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RESEARCH ARTICLE

Single Nucleotide Polymorphisms of Toll-Like Receptors and Association with *Haemonchus contortus* Infection in Goats

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ABSTRACT

Haemonchus contortus is blood sucking nematode and causal pathogen of intestinal infection. Such type of infection causes serious constraint to goat production. Toll-like receptors (*TLR*s) are known to induce immune response predominantly through activate different signaling pathways that produce natural resistance against pathogens. Toll-like receptors are thought to be a critical ''bridge'' between innate and adaptive immunity to diverse pathogens. The current study was performed to investigate the association between polymorphisms of the *TLR*s gene and susceptibility to *H. contortus* infection in goat. Preliminary, 31 single nucleotide polymorphisms were identified in the *TRLs* gene using both pooled DNA and randomly selected DNA sequencing. The identified single nucleotide polymorphisms (SNPs) were genotyped by *MALDI-TOF MS* (Matrix-assisted laser desorption/ionization time of flight mass spectrometry) methods from 245 individuals. Out of 31, nine SNPs individually showed statistical significance association with *H. contortus* infection, of which, three were non synonymous resulted to amino acid replacement. Seven haplotype blocks were observed in this study and of those, four blocks are found to be significantly associated with *H. contortus* infection. Among the blocks, block 6 containing haplotypes GAGCATC, GAACATC, TGGGGGT, GGGGGGC and GGGGGTC were associated ($P<0.002$) with a higher risk of *H. contortus* infection in goat. Our results indicate polymorphisms detected in *TLR*s might have an impact on the structure and/or function of *TLRs*; goat *TLR*s are predicted to be associated with responses to gastrointestinal nematode infection including *H. contortus* and hence affect the immune response to pathogens.

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INTRODUCTION

Barber's pole worm (*Haemonchus contortus*) is a hematophagous (blood feeding) nematode, which parasitizes the abomasum and presents a serious constraint to goat production in regions with warm climate and predominantly summer rainfall such as southern China. Each mature *Haemonchus* suck 0.05 ml blood per day. Chronic blood loss causes anemia, anorexia, reduction in body weight and wool growth, depression and death (Simpson, 2000). Infection causes economic loss by decreasing production and increasing cost of control measure (Bishop 2012; Silva *et al.*, 2012). To develop improved and/or alternative methods for controlling *H. contortus* such as marker assisted selection (MAS) of resistant goat is necessary. There is also increasing public awareness about the product come directly from animals and animal by products that are free of contaminating drug residues including anthelmintics, while being raised in a form that control disease and stress (Waller, 2006). Among alternative control measures, one includes the development of resistant populations of goat through genetic modification and another, the use of therapeutics such as vaccines that can increase flock resistance (Smith and Zarlenga, 2006).

Toll-like receptors (*TLR*s) are recognized as invading pathogens through detection of highly conserved pathogen associated molecular patterns (PAMPs) derived from a wide range of pathogens and, subsequently, activate the innate immune response through different signaling

pathways in mammals (Akira *et al.,* 2006). A highly effective mucosal immune response is needed for the development of resistance to GIN in animal. This type of immune response is developed through the activation of Th2 profile and the production of optimum levels of IgG1 and IgE antibodies, eosinophilia, mucosal mastocytosis, and goblet cell hyperplasia (Meeusen *et al.,* 2005). As a result, a number of functional signaling pathways are activated for the development of protective mucosal responses. The development of an acquired immune response is produced by the *TLR* family and associated signaling pathways.

Over the recent years, there has been consistent development in the identification and characterization of different *TLR* genes in different farm animal species, which has helped in clarification of their role in the disease outcome. Further, mutation in the *TLR*s has been found to be associated with disease susceptibility and resistance traits which also indicate the economic importance of these genes (Beutler *et al.,* 2006). More recently, all genes (*TLR*1-10) of *TLR* family of goat have been characterized by mRNA sequencing (Raja *et al.,* 2011); however, the information on gene structure and polymorphism of goat *TLR*s is still limited.

Several studies using different approaches, breeds and nematode species have already been published, and many QTL, more than ovine 20 chromosomal regions, associated with nematode resistance have been detected as reviewed by Bishop and Morris (2007). Some of these QTL were detected near candidate genes including *TLR*s gene family. However, QTL studies have generally been performed in sheep, and till to date only two studies have been conducted with candidate-gene approach in Australian goats (Bolormaa *et al.,* 2010) and a genome-wide quantitative trait loci (QTL) scan approach (de la Chevrotiere *et al.,* 2012) in Creole goat. Selection of animals that is resistant to gastrointestinal nematode is recently based on the application of phenotypic measures including faecal egg count (FEC) or worm egg counts (WECs), PCV, eosinophil count, body weight (BW) and immunoglobulin A and E (IgE and IgA) activity determined after infection which are indirect measures of resistance. Selection for phenotypic traits has been successfully exploited in Australia and New Zealand; however, it is expensive and time-consuming as it requires animals to be challenged with parasites. Considering the cost and time, for goat breeding and genetics, MAS is now considered the optimal choice (Weller and Ron, 2011). Unfortunately, little information about the candidate genes affecting diseases resistance traits is available. For this reason, excavating the suitable candidate DNA markers that correlate with diseases resistance traits in goat breeds has become a major objective.

MATERIALS AND METHODS

Animal selection and phenotypic data: A total of 360 goats were selected from southern China (Enshi, Nanjiang and Yichang) including three local goat breed named as Enshi black goat, Nanjiang yellow goat and Yichang white goat. Feces and ear tissues were collected from each goat. Total eggs of *Haemonchus contortus* were counted using McMaster egg counting technique. Genomic DNA was extracted from ear tissue samples of the goats.

Nematode challenge trials: Nematode challenge trials were performed in the animal experimental house of the University (Huazhong Agricultural University, Wuhan, China). Upon arrival in the goat house, all 8 goats around one year of age were drenched with double doses of anthelmintic treatment. The goats were acclimatized for one month before infections commenced. All animals were weighed weekly. It was maintained in goat and L3 larvae of *H. contortus* were cultured from infected feces, collected and stored in the laboratory. Activities of L3 larvae were confirmed by microscopic examination. After one month, infections were given in four goats and another four were kept as control. Faecal samples were collected at 28, 35 and 42 days after infection from all goats for FECs.

Detection and genotyping of the polymorphisms: A DNA pool (50ng/µL/goat) was constructed from the DNA of all selected animals. Primers (Supplementary Table was not shown) were designed to amplify complete cds sequences based on the reference sequence of the ovine *TLRs* gene (Supplementary Table was not shown). PCR amplifications for pooled DNA and randomly selected 10 DNA samples were performed in a final reaction volume of 50 µl consisting of 50 ng genomic DNA of 2 µl, 1.5 µl of each primer and 25µl premix (TaKaRa, Dalian. China). The PCR protocol was 5 min at 95ºC for initial denaturing followed by 30 cycles at 95ºC for 30 s; annealing at Tm (ºC) (S1) for 30 s; 72ºC for 40 s; a final extension at 72ºC for 5 min for all the primers. Then, 40 µl of each PCR product was sequenced using the ABI3730XL (Applied Biosystems, Foster City, CA). Both forward and reverse sequences were aligned to determine the presence of any polymorphisms. The identified SNPs were then genotyped in the 245 Chinese local goat using *MALDI-TOF MS* assay [Squenom MassARRAY®, Bioyong Technologeies Inc. HK].

Statistical analysis: With tracing back of gastrointestinal nematode infection information for last few years in the different region of Southern China, the total number of animals included in the statistical analysis was 360. For the association studies, the traits of interest were analyzed using PLINK v1.06 software. Preliminary, to identify the most associated SNPs, case-control association program of PLINK with Bonferroni adjustment of their raw p value was used. Finally, to assess single SNP associations, allele or genotype frequencies were compared between *H. contortus* infected goat and control goat in a different model. The allelic model, the genotypic model, the additive model, dominant model and recessive model were included in this analysis. All test statistics were distributed as $\chi 2$ or Fisher's exact test with 1 df under the null, with the exception of the genotypic test which has 2 df. The goodness of fit to Hardy–Weinberg Equilibrium (HWE) was performed using a chi square ($\chi 2$) test. Pairwise linkage disequilibrium (LD) among the polymorphisms was also calculated using the PLINK. Haplotype block/phases (computed using expectation–maximization algorithm) preparation and haplotype associations analysis both were determined using PLINK v1.06 software, haploview version 4.2 only used for pictorial presentation of haplotype block.

RESULTS

Out of 360 selected goats, 245 were found to be infected with *Haemonchus contortus*. The average infection rate was 71.85%. The infection status was shown in Fig.1. Relative comparison study showed that Enshi Black goat was highly susceptible to *H. contortus*. Thorough screening of amplified DNA sequences (Fig. 2), a total 31 SNPs were detected (Supplementary Table was not shown) in the *TLR* genes (*TLR* 1-10) of Chinese local goat. Fifteen non synonymous SNPs were detected out of 31 that was predicted to result in an amino acid replacement in the *TLR*s protein. Individual genotyping against each SNP with *MALDI-TOF MS* assay method showed 96.4% success rate, as a result the total number of SNPs and individuals included in the analysis was 27 and 200 respectively. After performing the association program, *TLR*8_14226 C>T was excluded from analysis due to unexpected χ^2 result. The genotypic, allelic frequencies and model based associations are summarized in Table 1 and Table 2. Based on a Chi-square test (χ^2), most of the allelic distribution of the *TLR* gene loci in the population (data not shown) was in Hardy-Weinberg equilibrium (HWE) (P>0.05), only four loci were found to be significantly deviated from the HWE in the population investigated (P<0.05) (Table 1). The association effects of the *TLR*s gene SNPs on *H. contortus* infection are shown in Table 1 with their raw P values. Nine SNPs (*TLR*2_9977 G>A, *TLR*4_8865 C>G, *TLR*4_8980 G>A, *TLR*4_9091 C>T, *TLR*4_9396 G>A, *TLR*8_12327 T>C, *TLR*8_14190 T>C, *TLR*9_11365 C>T and *TLR*9_11911 C>T) were found to be associated (P=<0.03~0.000009) with *H. contortus* infection in goats. Of these SNPs, *TLR*2_9977 G>A was found highly significant (P=0.000009) and associated with *H. contortus* infection, such association remained significant even after Bonferroni correction for multiple F-testing. To assess the linkage disequilibrium (LD) among the *TLR*s polymorphisms, pair wise LD coefficients (r2) were calculated (Table 4). The results showed that most of SNPs were in strong LD (0.6-1), indicating, these polymorphisms were strongly associated and frequently inherited together (Table 4). To determine the coinheritance of *TLR*s polymorphisms, the association between *TLR*s haplotypes and *H. contortus* infections were examined. Seven haplotypes block (Fig. 3) were detected among the identified SNPs (Table 3). The frequencies of haplotypes and association results are summarized in Table 1. Haplotype block 6 was found to be highly associated (P=<0.002) and other three haplotype block (Block 4, Block 5 and Block 7) were found to be mildly associated (P=0.03~0.005) with *H. contortus* infection with their raw P values <0.05 (Table 3). Within these four blocks, haplotypes CTAA, CGCG, GGGGGTC and TC were found to be significantly (P<0.05) associated and were found more frequent (47, 61, 7 and 79% respectively) in *H. contortus* infected goat than the control (37, 48, 1 and 68% respectively). Thus, individuals carrying the haplotype CTAA, CGCG, GGGGGTC and TC had a higher risk of *H. contortus* infection in Chinese goat. In contrast, haplotypes CTCA, GATA and CT were found more frequent (8, 47 and 30%) in control goat than *H. contortus* infected goat (2, 35 and 17%, respectively). This result suggested the possibility of a protective effect.

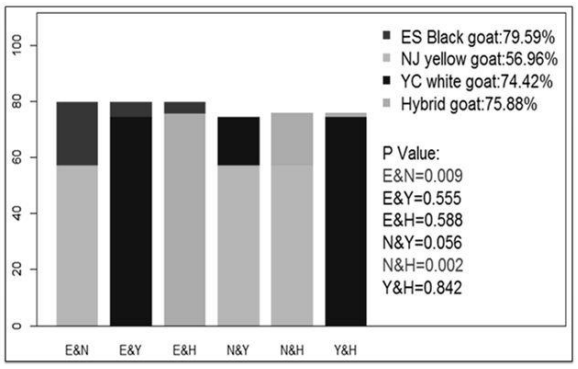

Fig. 1: Status of infection cased by *Haemonchus contortus* in Chinese local Enshi Black goat, Nanjiang yellow goat, Yichang white goat and Hybreid goats breeds

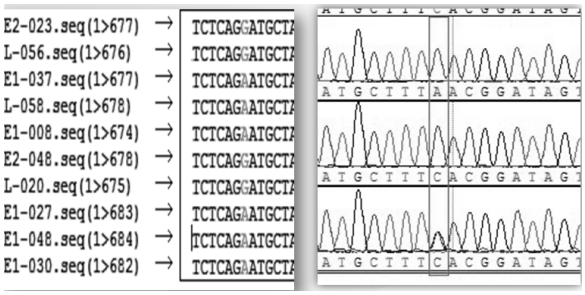

Fig. 2: Sequence analysis, polymorphisms detection and genotyping of individual polymorphisms of *TLR*s gene in Chinese local goat breeds.

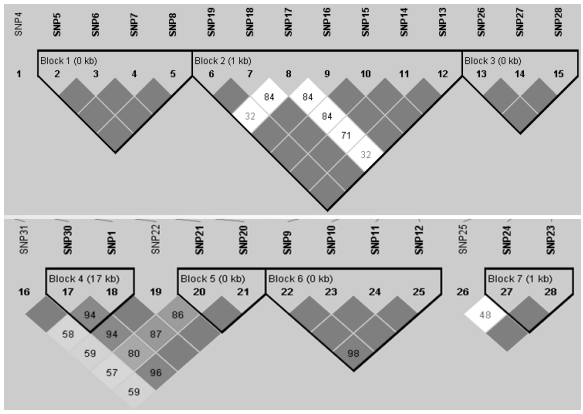

Fig. 3: Haplotypes Blocks and pair wise linkage diseqiulibrium (LD) in the *TLR*s polymorphisms. Bright dark color indicates strong LD, shades of dark color indicates uninformative LD and white color indicates the evidence of recombination.

DISCUSSION

The recent identifications of Toll-like receptors in mammals are considered as receptors of the innate immune system and *TLR*-mediated signals are central players in the induction and differentiation of adaptive immune responses (Iqbal *et al.,* 2005). Studies on *TLR*s immune function are quite advanced in Drosophila, mouse and human a series of *TLR*s have already been detected in other species including fish (Meijer *et al.,* 2004), chickens (Leveque *et al.,* 2003), cattle (Werling and Jungi, 2003), and goat (Raja *et al.,* 2011). Knowledge about innate immune mechanism and signaling mediated through *TLR*s could provide more useful information about the disease resistance of goats. Most often, non-synonymous single nucleotide polymerphism (NSSNP) have been listed in the *TLR*s gene family

without association of specific disease (Jungi *et al.,* 2011) and till to date, association between NSSNP and infection caused by *H. contortus* in goat has not been published. A recent publication of Tirumurugaan *et al.,* (2010) showed details of *TLR* expression profile in different tissues in an Indian breed of goat. This article also supported the earlier statement.

Polymorphisms in the *TLR* genes have been shown associated with various disease conditions in human and animals (Ogus *et al.,* 2004). In our study, out of nine associated polymorphisms with *H. contortus* infection, three (*TLR2*_9977 A>G, *TLR*4_8865 C>G, *TLR*4_9396 A>G) were predicted to result in amino replacement (Thr578Ala, Thr389Ser, Asp566Ser respectively) in the *TLRs* protein. Of these SNPs, one was detected in *TLR*2 and other two were detected in *TLR*4. *TLR*2 and *TLR*4 are known to stimulate innate and adaptive immune responses (Hahm *et al.,* 2007) and various diseases. A transitional non synonymous polymorphism was detected at position *TLR2*_9977 A>G in *TLR*2 gene that showed highest

Table 1: Allele frequencies of *TLR*s polymorphisms in *Haemonchus contortus* affected goats and normal control goats and single SNP association

*TLR*s	*Haemonchus contortus* affected goats			Control goats			Single SNP association
Polymorphisms	Allele	Frequency	HWE χ^2 test	Allele	Frequency	HWE χ^2 test	P-value
*TLR*1_7599 T>C	T	0.30	0.636	C	0.30	0.156	1
*TLR*2_9977 G>A	A	0.34	0.124	G	0.17	0.151	9.61E-05**
*TLR*3_8105 C>G	C	0.48	0.074	G	0.46	0.686	0.547
*TLR*3_8763 T>C	T	0.48	0.045	C	0.45	0.540	0.543
*TLR*3_8790 C>A	A	0.47	0.017	C	0.38	0.001	0.054
*TLR*3_9022 G>A	A	0.48	0.074	G	0.46	0.686	0.547
*TLR*4_8865 C>G	G	0.38	0.834	C	0.52	0.552	0.008**
*TLR*4_8980 G>A	A	0.35	0.662	G	0.48	1	0.014**
*TLR*4_9091 C>T	T	0.35	0.665	C	0.47	1	0.014**
*TLR*4_9396 G>A	A	0.36	0.828	G	0.48	1	0.019**
*TLR*5_11351 T>C	T	0.14	0.377	C	0.19	0.181	0.172
*TLR*5_11606 G>T	G	0.18	0.172	T	0.19	0.513	0.697
*TLR*5_11894A>G	G	0.25	0.603	A	0.20	0.232	0.283
*TLR*5_12236 C>G	G	0.25	0.603	C	0.20	0.232	0.283
*TLR*5_12347G>A	A	0.12	0.626	G	0.09	1	0.327
*TLR*5_12981A>G	G	0.25	0.603	A	0.20	0.232	0.283
*TLR*5_12933 G>T	T	0.14	0.378	G	0.18	0.181	0.172
*TLR*6_21031 C>A	C	0.35	1	A	0.32	0.489	0.595
*TLR*6_21211 C>T	C	0.28	1	T	0.26	0.042	0.821
*TLR*6_21664 C>T	T	0.29	0.629	C	0.30	0.156	0.912
*TLR*8_12327 T>C	T	0.18	0.234	C	0.31	0.136	0.003**
*TLR*8_14190 T>C	C	0.21	0.728	T	0.31	0.225	0.021*
*TLR*9_11365 C>T	T	0.06	1	C	0.12	1	0.036*
*TLR*9_11606 C>T	C	0.11	0.601	T	0.15	1	0.234
*TLR*9_11911 C>T	T	0.06	1	C	0.12	1	0.036*
*TLR*10_4642A>G	G	0.22	0.077	A	0.20	0.552	0.713
*TLR*10_4642A>G	G	0.39	0.673	A	0.36	0.515	0.605

Note: *P<0.05 and **P<0.01.

Table 2: Genotype frequencies of *TLR*s polymorphisms in *Haemonchus contortus* infected goats and normal controls

Polymorphisms		*Haemonchus contortus* affected goats (n=100)	Control goats (n=100)	Genotypic model P-value	Additive model P-value	Dominant model P-value	Recessive model P-value
	AA	15	5				
*TLR*2_9977G>A	AG	38	24	0.0013**	0.0001**	0.008**	0.032*
	GG	47	71				
	GG	14	27				
*TLR*4_8865 C>G	GC	49	47	0.028*	0.011*	0.092*	0.023*
	CC	37	25				
	AA	11	22				
*TLR*4_8980 G>A	AG	49	51	0.048*	0.019*	0.071*	0.055*
	GG	40	27				
	TT	11	22				
*TLR*4_9091 C>T	TC	48	50	0.051*	0.019**	0.073*	0.055*
	CC	41	28				
	AA	12	22				
*TLR*4_9396 G>A	AG	48	51	0.063	0.025	0.071*	0.089
	GG	40	27				
	TT	8	15				
*TLR*8_12327 T>C	TC	20	32	0.024**	0.003**	0.012*	0.069
	CC	71	53				
	CC	8	12				
*TLR*8_14190 T>C	CT	25	34	0.058	0.014**	0.046*	0.068
	TT	67	54				
	TT	0	1				
*TLR*9_11365 C>T	TC	12	22	0.061	0.053	0.061	1.00
	CC	88	77				
	TT	0	1				
*TLR*9_11911 C>T	TC	12	22	0.061	0.053	0.061	1.00
	CC	88	77				

Note: *P<0.05 and **P<0.01.

Table 3: Haplotype frequencies of the *TLR*s polymorphisms between *Haemonchus contortus* infected goats and normal controls

Haplotypes block	Haplotypes	Haplotype frequency		χ^2	df	P-value
		Haemonchus contortus infected goats	Control goats			
Block 1	-	-	-	5.184	2	0.075
H1	TCT	0.06	0.12	4.396	1	0.036
H1	CCC	0.05	0.03	1.042	1	0.307
H1	CTC	0.89	0.85	1.415	1	0.234
Block 2	-	-	-	0.155	2	0.925
H2	GT	0.21	0.20	0.074	1	0.784
H2	AT	0.09	0.10	0.105	1	0.745
H2	AC	0.69	0.69	0.001	1	0.973
Block3	-	-	-	0.490	2	0.782
H3	CC	0.27	0.26	0.051	1	0.821
H3	TC	0.07	0.05	0.384	1	0.535
H3	TA	0.65	0.68	0.282	1	0.595
Block4	-	-	-	11.2	2	0.004**
H4	CTAA	0.47	0.37	3.699	1	0.054
H4	CTCA	0.02	0.08	9.338	1	0.002
H4	GCCG	0.52	0.54	0.361	1	0.547
Block5	-	-	-	6.649	2	0.035*
H5	GATA	0.35	0.47	5.78	1	0.016
H5	GGCG	0.03	0.04	0.285	1	0.592
H5	CGCG	0.61	0.48	6.649	1	0.009
Block6	-	-	-	16.14	4	0.002**
H6	GAGCATC	0.65	0.72	2.306	1	0.128
H6	GAACATC	0.11	0.08	0.878	1	0.348
H6	TGGGGGT	0.13	0.17	1.919	1	0.166
H6	GGGGGGC	0.04	0.005	5.592	1	0.018
H6	GGGGGTC	0.07	0.01	7.874	1	0.005
Block7	-	-	-	10.37	2	0.005**
H7	CT	0.17	0.30	8.777	1	0.003
H7	CC	0.03	0.01	2.271	1	0.131
H7	TC	0.79	0.68	5.698	1	0.016

Note: *P<0.05 and **P<0.01.

Table 4: Pair wise linkage disequilibrium (LD) between SNPs in *TLRs* gene

SNP_A	SNP_B	R2	SNP_A	SNP_B	R2
*TLR1*_7599 T>C	*TLR6*_21031 C>A	0.98	*TLR6*_21664 C>T	*TLR6*_21031 C>A	0.86
*TLR1*_7599 T>C	*TLR6*_21211 C>T	0.69	*TLR8*_14190 T>C	*TLR8*_12327 T>C	0.90
*TLR1*_7599 T>C	*TLR6*_21031 C>A	0.87	*TLR9*_11365 C>T	*TLR9*_11606 C>T	0.61
*TLR4*_8980 G>A	*TLR4*_9091 C>T	0.97	*TLR9*_11365 C>T	*TLR9*_11911 C>T	1
*TLR4*_8980 G>A	*TLR4*_9396 G>A	0.98	*TLR9*_11606 C>T	*TLR9*_11911 C>T	0.61
*TLR4*_9091 C>T	*TLR4*_9396 G>A	0.96	*TLR10*_4642 A>G	*TLR1*_7599 T>C	0.63
*TLR5*_11606 G>T	*TLR5*_11351 T>C	0.82	*TLR10*_4642 A>G	*TLR6*_21664 C>T	0.63
*TLR5*_11894 A>G	*TLR5*_11606 G>T	0.75	TLR10_4642 A>G	TLR6_21211 C>T	0.54
TLR5_11894 A>G	TLR5_11351 T>C	0.61	TLR10_4642 A>G	TLR6_21031 C>A	0.56
TLR5_12236 C>G	TLR5_11894 A>G	1	TLR10_4642 A>G	TLR10_4642 A>G	0.42
TLR5_12236 C>G	TLR5_11606 G>T	0.75	TLR10_4642 A>G	TLR1_7599 T>C	0.27
TLR5_12236 C>G	TLR5_11351 T>C	0.61	TLR10_4642 A>G	TLR6_21664 C>T	0.28
TLR5_12981 A>G	TLR5_12236 C>G	1	TLR10_4642 A>G	TLR6_21211 C>T	0.23
TLR5_12981 A>G	TLR5_11894 A>G	1	TLR10_4642 A>G	TLR6_21031 C>A	0.32
TLR5_12981 A>G	TLR5_11606 G>T	0.75	TLR3_8105 C>G	TLR3_8763 T>C	1
TLR5_12981 A>G	TLR5_11351 T>C	0.61	TLR3_8105 C>G	TLR3_8790 C>A	0.86
TLR5_12933 G>T	TLR5_12981 A>G	0.61	TLR3_8105 C>G	TLR3_9022 G>A	1
TLR5_12933 G>T	TLR5_12236 C>G	0.61	TLR3_8763 T>C	TLR3_8790 C>A	0.86
TLR5_12933 G>T	TLR5_11894 A>G	0.61	TLR3_8763 T>C	TLR3_9022 G>A	1
TLR5_12933 G>T	TLR5_11606 G>T	0.82	TLR3_8790 C>A	TLR3_9022 G>A	0.86
TLR5_12933 G>T	TLR5_11351 T>C	1	TLR4_8865 C>G	TLR4_8980 G>A	0.87
TLR6_21211 C>T	TLR6_21031 C>A	0.77	TLR4_8865 C>G	TLR4_9091 C>T	0.86
TLR6_21664 C>T	TLR6_21211 C>T	0.68	TLR4_8865 C>G	TLR4_9396 G>A	0.86

association with *H. contortus* infection. This polymorphism resulted to replace the amino acid (Tre578Ala) threonine to alanine. Two non-synonymous mutations, transversion at position *TLR4*_8865C>G and transition at position *TLR4*_9396 G>A, in *TLR*4 gene were found to be significantly associated with *Haemonchus* infection in the population analyzed. Polymorphism *TLR4*_8865C>G led to amino acid replacement (Thr389Ser) threonine to serine in the *TLR* protein in goat. Polymorphism at the position of 389 amino acid was previously reported in cattle and was found to be significantly associated with increased *Mycobacterium avium subsp. paratuberculosis* (MAP) susceptibility in Holstein cows (Mucha *et al.*, 2009). Instead of Gly289Ser detected in cattle, the missense mutation Thr389Ser in *TLR*4 gene was found in *H. contortus* infected goat in this study. The increased incidence of *H. contortus* infection in goat bearing the Ser566Asp mutation was also observed in this study. In this position, sheep, cattle and buffalo possesses aspapragine whereas histidine is found in pig and human. However, a noteworthy association between *TLR*s haplotypes and increased susceptibility to *H. contortus* infection was found. Such haplotypes association ever not described before. This study is the first to present an association of goat genetic polymorphism with gastro-intestinal nematode infection in a goat population of Southern China.

Conclusions: Our studies provide conclusive evidence that *TLR*s gene family play a key pathogenic role in gastrointestinal nematode (*H. contortus*) infection. Considering the highly conserved nature of *TLR*s gene family among mammals, these mutations may play a significant role in *H. contortus* infection not only in goat but also in other mammals including humans. Although whether the identified mutations affected gene expression remain to be explored. Further investigation using *in vitro* biological data from large number of animals and *TLR*s gene in a cell culture transient expression assay for analyzing the transcriptional activity of mutations would help establish whether the identified mutations are responsible for the alterations in the expression pattern of the *TRLs* gene.

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