PCR-RFLP based identification of duplicated DQA loci in riverine buffalo

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ABSTRACT

Major histocompatibility complex (MHC) DQ genes are highly polymorphic and duplicated in bovines including buffalo. Duplication of DQ genes seems to generate greater genetic diversity, which causes better recognition of wider range of pathogens. In this study, we devised the PCR-RFLP based genotyping method for rapid identification of duplicated haplotypes at DQA locus in riverine buffaloes. Genomic region corresponding to Bubu-DQA exon 2 to 3 was amplified from 152 buffaloes belonging to Murrah and Bhadawari breeds. Three primers were multiplexed for PCR amplification to amplify both loci. RFLP analysis obtained through digestion of amplified products by *Ava II* restriction enzyme revealed five different patterns. A total of three major haplotypes carrying either DQA1 only or DQA2 only or both DQA1 and DQA2 were identified. About more than half of the buffalo population was found to have duplicated haplotype carrying DQA1 and DQA2 genes. This simple, rapid and reliable PCR-RFLP technique can be used for the identification of duplicated DQA haplotypes in buffalo population.

Key words: Water buffalo, MHC, DQA1, DQA2, duplicated haplotype, PCR-RFLP

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INTRODUCTION

The major histocompatibility complex (MHC) class II genes are known to be associated with resistance and susceptibility to certain diseases in most of the vertebrates (Trowsdale 1995, Glass et al., 2000). MHC class II molecules 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 (Glass et al., 2000). DQ genes (DQA and DQB) of MHC class II region are highly polymorphic and duplicated in bovines (Andersson and Rask, 1988, Ballingall et al., 1997 Niranjan et al., 2010). Duplication of the DQ genes along with polymorphism markedly increases the variation at the cell surface by inter-and intra-haplotype pairing.

Water buffalo *(Bubalus bubalis)* is major milk producing species in South-East Asia and known to have higher resistance comparatively to certain endemic infections (Gibson, 2002). The DQA gene has also been found to be highly polymorphic and duplicated in buffalo (Niranjan et al., 2009, 2010). Evidence shows that duplicated DQ haplotypes also found to be expressed, which might contribute to higher disease resistance in buffalo (Niranjan et al., 2009). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based genotyping of DQA haplotype has been carried out in cattle (Sigurdardottir et al., 1991). However, neither type of such genotyping information is available in water buffalo. Therefore, we conducted a study to explore easy and rapid method for genotyping the DQA haplotype in water buffalo population.

MATERIAL AND METHODS

Blood was collected from 152 riverine buffaloes selected randomly belonging to Murrah (n=122) and Bhadawari (n=30) breeds from different governmental farms. Genomic DNA was isolated by using standard protienase K treatment followed by phenol-chloroform extraction protocol (Sambrook et al., 1989). The purity of the genomic DNA was assessed by spectrophotometer. For amplification of buffalo genomic DQA exon 2-3 region, two forward primers (A1GF: 5'-CTCCGACTCAGCTGACCACATTGG-3' and A2GF: 5'-CCTCAATTATCAGCTGACCACGTTGG-3') were multiplexed with a single reverse primer (DQAR: 5'-GTCATCATCAGAAGGGAGGAAGGTG-3'). The forward primers were taken as described by Traul and coworkers (2005), whereas, the reverse primer was based on conserved exon 3 region of cattle DQA sequences (Acc. No. Y07819, Y07820). Three primers (two forward and one reverse) were taken to amplify different DQA alleles (DQA1 and DQA2 alleles) in a single PCR so that polymorphism as well as haplotype study could be carried out simultaneously. Among forward primers, A1GF was specific to DQA1 locus and other A2GF was specific to duplicated locus DQA2. The optimized concentrations for 50 µl volume of reaction mixture were 1.5 mM of MgCl2, 200 µM of dNTPs and 30 pmol of primers each, 140 ng of genomic DNA as template, 1x PCR assay buffer and 1.25 units of Taq DNA polymerase. Amplification conditions were: initial denaturation at 94^{0}C for 3 minutes, 35 cycles of denaturation at 94^{0}C for 30 seconds, annealing at 66^{0}C for 30 seconds and extension at 72^{0}C for 1 minute 10 seconds and final extension at 72^{0}C for 10 minutes. Amplified PCR products were digested with *Hae III* and

Ava II restriction enzymes for RFLP analysis to differentiate between the DQA1 and DQA2 genes in buffalo. Digested products were analyzed by gel electrophoresis by using 1.5% agarose gel electrophoresis at 50V for 3 hour. The frequency of different patterns was estimated by using standard method.

RESULTS AND DISCUSSION

Buffalo genomic DQA exon 2-3 region was amplified successfully with expected 930 bp long fragment size. Amplified products of Bubu-DQA were digested with *Hae III* and *Ava II* restriction enzymes. These restriction enzymes were chosen specifically to identify duplicated haplotypes and to discriminate between DQA1 and DQA2 alleles on the basis of restriction patterns in different DQA alleles of cattle (Acc Nos. M30117, NW_345697) and bison (Acc.Nos. AY805077-AY805084). Digestion of amplified PCR products with *Hae III* revealed distinct and identifiable restriction patterns (Figure 1), however, most of these patterns were unable to reveal the discrimination between DQA1 and DQA2 haplotypes in buffalo. This was probably due to absence of *Hae III* restriction sites in some of the Bubu-DQA alleles. However, the digestion of Bubu-DQA with *Ava II* restriction enzyme revealed a total of five distinct patterns AA, CC, AACC, ABCC and BBCC (Figure 2, Table 1). Among five different patterns the frequency of AACC was highest (0.467) in buffaloes. Analysis based on *Ava II* restriction pattern revealed that these observed patterns were found to be clustered into three groups, first group was carrying alleles of DQA1 locus only, second group was carrying of DQA2 locus only, where as third group was carrying the alleles of both loci i.e. DQA1 and DQA2. Patterns AA and CC were belonging to first and second groups respectively, while other three patterns (AACC, ABCC, and BBCC) were showing DQA1 as well as DQA2 haplotypes, hence grouped in third type. Presence of three haplotype patterns was also comparable with Sigurdardottir et al. (1991), who described cattle haplotypes carrying either a single DQA1 locus, or a single DQA1 locus together with a DQA2 or two DQA2 loci. The frequency of haplotype carrying both DQA1 and DQA2 genes was highest (0.593) in buffalo population followed by haplotype (0.296) carrying only DQA1 gene and haplotype (0.111) carrying only DQA2 gene. Presence of duplicated haplotypes in more than half of the buffalo population was analogous to similar finding in cattle. Ballingall and co-workers (1997) reported that about half of the DQ haplotypes were duplicated in cattle.

Pattern	Frequency of pattern			DQA1	DQA2	Haplotype
	Murrah	Bhadawari	Total			
AA	0.221	0.600	0.296	*Ava* II A-426, 396,56,53bp		DQA1
CC	0.131	0.033	0.111		*Ava* II C-475,337,56,53 bp	DQA2
AACC	0.508	0.300	0.467	*Ava* II A-426,396,56,53bp	*Ava* II C-475,337,56,53 bp	DQA1, DQA2
ABCC	0.024	0.00	0.019	*Ava* II A-426,396,56,53bp *Ava* II B-437,270,125,56,53bp	*Ava* II C-475,337,56,53 bp	DQA1, DQA2
BBCC	0.114	0.066	0.105	*Ava* II B-437,270,125,56,53bp	*Ava* II C-475,337,56,53 bp	DQA1, DQA2

PCR-RFLP based analysis of bubu-DQA genes revealed the presence of distinct loci for DQA1 and DQA2 similar to cattle (Sigurdardottir et al., 1991). Ballingall et al. (1997) also described the PCR-RFLP as best rapid and effective method to analyse genetic diversity as well as haplotypic pattern i.e. to detect the presence or absence of DQA1 and most duplicated DQA2 genes. However, they used *Hinf I, Mse I,* and *Taq I* restriction enzymes to detect diversity and duplication.

Restriction patterns based on cattle and bison sequences could identify only three alleles Ava II A and Ava II B, alleles of DQA 1 gene and Ava II C allele of DQA2 gene in buffalo. These Ava II restrictions sites were also found in DQA alleles of cattle and bison. The frequencies of Ava II A, B and C alleles were 0.450, 0.083 and 0.465 in Murrah and 0.658, 0.048 and 0.292 in Bhadawari buffaloes, respectively. In pooled buffalo population, the frequency of Ava II A allele was highest (0.485), followed by Ava II C (0.436) and Ava II B (0.077) alleles. However, no new allele could be detected in buffalo by Ava II digestion.

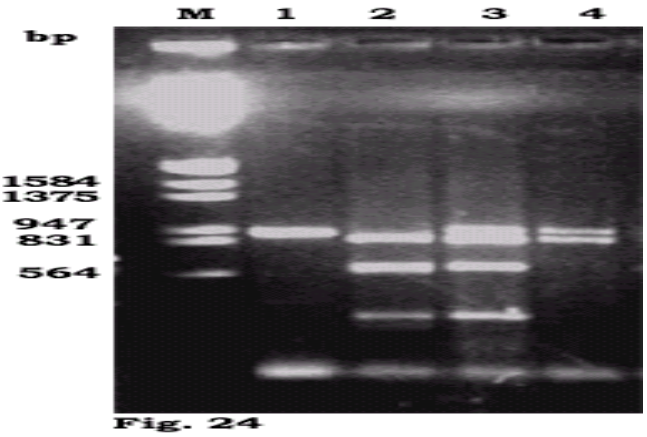

Figure 1: PCR-RFLP patterns after Hae II digestion of Bubu-DQA exon 2-3 region; Lane M =λ DNA EcoR I + Hind III marker, Lane 1 : Undigested PCR product, Lane 2 = DQA1 and DQA2 haplotype, Lane 3-4 = undefined haplotypes

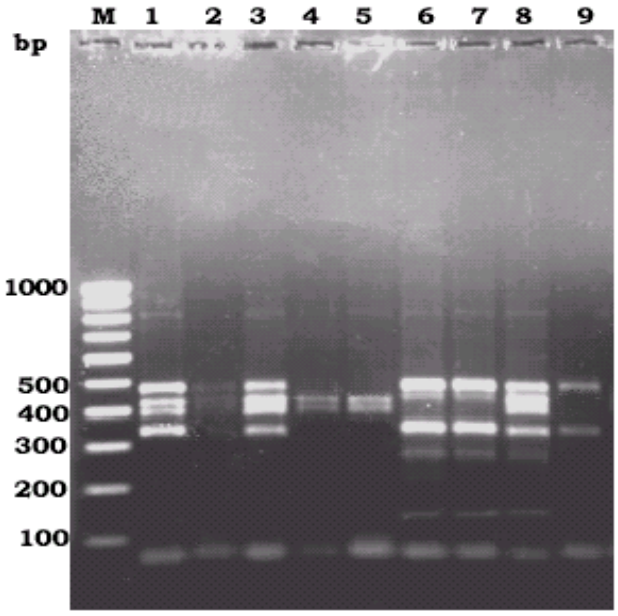

Fig. 26

Figure 2: PCR-RFLP patterns after Ava II digestion of Bubu-DQA exon 2-3 region; Lane M = 100 bp DNA Ladder (Marker), Lane 1-3=AACC pattern, Lane 4-5 = AA pattern, Lane 6-7 = BBCC pattern, Lane 8 = ABCC pattern, Lane 9 = CC pattern.

In present study, PCR-RFLP of Bubu-DQA by Ava II restriction enzyme could identify three different DQA haplotypes in buffalo. This simple, rapid and reliable PCR-RFLP technique can be used for the identification of duplicated DQA haplotypes in buffalo population. Further, greater presence of duplicated haplotypes in the population, which seems favorable under natural selection, may contribute to the higher host resistance against wider range of pathogens by generating greater genetic diversity.

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