2 markers with mastitis.
Amplification of MHC-DRB3 exon 2 was carried out in a programmable thermocycler (Eppendorf Master Cycler Gradient™). The amplicons (304 bp) were run on 1% agarose gel to confirm amplification. After confirmation, the PCR products were digested with restriction enzymes viz. PstI, Rsal (MBI Fermentas) and HaeIII (New England Biolabs) and the restriction fragments were resolved on 4% agarose gel. The purity and concentration of DNA was evaluated by running the DNA samples in 0.7% agarose gel. The quality of DNA was checked by spectrophotometry. The DNA samples with OD ratios (260/280 nm) between 1.7 and 1.9 were used for further analysis.

Digestion with PstI revealed the least polymorphism in the population, obviously due to few restriction sites in the DRB3.2 locus. It yielded 4 alleles (x, y, z & s) with 7 different genotypes (x/x, y/y, z/z, x/y, x/z, y/z & s/z) (Table 3). The information available in the literature on PCR-RFLP of DRB3.2 with PstI in buffaloes is too scanty for comparison with the findings of present study. However, restriction site at 216 bp was observed when 284 bp PCR product of DRB3.2 gene digested with HaeIII enzyme in Murrah buffaloes (Table 2). These patterns were also reported in cattle and in Murrah buffalo. HaeIII alleles c, f, g and h reported in cattle were not observed in buffaloes. In Murrah breed, allele a had frequency 0.46, which might be due to natural selection favouring the fixation of allele.

The RFLP analysis of Rsal digest was very complex with 10 alleles (a, b, c, f, g, i, l, o, s & t) and 12 different genotypes (a/a, b/b, c/c, f/f, i/i, l/l, o/o, s/s, t/t, a/g, i/f, l/h & i/s) in Murrah buffalo (Table 1). Similar patterns were also reported in cattle (21 different genotypes) and in Murrah buffalo (13 different genotypes).

HaeIII revealed 5 alleles (a, b, d, e, & i) with 8 different genotypes (a/a, b/b, d/d, e/e, a/e, a/b, a/d & b/i) in Murrah buffalo. These patterns were also reported in cattle and in Murrah buffalo. HaeIII alleles c, f, g and h reported in cattle were not observed in buffaloes. In Murrah breed, allele a had frequency 0.46, which might be due to natural selection favouring the fixation of allele.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Genotype frequencies</th>
<th>Alleles</th>
<th>Allele frequencies</th>
</tr>
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<tbody>
<tr>
<td>Healthy</td>
<td>0.08 0.12 0.04 0.08 0.20 0.04 0.20 0.04 0.20 0.04 0.04 0.04</td>
<td>a b c f g i l o s t</td>
<td>0.20 0.10 0.10 0.20 0.20 0.10 0.067 0.10 0.04 0.04 0.04 0.04</td>
</tr>
<tr>
<td>Mastitis</td>
<td>0.20 0.10 0.10 0.20 0.20 0.10 0.067 0.10 0.04 0.04 0.04 0.04</td>
<td>a b c f g i l o s t</td>
<td>0.20 0.10 0.10 0.20 0.20 0.10 0.067 0.10 0.04 0.04 0.04 0.04</td>
</tr>
<tr>
<td>Overall</td>
<td>0.08 0.12 0.04 0.08 0.12 0.08 0.20 0.04 0.20 0.04 0.12 0.04 0.04 0.04</td>
<td>a b c f g i l o s t</td>
<td>0.20 0.10 0.10 0.20 0.20 0.10 0.067 0.10 0.04 0.04 0.04 0.04 0.04</td>
</tr>
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</table>

Table 2—Genotype and allele frequencies of DRB3.2 gene digested with HaeIII enzyme in Murrah buffaloes

Farm, Hisar. Genomic DNA was isolated from 10 mL of venous blood. The quality of DNA was checked by running the DNA samples in 0.7% agarose gel. The purity and concentration of DNA was evaluated by spectrophotometry. The DNA samples with OD ratios (260/280 nm) between 1.7 and 1.9 were used for further analysis.

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heterogeneity available in healthy animals might be the reason for their resistance than mastitis animals. However, relatively due to small sample size, it was less informative and nothing conclusive could be said. Nonetheless, it initiated the study on the possibility of association between BuLA and mastitis that needs to be explored in a large population.

References


| Table 3 Genotype and allele frequencies of DRB3.2 gene digested with PstI in Murrah buffaloes |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Genotypes x/x   y/y   z/z   x/z   y/z   x/z   y/z   Genotype frequencies |
| Healthy         0.07   0.13   0.46   0.07   0.20   0.07   0 |
| Mastitis        0       0      0.50   0      0.10   0      0.40  |
| Overall         0.04   0.08   0.48   0.04   0.16   0.04   0.16  |
| Allele frequencies x  0.20  0.17  0.60  0.03  |
| Healthy         0.05   0.20   0.75   0  |
| Mastitis        0.14   0.18   0.66   0.02  |
| Overall         0.16   0.18   0.66   0.02  |

Table 3—Genotype and allele frequencies of DRB3.2 gene digested with PstI in Murrah buffaloes.