

Short Communications

Polymorphism in DRB3 exon 2 by PCR-RFLP and its association with mastitis in Nili-Ravi breed

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The present investigation was undertaken to study the genetic polymorphism in exon 2 of DRB3 gene in Nili-Ravi (n=25) buffalo breed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and its association with mastitis. The gDNA was isolated from whole blood samples. When 304 bp PCR product of exon 2 of DRB3 gene was digested with *Rsa*I, 11 genotypes, viz., *b/b*, *c/c*, *f/f*, *o/o*, *s/s*, *f/o*, *b/f*, *b/o*, *o/s*, *b/l* and *l/s*, with frequency range 0.04-0.16 and 6 alleles, viz., *b*, *c*, *f*, *l*, *o* and *s* with frequency range 0.08-0.26 were observed. *Hae*III detected 6 genotypes, viz., *a/a*, *e/e*, *d/d*, *a/b*, *b/d* and *b/e* with frequency range 0.04-0.28 and four alleles, viz., *a*, *b*, *d* and *e* with frequency range 0.08-0.6. However, *Pst*I revealed 5 genotypes, viz., *y/y*, *z/z*, *x/y*, *x/z*, *s/z* and *y/z* with frequency range 0.08-0.32 and four alleles, viz., *x*, *y*, *z*, and *s* with frequency range 0.04-0.42. These results revealed that exon 2 of DRB3 gene was highly polymorphic in Nili-Ravi breed. Certain genotypes (*c/c*, *f/l*, *b/f*, *o/o*, *l/s*, *a/a* and *e/e*) were observed only in healthy animals, while others (*b/f*, *b/o*, *f/o* and *y/z*) in mastitis cases. Result to be tested on large sample size before its practical application.

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

For long time, animal breeders have given main emphasis on improvement of production traits with little or no attention for improvement of disease resistance traits. Major histocompatibility complex (MHC) of farm animals comprises of 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. Bovine leucocyte antigen (BoLA) is classified into four regions namely class IIb, class IIa, 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 DRB3¹. Class IIa region genes encode heterodimeric glycoproteins, which are composed of α and β chains, and 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 individuals in the immune response to different infectious agents. Expression of MHC genes is, thus, an essential component of studies on immune response and resistance/susceptibility to diseases². An analysis of MHC polymorphism is of interest because associations have been found between MHC and various diseases in farm animals, like B²¹ haplotype with Marek's disease. DRB3.2 alleles have found to be associated with SCC (somatic cell count) and mastitis resistance in cattle³⁻⁸. The MHC of buffalo is called BuLA, i.e. buffalo lymphocyte antigen⁹.

Of the various methods used to investigate the genetic polymorphism of MHC loci, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) has been found useful for DRB3 typing in cattle¹⁰. However, there is a paucity of information on MHC polymorphism in buffaloes. Considering the above, the present investigation was undertaken to study the polymorphism in exon 2 of DRB3 gene in Nili-Ravi buffaloes using PCR-RFLP and its association with mastitis.

The present investigation was conducted on 25 unrelated animals of Nili-Ravi breed (*Bubalus bubalis*) maintained at Central Institute for Research on Buffaloes, sub-campus Nabha (Punjab, India). Approximately 10 mL of venous blood sample was collected in 50 mL sterile polypropylene centrifuge tubes containing 0.5 mL of 0.5 M EDTA as anticoagulant and gDNA was isolated. The 304 bp PCR product of exon 2 of DRB3 gene was amplified by using the following primer pair¹¹.

Forward primer:

LA31 [5'-GATGGATCCTCTCTCTGCAGCACATTTCTCT-3']

Reverse primer:

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LA32 [5'-CTTGAATTCGCGCTCACCTCGCCGCTG3']

The master mix was prepared by adding 18.7 µL of nuclease free water (autoclaved triple glass distilled water), 2.5 µL Taq DNA polymerase buffer, 1.5 µL 25 mM MgCl₂, 0.5 µL (100 µM) dNTPs, 0.25 µL (40 ng) each primer and 0.3 µL Taq DNA polymerase (MBI Fermentas) for each sample, and after proper mixing, it was distributed to 25 tubes (24 µL to each tube). To each sample, 1 µL (50 ng) gDNA was added. The 25 µL reaction mix was kept for amplification in programmed thermocycler. After an initial denaturation for 2 min at 94°C, 35 cycles were given, each comprising of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C followed by a final extension of 72°C. After the final extension step, the PCR products were resolved in 1% agarose gel and stored at 4°C for further analysis.

For RFLP analysis, 5 µL of PCR product was digested with 5 units of *Pst*I/*Rsa*I (MBI Fermentas)/*Hae*III [New England Biolabs] in a final volume of

10 µL at 37°C overnight. The restriction fragments were resolved using 4% agarose gel (Sigma, USA) in 1× TAE buffer at 100V for 90 min. Two ladders of 25 bp and 50 bp [New England Biolabs] were used as size marker. After staining with ethidium bromide, the fragments were visualized by UV transilluminator and documented by photography.

RFLP analysis of exon 2 by *Rsa*I was complex as it yielded maximum genotypes (11), viz., *b/b*, *c/c*, *ff*, *o/o*, *s/s*, *f/o*, *b/f*, *b/o*, *o/s*, *b/l* and *l/s* with frequency range 0.04-0.16, and 6 alleles, viz., *b*, *c*, *f*, *l*, *o* and *s* with frequencies range 0.08-0.26 (Table 1). All these genotypes were reported in cattle¹² and buffalo¹³. The digestion of 300 bp PCR product with *Hae*III resulted in 6 genotypes, viz., *a/a*, *e/e*, *d/d*, *a/b*, *b/d* and *b/e* with frequency range 0.04-0.28, and 4 alleles, viz., *a*, *b*, *d* and *e* with frequency range 0.08-0.6 (Table 2). All these genotypes were reported in cattle¹² and buffalo¹³. However, alleles *c*, *f*, *g* and *h* were reported in cattle only¹².

Enzyme *Pst*I revealed 6 genotypes, viz., *y/y*, *z/z*,

Table 1—Genotypic and allelic frequencies of BuLA–DRB3.2 gene with *Rsa*I enzyme in Nili-Ravi Breed

Animal	Genotypic frequencies										
	<i>b/b</i>	<i>c/c</i>	<i>ff</i>	<i>o/o</i>	<i>s/s</i>	<i>b/f</i>	<i>b/l</i>	<i>b/o</i>	<i>f/o</i>	<i>l/s</i>	<i>o/s</i>
Healthy	0.13	0.13	0.06	0.06	0.06	0	0.13	0	0	0.20	0.20
Mastitis	0.10	0	0	0	0.20	0.20	0	0.10	0.30	0	0.10
Total	0.12	0.08	0.04	0.04	0.12	0.08	0.08	0.04	0.12	0.12	0.16
Animals	Allelic frequencies										
	<i>b</i>	<i>c</i>	<i>f</i>	<i>l</i>	<i>o</i>	<i>s</i>					
Healthy	0.20	0.13	0.26	0.16	0.16	0.06					
Mastitis	0	0.25	0.25	0	0.25	0.25					
Total	0.22	0.08	0.26	0.10	0.20	0.14					

Table 2—Genotypic and allelic frequencies of DRB3.2 gene with *Hae*III in Nili-Ravi breed

Animal	Genotypic frequencies						Allelic frequencies			
	<i>a/a</i>	<i>d/d</i>	<i>e/e</i>	<i>a/b</i>	<i>b/d</i>	<i>b/e</i>	<i>a</i>	<i>b</i>	<i>d</i>	<i>e</i>
Healthy	0.06	0.33	0.06	0.26	0.20	0.06	0.20	0.60	0.10	0.10
Mastitis	0	0.20	0	0.30	0.10	0.40	0.15	0.60	0.05	0.20
Total	0.04	0.28	0.04	0.28	0.16	0.20	0.18	0.60	0.08	0.14

Table 3—Genotypic and allelic frequencies of DRB3.2 gene with *Pst*I in Nili-Ravi breed

Animal	Genotypic frequencies						Allelic Frequencies			
	<i>y/y</i>	<i>z/z</i>	<i>x/y</i>	<i>x/z</i>	<i>s/z</i>	<i>y/z</i>	<i>x</i>	<i>y</i>	<i>z</i>	<i>s</i>
Healthy	0.07	0.40	0.20	0.20	0.13	0	0.40	0.16	0.56	0.07
Mastitis	0.10	0	0.50	0.10	0	0.30	0.30	0.50	0.20	0
Total	0.08	0.24	0.32	0.16	0.08	0.12	0.24	0.30	0.42	0.04

x/y , x/z , s/z and y/z with frequency range 0.08-0.32, and 4 alleles, viz., x , y , z , and s with frequency range 0.04-0.42 (Table 3). This enzyme revealed the least polymorphism in the DRB3.2 locus as it is hexacutter. The information available on PCR-RFLP of DRB3.2 with *PstI* in buffaloes is scanty to compare with the finding of present study. Restriction site at 216 bp was observed^{14,15}, when 284 bp PCR product of Murrah was digested with *PstI*. This is in consistent with the finding of the present study.

Genotypes c/c , f/l , b/f , o/o , l/s (*RSA1*), a/a , e/e (*HaeIII*), z/z and s/z (*PstI*), and likewise alleles b , l (*RSA1*) and s (*PstI*) were observed in healthy animals only. These genotypes may be involved in resistance to mastitis. However, Genotypes b/f , b/o , f/o (*RSA1*), and y/z (*PstI*) were observed in mastitis cases only and, therefore, may be susceptible to mastitis. For small sample size, the present results need to be tested on large sample size before its practical application.

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