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Short communication

# Allelic diversity at MHC class II DQ loci in buffalo (*Bubalus bubalis*): Evidence for duplication☆

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ABSTRACT

The genetic diversity of MHC class II DQ genes was investigated in riverine buffalo (*Bubalus bubalis*) by PCR-RFLP and sequencing. Highly variable regions (exons 2–3) of DQ genes were amplified from 152 buffaloes and genotyped by PCR-RFLP. Alleles identified by differential restriction patterns were sequenced for the characterization. PCR-RFLP was a rapid method to discriminate between DQA1 and duplicated DQA2 genes in buffalo, however, the method appeared to be inadequate for determining the more complicated DQB genotypes. A total of 7 and 10 alleles were identified for DQA and DQB loci, respectively. Nucleotide as well as amino acid variations among DQ alleles particularly at peptide binding regions were high. Such variations were as expected higher in DQB than DQA alleles. The phylogenetic analysis for both genes revealed the grouping of alleles into two major sub-groups with higher genetic divergence. High divergence among DQ allelic families and the isolation of two diverse DQA and DQB sequences from individual samples indicated duplication of DQ loci was similar in buffalo to other ruminants.



## 1. Introduction

MHC molecules play a major role in immunological defense against pathogens. These molecules loaded with peptides derived from invading pathogen are recognised by the immune system to produce a highly effective and specific response against foreign pathogens (Benacerraf, 1981). MHC genes, encoding the MHC molecules are prime candidates for the investigation of genetic variation in the host resistance to infection. The MHC class II genes are associated with resistance to the diseases and are extremely polymorphic in most vertebrates (Trowsdale, 1995). These genes have attracted much attention in farm animals due to the need of improved methods of disease control through the design of novel vaccines and selection of disease resistant animals. DQ genes of class II region encode for α (DQA) and β (DQB) chains of the molecule and are highly polymorphic. The DQ genes have been extensively studied for their allelic variation and haplotypic pattern in most of the farm animals (Andersson and Rask, 1988; Sigurdardottir et al., 1992; Marello et al., 1995; Ballingall et al., 1997; Russell et al., 1997; Snibson et al., 1998). In cattle, both DQA and DQB have approximately 51 and 74 characterized alleles, respectively (IPD: A Bovine MHC data base: http://www.ebi.ac.uk/ipd/mhc/bola/nomenclature.html). The DQ genes are a single copy gene in mouse, rat, pig and rabbit, whereas in human and other primates, multiple DQ genes have been identified (Kappes and Strominger, 1988). However, as a unique feature, certain variability

☆ Nucleotide sequences are available in GenBank database under the Accession Nos. DQ822570–DQ822573, DQ868979–DQ868981 and EU025857–EU025866.

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**Table 1**
Primers used for amplification of genomic *Bubu*-DQA and DQB sequences.

| Locus | Primer | Primer sequence | Primer location |
|---|---|---|---|
| DQA | A1G-F | 5′-CTCCGACTCAGCTGACCACATTGG-3′ | Intron 1/exon 2 boundary |
| | A2G-F | 5′-CCTCAATTATCAGCTGACCACGTTGG′ | Intron 1/exon 2 boundary |
| | DQA1G1R | 5′-GTCATCATCAGAAGGAGGAAGGTG-3 | Exon 3 |
| DQB | B1G-F | 5′-TCCCCCGCAGAGGATTTCGTG-3′ | Intron 1/exon 2 boundary |
| | B2G-F | 5′-CTCCCCGCAGAGGATTTCGTG-3′ | Intron 1/exon 2 boundary |
| | BG-R | 5′-CGCACTCACCTCGCCGCTGC-3′ | Exon 2/intron 2 boundary |

in number of the DQ loci has been observed in ruminants (Andersson and Rask, 1988; Sigurdardottir et al., 1992; Traul et al., 2005). In cattle, most haplotypes carry duplicated DQ genes, which are also expressed (Ballingall et al., 1997; Russell et al., 1997). Duplication combined with polymorphism of the DQ genes has the potential to markedly increase the variation at the cell surface by inter- and intra-haplotype pairing.

Water buffalo (*Bubalus bubalis*) is a major source of milk and meat in many of the tropical countries. Although they thrive and adapted to tropical conditions buffalo are susceptible to many of the diseases that also affect cattle. There are few reports describing which buffalo MHC-DQ genes are responsible for susceptibility or resistance to disease. We have previously shown the presence of two diverse DQA alleles in buffalo, which are also expressed (Niranjan et al., 2009). However, information about the genomic diversity as well as duplication of DQ genes is needed. In the present study, we carried out the investigation on genomic diversity and duplication of DQ loci in water buffalo.

## 2. Materials and methods

### 2.1. DNA isolation and amplification of DQ alleles

Blood samples were collected from 152 riverine buffaloes, selected at random from a herd of nearly 400 animals. DNA was isolated from blood leukocytes as per the standard protocol (Sambrook et al., 1989). The primers for the amplification of *Bubu*-DQ genes were synthesized similar to primer sequences used in bison (Traul et al., 2005, Table 1). PCR amplification for both sequences was carried out in 50 μl volume of reaction mixture containing optimized concentrations of 1.5 mM of MgCl2, 200 μM of dNTPs and 30 pmol of primers each, 140 ng of genomic DNA as template and 1.25 units of Taq DNA polymerase. Amplification was carried out for 35 cycles with following conditions: denaturation at 94 °C for 30 s, annealing at 66 °C for 30 s for DQA and at 65 °C for 45 s for DQB; and extension at 72 °C for 70 s and final extension at 72 °C for 10 min.

### 2.2. PCR-RFLP analysis

Amplified products were subjected to PCR-RFLP for the analysis of different haplotypes/genotypes at *Bubu*-DQ region through differential restriction patterns. Enzymes were selected by restriction map analysis on the basis of cattle and bison sequences. *Hinf* I restriction enzyme was found to be differentiating the DQA1 and DQA2 alleles. Similarly, amplified products of DQB exon-2 region were digested with *Hae* III to identify allelic patterns. Digested products were differentially visualized by polyacrylamide gel electrophoresis (PAGE) at 8% concentration and analysed for genotyping on the basis of differential restriction patterns.

### 2.3. Cloning and identification of DQ alleles by PCR-RFLP

The amplified products showing different restriction patterns were cloned into the pGEM-T-easy vector (Promega, Medison, WI). Positive clones were identified after releasing the insert by *EcoR* I enzyme. At least 10 positive clones selected randomly for each amlicon were further amplified through PCR. The reaction mixture and conditions were followed the same as above except template. The amplified clone products were subjected to RFLP analysis to discriminate between the clones of different alleles. *Hinf* I and *Hae* III enzymes were employed separately in colony PCR products of DQA. Similarly for DQB, *Hae* III, *Msp* I and *Tru* 1I enzymes were used. Different subclones having specific allele were sequenced.

### 2.4. Sequence and phylogenetic analysis

Three sub-clones for each allele were sequenced from both sides using the automated dye-terminator cycle sequencer. Obtained sequences were determined on the basis of orthologous sequences of different species using public database search. The nucleotide and deduced amino acid sequences were compared with orthologous DQ sequences. Phylogenetic trees were derived for nucleotide sequences (exon 2 region) of DQA and DQB alleles based on Neighbor-joining method using MEGA 4 programme (Tamura et al., 2007). Bootstrap values were also obtained using 500 replicates (Felsenstein, 1985). The evolutionary distances for nucleotide and amino acid were computed using the Kimura 2-parameter based on nucleotide (Kimura, 1980) and P-distance models, respectively.

## 3. Results and discussion

### 3.1. Amplification and genotyping of Bubu-DQ alleles

Genomic fragments with expected sizes of 930 bp corresponding to DQA exons 2–3 and 289 bp corresponding to DQB exon 2 regions were obtained by PCR. For genotyping of DQA locus in the population, PCR-RFLP using *Hinf* I enzyme revealed six patterns. Analysis of obtained restriction patterns in the population revealed three genotypes *viz.* genotypes carrying only DQA1 (0.297) alleles or only DQA2 (0.111) alleles or genotypes carrying both DQA1 and