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ASSOCIATION OF GENETIC RESISTANCE TO GASTROINTESTINAL NEMATODES AND THE POLYMORPHISM AT *CAHI*-DQA1 EXON 2

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Abstract: The Major Histocampatability Complex (MHC) is an organised cluster of tightly linked genes having immunological functions. The MHC has been consistently associated with nematode resistance and the polymorphism in MHC class II genes is playing important role in resistance to gastrointestinal nematodes. The widest polymorphism among the MHC genes is found in locus DRB and DQA. The most frequently investigated fragment of the DRB and DQA gene covers exon 2, which codes the binding site for a foreign protein. The present study was to know the polymorphism in the *cahi*-DQA1 gene in resistance to Kumaon hill goats. A total of 60 animals were used for analysing resistance of Kumaon hill goats. The blood samples were collected and genomic DNA was isolated. The *cahi*-DQA1 gene was amplified from the genomic DNA, purified and sequenced for the polymorphism. The nested PCR amplicons of DQA1.2 revealed 349bp in agarose gel electrophoresis. The sequence obtained was analysed and found the existence of five *cahi*-DQA1 alleles in Kumaon hill goats. The occurrence of alleles varied with different proportion among the population. The variation in *cahi*-DQA1 gene was correlated with the published alleles.

Keywords: MHC, FEC, DNA, PCV, DRB, DQA, Polymorphism.

Introduction

The parasitic infection is one of the main problems causing considerable losses in ruminants causing decrease in productivity (Perry and Randolph, 1999), mortality (Sykes, 1994), and high economic losses (Iqbal *et al*., 1993) in small and marginal farming communities. The control of the nematode infestations in ruminants relies mainly on the proper organization of grazing and (or) use of anthelmintic agents. One of the novel approaches is the characterization and utilization of host genetic variation for resistance or resilience to endoparasites. It is carried out by selection and breeding of animals genetically resistant to nematodes (Baker *et al.,* 1999; Bishop and Stear, 1999).

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A number of studies show that, the QTLs (Quantitative Traits Loci) for resistance to gastrointestinal nematodes in ruminants are located on chromosomes. The polymorphism in MHC class II genes is playing important role in resistance to helminths, in which *DRB* and DQA locus are important in small ruminants. They encodes for beta chain and α-chain of the DR and DQ molecules respectively found in high concentrations on the surface of antigen-presenting cells. The MHC Class II genes are members of the immunoglobulin super family, which are functionally specialised for presentation of antigenic peptides. The major histocompatibility complex plays an important role in immune responsiveness, disease resistance, autoimmunity and reproductive success (Tizard, 2008). The MHC class II molecules are found on the surface of antigen-presenting cells (APC), mainly on B lymphocytes, macrophages and dendritic cells and are responsible for presenting exogenous antigens to $CD4^+$ T lymphocytes. The MHC of goat (Cahi/GoLA/CLA) is located on chromosome 23 (Vaiman *et al.,* 1996). A number of studies showed that the MHC region had a statistically significant association with gastrointestinal nematode parasite resistance (Schwaiger *et al*., 1995; Outteridge *et al*., 1996; Paterson *et al*., 1998; Van Haeringen *et al*., 1999).

Materials and Methods

Animals for study

The experimental local hill goats used in present study belonged to Indian Veterinary Research Institute herd maintained at IVRI, Mukteshwar (Nainital, Uttarakhand) is located in the temperate Himalayan region of India at $29^0 28' 20'' N$, $79^0 38' 52'' E$, and has an average elevation of 2,171 metres (7,123 feet) above the mean sea level (msl).

Blood sample collection

The Blood samples (5ml) were collected aseptically in plastic containers with anticoagulant (0.1% ethylene diamine tetraacetic acid (EDTA)) from the jugular vein from 60 local hill goats.

Isolation of Genomic DNA

The genomic DNA was isolated using the 'Genomic DNA Purfication Kit' supplied by Qiagen using standard protocol. The isolated DNA was resolved by 0.8% agarose gel and checked for concentration and quality. The quality (ratio of A_{260}/A_{280}) and quantity ($ng\mu l^{-1}$) of genomic DNA was estimated using NanoDrop (NanoDrop 1000-Thermo Scientific Spectrophotometer, USA).

Amplification of *cahi-DQA1* exon 2

Initially cahi-DQA1exon 2 was amplified from genomic DNA by using published primers. To avoid amplification of non specific products, the first PCR amplicons were subjected to nested PCR. The initial PCR was standardised with annealing temperature of 54°C. The amplicons of first PCR used for nested PCR. The nested PCR was also standardised with annealing temperature of 54°C.

Table 1: Primers for the PCR amplification and sequencing of DQA1 Gene

Orientation	Primer	Sequence (5′ - 3′)	Length (bases)
Primers for initial PCR			
Sense	DQA intL2	CAC CAA ATG AAG CCC ACA AAT G	20
Antisense	DQA1-677R	CCC TAG GGA AAA AGG GAG TGA	20
Primers for Nested PCR			
Sense	DQA Int L3	GCC CAC AAT GTT TGA TAG TC	20
Antisense	DQA1 ex2 REV ver2.1	GGG RAC CAC ATA CTG TTG GTA G	21

Sequence based typing and analysis

The sequences obtained from the forward and reverse reactions of each gene were analysed using GENE TOOL and Lasergene program (DNASTAR, Madison, WI) software as well as visually using chromogram of corresponding gene. The nucleotide sequences were compared with sequences in the GenEMBL database using the BLAST algorithm. The heterozygous positions were assigned ambiguity codes as recommended by the IUPAC-IUB Biochemical Nomenclature Commissions. A consensus of nucleotide sequence containing ambiguity codes at heterozygous positions was generated.

Results

The nested PCR amplicons of DQA1.2 revealed 349bp in agarose gel electrophoresis (Fig.1). The amplified products consisted of 249bp exon 2 and both side of exon flanked by introns. The nested PCR samples were purified and sequenced using same primers.

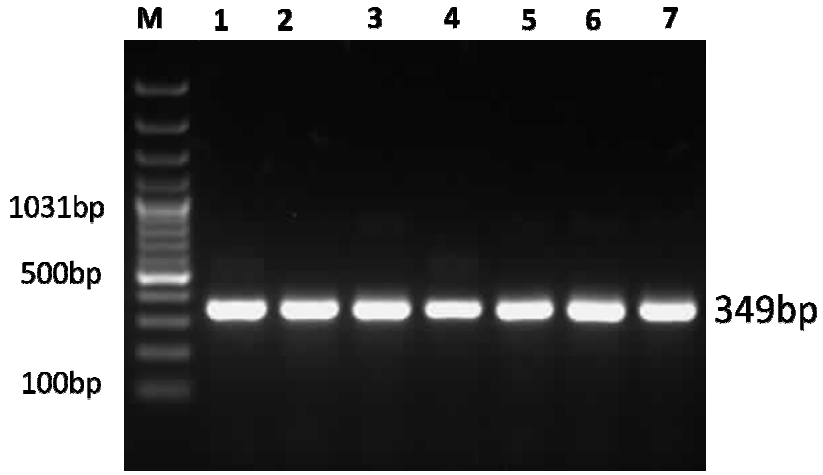

Fig 1. The amplification of *Cahi*–DQA1 exon 2 showing a band at 349bp in Kumaon Hill goats

Lane M: 100bp plus DNA ladder

Lane 1-7: 349bp PCR product of *DQA1* exon 2

Sequence results

The sequencing of the DQA1.2 revealed 349bp gene including exon of 249bp encoding 83 amino acid protein. The nucleotide identity between DQA1.2 and published alleles is between 85 to 100% for hill goats. Among the 249 nucleotides and 83 amino acid positions, 37 (14%) nucleotide positions and 27 (32.5%) amino acid residues were variable (Fig. 2). Many of the variations were localized to the specific regions of peptide binding. The analysis of the DQA1.2 sequences of hill goat population revealed existence of four alleles namely DQA1.1, 1.4, 1.5 and 1.6 as well as one new allele (showing 98% similarity to allele 1.4. The frequency of allele DQA1.1 is 12.5%, allele 1.4 is 25%, allele 1.5 is 12.5% and allele 1.6 is 25% as given in Table. 2. The allele 1.2 and 1.3 were completely absent in the hill goat population. The new allele (98% homology with known allele 1.4) frequency was 25% of the hill goats. The phylogenetic analysis (Fig.4) reveals clustering of majority of animals around alleles such as DQA1.4, 1.5 and 1.6. The aminoacid identity with published alleles was between 74-100% (Fig. 2).

Table 2: The frequency of different *DQA1* alleles in Kumaon hill goats

Allele	**Frequency**	**Percent**
DQA1.1	3	12.5
DQA 1.4	6	25
DQA 1.5	3	12.5
DQA 1.6	6	25
Allele 98% of **DQA** 1.4	6	25
Total	**24**	**100.0**

The deduced amino acid alignments showed more or less similar to the nucleotide homology. However, the frequency of occurrence new allele (98% homology with known allele 1.4) in the population is more along with known allele 1.4, as per deduced amino acid sequences. A total of five DQA1.2 alleles were found among the hill goat population. After thorough analysis of sequences, it was found that majority of the animals with in the population were homozygous (75%).

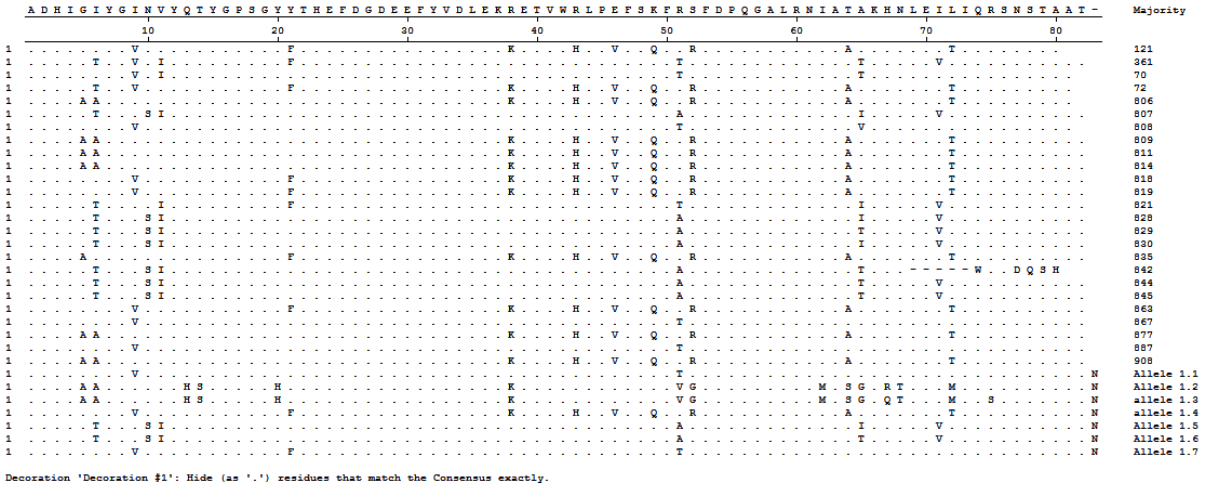

Fig. 2: The aminoacid sequence alignment of *cahi*-DQA1 with published alleles

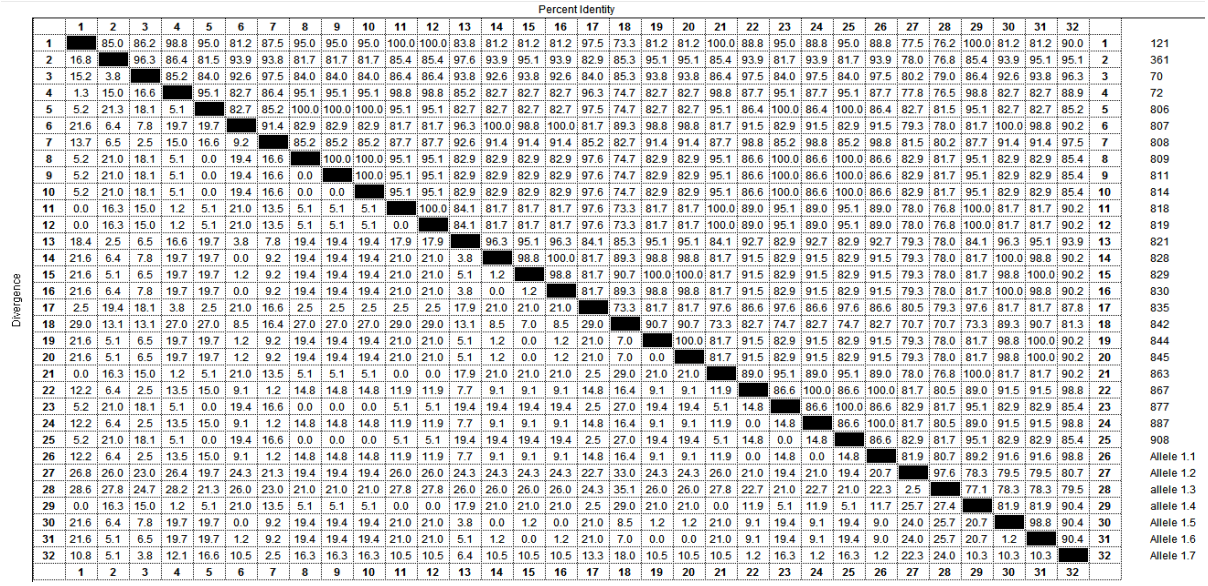

Fig. 3: The aminoacid sequence homology of *cahi*-DQA1 with published alleles

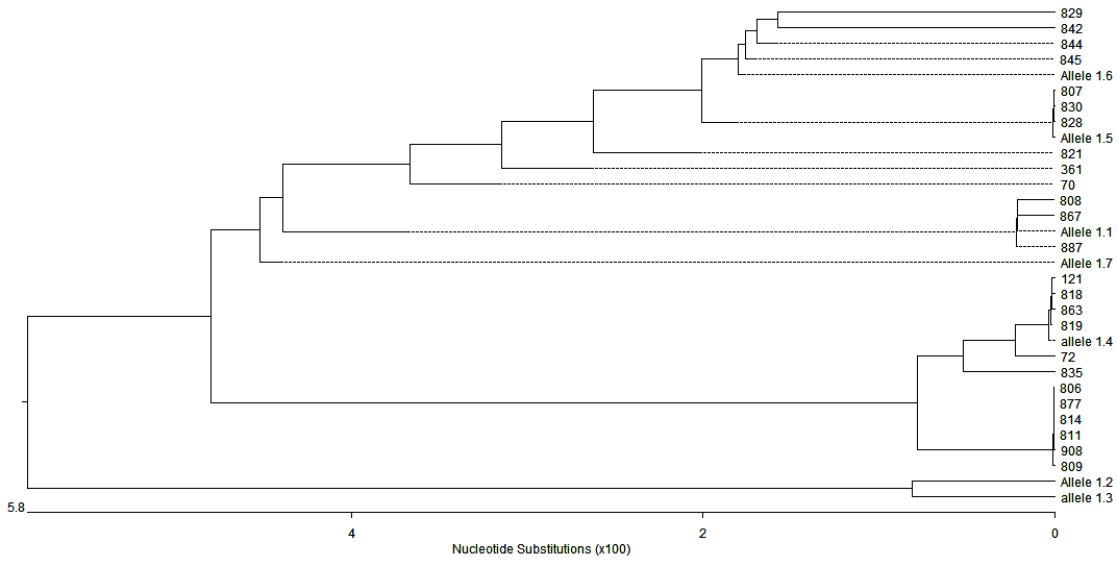

Fig. 4: The phylogenetic analysis of *cahi*-DQA1 with published alleles

Discussion

It is well known fact that members of a given species are not all equally susceptible to infectious diseases though all individuals are equally exposed. Evidence showed that domestic animals show variation in the ability to resist infection with protozoan and helminth (worm) parasites, which have been documented. Such variation in response is now known to occur both within and between breeds of cattle, chicken, goat, pig and sheep (Owen and Axford, 1991). The genetic basis of variation in resistance is evident from observations on breed differences, from the results of selective breeding experiments and from laboratory studies. The enhanced capacity to resist infection is clearly inherited and can be passed from parent to offspring, usually as a dominant trait (Wakelin and Blackwell, 1993). The nematode resistance includes the initiation and maintenance of a host response that prevents, reduces or clears parasitic infection (Hooda *et al*., 1999; Bricarello *et al.,* 2004). The resistant animals do not completely reject the disease, but they have a lower parasitic load than susceptible animals, as measured by fewer eggs in their faeces. This resistance is based on the immunological capabilities of each individual when challenged with parasitoses. Resilience is the capacity of an animal to compensate for the negative effects of parasitism by the maintenance of productive parameters (Paolini *et al*., 2005). Some breeds have moderate or low resistance with relatively high resilience, allowing them to have productivity similar to those that are naturally resistant (Alba-Hurtado *et al*., 2010). Low or reduced FEC has been used as a parameter for sheep selection in Australia (Wooleston, 1993; Eady *et al.,* 1996) and New Zealand (Pernthaner *et al*., 1995). The Genetic markers associated with resistance could

also be used to select sheep within a breed. Alleles OMHC1-188 and OLADRB2-282 of the major histocompatibility complex (MHC) (Figueroa et al., 2010) and several quantitative trait loci (QTL) that contain diverse significant loci have been associated with FEC reduction.

The polymorphism in MHC gene is strongly influence the outcome of infection and lead to genetic resistance to infections. The main function of MHC molecule is the presentation of peptide antigen to T lymphocytes. Special attention is paid to the MHC class II molecules that induce the immune response in case of extracellular infection. The present study is designed to study the genetic diversity in hill goats of Uttarakhand with respect to MHC class II genes DQA1.2, hitherto no one studied any of the genetic study in these goats. The major histocompatibility class II DQ molecules are dimeric glycoproteins involved in antigen presentation to CD4+ T cells. The sequencing of the hill goat DQA1.2 revealed 349bp gene including exon of 249bp encoding 83 amino acids. The total reading frame of DQA1 gene is 768bp consisting of four exons and encoding a 255 amino acid protein (GenBank accession no. AY464652). The sequencing of exon 2 amplicons revealed the existence of five DQA1 alleles in hilly goats of Uttarakhand. The amino acid positions involved in peptide binding were identified according to Reche and Reinherz (2003). Among the 349 nucleotides and 116 amino acid positions, 37 nucleotide positions and 28 amino acid residues were variable. Many of the variations were localized to the specific regions of peptide binding. We identified five Cahi-DQA1 alleles that likely correspond to four different allelic lineages. There are seven DQA1 alleles are reported from Capra hircus with at least three amino acid substitutions. Ten of the 23 polymorphic amino acid sites were included in the peptide binding region and consequently they might play a crucial role in immunological processes modulating disease pathogenesis (Amills et al., 2005). This gene is known to be extremely polymorphic in many species, and 10–18% of sheep are reported to completely lack a DQA1 gene (Zhou and Hickford, 2004). The Ovar-DQA1 gene was 8.4-fold more highly expressed in resistant animals. In our study all animals expressed DQA1 gene of major histocompatability complex. There is a higher frequency of DQA1 null alleles in susceptible animals (Keane et al., 2007). The null allele of DQA1 was also associated with susceptibility in a separate selection flock, presenting the hypothesis that failure to present parasite antigens to immune cells led to nematode susceptibility.

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