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Research Article

5' UTR OF IGE EPSILON (E) HEAVY CHAIN AND ITS ASSOCIATION WITH MIXED NATURAL INFESTATION OF *Haemonchus contortus* IN ROHILKHANDI GOAT

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Abstract- Immunoglobulin E has a prominent role in allergic reactions specially those launched against parasites. It has been thought that IgE dependent immune response has been evolved to target specifically metazoan parasites that are too large for phagocytosis. In the current manuscript, we explored the possibility of association between polymorphism at 5' UTR of IgE gene and faecal egg count in case of natural exposure to *Haemonchus contortus* in Rohilkhandi goat breed. We studied the polymorphism by means of direct sequencing and made four haplotypes. Among these, the haplotype B was having significantly highest FEC value (575.00 ± 450.00 epg) at P< 0.05.

Key words- *IgE, 5' UTR, Haemonchus contortus, Rohilkhandi goat, FEC*

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Introduction

Immunoglobulin E (IgE) is the primary antibody involved in hypersensitivity (type I) and allergic reactions, it contains two heavy chains (called epsilon heavy chain) and two light chains, the epsilon (ε) heavy chain contains four immunoglobulin like constant domains (*Cε* 1 to *Cε* 2). The other most important role of IgE is in parasitic/ helminth infestations. The characteristic feature of any helminth infestation is the strong Th2 inclined immune response with mastocytosis, eosinophilia and immunoglobulin class switching to produce IgE [1]. This response is peculiar to parasitic infestation and it has been thought that IgE dependent immune response has been evolved to target specifically metazoan parasites that are too large for phagocytosis [2]. Therefore, studies relating parasitic resistance have been targeting levels of IgE for identification of susceptible or resistant animal. The increased numbers of mast cells are a hallmark of many nematode infections and they have been implicated in the control of worm numbers in some infections like *Trichinella spiralis, Haemonchus contortus* [3]. Resistant or responder animals have a high number of IgE bearing cells and might be used as an additional marker for resistance [4]. Huntley [5] indicated that IgE may be involved in the rapid expulsion of incoming larvae from the gut but the mechanism is not clear. IgE leads to mast cell sensitization and consequent release of histamine via, degranulation of these cells, thus causing worm expulsion. Association between IgE plasma level and low faecal egg count has been previously reported in naturally infected crossbred sheep [6]. Clarke and Beh [7] reported a polymorphism at 5' end of ruminant *Cε* gene and it was postulated that sequence variation near 5' end of the ruminant *Cε* region might influence the efficacy of class switching to the *Cε* gene or affect the level of *Cε* gene expression. Clarke *et al*. [8] reported polymorphism at 5'end of ovine IgE gene and its subsequent association with *Trichostrongylus columbriformis* infection in one sheep flock however attempt to confirm this finding in two other flocks using linkage analysis and genotype association failed to identify any significance associations between IgE polymorphism and resistance to either *T. columbriformis* or *H. contortus* infection. However, looking into the importance of 5' untranslated region in class switching process the current study was planned to study this region in Rohilkhandi goats which were exposed to a mixed natural infection of *H. contortus.*

Materials and Methods

Population studied

Rohilkhandi goats maintained at Sheep and Goat Farm, LPM section ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, were used for the current study. Rohilkhandi is a small sized, dual purpose goat found in the districts Bareilly, Pilibhit, Shahjahanpur, Budaun, Moradabad, Bijnor and Rampur in the state of Uttar Pradesh, India.

Sample collection

Blood and faecal samples were collected for the current study. Blood was collected in sterile EDTA coated vacutainers and bought to lab on ice and stored at -21 oC till further processing. Rectal faecal samples were collected from each animal and stored in plastic zip-lock bags and bought on ice in lab. Due care was taken to process all the samples on the same day of collection.

Faecal egg count by McMaster technique

Faecal egg counting was done using McMaster egg counting technique and the results were expresses as eggs per gram (epg) of faeces. In brief, it involves the counting of parasitic eggs by subjecting a weighed amount (2 gm) of faeces to egg

floatation technique using saturated salt solution, the nematode eggs being lighter floats on top whereas the debris settles down. The supernatant fluid containing the eggs was then gently mixed and charged in to McMaster egg counting chamber. It was allowed to settle down for two minutes and eggs were counted inside the gridlines of the chamber under 4X magnification. Number of eggs counted were then multiplied by 50 (dilution factor) to get eggs per gram (epg) of faeces [9]. For better statistical accuracy we used the average of four readings per sample.

Dot ELISA

To ascertain the presence of *Haemonchus* Dot ELISA test was done on serum samples separated from each animal. Dot ELISA test was performed as per the protocol described by Prasad *et al*. [10], using the ES (Excretory – Secretory) antigen against the test and control (positive and negative) serum samples.

Animal selection for genotyping

Animals were exposed to mixed natural infection of *H. contortus* by delaying the scheduled deworming. All the animals were first screened for the presence of gastrointestinal nematodes by McMaster faecal egg counting technique. Presence of *Haemonchus* was confirmed by larval culture of pooled faecal samples in Baermann's apparatus and dot ELISA test. Once it was confirmed that the only *Haemonchus* is present, the strongyle group eggs were taken for FEC and statistical analysis. For genotyping, animals were selected based on FEC and dot ELISA results. Two groups were made, the high epg group (FEC values > 150 epg and dot ELISA positive) and the lower epg group (FEC values < 149 epg and dot ELISA negative).

DNA Isolation and PCR amplification

DNA was isolated from whole blood using the technique as described by Sambrook and Russel [11]. The quality and quantity of DNA was checked using 0.8% agarose electrophoresis and nanodrop spectrophotometer (ND-2000). As the data on polymorphism at 5' UTR of IgE gene in goats is not available genotyping was done by direct sequencing. PCR amplification was carried out using 80ng of genomic DNA, in a 25 µl reaction using 10 pmoles of each primer as reported by Clarke *et al*. [8], 12.5 µl of PCR master mix (Thermo scientific) and nuclease free water to make the volume. The amplification was carried out using a pre-programmed thermal cycler (ABI thermocycler) with the following conditions: Initial denaturation at 95°C for 5 min, followed by 39 cycles of denaturation at 94°C for 1 min, annealing at 59.0°C for 45 s and extension at 72°C for 1 min, and finally the last extension at 72°C for 5 min. The PCR products were checked by agarose gel electrophoresis using 2.0% agarose gel in 1X Tris-Borate-EDTA (TBE) buffer at 6 V/cm for 1 h. The PCR products were then cleaned by using GeneJet PCR purification kit following the manufacturer's instructions and the cleaned samples were sent for automated sequencing.

Sequence analysis

The raw sequences obtained for different animals were assembled using DNA Baser software to obtain final sequences. Final checking of variations between sequences and the reference sequence was done by looking the chromatogram peaks for each individual variation. The final assembled sequences were then blasted (www.ncbi.nlm.nih.gov/BLAST) to ascertain the accuracy of the different regions and to obtain the correct annotation. The obtained final sequences were then aligned in MEGA 7.0 software using ClustalW algorithm. Haplotypes were made based on difference between the sequences. The haplotypes were then analysed using Mega 7.0 to find dissimilarities between them and were then submitted to NCBI database. SAS 9.3 software was used to find association of these haplotypes with FEC.

Statistical analysis

Statistical analysis was performed using SAS 9.3 (SAS® Software Version 9.3, 2011). The FEC values showed skewness hence were log10(x + 2) transformed before analysis to normalize the distribution. To find the effect of haplotypes on FEC, one-way analysis of variance performed in a general linear model using the PROC GLM procedure of SAS 9.3.

Results and discussion

The primers amplified a 617 bp fragment in Rohilkhandi goats. Raw sequences obtained for each animal were aligned by NCBI BLAST and DNA baser programme to assemble the final contig. The final sequences obtained were then aligned by MEGA 7.0 software and based on 100% sequence similarity different haplotypes were made. Total four haplotypes were made based on above criteria and each animal was assigned a single haplotype according to sequence similarity. These four haplotypes were submitted to NCBI gene bank database and accession numbers were assigned as KT725637 (Haplotype A), KT737378 (Haplotype B), KT737379 (Haplotype C) and KT737380 (Haplotype D). As the sequence consisted of untranslated region, the haplotypes were only analyzed for nucleotide variations, not for amino acid variations and were tested for statistical association with faecal egg count (EPG and log EPG). Clarke *et al*. [8] have characterized the 5' un- translated region (UTR) of immunoglobulin E in sheep. They reported an insertion deletion polymorphism in the 640 bp fragment of 5' UTR of IgE gene and one of the alleles were found to be significantly associated with intestinal nematode parasite *T. columbriformis* [8]. In the current study, we used primers for the 5' UTR of IgE gene as described by Clarke *et al*. [8] and genotyped each individual by direct sequencing and three variable sites were found on the basis of which four haplotypes were made. The sites of dissimilarities were at position 50th, 607th and 608th. As per the expectation the mean overall distance between the sequences was low (0.003) and divergence between the haplotypes ranged from 0.2 to 0.5. The phylogenetic tree was prepared using maximum likelihood method based on JTT matrix-based model in Mega 7.0, all positions with gaps were excluded from analysis. This clubbed the haplotype A and D together and haplotype B and C together in two different clusters [Fig-1].

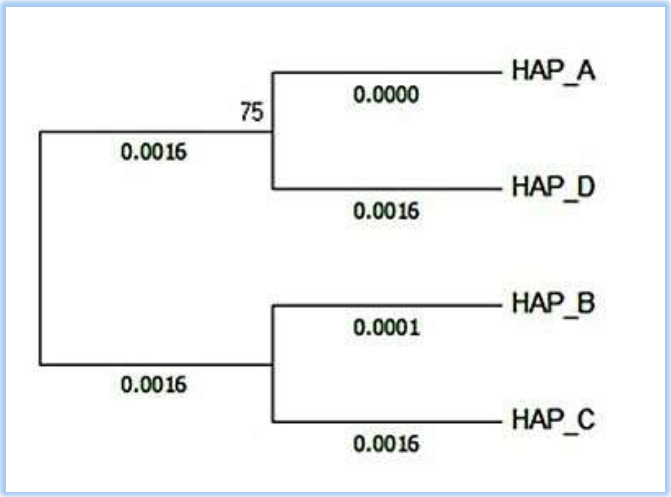

Fig-1 Phylogenetic tree constructed using maximum likelihood method of all four haplotypes. Values above/below the branches indicated the divergence.

Table-1 Analysis of variance carried out to establish association between FEC, LFEC and haplotypes

Trait	Source of variation	Sum of Squares	Mean Square	F	Sig.
FEC	Between Groups	448976.936	149658.979	3.729	0.024*
	Within Groups	1043365.864	40129.456		
	Total	1492342.800			
LFEC	Between Groups	8.140	2.713	2.894	0.054
	Within Groups	24.377	0.938		
	Total	32.517			

**Indicates significant at P<0.05*

The haplotypes A and C were found in maximum frequency (0.367), followed by haplotype D (0.200) and haplotype B was found in least frequency (0.066). The mean FEC value in the population studied was 131.80 ± 41.42 epg. The haplotype B had the highest FEC value of 575.00 ± 450.00 epg, followed by haplotype C (136.55 ± 37.85 epg), haplotype D (97.50 ± 95.50 epg) and haplotype A (65.18 ± 43.40 epg).

Due to skewness in data, the FEC values were log transformed (LFEC), however for comparative purposes analysis of variance was performed with both FEC and LFEC. Because of the prominent role of immunoglobulin E (IgE) in hypersensitivity reactions and in immune response against parasitic infestations, the IgE gene has been considered as a candidate gene for resistance/susceptibility against gastro-intestinal nematodiasis. IgE levels has been associated with resistance to gastrointestinal – nematodiasis *viz*., *Trichostrongylus columbriformis* in sheep [12] [13], H. *contortus* [14]. There was significant association between the haplotypes and FEC values at P<0.05 however, no association could be established with LFEC (P<0.05) (Table 1). The multiple comparison procedure showed haplotype B (Hap_B) to be significantly different from other haplotypes with highest EPG value. The one-way analysis of variance showed significant association with FEC but no association with LFEC at P<0.05. One of the haplotype was having significantly higher FEC value (haplotype B) as compared to others. Regardless of the fact that the analysis was carried out in relatively less number of individuals, the genotyping was done for extreme groups (low and high FEC), hence the results suggests that the variations at 5' UTR region of IgE gene may play a role in susceptibility/resistance towards *H. contortus* (or gastro-intestinal nematodiasis in general) infestation in Rohilkhandi goat.

Conclusion

Immunoglobulin E plays significant role in allergic response specially which are of parasitic origin, hence, its role in conferring resistance to gastrointestinal nematodiasis is unequivocal. Previous results at expression level have shown increased IgE levels in resistant sheep, based on faecal egg count. Current study underscores the fact that in goats, this region and the polymorphism within, may play significant role in imparting resistance towards mixed natural infestation of *H. contortus*.

Application of research

The current research can be applied on larger sample size to characterize the polymorphism in 5' UTR of goat, this polymorphism can later be used in development of marker for gastro-intestinal nematodiasis in goat.

abbreviations

FEC – Faecal egg count
LFEC – log transformed faecal egg count
Epg – eggs per gram
UTR – Untranslated region
ELISA – Enzyme linked immune-sorbent assay
IgE – Immunoglobulin E

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