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Lack of association of resistance towards *Haemonchus contortus* infection with PCR-RFLP of MHC-DYA gene in Rambouillet crossbred sheep*

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ABSTRACT

The present study was carried out to find class I molecular marker for resistance and susceptibility against *Haemonchus contortus* infection in Rambouillet crossbred sheep. Random samples of blood and faeces (50) were collected from adult sheep maintained at Sheep Breeding Farm, Reasi, Jammu and Kashmir, India. Genomic DNA was isolated and a 719 bp fragment from exon IV-V of MHC-DYA was amplified using specific primers. The PCR product was digested with restriction enzyme *Alu* I and *Taq* I. *Alu* I digestion revealed only one type of banding pattern with 3 bands of size 386 bp, 323bp and 10 bp. *Taq* I digestion of PCR product also revealed only one type of banding pattern with two bands of size 670 bp and 49 bp. Hence the exon IV-V of MHC-DYA gene was monomorphic for restriction enzyme *Alu* I and *Taq* I. Faecal egg count was done by modified McMaster's method following faecal larvae culture to identify the% of *Haemonchus contortus*. All the eggs counted were of strongyles out of which 90±5% (85–95%) of larvae were of *Haemonchus contortus.* Twenty one animals with epg of 0 were grouped as resistant. Nine animals were grouped as susceptible with epg of 400 and above. Since no polymorphism was detected, no association could be established.

Key words: *Haemonchus contortus,* Host Resistance, MHC-DYA, Sheep

One of the major constraints in sheep husbandry is gastrointestinal nematode infection. The nematode of particular concern is *Haemonchus contortus,* which can cause severe blood loss resulting in anemia, anorexia, depression, loss of condition, and eventual death. In India, annual treatment costs of *Haemonchus contortus* infection was estimated to be $103m (McLeod 2004). Dukkipati *et al.* (2006) investigated the polymorphisms of genes within the Ovar-MHC and their association with resistance to infectious diseases. In Rhonschaf sheep markers *OarCP73, DYMS1* and *BM1815* have a significant association with haematocrit level, IgL (L3 stage larvae specific immunoglobulin) level and log transformed FEC respectively following an artificial challenge with *Haemonchus contortus* (Janssen *et al*. 2002). The MHC-*DYA* closely linked to the microsatellite *DYMS1*, is a possible candidate gene for resistance to *Haemonchus contortus* infection in sheep (Charon 2004). Also in an extensive whole-genome QTL analysis for resistance to *H. contortus* it was found that in one family weak linkage between egg counts and the *Ovar-DYA* region in the MHC class IIb region was there (Marshall *et al.* 2009).

Therefore considering the potential role of MHC in determining genetic resistance against *Haemonchus contortus* infection, the present study was carried out with the objective to find genetic polymorphism within MHC-DYA gene and its association with resistance towards *Haemonchus contortus* infection.

MATERIALS AND METHODS

Animals: The present study was carried out on 50 adult Rambouillet crossbred sheep maintained at Sheep Breeding Farm, Reasi, Jammu and Kashmir, India. Both blood samples and faecal samples were collected from same 50 animals. The samples were taken at random in order to estimate gene frequency. The same samples were used for association study.

Isolation of genomic DNA: Blood (5 ml) was collected from jugular vein of sheep in a 15 ml polypropylene tube containing 250µl of 0.5 M EDTA as anticoagulant. Genomic DNA was isolated using standard phenol chloroform extraction method (Sambrook *et al.* 2001).

Primer designing: A pair of PCR primers for MHC-DYA

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gene was designed based on the available sequences of sheep (Acc.no. AJ812235). The forward primer was of 23 bp (TGGTGGGCAC- TGTCCTCATCCTC) and reverse primer was of 28 bp (ATCCCCCTAGCTTCCCTTT-CAGTGTTAC) with Tm 73.6°C and 70.8°C, respectively. The PCR primers were designed as such to amplify a part of exon IV, complete intron IV and a part of exon V.

Polymerase chain reaction (PCR): PCR was carried out in a final volume of 50 µl reaction mixture containing 100ng of template DNA, 1X PCR assay buffer, 2.0 mM of Mg^{2+}, 200 µM of dNTPs, 10 pM of each primer and 1U of *Taq* DNA polymerase. Amplification was carried out in thermal cycler. Different annealing temperature was tried to optimize PCR cycle condition. PCR condition were: initial denaturation at 94°C for 5 min; followed by 94°C for 40sec, 65°C for 40sec, 72°C for 1 min, and a final extension of 72°C for 5min.

PCR-RFLP: To detect the PCR RFLP, the amplicon was digested independently with *Alu I at* 37°C and *Taq I* at 65°C. The restriction digestion was carried out in 25 µl volume. For setting a large scale restriction digestion a master mix was prepared on ice by adding the different components in following order: For *Alu I* R.E Assay Buffer C (10x) 2.5µl, *Alu I* enzyme (5U/µl) 3.0µl and autoclaved distilled water up to 25µl was taken. For *Taq I* R.E assay buffer C (10x) 2.5µl, *Taq I* enzyme (10U/µl) 1.5µl and autoclaved distilled water up to 25µl was taken. *Alu I and Taq I* digested product was run on the 2.5% agrose gel, respectively in 1×TAE buffer for 2–3 h at 5 V/cm

Faecal egg count: Faecal sample (5–10 g) was collected from the rectum of the same sheep from which blood samples was collected. Eggs were counted by modified McMatser method (FFMA 1986) and the animals were grouped as resistant and susceptible based on the relative egg count.

Faecal culture/coproculture: The culture of egg to produce larvae was performed according to Soulsby (1982a). The percentages of different types of nematode larvae were recorded after studying their morphological details.

RESULTS AND DISCUSSION

Polymerase chain reaction: Agarose gel electrophoresis of the amplicon revealed an amplification of a fragment of 719 base pair (Fig. 1).

*Alu I/PCR – RFLP: Alu*I digestion of the PCR product revealed only one type of genotypic pattern. The PCR product of 719 bp was digested in to restriction fragments of molecular sizes 386 bp, 323bp and 10 bp (Fig. 2). The sizes of the fragments were ascertained with available sequence of ovine MHC DYA gene (Acc. no. AJ812235) and was confirmed by running 100 bp ladder in the gel. The band of 10 bp was not visible because of the limitation of agarose gel. There was no variation or polymorphism in 50 sample and same type of banding pattern was revealed and hence the exon IV-exon V of Sheep MHC DYA gene is monomorphic with respect to *Alu* I restriction enzyme.

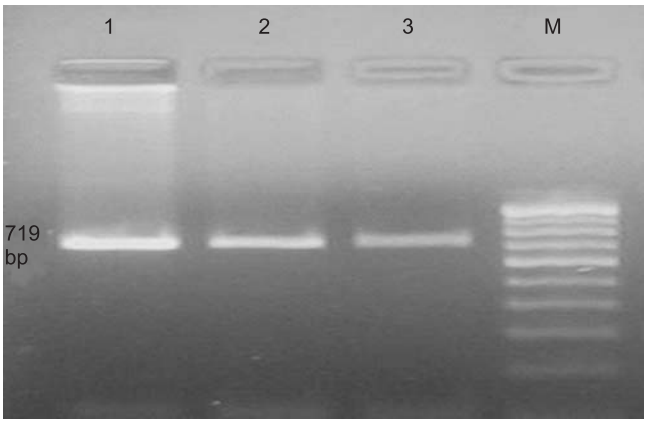

Fig 1. Amplification of PCR product; Lane1–3: PCR product; lane M, 100bp DNA ladder.

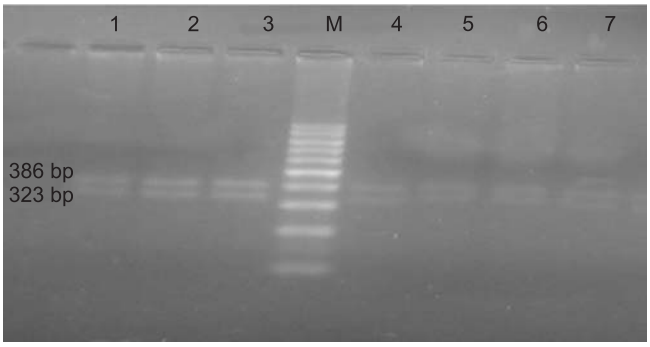

Fig 2. Genotypic patterns of MHC-DYA/*Alu* I PCR-RFLP; Lane M, 100 bp DNA Ladder; lane 1–7, digested PCR product.

Taq I/PCR – RFLP: The PCR product was digested in to restriction fragments of molecular sizes 670 bp and 49 bp (Fig. 3). The band of 49 bp. was not visible because of the limitation of agarose gel. The sizes of the fragments were ascertained with available sequence of ovine MHC DYA gene (Acc.no. AJ812235). There was no polymorphism in 50 sample and same type of banding pattern was observed

Faecal egg count (FEC) and faecal culture/coproculture: All the eggs counted were of strongyles and no other infection was present. In faecal culture, 100 larvae were counted on the glass slide at random and the recording of % of different types of nematode larvae revealed that about 90±5% (85–99%) of larvae were of *Haemonchus contortus.*

The mean of the FEC was 168. Based on the mean of the FEC the animals were grouped as resistant and susceptible towards *Haemonchus contortus* infection. All animal with epg of 0 eggs per gram were grouped as resistant. A total of 21 such animals were grouped as resistant. Next, all animals with epg of 400 and above are grouped as susceptible. A total of 9 such animals were grouped as susceptible (Fig. 4).

Association study: Though variation in response to infection with *Haemonchus contortus* was detected, no association was established. The reason being that exon IV

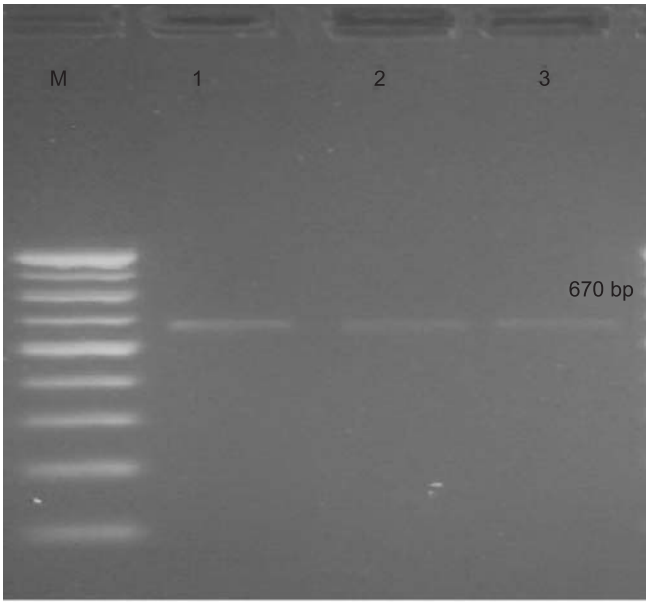

Fig. 3. Genotypic patterns of MHC-DYA/*Taq* I PCR-RFLP. Lane M, 100 bp DNA Ladder; lane 1–3, digested PCR product.

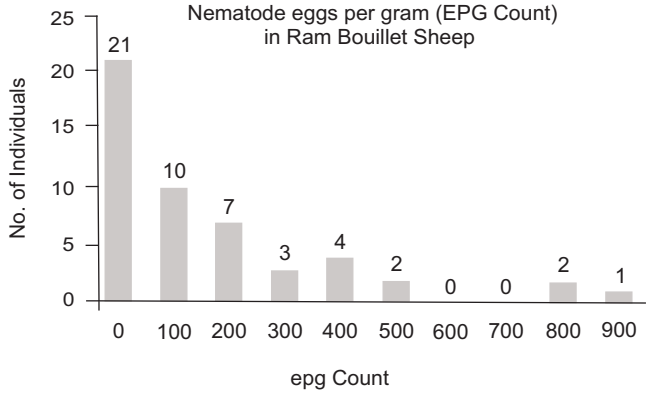

Fig. 4. Faecal egg count of strongyles.

–exon V of MHC DYA gene was monomorphic with respect to *Alu* I and *Taq* I restriction enzyme.

This is the first study reporting exon IV-V of MHC DYA gene PCR RFLP. Though, no polymorphism was detected enough variation in sheep resistance against strongyles (*Haemonchus contortus)* infection was detected. However several studies showed significant association between gastrointestinal nematode infection and polymorphism in MHC and non MHC genes. According to Sayers and Sweeney (2005) quantitative trait loci for resistance against nematode infection include the MHC genes, interferon gamma gene, IgE gene and microsatellites on chromosome 1, 5 and 6. An allele at a microsatellite locus located in the first intron of interferon gamma gene in Soay sheep was associated with reduced faecal egg count. The same allele was associated with increased *T. circumcincta* IgA level in Soay sheep lambs (Coltman *et al.* 2001). Ovar-DRB1 gene plays an important role in resistance to nematode infection in the Suffolk breed (Sayers *et al.* 2005). These results suggested that resistance/ susceptibility against gastrointestinal nematode infection is well under control by genes responsible for immune response of host.

In a similar study polymorphic bands were detected within the DQB and DRB regions of the ovine major histocompatibility complex by probing *Taq* I digested DNA from 3 large sheep half-sib families derived from a highly resistant ram. All animals were phenotypically assessed for resistance towards *Haemonchus contortus* infection by faecal egg counts and associations with RFLP bands and haplotypes, and found that there is no significant association between any band or haplotype and faecal egg count (Blattman *et al.* 2009). However, Castillo *et al*. (2011) reported that MHC polymorphisms have an important role in resistance or susceptibility against parasite in Pelibuey sheep and could be used as genetic markers to assist selection and improve parasite resistance to *H. contortus* infection. There is a genetic component to nematode resistance, and the MHC is one of the most important components of genetic resistance. QTL analyses have shown a link between the MHC region and FEC in mouse models, and in sheep and cattle (Lee *et al.* 2011).

Janssen *et al*. (2002) also found significant association of microsatellite *DYMS1 and* resistance to *Haemonchus contortus infection* in sheep. The MHC DYA gene is closely linked to the microsatellite *DYMS1* and hence a possible candidate gene for resistance towards *Haemonchus contortus* infection (Charon 2004). However we could not find any association due to lack of polymorphism. This is a preliminary work and does not imply that MHC-DYA has no role in resistance towards *Haemonchus contortus* infection. One of the reasons of detecting no association is due to nature of infection. Unlike artificial challenge model as in Janssen *et al*. (2002), the dose of infective organism or duration of exposure cannot be determined in a naturally infected herd. Another possible reason could be due to the difference in genetic background of sheep population under study. It is also plausible that more than a single gene is responsible for determining resistance/susceptibility to *Haemonchus contortus* infection.

In the conclusion though we find variation in resistance against *Haemonchus contortus* infection we could not establish any association due to lack of polymorphism. However it would be interesting to study exon IV-V polymorphism in other Indian breeds of sheep. Future studies need to be directed to explore polymorphism throughout the entire MHC-DYA gene and to ascertain their suitability as potential class I genetic marker for resistance towards *Haemonchus contortus* infection.

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