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SINGLE NUCLEOTIDE POLYMORPHISMS IN DRB1, IGF1 AND ILs ASSOCIATED WITH FECAL EGG COUNT CONFERS RESISTANCE AGAINST *Haemonchus contortus* INFECTION IN GOATS

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Haemonchus contortus is a blood sucking gastrointestinal (GI) parasite that causes heavy production losses in small ruminants due to their poor immune response and rapid development of anthelmentic resistance. Identifying genes involving in immune related pathways against GI nematode infection will assist in better responding and curing the ill effects of infection with *H. contortus*. Fecal egg counts and ear tissues were collected from 200 goats belonging to four breeds and PCR sequencing method was used for identification of 25 seven single nucleotide polymorphisms (SNPs) and found two SNPs of dopamine receptor binding 1 (DRB-1) (37 C/G; 10375 C/T), one from insulin-like growth factor1 (IGF-1) (58110 C/G), three from interleukin (IL) 32 (7638 G/C; 9375 A/G; 1158 A/G) and one from IL-33 (70589 C/A) significantly ($P<0.02$) associated with fecal egg count (FEC). Out of those seven SNPs, 5 were missense and changed the amino acids from (Pro Leu) in DRB1, IL32 (Arg Trp and Ile Val) and in IL33 (Arg Trp). These missense SNPs may change the function of genes which play a crucial rule in immunity of host and expulsion of GI nematode. Models of the SNPs of DRB-1, IL-32, IL-33 and IGF-1 genes were found significant ($P<0.02$) indicating a strong association with FEC of *H. contortous* in goat. The haplotypes CCC and GCT of DRB-1, AAG, AGG, GGC, GCA, and GGG of IL-32 gene were found significantly ($P\leq0.03$) associated with FEC. The results of linkage disequilibrium (LD) showed that most of the SNPs were in the range of (0.29-0.98). Therefore, the present study investigated the role of these candidate gene SNPs in innate immunity and concluded that these SNPs were potentially important for future goat breeding plans against *H. contortus* infection in goats.
Keywords: Goat, single nucleotide polymorphisms, association analysis, immune response, interleukins.

INTRODUCTION

The goat production is generally important for their potential to produce meat and milk, while remains a useful source of hair, leather and manure production worldwide (Xie *et al*., 2015. This makes it a good source of income for the rural areas and brings up gradation of living standards (Bressani *et al*., 2014; Butt *et al*., 2014). Parasitic infections affect livestock severely and often reduce their potential to yield for which they are reared (Nieuwhof and Bishop, 2005). The parasite causes anemia, weight loss, lethargy, diarrhea that leads to intensive production losses and ultimately death. Parasitic disease management in sheep goat has been more complex due to emergence of anthelmintic drug resistance strains of parasites (Kenyon *et al.,* 2009; Molento *et al.,* 2011; Desoky *et al.,* 2015). Presently dominant nematodal species affecting small ruminants: *Trichostrongylus colubriformis*, *Teledorsagia circumcincta* and *H. contortus. H. contortus* generally recognized as a GI parasite that sucks blood from the abomasum of ruminant compound stomach particularly in sheep and goat (Mortensen *et al*., 2003; Saminathan *et al.,* 2015; Asif *et al.,* 2016). The mechanism of innate immunity is a crucial part of immune system that consists of cells which overcome the host from infection of pathogens. Innate reflexes are very important part of host defense system against posed by pathogens in daily life. Most of the host cell defending the pathogens by activating the local defense system (Clark and Kupper, 2005). On the other hand the mucosal barrier of intestine consistently examined by the mucus layer. It act as a primarily barrier for GI nematode

parasite which comes in contact during the process of infection. So, the mucus layer is defined as a first phase of innate immune system, but data showed that the mucus production is controlled by many other part of immune system including adaptive immunity. Specially cytokine type-2 IL-4 and IL-13 latter play role in differentiation and proliferation of goblet cells (Grencis *et al*., 2014).

IL-32 was initially illustrated as mRNA that called as nature killer (NK cells) which determined as a protein with several functions of cytokine (Dahl *et al*., 1992). It is a proinflammatory cytokine concerned with numerous diseases involving chronic inflammation and cancer. IL-32 secreted from NK cells and T cells, epithelial cells show IL-32 stimulation with interferon gamma (INF-γ), (tumor necrosis factor) TNF-α, IL-β and IL-18 (Dinarello and Kim, 2006). It played a key role in several diseases, inflammation, host defense and immune functions (Akdis *et al.,* 2011). IL-33 belongs from cytokine family and found to be similar in structure with IL-1. It trigger signaling pathways in target cells, mast cells and helper 2 cells (Th2) which produce large amount of IL-5 and IL-13 in response of IL-33. After GI parasite infestation IL-33 activates the secretion IL-13 that induces the worm expulsion in lack of adaptive immunity (Cayrol and Girard, 2014; Moro *et al.,* 2010).

DRB1 belong from Major Histocomaptibilty (MHC) group of genes and also play crucial role in pathogen presentation to T-lymphocyte. In addition, DRB-1 gene and GI nematode infection is associated with each other (Charon, 2004). So, the DRB-1 gene has the significant concern as a candidate genetic marker of the GI nematode parasite resistance (Preston *et al.,* 2014). Presently, there is little information about the variants of innate immune genes related to the host resistance to parasite infection in goats. This study was aimed to identify SNPs of these four innate immune genes including IL-32, IL-33, IGF1 and DRB-1 (Heemskerk *et al.,* 1999; Saenz *et al.,* 2008), and to determine their association with FEC of GI parasite. The identification of resistance-associated genetic markers may contribute to the implementation of marker-assisted selection in animal breeding programs.

MATERIALS AND METHODS

***Ethics statement*:** All the experimental protocols were approved by the Law of Animal Husbandry in People's Republic of China (Dec 29, 2005). The whole experimental procedures for collection of ear tissue sample of experimental individuals were reviewed and permitted by the Biological Studies Animal Care and Use Committee of National Animal Husbandry Service, Hubei, PR China. All efforts were made to minimize any discomfort during sample collection.

***Animal selection and phenotypic data*:** A total of 200 goats (99 infected and 101 healthy with no infection) were selected from southern China goat breeding farm in Yichang, Hubei province and these goats were belonging to four breeds namely as Yichang white goat, Hybrid white yellow, Nanjiang yellow goat and Enshi black goat. FEC used as phenotype trait for the *H. contortus*. We collected feces and the ear tissue from each animal and McMaster egg counting technique (Da Silva *et al.,* 2013) was used counting the total eggs of *H. contortus*. Genomic DNA was extracted from ear tissue of each goat by using Genomic DNA kit (TianGen, Beijing, China).

***Genotyping and detection of polymorphisms*:** In order to identify 25 SNPs, primers were designed to amplify complete coding regions sequences (cds) based on the reference sequence of the caprine ILs gene with Primer5 web Program (v.0.4.0) (Lalitha, 2000). For PCR amplifications pooled DNA and randomly selected 10 DNA samples were taken in a final reaction volume of 50 µl consisting of 1.5 µl of each primer 50 ng genomic DNA of 2 µl, and 25µl premix (TaKaRa, Dalian, China). The protocol of polymerase chain reaction (PCR) was 5 min at 95°C for initial denaturing followed by 30 cycles at 95°C for 30 s; annealing at Tm (55-65°C) for 30 s; 72°C for 40 s; a final extension at 72°C for 5 min for all the primers. From the each PCR product 40 µl of was sequenced using the ABI3730XL (Applied Biosystems,

Table 1. Primers used for PCR and sequencing of genes.

Gene	Primer sequences 5'- 3'	Annealing temperature (°C)	Product size(bp)	GeneBank accession No.
DRB1	F-TCAGATGCAACAGGGTCAAA R-TGGCCTGGATGTAGTGACAA	55.7	473	XM_013976917.1
DRB1	F-ATGCTGTCAGTGTTTGCCTT R-CAGATGATTTTCACTCCGTA	54.5	821	XM_013976917.1
IGF1	F-AAGGTGTGGGTTGACATGGT R-ATTCGGATGCTGCTGCTACT	59.0	384	XM_005680539.2
IL33	F-TTAACTCTGAACTTCCCACTC R-GTCAATCTGTGTAGCTTCCA	52.0	703	XM_005683684.2
IL32	F-CAGATTCCAGGACGCTTG R-GGCATTCAGAGGACTTCAG	56.8	559	XM_013974707.1
IL32	F-TAGGCATTACTGTCATCTCC R-AATCGCACCACCATGAAG	55.9	505	XM_013974707.1
IL32	F-TTCAGAGGTCAGTCCATCT R-TTAGGACATCAGCACTACAG	57.1	798	XM_013974707.1

Foster City, CA). The sequences were aligned with SeqMan (Swindell, 1997) program to determine the presence of any polymorphisms. Then the identified SNPs were genotyped in the 200 Chinese local goats using Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) assay (SquenomMassARRAY®, Bioyong Technologeies for spacing Inc. HK). The exonic region of DRB-1, IGF-1 IL-32 and IL-33 gene were sequenced. The size of the fragments and primers are shown in (Table 1).

***Statistical analysis*:** The genotypic and allelic frequencies of the identified SNPs were calculated using Excel program. PLINK v1.06 software (Purcell *et al*., 2007) used for analysis of association studies for the traits of interest. To identify associated SNPs, case-control association and Bonferroni corrections were designed to calculate raw p value. The genotypic, allelic, additive (Cochran–Armitage trend test), recessive and dominant models were included in this analysis. All test statistics were examined as Chi-square (χ^2) or Fisher's exact test under the null, with the segregation of the genotypic test which has 1 degree of freedom (df). The goodness of Hardy–Weinberg Equilibrium (HWE) was executed using χ^2 test. The analysis of Pairwise linkage disequilibrium (LD) among the polymorphisms was performed using the PLINK v1.06 software. Haplotype associations analysis was determined using PLINK v1.06 and Haploview (Barrett *et al.*, 2005) software.

RESULTS

A total of 200 goats from four different breeds were selected out of which 99 goats were found infected with *H. contortus*. In search of a good breeding plan to make goat population resistant against *H. contortus* important SNPs were found in immune related genes DRB-1, IGF-1 and ILs which shows high significance with FEC assumed to be a positive indicator for *H. contortus* resistance.

***The identification of SNPs and association effects of immune related genes with FEC*:** Our test of PCR and sequencing of pool DNA resulted in 25 SNPs in exonic regions of 12 different genes. Out of 25, seven SNPs in DRB-1, IGF-1, IL-32 and IL-33 genes found to be significant (P<0.02) with FEC of *H. contortus* (Table 3). The detailed

Table 2. Information of SNPs.

S. No.	Gene	SNPs position	Chromosome (chr)	Code	Encode
1	IL32	IL32_1158	chr25	[C/T]GG	R=>W
2	IL32	IL32_7638	chr25	[A/G]TC	I=>V
3	IL32	IL32_9375	chr25	GC[G/A]	A=>A
4	IL33	IL33_70589	chr8	[C/T]GG	R=>W
5	DRB1	DRB1_37	unknown	C[C/T]G	P=>L
6	DRB1	DRB1_10375	unknown	C[C/T]G	P=>L
7	IGF1	IGF1_58110	chr5	AA[C/T]	N=>N

Amino acid symbles: R=>Arg, W=>Trp, I=>Ile, A=>Ala, P=>Pro, N=>Asn, L=>Leu and V=>Val

Table 3. Allele, gene frequencies, χ^2 test and association of SNPs with FEC.

SNPs	Gene frequency			Allele frequency		χ^2 test		Significance of FEC
						Affected goats	Normal goats	
	AA	AT	TT					
DRB1_37C>G	0.69 (n=139)	0.24 (n=49)	0.03 (n=7)	C (0.58)	G (0.37)	0.191	0.498	0.0000*
	AA	AT	TT					
DRB1_10375C>T	0.02 (n=4)	0.45 (n=91)	0.51 (n=103)	C (0.30)	T (0.19)	0.459	0.375	0.0110*
	CC	CT	TT					
IGF1_58110C>G	0.29 (n=59)	0.43 (n=86)	0.24 (n=49)	C (0.25)	G (0.37)	0.397	0.427	0.0170*
	TT	TG	GG					
IL33_70589C>A	0.53 (n=106)	0.37 (n=75)	0.06 (n=13)	C (0.18)	A (0.07)	0.186	0.216	0.0014*
	AA	AG	GG					
IL32_1158A>G	0.16 (n=32)	0.37 (n=75)	0.46 (n=92)	A (0.47)	G (0.22)	0.376	0.454	0.0000*
	CC	CG	GG					
IL32_7638G>C	0.35 (n=70)	0.44 (n=88)	0.2 (n=40)	G (0.54)	C (0.31)	0.444	0.488	0.0000*
	AA	AG	GG					
IL32_9375A>G	0.1 (n=21)	0.39 (n=78)	0.49 (n=99)	A (0.21)	G (0.38)	0.393	0.422	0.0003*

Note: * represent significance with p<0.02 with FEC.

information of these SNPs are DRB-1 (37 C/G; 10375 C/T), IGF-1 (58110 C/G), IL-32 (1158 A/G; 7638 G/C; 9375 A/G) and IL-33 (63854 C/T) (Table 2). All these SNPs are novel, while polymorphisms at locus 37C/G, 10375C/T of DRB-1, 1158A/G, 7638G/C of IL-32 and 63854C/T of IL-33 genes were found missense and were predicted to change the amino acid from Proline to Leucine, Arginine to Tryptophan and Isoleucine to Valine (Table 2). The change in amino acids may change the function of gene that play a major role in immunity as leucine and valine work in the mTOR signaling pathway and Tryptophan plays a role in immune responses by producing a local immunosuppressive environment that is able to control T-cell homeostasis and self-tolerance during inflammation (Platten *et al.,* 2005; Li *et al.,* 2007) respectively. Genotypic and allelic frequencies and χ^2 test are shown in (Table 3). The results revealed that FEC was in significant association with *H. contortus* infection. The missense mutations are also mandatory for this association study, because the change in amino acid may take a major role to change the functions of genes which enhance the resistance of parasite infection in goats.

***Determination of different genetic models with FEC*:** In association study the different genetic models were used to analyze genotype and phenotype for the comparison of analytical strategies. When the causal allele of inheritance is unknown slightly more powerful results were found in genetics models (Lettre *et al.,* 2007). The genetic models of 7 significant SNPs in four genes were subdivided into genotypic, allelic, dominant and recessive models effects determined through PLINK software. In DRB-1 gene (37 C/G) and IL-32 (7638 G/C; 9375 A/G) all of the models were significant (P<0.02), whereas in DRB-1 (10375 C/T), IL33 (70589 C/A) only allelic and in IGF-1 (58110 C/G) genotypic, allelic and recessive models were significant (P<0.02) indicating a strong association among these genes and the FEC of *H. contortous* infection in goat (Table 4) .

***Determination of haplotype frequencies and association with FEC*:** Haplotype is a tool used in genetics for the investigation of diseases in which specific collection of alleles inherited together from those genes which are present on same chromosome (Gibbs *et al.,* 2003). The eleven potential haplotype frequencies of the seven SNPs are given in (Table 5). These haplotypes CCC and GCT haplotypes of DRB-1 gene (14.3% and 8.2% of the FEC affected verses 6.7 % and 19.7% of the control goats respectively), interferon gamma (IFN-γ) and IGF-1 gene haplotype CGG (43% of affected and 32% of control goats) and IL-32 gene haplotypes AAG, AGG, GGC, GCA, and GGG (20%, 27%, 33%, 17%, 31% of FEC affected and 10%, 12%, 47%, 29% and 18% of control goats) and IL-33 gene haplotype TCC (9% of FEC affected and 2% of control goats) were found significantly (P≤0.03) associated with FEC.

***Linkage disequilibrium analysis among SNP*:** Linkage disequilibrium (LD) acts as a vital component in mapping of diseased gene and in identifying the haplotype blocks. LD is used recurrently to enumerate the association among two loci. In this study pair wise LD coefficients (R^2) were calculated to determine the (LD) among ILs and DRB-1 genes. The results

Table 4. Genetic models of significant SNPs.

Polymorphism		Affected goats	Control goats	Genotypic model	Allelic model	Dominant model	Recessive model
	GG	47	26				
DRB1_37C>G	GC	19	18	0.006*	0.000*	0.004*	0.003*
	CC	31	49				
	TT	2	2				
DRB1_10375C>T	TC	55	32	0.000	0.011*	0.000	0.000
	TT	39	59				
	GG	6	16				
IGF1_58110C>G	GC	38	37	0.042*	0.017*	0.100	0.017*
	CC	53	40				
	AA	5	1				
IL33_70589C>A	AC	25	11	0.000	0.001*	0.000	0.000
	CC	67	80				
	GG	22	15				
IL32_1158A>G	GA	42	40	0.470	0.210	0.370	0.260
	AA	32	36				
	CC	30	9				
IL32_7638G>C	CG	45	40	0.000*	0.000*	0.001*	0.0003*
	GG	22	43				
	GG	5	13				
IL32_9375A>G	GA	32	45	0.002*	0.000*	0.001*	0.035*
	AA	60	34				

Note: * represent significance with p<0.02 with FEC.

Table 5. Haplotype frequencies of the significant SNPs.

SNPs	Haplotype	Haplotype frequency		χ^2	df	P-value
		Affected goats	Control goats			
DRB1		-	-	21.39	3	0.003*
1	CCC	0.143	0.067	5.888	1	0.015*
2	GCT	0.082	0.197	10.50	1	0.001*
IFNG,IGF1	-	-	-	5.955	2	0.114
1	CGG	0.431	0.326	4.331	1	0.037*
IL33	-	-	-	10.77	2	0.013*
1	TCC	0.091	0.027	6.619	1	0.010*
IL32	-	-	-	27.22	5	0.000*
1	AAG	0.208	0.101	8.287	1	0.004*
2	AGG	0.270	0.124	12.75	1	0.004*
3	GAC	0.125	0.208	4.698	1	0.030*
4	GGC	0.332	0.478	8.326	1	0.004*
IL32	-	-	-	21.61	4	0.001*
1	GCA	0.170	0.297	8.592	1	0.003*
2	AGG	0.229	0.130	6.253	1	0.012*
3	GGG	0.311	0.184	8.109	1	0.004*

Note: * represent significance with p ≤0.03 FEC.

Table 6. Linkage disequilibrium (LD) among SNPs.

SNP-A	SNP-B	R2
DRB1-37C>G	DRB1-10375C>T	0.43
IL8-2498T>C	IL8-2739T>C	0.29
ILβ-3568A>G	ILβ-5005G>A	0.98
ILβ-3568A>G	ILβ-7928G>T	0.61
ILα-3721G>A	IL32-7638G>C	0.33
ILβ-5005G>A	ILβ-7928G>T	0.59
IL32-1158A>G	IL32-7638_G>C	0.78
IL32-1158A>G	IL32-9375A>G	0.27
IL32-7638G>C	IL32-9375A>G	0.36

Note: LD range (0.29-0.98) associated with FEC.

indicated that most of the SNPs were in the ranged from (IL32 0.29 to ILβ 0.98), which indicated that these SNPs were strongly associated and commonly inherited together (Table 6).

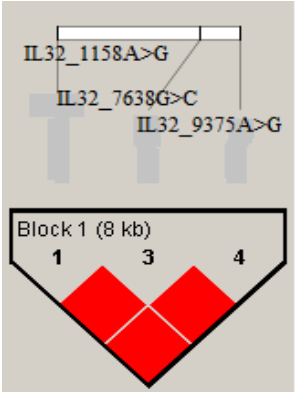

Figure 1. Pairwise linkage disequilibrium IL-32 (SNPs) genes.

The structure of linkage disequilibrium between the single nucleotide polymorphisms shows LD block comprising of SNPs of IL32 gene at locations (1158C/T), IL-32 (7638A/G) and IL-32 (9375G/A) (Fig. 1). Among IL-32 following (1158), (7638) SNPs were missense which can play a vital role in controlling the parasitic infections by interacting with the immune system.

DISSCUSION

The discovery of genes responsible for the disease helps in better understanding of physiological mechanisms of vulnerability to infection. Immunity related genes can play a vital role in developing novel tools to fight and control the diseases. The effective host resistance genes are reported as functional candidate genes, such as DRB-1, IL-32, IL-33 and IGF-1 among different species (Zaros *et al.*, 2010; Ibelli *et al.*, 2012). Our study revealed 25 SNPs out of which seven SNPs were found significantly associated with the FEC and five of these SNPs were missense resulting in substitution of amino

acid from Proline to Leucine, Arginine to Tryptophan and Isoleucine to Valine. These missense SNPs can play key role by enhancing immunity against *H. contortus* infection.

Schwaiger *et al.* (1995) and McManus *et al.* (2014) worked on the DRB-1 gene association with FEC through natural infection in sheep and they found this gene in significance with the FEC. The results of their study coincides with the finding of this study as in following study DRB-1 gene was also found significant with the FEC of *H. contortus* infection. The genetic variation of this gene might play a key role in the resistance against nematodal infection because DRB-1 gene is linked with the innate immunity so it can be helpful in controlling the infection caused by the parasites and will help in reduction of anthelmintics usage in animals.

Some studies of Charon, (2004), Van Haeringen *et al.* (1999) and Paterson *et al.* (1998) proved that the polymorphism in DRB-1 highly associated with the resistance of GI nematode in ruminants. DRB-1 gene encodes proteins which play key role in the immune system to control the parasite infection. In the present study the DRB-1 is significantly associated with FEC and its mutation also changed amino acid from proline to leucine which may lead to functional alteration of DRB-1 and can enhance the immunity against GI parasite.

Bressani *et al.* (2014) worked on the functional candidate genes of cytokine family IL (IL-2, IL-4, IL-13, and IFN-γ) for identification of SNPs and their association with resistance to GI endoparasites and found one missense mutation in IFN-γ gene. We also worked on candidate genes related to host resistance i.e. IL-32, IL-33, DRB-1 and IGF-1 and identified five missense substitutions i.e. 2 in each DRB-1, IL-32 and 1 in IL-33 gene. These missense substitutions were found significantly associated with FEC. So the results of our study coincide with the above mentioned study.

Yang *et al.* (2013) reported that type 2 immunity is essential for host prevention against nematode infection. They demonstrated that IL-25/IL-33 are the receptive cells which play a key role in inducing type 2 immunity by in vitro and in vivo approaches. The significance of the IL-33 gene in controlling parasitic infections also confirmed in *Nippostrongylus brasiliensis* infection, IL-33 was necessary in secondary and primary infections for enhancing the parasitic expulsion (Hung *et al.*, 2013). In the present study the IL-33 was found significantly associated with FEC of *H. contortus*. The amino acid substitution in IL33 was from arginine to tryptophan, this substitution might be of pivotal role in the immunity by altering the function of gene because IL-33 is interacts with host cell immune system through type 2 immunity so it might play crucial role for genetic control of parasitic infections.

The identification of missense mutations and their association with FEC signifies the importance of these genes. Genetic variations of IL-32, IL-33, DRB-1 and IGF-1 genes are of utmost important because of these genes interaction with the immune system. IL32 act as a pro-inflammatory cytokine and induces the IFN-γ in monocytes and epithelial cells (Kim *et al.*, 2005; Akdis *et al.*, 2011). IL-32 play key role in auto immune infections and host defense mechanism against the microbes (Joosten *et al.*, 2006) so, the significant genetic association of IL-32 gene with FEC shows that it can play a vital role in controlling the parasitic infestations by interacting with the innate immunity and it favors the finding of our study.

The IGF-1 gene enhances the immune system against the nematode by inducing the Th2 immune response in murine model (Chen *et al.*, 2012; Sallé *et al.*, 2014). In the present study IGF1 gene was significantly associated with FEC of *H. contortus*. However, IGF-1 can play a key role in the immunity against nematodal infection.

For the determination of association study the application of genetic models (genotypic, allelic, dominant and recessive) are necessary. We can categorize the dominant effect of alleles by applying the homozygote and one of the heterozygote genotypes as a single group. The classification of SNPs genotype forces homozygotes has the same risk as one of heterozygotes against phenotype (Lunetta, 2008). In the present study we also apply the genetic model to check the effect of heterozygotes and homozygotes genotypes against the FEC phenotype. We found the genetic models significant with phenotype trait. The findings of this researcher favour the results of our study.

Haplotypes is a special thought in the field of genetics to investigate the diseases. A set of few specific alleles in the sequence can be identified by such polymorphic sites in surrounding genes on the same chromosome (The International HapMap Consortium, 2005). In the current study various sets of haplotypes in different genes were identified and found to be associated with the FEC of *H. contortus*. These haplotypes can be helpful in enhancing the immunity and to overcome load of parasite when inherited together in next generation. In this way the current study is boosted by the statement of the international Hap-Map consortium.

This study summarizes the significance of immune related genes their association with FEC and the findings are the evidence of key role of IL-32, IL-33, IGF-1 and DRB-1 genes in immunity against the *H. contortus* infections. Haplotype and genetic models of the significant SNPs are also associated with FEC of *H. contortus.* Further studies with larger number of animals and animals from different locations can be more useful in future prospects for these genes in parasitic infections.

***Conclusion*:** The association of missense SNPs in DRB-1, IGF-1, IL-32 and IL-33 genes with FEC was found to demonstrate their probable key role in genetic control of *H. contortus* infection in goats. It would be interesting to study these SNPs as a probable therapeutic target to counter *H. contortus* infection. Further investigation of the function of

these genes including expression pattern, in large number of animals as well as the role of these mutations in vitro studies would might improve the current understanding and find its novel interaction in immune related pathways that might help to develop the genetic tools to control parasitic diseases.

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