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ORIGINAL PAPER

# Putative SNPs in *Ovar-DRB1* and *GALNTL6* Genes Conferring Susceptibility to Natural Infection of *Haemonchus Contortus* in Southern Indian Sheep

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## Abstract

**Aim** To explore associations between phenotypic traits and polymorphisms in the *DRB1* and *GALNT6* gene in Nellore, Deccani and Kenguri sheep naturally infected with *Haemonchus contortus*.

**Materials and Methods** Blood and faecal samples were collected to evaluate fecal worm egg counts (FEC), packed cell volume (PCV), hemoglobin (Hb), eosinophilia and for DNA isolation.

**Results** Animals were grouped into susceptible and resistant groups based on EPG counts. FEC and circulating eosinophilia were higher in a susceptible group. Log FEC was negatively correlated ($P < 0.01$) with PCV, and Hb estimates. The second exon of *DRB*1 and intron variant of *GALNTL6* genes were amplified from DNA samples of resistant and susceptible sheep. Characterization of *Ovar-DRB1* amplicon by RFLP revealed two genotypes ('bb' and 'ab'). The genotype frequencies differed significantly between both groups ($P < 0.05$). The 'bb' genotypes had higher ($P < 0.05$) log FEC value than 'ab' genotypes and 'b' allele was linked with susceptibility to haemonchosis in sheep. The mean FEC of Nellore sheep was high indicating susceptibility of the breed and also in which the frequency of 'b' allele was more compared to the other two breeds. *OVAR-DRB1* genotypes associated with FEC did not affect PCV and Hb. PCR–RFLP assay developed to determine the genotypes with respect to SNP rs424521894 of *GALNTL6* revealed monomorphic nature at the locus in the breeds studied.

**Conclusion** MHC polymorphism could be used as a genetic marker for the selection of sheep resistant to *H. contortus*. However, a more intensive study, involving controlled infections and other *GALNTL6* SNPs may be enforced to make any decisive assertion.

**Keywords** Association · Faecal egg counts · *GALNTL6*, *Haemonchus contortus* · *OVAR-DRB1* · Sheep · Susceptibility

## Introduction

*Haemonchus contortus*, one of the pathogenic gastrointestinal nematodes (GIN) is an important health restraint in sheep production in India causing significant economic losses. Chemotherapy is the first line of defense against *H. contortus* infections due to the lack of commercial vaccines. Yet, widespread use and abuse of anthelmintics led to the development of anthelmintic-resistant parasites throughout the world [17] and forced a search for alternatives to chemotherapy. Selecting sheep, which are genetically resistant to *H. contortus*, is one of the viable alternative strategies because it is long lasting [38].

Host resistance to GIN is a moderately heritable trait [11] and is measurable through the performance of individuals after parasite challenges by phenotypic traits such as packed cell volume (PCV), serum antibodies, peripheral eosinophilia, FAMACHA visual indicator scores of anaemia and fecal egg counts (FEC). Use of both FEC and PCV values as indicators of GIN infections is limited because of the inability to store samples for a long time and laborious procedure of examination. However, FEC is far less invasive

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to measure and is the preferred method in most studies. Recognizing genetic markers related to disease resistance would probably improve the selection.

It is hypothesized that inherited GIN resistance is polygenic and related to immune system resistance [1]. Variation within these genes is related to resistance or susceptibility to *H. contortus* infection in sheep. Polymorphisms related to the DRB locus of major histocompatibility complex (MHC) and interferon gamma (IFN**γ**) genes have been the most frequently reported markers associated with *H. contortus* infection. The MHC genes are highly polymorphic [18] and play an important role in presenting processed antigen to host T-lymphocytes, causing T cell activation and consequent immunological cascade of events building host immunity. The polymorphism of MHC-DRB1 gene in sheep has been studied as a genetic marker for resistance to *H. contortus* [32]. Goats susceptible to *H. contortus* infection showed up regulation of IFNγ [30].

Recent genome-wide studies stated that not only immune mechanisms are important determinants of host resistance but also the genes involved in the gastrointestinal mucus production (*MUC* or *GALNT*), and hemostasis (*TAL*1 or *PPAP2B* or *LRP*8) regulation may also simulate [5]. Mucin biosynthesis plays an important role in gastric mucosal protection, acting as a barrier and triggering swift parasite expulsion. Exploration of genetic variation either within specific regions of the genome or more specifically in candidate genes involved in these pathways may help to identify a set of DNA markers significantly associated with parasite resistance characteristics. The effects of these established gene markers on phenotypic traits such as FEC and PCV are measured to select resistant individuals. The study is aimed to assess the resistance status of sheep against *H. contortus* using phenotypic traits such as FEC, peripheral eosinophilia and PCV in relation to variation in the genomic region of immune pathway and mucus production.

## Materials and Methods

### Sample Collection

Sheep (excluding the advance pregnant ewes and lambs up to 4 months age) grazed freely in native pastural land were included in the study to maximize and confirm the likelihood of natural parasite infection. Nellore sheep ($n=95$) maintained at the sheep farm of Livestock Farm Complex, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh, Deccani ($n=28$) and Kenguri sheep ($n=34$) maintained at sheep farm in Bidar district, Karnataka were included in the study. Faecal samples were collected directly from the rectum of 157 sheep in plastic zip-lock bags and were brought to the laboratory for further processing. From each animal, blood was also collected into a sterile vaccutainer containing K3 EDTA under aseptic conditions for haematological parameters estimation and genomic DNA extraction on the same day. The samples were labeled and transported to the laboratory in an ice-packed container immediately.

### Phenotyping of Animals

FEC, PCV, hemoglobin (Hb) and eosinophil count were performed for the phenotyping of animals. FEC measurements for strongyle eggs were determined by floating the faeces samples in zinc sulphate ($d=1.33$) solution on McMaster slide and counting the eggs. The results were interpreted as eggs per gram (epg) of faeces. Two chambers of McMaster slide were counted for each sample and their mean was considered for analysis. The presence of *H. contortus* was determined by culturing the pooled faecal samples and subsequent third-stage larvae identification.

PCV and Hb were determined using the standard Wintrobe's tube method and Sahil's hemoglobinometer respectively, on the same day of collection. Blood smears were stained with Leishman's stain and differential leukocyte counts (DLC) were carried out by battlement method. An absolute eosinophil count was estimated from the DLC and TLC values. The counts were expressed as a number of cells per microliter of blood.

### Genotyping of Animals

DNA was isolated from each blood sample (500 µL) using a modified high salt method [22]. The exon 2 of *Ovar-DRB1* gene was amplified using the primers and cycling conditions of Sankhyan *et al.* [27]. A total of 15 µL reaction mixture containing 30 ng DNA template, 5 pm of each primer, and 7.5 µL of master mix (Green dye PCR master mix (2x), Takara) was set up for amplification.

The polypeptide N-acetylgalactosaminyltransferase like-6 (*GALNTL6*) gene of sheep was amplified using the forward (5′ -AGACATACCTGGGACCACTTC-3′) and reverse (5′ -CCC ACTCTTAGCAACCCCATAG-3) primers that were designed using Primer Quest tool of Integrated DNA Technologies against an intronic variant of *GALNTL6* gene capturing the SNP rs424521894 identified as having significant association with parasite resistance in sheep from the genome-wide association studies (GWAS) [2]. The amplification reaction comprised of an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 94 °C for 45 s, 60 °C for 40 s and 72 °C for 60 s with a final extension of 7 min at 72 °C. A negative control was run along with the samples at every PCR setup. PCR amplicons were analysed on 2% (w/v) agarose gel containing ethidium bromide (0.5 µg/mL) and visualized under

a UV transilluminator (Omega Fluor™ Plus Documentation Systems, BioExpress, USA).

RFLP was employed to detect the polymorphic patterns and thus genotype the sheep at the *Ovar-DRB1*and *GALNTL6* (*polypeptide N-acetyl galactosaminyl transferase-like 6*) genes. The PCR amplicons of *Ovar-DRB1*genewere digested using REase *PstI* (5 U) (Himedia, Mumbai) with the appropriate buffer at 37 °C for 16 h. whereas the PCR amplicons of *GALNTL6* gene were digested using REase*Alu*I (10 U) (Himedia, Mumbai) with the appropriate buffer at the same conditions. The digested products were analyzed on 2% agarose gel to observe the polymorphic patterns and thus the genotypes of the *Ovar-DRB1* and *GALNTL6* genes. To monitor *Pst*I enzyme activity the PCR product of 285 bp without restriction enzyme was used as positive control. To monitor *Alu*I enzyme activity the PCR product of 543 bp without restriction enzyme was used as internal control. A 485 bp PCR product of TLR1 (F: 5′-TTTAGCAGCCTTTCCATACT-3′, R: 5′-CAGAATCGT GCCCACTATATGA-3′) selected from department laboratory archives having a known cleavage site for *Alu*I is used as positive control. Negative control was a mix inoculated with ultrapure water in substitution of PCR product and enzyme was used.

### Statistical Analysis

The descriptive statistics were derived on FEC, PCV, and Hb. Normalcy of the data was assessed with the Shapiro-Wilks test, kurtosis and skewness analyses conducted in SPSS17. The FEC data of this study was observed to be not normally distributed and showed positive skewness. As a result, a logarithmic transformation [LFEC = log10(FEC + 200)] was applied to normalize the FEC before analysis. No transformation was necessary for PCV, or Hb.

Genotypic and allelic frequencies were assessed for each candidate gene in each population [13]. Population genetic parameters including gene heterozygosity (He), polymorphism information content (PIC), and effective allele numbers (Ne) were calculated [3, 24]. A generalized linear model (GLM) was tested using the SPSS17 software to determine associations between SNP and phenotypic indicator, as measured by FEC/PCV/Hb. The statistical model was: $y = \mu + B + G + e$, where $y$ is the trait measured upon, $\mu$ is the overall population mean, $B$ is fixed effects of breed and $G$ is genotype, which was tested for an association with LFEC/PCV/Hb, and e is a residual error. Statistical analyses were performed, at a 5% significance level. All the graphs were plotted using the online version of chart maker [31].

## Results

### Phenotypic Data for Haemonchosis Status

The phenotypic indicator traits were assessed in 157 sheep (Supplementary material I). The faecal cultures revealed the presence of exclusively *H. contortus* infective larvae in the studied sheep. Hence, for EPG estimation only the strongyle eggs were counted. Sheep were grouped into high FEC (> 500 EPG; $n = 119$) and low FEC (0–500; $n = 38$) groups based on EPG counts corresponding to susceptible and resistant groups, respectively. The mean value of FEC in resistant sheep was lower ($48.7 \pm 14.3$ epg) than in susceptible sheep ($2675.2 \pm 280.6$ epg). The mean of log-transformed FEC (LFEC) was $3.3 \pm 0.1$ and $2.4 \pm 0.1$ in susceptible and resistant sheep, respectively. Among breeds, Nellore sheep had the highest log FEC than Deccani and Kenguri sheep. The mean value of eosinophilia was high in susceptible sheep with a significantly higher level of FEC.

The mean value of PCV and Hb in resistant sheep was high compared to that of susceptible sheep. The distribution of FEC, PCV and Hb among resistant and susceptible populations is depicted in Supplementary material II: Fig. 1–3. Log FEC was negatively correlated ($P < 0.01$) with PCV (0.71), and Hb (0.73) estimates. However, the correlation between PCV and Hb was positive ($P < 0.01$) in all sheep (0.95).

### Genotyping of Ovar-DRB1 and GALNTL6 Genes

The amplification of *Ovar-DRB1* gene and *GALNTL6* gene by PCR yielded the expected fragment of 285 bp and 543 bp, respectively. Oligonucleotide primers did not yield PCR products with the negative control. Following digestion of *Ovar-DRB1*amplicons with REase *Pst*I, two patterns were observed, one with 226 bp, 44 bp and 15 bp, and the second with 270 bp, 226 bp, 44 bp and 15 bp fragment which were referred to 'bb' (homozygous) and 'ab' (heterozygous) genotypes, respectively (Fig. 1). 'aa' (homozygous) pattern was not observed. The Renase *Pst*I recognizes the nucleotide sequence CTGCA↓G.

Considering the whole population together, the 'bb' genotype frequency was found to be non-significantly ($P > 0.05$) more (0.78) compared to that of 'ab' genotypes and the frequency of 'b' allele (0.89) was more than 'a' allele (0.11). Minor allele frequency (MAF) across the breeds was ranging between 0.09 and 0.16. The expected and observed genotypic frequencies for *DRB1*exon2/*Pst*I locus in the sheep groups were almost similar and were consistent with Hardy–Weinberg equilibrium ($P > 0.05$)

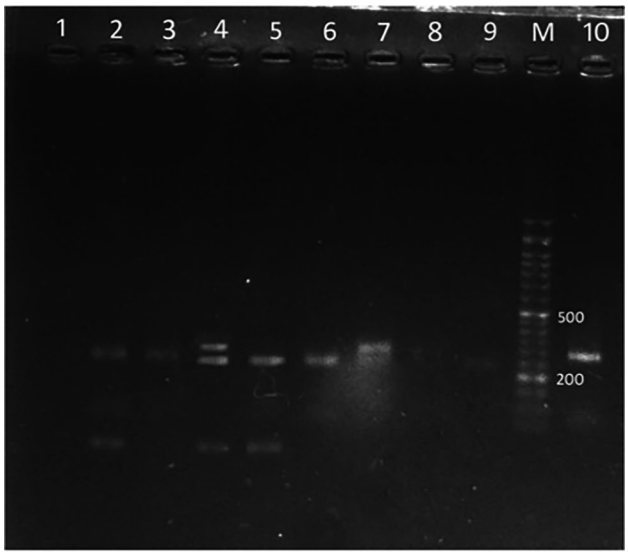


**Fig. 1** Electrophoretic patterns of exon 2 of *Ovar-DRB1* digested with *Pst*I. Lane M: 50 bp ladder; Lane 1: Negative control; Lane 2, 3, 5, 6: 'bb' homozygous genotypes at 226 bp, 44 bp and 15 bp; Lane 4 and 7: 'ab' heterozygous genotypes at 270 bp, 226 bp, 44 bp and 15 bp; Lane 10: Positive control (amplicon of *Ovar-DRB1* exon 2)

(Table 1). The observed heterozygosity value was in accordance with the expected heterozygosity (Supplementary material I). The PIC values are indicative of low polymorphism (0.15–0.23) in all the populations studied. Fixation index is −0.10 to −0.19.

With the significant influence of rs424521894 (*GALNTL6*) on resistance to GIN, the restriction enzyme (*AluI*) was identified to determine the SNP, thus enabling to screen sheep population by RFLP. Digestion of PCR fragment of *GALNTL6* with REase *AluI*, undigested single fragment (543 bp) referred to "aa' genotype (Fig. 2) was observed.

## Association of Ovar-DRB1 Genotypes and Breeds with Infection Traits

Least squares means and standard deviations for FEC, PCV and Hb levels involving breed and *DRB1* polymorphs as independent factors are shown in Table 2. The overall mean log FEC in the sampled sheep was 2089 ± 0.04. The pattern of distribution of FEC among the DRB genotypes of sheep is portrayed in Fig. 3. The 'bb' genotypes had significantly ($P < 0.05$) higher log FEC value than 'ab' genotypes. The mean FEC of Nellore sheep was high ($P < 0.01$) compared to Deccani and Kenguri breeds. No differences were observed in FEC between Deccani and Kenguri sheep ($P > 0.05$). The PCV and Hb was highest in the Deccani breed and statistically significant differences in mean PCV ($P < 0.01$) and Hb ($P < 0.05$) were observed among breeds. Eosinophil count is more in a susceptible group (0–7) than in the resistant group (0–2). The pattern of distribution of eosinophil, FEC, PCV and Hb among the DRB genotypes of sheep is portrayed in Supplementary material II: Fig. 4–6.

Considering the significant association of genotypes with log FEC the distribution pattern of genotypes between susceptible (high FEC group) and resistant (low FEC group) sheep were verified. The 'ab' and 'bb' genotype frequency in the resistant group was 0.32 ($n = 12$) and 0.68 ($n = 26$) and the corresponding values in the susceptible group were 0.19 ($n = 23$) and 0.81 ($n = 96$). The 'a' and 'b' allele frequencies in the resistant group were 0.16 and 0.84, respectively, and 0.1 and 0.9 respectively, in the susceptible group. Both the groups were in accordance with Hardy–Weinberg equilibrium. The genotype and allele frequencies between resistant and susceptible group was subjected to chi-square analysis. The genotype frequencies differed significantly ($\chi^2 = 4.45$; $df = 2$) between both groups ($P < 0.05$). As GLM analysis (Table 2) revealed a difference ($P < 0.01$) in mean log FEC among breeds, the data was subjected to analysis to test for the significant difference in genotype and allele frequencies between the breeds (Table 3). The genotype frequencies of Nellore sheep differed significantly ($P < 0.05$) with Deccani.

**Table 1** Distribution of genotypes and allele frequencies at *DRB1/Pst*I locus in sheep

| Genetic group | Total number of animals (*n*) | Observed genotype frequency | | | Allele frequency | | Expected genotype frequency | | | $\chi^2$ value | *P* value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | aa | ab | bb | a | b | aa | ab | bb | | |
| Nellore | 95 | 0.00 (0) | 0.19 (18) | 0.81 (77) | 0.09 | 0.91 | 0.01 (0.81) | 0.17 (16.38) | 0.82 (77.81) | 0.98 | $0.32^{NS}$ |
| Deccani | 28 | 0.00 (0) | 0.32 (9) | 0.68 (19) | 0.16 | 0.84 | 0.02 (0.65) | 0.27 (7.69) | 0.71 (19.65) | 0.89 | $0.34^{NS}$ |
| Kenguri | 34 | 0.00 (0) | 0.24 (8) | 0.76 (26) | 0.12 | 0.88 | 0.01 (0.42) | 0.21 (7.16) | 0.78 (26.42) | 0.52 | $0.47^{NS}$ |
| Total | 157 | 0.00 (0) | 0.22 (35) | 0.78 (122) | 0.11 | 0.89 | 0.01 (1.90) | 0.20 (31.20) | 0.79 (123.90) | 2.39 | $0.12^{NS}$ |

Figures in parentheses are the number of animals

*NS* Non significant ($P > 0.05$)

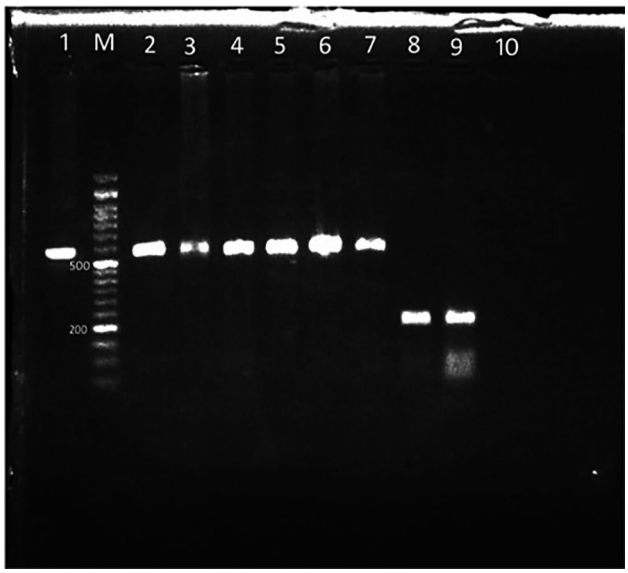


**Fig. 2** Electrophoretic patterns of *GALNT6* digested with *Alu*I. Lane M: 50 bp ladder; Lane 1: internal control (amplicon of *GALNT6* without enzyme); Lane 2–7: 'aa' (homozygous genotypes) at 543 bp; Lane 8 and 9: Positive control (digested amplicon of *TLR1*)

**Table 2** Least-squares mean (±SE) of Log-FEC, PCV, and Hb for effects of breed and *DRB1* genotypes in sheep

| Main effect/subclass | *n* | Log-FEC | PCV | Hb |
|---|---|---|---|---|
| Overall mean | | 2.89 ± 0.04 | 24.12 ± 0.53 | 7.98 ± 0.19 |
| Genotype | | * | NS | NS |
| Aa | – | – | – | – |
| Ab | 35 | 2.80 ± 0.07 | 24.48 ± 0.87 | 8.09 ± 0.31 |
| Bb | 122 | 2.98 ± 0.04 | 23.75 ± 0.52 | 7.86 ± 0.18 |
| Breed | | ** | ** | ** |
| Nellore | 95 | $3.29 \pm 0.05^{a}$ | $20.26 \pm 0.60^{a}$ | $6.63 \pm 0.21^{a}$ |
| Deccani | 28 | $2.68 \pm 0.08^{b}$ | $28.02 \pm 0.97^{b}$ | $9.22 \pm 0.34^{b}$ |
| Kenguri | 34 | $2.68 \pm 0.07^{b}$ | $24.08 \pm 0.91^{c}$ | $8.07 \pm 0.32^{c}$ |

Means with different superscripts within classes differ significantly

*n* number of observations

$^{*}P < 0.05$; $^{**}P < 0.01$; *NS* non-significant

Characterization of *GALNTL6* with PCR–RFLP revealed an undigested single fragment (543 bp) referred to "aa' genotypes. Since monomorphic no further association studies could be conducted for the investigated SNP.

## Discussion

Selection of resistant sheep mostly relies on indirect criteria including a number of nematode eggs passed in the sheep faeces (FEC), PCV and less commonly eosinophilia. It can be evaluated by both challenge and natural infections, but

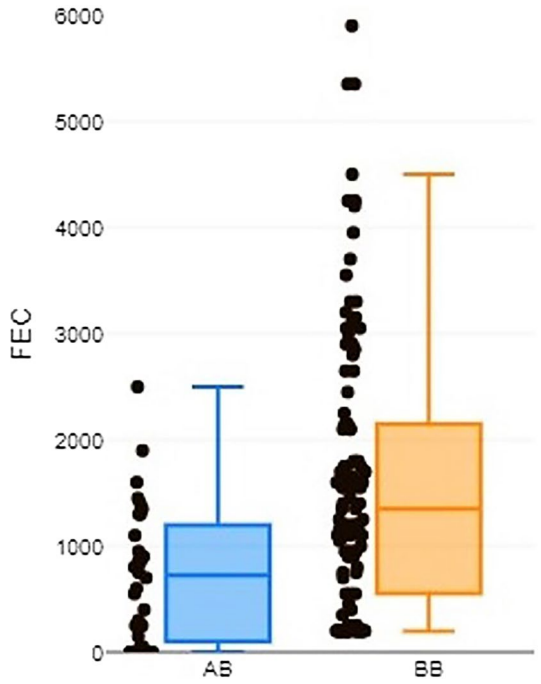


**Fig. 3** Distribution of FEC in DRB1 genotypes of sheep

**Table 3** Chi-square difference in genotypic and allelic frequencies among the breeds

| Breed | *DRB* locus | | |
|---|---|---|---|
| | Nellore | Deccani | Kenguri |
| Nellore | – | 4.45* ($P = 0.03$) | 0.74 ($P = 0.39$) |
| Deccani | 2.24 ($P = 0.13$) | – | 1.59 ($P = 0.21$) |
| Kenguri | 0.48 ($P = 0.49$) | 0.66 ($P = 0.41$) | – |

Above the diagonal: $\chi^2$ (*P* value) for differences in genotypic frequencies ($df = 2$) between the two breeds; Below the diagonal: $\chi^2$ (*P* value) for differences in allelic frequencies ($df = 1$) between the two breeds

*Significant ($P < 0.05$)

natural infection is better and more feasible while the heritability of infection based on FEC and PCV was greater with natural infection. For example, Red Maasai showed a better response to natural infection compared with experimental infection with *H. contortus*. Among phenotypic indicators, FEC has been the phenotype of choice to evaluate resistance in sheep experiencing similar parasite challenge as it is correlated to worm burden [20]. Variation in FEC of studied sheep could be because of a number of factors such as hypobiosis of larvae, suppression of egg production or marked differences in the proportion of female and male worms.

*Haemonchus contortus* is a blood-feeding parasite causing anaemia an important clinical sign; thence PCV percentage is another useful marker [34] and is negatively correlated

with FEC. In the studied sheep, FEC was negatively correlated ($P < 0.01$) with PCV, and Hb estimates [9, 19]. Low values for PCV are therefore commonly associated with high FEC due to the sucking of large amounts of blood from the abomasum by worms. PCV has also been reported to be higher in resistant breeds infected with *H. contortus* than in susceptible breeds [23].

Eosinophilia has been reported to be another indicator of parasite infection and naturally resistant breeds; Red Maasai and Scottish Blackface had high circulating eosinophil counts indicating a negative correlation with FEC [33]. Contrary, the association between resistance to *H. contortus* and eosinophilia in sheep did not indicate an inverse relationship [15]. No significant correlation between FEC and eosinophil counts in resistant Romney lambs [26] was found. Current results showed that eosinophilia is more pronounced in susceptible sheep with a significantly higher level of FEC. The eosinophilia appeared to be related to the level of infection instead of the resistance status. Confirming the present findings, FEC and circulating eosinophilia were higher ($P < 0.05$) in susceptible Creole kids [4] however, from a phenotypic view, the relationship was insignificant. The differences shown between eosinophilia and FEC may be associated with the species of parasite involved, though genetic variation in the ability to mount eosinophil response after parasite infection is evidenced in mice [36]. Anyhow, the contradictory results demonstrate that peripheral eosinophilia is not a reliable indicator of parasite burden in sheep.

Identification of genes responsible for parasite resistance could enhance the precision of genomic prediction that in turn resulting genetic improvement for the FEC trait. Characterization of *Ovar-DRB1* with REase *Pst*I two genotypes and two distinct DRB1 alleles were observed, indicating polymorphism at the loci [35]. However, three genotypes for the same region were observed in a population of Raeini Cashmere goats [3] and small ruminant breeds of North India [27]. The difference in fragment pattern and number of alleles could be due to the variation in the length of nucleotide sequences and the restriction enzymes used. The frequency of 'bb' genotypes and 'b' allele was high in the entire sheep population under study. Contrary to the present observations, 'ab' (70.5%) genotype (restriction site was alike, but genotype was referred as 'A1A2') and 'a' allele was frequent in Iranian sheep [35].

The studied sheep exhibited low variation (PIC = 0.15–0.23) at the *Ovar-DRB1* locus similar to the populations of Suffolk and Texel sheep [29]. Fixation index in the studied groups is indicative of heterozygote excess suggesting avoidance of inbreeding that might have occurred in the population. Heterozygotes recognize parasitic antigens more probably than homozygotes, hence susceptibility to worms may be influenced not simply by the effects of a particular gene as well as by individual MHC heterozygosity [25]. Wild-derived mice, which were MHC heterozygotes did not display better resistance to *Salmonella* or better survival in semi-natural enclosures [16]. Reduced heterozygosity may be a signature of selection in sheep for resistance to haemonchosis and trypanosomosis [37]. This obvious inconsistency could be partly explained by heterozygous animals being more likely to possess either ''resistance'' or ''susceptibility'' alleles. These results emphasize the importance of studies on wild, outbred species.

The 'bb' genotypes had significantly higher log FEC value, hence 'b' allele is linked with susceptibility to haemonchosis in sheep which was further supported by the observation of increased frequency of 'bb' genotypes in the high FEC group. Moreover, difference ($P < 0.05$) in genotype frequency between high and low FEC groups clearly stated the association of 'b' allele with high FEC. Homozygosity at the *Ovar-DRB1* locus impair the ability to respond effectively to disease [28]. The *Ovar-DRB1* locus was associated with low FEC in sheep infected with *H. contortus* [12]. However, the absence of an association between *Ovar-DRB1* alleles and FEC was observed in Texel sheep [29], due to the absence of linkage disequilibrium.

The mean FEC of Nellore sheep was high (Table 2) specifying the susceptibility of the breed and also in which the frequency of 'b' allele was more compared to Deccani and Kenguri breeds. The difference in FEC between these breeds may be attributed to the different allele profile at the DRB1 locus. The present results validated the evidence of an observation of the association of 'b' allele with increased FEC in Iranian sheep [35]. Though higher mean PCV and Hb was observed in 'ab' genotype sheep, the difference was not significant ($P > 0.05$). GWAS in a Red Maasai X Dropper back cross population revealed that genotypes associated with FEC did not affect PVC [6]. Similarly, the effect of genotypes on log FEC was found statistically significant at DRB1 exon 2, without association with PCV [30]. Consequently, selection for resistance to *H. contortus* infection has been based predominately on FEC [8], with the main objective of reducing FEC.

Gastrointestinal mucus and its constituents, mucins are found to be crucial in protecting the gut against enteric pathogens. Previous studies noticed that host-derived mucus was found to be present in the intestine of the nematode parasites themselves [21] and parasite feeding and motility were restricted when larvae were co-cultured with sheep intestinal mucus [10]. On this account, mucin is regarded to be the first line of host defense against invading pathogens and is an essential anti-parasite effector molecules [14].

The *GALNTL6* gene is a member of a highly conserved family of proteins and is accountable for the synthesis of mucin-type *O*-glycans. PCR–RFLP assay was developed to genotype the sheep with respect to SNP rs424521894 of *GALNTL6* that revealed monomorphic nature in the breeds

studied. Several genes from this family, such as *GALNT1*, *GALNT4* and *GALNT8*, have also been reported as being of importance for sheep resistance to GIN infections [5]. *GALNTL6* harbours the most significant SNP (rs424521894) detected by GWAS analysis [2] and contains haplotype block6 (107.33–107.38 Mbp), the only haplotype block having a significant effect on parasite resistance.

MHC polymorphism has a crucial role in parasite resistance or susceptibility in sheep and could be used as genetic markers to assist selection and improve parasite resistance to *H. contortus*. Screening of *H. contortus* FEC under natural infection is informative because it agrees with typical conditions in the production environment. *Ovar-DRB1* genotypes associated with FEC did not cause significant differences in PCV nor Hb in the studied sheep. However, more intensive studies, involving controlled infections coupled with clinical parasitology and other *GALNTL6* SNPs may be required to make any conclusive statement as host resistance to internal nematode parasites is likely to be controlled by a number of loci of moderate to small effects.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11686-023-00778-8.

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**Author Contributions** Dr. RP: Conceptualization, methodology, investigation, final analysis; Dr. SC: Conceptualization, original draft preparation; Dr. SK: Methodology, final analysis; Dr. JC: Supervision; Dr. RKP: Review and editing.

## Declarations

**Conflict of Interest** The authors declare no conflict of interest.

**Ethical Approval** The present study does not involve any animal testing or experimental studies with animals. Ethical review and approval were not warranted for this study, as the faecal material and blood samples collected for routine diagnostic screening were included in the study. The samples were collected aseptically from sheep by a qualified veterinarian after written informed consent from the organization / owners following the applicable guidelines for the care and use of animals.

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