Short communication

Exploring the molecular basis of resistance/ susceptibility to mixed natural infection of *Haemonchus contortus* in tropical Indian goat breed

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ABSTRACT

The present investigation was carried out with the objective to identify putative candidate genes / Quantitative trait loci for resistance / susceptibility towards *Haemonchus* infestation in tropical goat breed (Rohilkhandi goat) of India. The mean faecal egg count (FEC) and packed cell volume (PCV) of the population were 142.78 ± 22.54 epg (eggs per gram) and $31.73\% \pm 0.49$, respectively. Grouping of animals as per dot ELISA test showed 41.33% (n = 124) positive and 58.66% (n = 176) negative for *Haemonchus* infestation. The microsatellite loci DYA and ODRB1.2 were significantly associated ($P \leq 0.05$) to parasite resistance. The locus DYA showed significant association with log FEC and dot ELISA and the locus ODRB1.2 showed significant association with log FEC, PCV and dot ELISA at $P \leq 0.05$. Real time expression profiling revealed that the susceptible group (high FEC group) had 11.1-fold more expression of IFNγ mRNA (Th$_1$ cytokine) and 0.11-fold lower expression of IL-10 mRNA (Th$_2$ cytokine), which was found to be statistically significant ($P \leq 0.05$).

1. Introduction

The gastrointestinal nematodes (GINs) are one of the major threats in small ruminant (sheep and goat) production systems, because of their high prevalence and worldwide distribution. Among these GINs, *Haemonchus contortus*, commonly known as Barber's pole worm is one such important parasite which parasitize the abomasum of small ruminants causing haemorrhagic lesions, massive blood loss and high mortality specially in young sheep and goat (Emery et al., 2016). Due to high level of commercialization in sheep industry, there has been dominance of studies concerning host - parasite relationship and parasitic control strategies, however, these areas are still lagging in goat industry (Hoste et al., 2010). Studies have shown that the browsing behaviour of goats has led to avoidance of infective L$_3$ larval stages, therefore, the immune response in goats is such that it is strongly expressed to reduce the growth, development and female fertility of parasite harboured in abomasum, however, for establishment of infective L$_3$ larva and persistence of adult worms in abomasum the immune response is weakly expressed (Hoste et al., 2010). Hence, goats in

principal, may act as carriers once they acquire the infection, therefore, also forming a potential source of pasture contamination.

The major areas of concern in current control strategies are the mounting evidence for development of resistance by gastrointestinal nematodes to anthelmintic treatments (Papadopoulos et al., 2012), environmental impact and rising cost of treatment and labour. Hence, presently the focus for control has shifted towards breeding for nematode resistance. Genetic selection for GIN resistance is advantageous because genetic changes are of permanent nature and it gives a manageable strategy to control nematode parasites (Zvinorova et al., 2016) without any risk of development of drug resistance. Reports have suggested that there are some goat breeds around the world that are inherently resistant or resilient toward gastro-intestinal nematodiasis, in particular to haemonchosis (Chiejina and Behnke, 2011).

The current study deals with the identification of specific genetic markers associated with resistance to indicator traits related with haemonchosis in goats using the Rohilkhandi breed as our model organism which was tested for within breed variability in haemonchosis related indicator trait(s) in a population naturally exposed to mixed *H.*

contortus infestation.

2. Material and methods

2.1. Experimental animals and sample collection

Rohilkhandi goats maintained at Sheep and Goat farm, Indian Veterinary Research Institute, Izatnagar, Bareilly, UP, India, were used for this study. Before screening and selection of animals the scheduled deworming was delayed, and animals were kept exposed naturally to parasitic infestation. Rectal faecal samples of 300 animals (including bucks and goat, excluding the advance pregnant does and kids up to 4 months age) were collected and stored in plastic zip lock bags and bought to lab for further processing. Blood was collected under aseptic condition for serum separation and genomic DNA isolation. Due care was taken to collect blood and faecal sample from each animal on the same day.

2.2. Phenotyping of animals

Faecal egg count (FEC), PCV and dot ELISA were performed for phenotyping of animals. FEC for strongyle eggs was estimated from weighed rectal faecal samples (2 g) using Mc Master egg counting technique and the results were expressed as eggs per gram (epg) of faeces (MAFF, 1986). The minimum detection level of assay is 50 epg, however, to reduce it to 25 epg, two McMaster slides (04 chambers) were counted for each sample and their mean was taken for final analysis. PCV estimates were used to ascertain the extent of anaemia developed in each subject animal, using the standard Wintrobe's tube method (Feldman et al., 2000). To determine the presence of *H. contortus,* pooled faecal samples were subjected to coproculture and larval identification as per the protocol described in RVC/FAO Guide to Veterinary Diagnostic Parasitology (Gibbons et al., 2015). Dot ELISA test was performed on serum samples as described by Prasad et al. (2008) and the test results were compared against standard positive and negative control.

2.3. DNA extraction and PCR amplification

Genomic DNA was isolated from blood using the standard protocol of Sambrook and Russel (2001). PCR amplification was carried out using primer sequence (as shown in Appendix I & II) in a standard PCR mixture (Thermo Fisher Scientific). The amplified products were checked on 3% (w/v) agarose gel electrophoresis using the standard protocol as described by Sambrook and Russel (2001).

2.4. Genotyping of animals

SNPs (Appendix I) and microsatellites (Appendix II) were used for genotyping of animals which were selected as per their reported association with the indicator traits. For SNP genotyping of animals, the PCR products were sequenced and suitable enzymes were selected using Neb cutter v 2.0 (http://nc2.neb.com/NEBcutter2/) (Appendix I). For microsatellite genotyping, the amplified products were first run on 2.5% (w/v) agarose gel electrophoresis to check for their amplification. The products were then resolved on ultra-high-resolution agarose to differentiate alleles as per their length (in base pairs). Visualizing of each gel was done under Gel Doc (Genesnap, Syngene) system and allele size was determined by using Gel Analyzer (2010) software.

2.5. Real time expression profiling

Real time expression profiling was done to establish differential gene expression between resistant and susceptible animals. For this, the animals were classified into two groups based on their faecal egg count estimates, high group (FEC ≥ 150 epg and dot ELISA positive) and low group (FEC ≤ 149 epg and dot ELISA negative). From each group, ten samples were used for RNA isolation and each sample was run in triplicate for expression analysis. RNA was extracted from freshly isolated PBMC (peripheral blood mononuclear cells) following Sambrook and Russel (2001). The purity of RNA was verified by measuring absorbance in UV-Spectrophotometer at 260 nm and 280 nm. From the cleaned RNA samples, cDNA was synthesized using High capacity RNA to cDNA kit (Invitrogen, Life Technologies, USA) as per manufacturers' protocol. The RT-qPCR was performed using Fast SYBR® Green Master Mix (Applied Biosystems, Life Technologies, USA) and primers as reported by Shakya et al. (2009) using GAPDH as internal control.

3. Statistical analysis

Statistical analysis was performed using SAS 9.3 (SAS® Software Version 9.3, 2011) and for population genetic data Popgene V 1.32 was used. The polymorphism information content (PIC) values were calculated by the formula of Botstein et al. (1980). The FEC values showed skewness hence were $\log_{10}(x + 1)$ transformed before analysis to normalize the distribution. To find the effect of various genotypes on indicator traits (log FEC, PCV, dot ELISA), one-way analysis of variance for indicator traits was performed in a general linear model using the PROC GLM procedure of SAS 9.3. The dot ELISA procedure yields result as positive or negative in binary format hence, the association between genotypes and dot ELISA results were analysed using PROC LOGISTIC procedure. The probability modelled against was dot ELISA negative taken as zero (positive taken as 1) at $P \leq 0.05$. In expression profiling, the target genes were compared against the housekeeping control (GAPDH) gene. The fold changes in target transcript levels were determined by using the method described by Livak and Schmittgen (2001) and was statistically tested using Mann Whitney test. Heritability (h^2) was estimated using paternal half sib correlation method, model II of Harvey (1990), taking sire as random effect, with the statistical model as:

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where, Y_{ij} = Log FEC; μ = population mean; S_i = effect of ith sire; e_{ij} = random error (NID ~ 0, σ_e^2).

4. Results and discussion

4.1. Phenotypic traits

FEC, PCV and dot ELISA were the phenotypic indicator traits that were studied in 300 Rohilkhandi goats. The coproculture on pooled faecal samples showed presence of only *Haemonchus* spp. (L₃) larva and hence for epg estimation only the strongyle eggs were counted. The mean value of FEC and PCV was, 142.78 ± 22.54 epg of faeces and 31.73% ± 0.49, respectively. Dot ELISA test showed 41.33% (n = 124) animals were positive and 58.66% (n = 176) were negative for *Haemonchus* spp. infestation. There was statistically significant (P ≤ 0.05) difference between the mean FEC values of dot ELISA positive (208.97 ± 49.71 epg) and negative groups (96.31 ± 14.48 epg). However, there was no significant difference (P ≤ 0.05) in the PCV values between the two groups. The estimate of heritability obtained using paternal half sib method was 0.26 ± 0.16 for log FEC and 0.48 ± 0.31 for PCV. Heckendorn et al. (2017) showed a low heritability estimates for both FEC (h² = 0.22) and PCV (h² = 0.22), in Saanen and Alpine goat. We obtained low to moderate h² estimate for log FEC and PCV respectively, however, the precision of estimate was low and hence for a reliable estimate of heritability a larger population is required.