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PCR-RFLP polymorphism in peptide binding region of MHC class II DRA gene in Ongole cattle

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Abstract
The variability of the major histocompatibility complex (MHC) molecules is correlated with the diversity of the T-lymphocyte receptors which in turn determine the disease and parasite resistance of an organism and thus may influence the long term survival probability of populations. In cattle, *DRA* has not been investigated in depth, and the extent of variation at the locus is not yet known. In this study, we used PCR-RFLP analysis to screen for potential variation in the second and third exon of bovine *DRA*, which encodes the antigen-presentation groove/peptide binding region. Two unique patterns were detected among 50 Ongole cattle screened from India with *Taq I* and single pattern with *Hae III* Restriction Enzymes. The polymorphism detected may be linked to variation elsewhere in the gene that affects its structure or function. Further investigations with larger sample size; including cloning and sequence analysis may identify the complete polymorphism and potential breed specific alleles if any, which will increase the MHC, Class-II, DR receptors diversity. Present results may also be important in the context of the associations between MHC variations at this locus, with susceptibility to disease for this breed of cattle in further studies.

Keywords: MHC DRA, PCR-RFLP, cattle, DR receptors diversity

1. Introduction
Major Histocompatibility Complex (MHC) has a determinant role in deciding the fate of antigen and initiating the immune response. MHC of cattle is known as BOLA system (for bovine lymphocyte antigens). It is divided into *BOLA-A* and *BoLA-D* which harbour class I and class II genes respectively. The class II region in cattle, designated *BOLA-D,* was first defined as the major locus controlling mixed lymphocyte reactions (MLR) (Usinger *et al.* 1977) [1].

MHC class II molecules are cell surface glycoproteins that bind antigenic peptides and present them to T lymphocytes for immune recognition. MHC class II molecules are αβ heterodimers with an Antigen Binding Site (ABS)/Peptide Binding Site (PBS), comprising the outermost domains of both subunits. An important feature of the ABS/PBS of MHC molecules is its very high level of polymorphism, with alleles differing by 10-20-amino acids within the 200-residue domain. This polymorphism appears to be functionally very important, as the residues that line the ABS define the properties of peptides that can be bound and presented to T cells. Different MHC alleles will therefore present to T cells different populations of peptides, which may or may not include peptides important for the development of an effective immune response (Russel and Gallagher, 1996) [2]. The variability of the MHC-molecules is correlated with the diversity of the T-lymphocyte receptors which in turn determine the disease and parasite resistance of an organism and thus may influence the longterm survival probability of populations (Hedrick *et al*, 2001) [3]. Loss of genetic diversity in the MHC genes is associated with reduced ability to cope with new and changed diseases (Frankham, 2003) [4].

MHC variants influence many important biological traits, including immune recognition, susceptibility to infectious and autoimmune diseases, individual odours, mating preferences, kin recognition, cooperation and pregnancy outcome (Simone Sommer, 2005) [5]. Polymorphism in the bovine leukocyte antigens (BoLAs) has been extensively investigated for identifying markers for bovine diseases and immunological traits. This approach requires identification and documentation of the allelic diversity of BoLA among different animals across the globe (Vandre *et al*, 2014) [6].

However, a detailed knowledge of the organization of the MHC and efficient tools for analyzing MHC polymorphism are prerequisites for powerful investigations on MHC disease associations. These prerequisites have, so far, not been acquired for farm animals.

In, *BOLA-D,* again there are two expressed groups of MHC class II receptors, DQ and DR. For DQ, the *α* and *β* chain encoding genes are highly polymorphic and duplicated in some individual animals, creating extensive diversity for the DQ molecule (Sigurdardottir *et al*., 1992) [7].

For DR, only one *α*-chain gene (*DRA*) was identified, and only one *β*-chain gene (*DRB3*) is known to be functional in cattle (Groenen *et al.*, 1990) [8]. In cattle, *DRA* has not been investigated in depth, and the extent of variation at the locus is not yet known

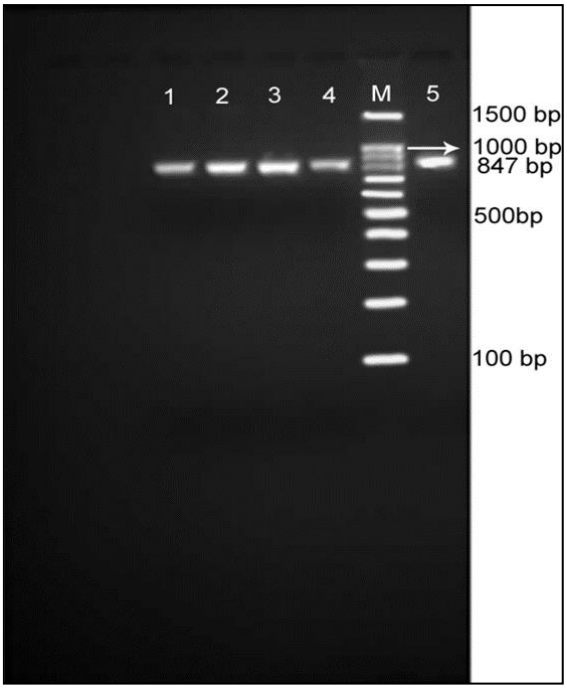

Fig 1: PCR Product of 847 bp fragment of DRA gene from genomic DNA of Ongole cattle

Lane 1-4 and 5: PCR product of 847 bp fragment of DRA gene in Ongole cattle.

Lane M: 100 bp DNA ladder used as marker

Before sequence variation was reported, the bovine DRA gene is thought to be monomorphic (Ellis and Ballingall, 1999) [9]. However, this may not be true, especially as DRA has not been well studied in cattle, and in sheep the homologue has 3 alleles identified by Southern hybridization (Escayg *et al*., 1993) [10]. Although DRA appears to be highly conserved in most mammalian species (Chu *et al*., 1994; Smith *et al*., 2005) [11, 12], polymorphism has been observed in equids (Brown *et al*., 2004) [13], rhesus macaques (de Groot *et al*., 2004) [14], and water buffalo (Sena *et al*. 2003) [15].

Hence in the present study, to study the polymorphism of DRA gene, we isolated the genomic DNA of white blood cells from the native cattle of Andhra Pradesh and world famous breed i.e., Ongole which is known for its disease resistance and hardiness. PCR amplification of exon 2 and 3 regions of MHC-II DRA locus and subsequent Restriction Fragment Length Polymorphism (RFLP) analysis showed, one variant with *Hae III* and two RFLP variants with *Taq I* Restriction Enzymes (RE), in which frequency of one variant is less than other, indicating less polymorphism of DRA locus in this breed.

2. Materials and Methods

2.1 PCR-RFLP of DRA gene

For a polymorphism study of DRA gene in Ongole cattle, venous blood was collected from 50 unrelated Ongole cattle from institutional herd. Genomic DNA was isolated by phenol-chloroform extraction method (Sambrook & Russel 2001) [16]. A 847 bp fragment of DRA gene corresponding to exon-2, intron-2 and exon-3 was amplified by using the primer pair DRAG1-F and DRAG1-R (fig. 1). Primers for PCR amplification (Forward:5'-DRAG1(F):5'GGAATCAGCCGAGTTTATGTTTG 3', Reverse: DRAG 1 (R):5'GGGCAGGAAGGGGAGGTAGT 3') were designed based on the available cattle sequence (Gen Bank Acc.No.D37956) by using the DNASTAR computer programme. Amplification was carried out in 25 µL reaction mixture containing 1x PCR buffer, 1.5mM $MgCl_2$, 250µM dNTPs, 30ng of each primer, 1U Taq DNA polymerase and genomic DNA template in a thermal cycler for 35 cycles (94 ºC/45 sec, 61 ºC/40 sec and 72 ºC/45 sec). The PCR product was digested with two restriction enzymes namely *Taq I and HaeIII* to study polymorphism for RE (Restriction Enzyme) sites in the genomic sequences.

3. Results and Discussion

3.1 HaeIII digestion of 847 bp DRA gene

HaeIII digestion was carried out at 37 ºC overnight and the digested products were run on PAGE and visualized in the gel doc system. It was found only one type of band pattern for this enzyme in the all samples screened (fig. 2).

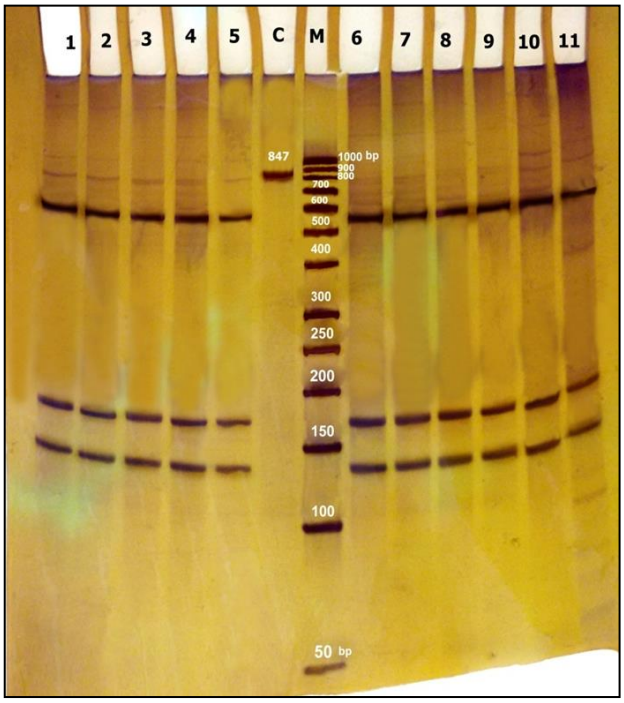

Fig 2: Hae III digestion of 847 bp fragment of DRA gene in Ongole cattle

Lanes 1-5 and 6-11: Hae III digested product of 847 bp fragment of DRA gene

Lane C: Undigested product (847 bp)

Lane M: 50bp ladder used as marker

Three band pattern in all 50 samples. Hence no polymorphism found for this restriction enzyme for the DRA gene in Ongole cattle. Several authors using conventional RFLP (hybridisation based) technique, reported monomorphism in DRA gene (Russel and Gallagher, 1996., Albright-Fraser *et*

al., 1996 and Kulski *et al*., 2003) [2, 17, 18]. Sakaram *et al*., (2013) [19] in their PCR-RFLP studies also reported no polymorphism of this gene. Present results correspond well with the study of (Russel and Gallagher, 1996., Albright-Fraser *et al*., 1996 and Kulski *et al*., 2003 and Sakaram *et al*., 2013) [2, 17, 18, 19] indicating the monomorphic and conserved nature of this gene, irrespective of methods followed.

3.2 Digestion of 847 bp DRA gene with *Taq I*

Taq I digestion was carried out with 10µl of PCR product of DRA gene incubated overnight at 65ºC and the digested products were run on PAGE and visualized in gel doc system. The digestion yielded two kinds of pattern corresponding to the two RFLP variants in the total fifty samples screened for polymorphism (fig. 3).

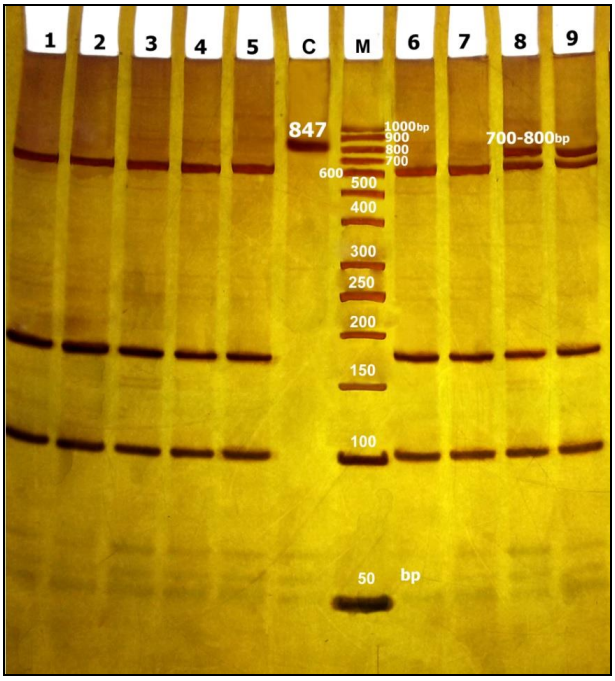

Fig 3: Taq I digestion of 847 bp fragment of DRA gene in Ongole cattle

Lanes 1-5 and 6-9: Taq I digested product of 847 bp fragment of DRA gene
Lane C: Undigested product (847 bp)
Lane M: 50bp ladder used as marker

The fact that very limited polymorphism was revealed with other enzyme implies that the extensive *TaqI* polymorphism is mainly due to restriction site polymorphism and not so called length polymorphism (i.e. insertions, duplications, etc.). *TaqI* has been reported to give a high frequency of polymorphism as its recognition sequence involves the doublet CpG (Barker *et al*. 1984) [20]. Furthermore, it is known that the CpG doublet occurs at a high frequency in the 5' part of MHC class I and class II genes (Tykocinski & Max, 1984) [21]. In the present study five samples out of fifty yielded a four fragment pattern and remaining 45 samples revealed three fragment (band) pattern with *Taq I* indicating only two kinds of RFLP variants for DRA gene suggesting the low polymorphic and the conserved nature of this gene in ongole cattle. The hybridization studies by Andersson *et al*., (1986b) [22] revealed three DRA types, Sigurdardottir *et al*., (1988) [23] reported 5 DRA types. Zhou *et al*., (2007) [24] reported four DRA types (SNP's) in cattle and those bovine *DRA* allele sequences detected were named as *01011, *01012, *01013, and *01014. All of these SNP were synonymous, and no amino acid polymorphism was predicted in the α1 domain of the bovine DRA molecule.The monomorphic views were given by some authors like Russell and Gallaghar, (1996) [2], Kulski *et al*., (2003) [18], Sakaram *et al*. (2013) [19] for DRA gene. These results indicated that the gene had not under gone much recombinations or gene conversions in the molecule. The present study findings are in agreement with the low polymorphic view of Andersson *et al*., (1986b) [22], Sigurdardottir *et al*., (1988) [23], and Zhou *et al*., (2007) [24], irrespective of the methods followed. Small population size of the present investigation might also be contributed to this less polymorphism. Large number of samples needs to be screened to elucidate the polymorphic pattern of this gene.

4. Conclusion

The present study led to the knowledge of genetic variation in the immune resistance gene (MHC, Class II-DRA) in Ongole cattle of India. The polymorphism detected may be linked to variation elsewhere in the gene that affects its structure or function. Further investigations are required with larger sample size; with cloning and sequence analysis to identify the complete polymorphism and potential breed specific alleles if any, which will increase the MHC, Class-II, DR receptors diversity. Present results may also be important in the context of the associations between MHC variation at this loci, and susceptibility to disease for this breed of cattle in further studies.

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