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ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN *NOD1* AND *NLRP9* GENES WITH FECAL EGG COUNT TRAIT OF CHINESE AND BANGLADESHI GOAT BREEDS BEING NATURALLY INFECTED BY *HAEMONCHUS CONTORTUS*

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ABSTRACT

The study was conducted to identify relationships between single nucleotide polymorphisms (SNPs) in the *NOD1* and *NLRP9* genes and resistance to *H. contortus* infestation in goats in China and Bangladesh. A total of 507 unrelated goats from 6 different populations were sampled. Fecal egg count (FEC) was determined and genomic DNA was extracted from those goats. Touchdown PCR assays were used to genotype each of the target SNP. An association study was carried out using a generalized linear model and R software after transforming the FEC as $\log_{10}(FEC + 25)$. All breeds of goat in Bangladesh had lower mean FEC than any of the Chinese breeds. Two single nucleotide polymorphisms (SNPs) viz. *NOD1*_146_A>G ($p<0.001$) and *NLRP9*_43_A>G ($p<0.003$) were significantly associated with FEC trait after *H. contortus* infection. Goats with the GG genotype for *NOD1*_146_A>G (904.43 ± 140.65epg) and the AA genotype for *NLRP9*_43_A>G (1129.57 ± 196.5 epg) had higher FEC than goats with the AA genotype (396.68 ± 67.22 epg) or the GG genotype (399.12 ± 106.4 epg) at the respective loci. *NOD1* and *NLRP9* genes were significantly associated with FEC and might be related to resistance to *H. contortus* infection in goats.

Keywords: Goat, *Haemonchus contortus*, fecal egg count, parasitic resistance, SNP.

INTRODUCTION

Among livestock species, the goat is considered to be one of the first domesticated farm animals and is used for daily activities of human life. Goat are utilized for supplying meat, milk, leather, hair, manure, and income (Gebresilassie *et al.* 2015; Asif *et al.* 2016). More than 90% of goats are reared in Asia and Africa. China possesses the highest number of goat population in the world followed by India, Pakistan, Bangladesh, and Nigeria making the top five goat producing countries. China has more than 58 native goat breeds scattered among different geographical zones and kept in mixed pastoral and agricultural regions, producing the highest amounts of goat meat, skin and hair in the world (Ijaz *et al.* 2015). The Black Bengal goat has three populations viz., BBW (Black Bengal goat of western part), BBC (Black Bengal goat of central part) and BBH (Black Bengal goat of hilly regions of eastern part) according to their distribution and morphology (Faruque *et al.* 2009). Goats contribute to the development of the rural economy in both countries.

Goats are affected by several gastrointestinal parasites. Among the different gastrointestinal parasitic diseases, haemonchosis, caused by the nematode species *Haemonchus contortus* is considered the most serious parasitic disease of goats and is associated with potentially substantial death losses, harmful effect on performance on the flock and then the reduction of income. High death rate of goats due to heavy infestation of *H. contortus* during summer season has been reported in humid and warm countries like southern China and Bangladesh (Gebresilassie *et al.* 2015; Alim *et al.* 2016). Several studies conducted in China and Bangladesh indicated that *H. contortus* was the major nematode species and most common parasite affecting goat production efficiency (He *et al.* 2008; Hassan *et al.* 2011; Nahar *et al.* 2012; Yin *et al.* 2013; Alim *et al.* 2016; Omar *et al.* 2017). In addition, the frequent and continuous use of anthelmintic drugs for deworming has allowed the parasite to develop resistance to the available anthelmintic drugs, making the goats even more susceptible to the infection (Sargison *et al*. 2011; Desoky *et al.* 2015; Asif *et al.* 2016).

The development of goat breeds that are resistant to nematode parasites, and especially to *H. contortus*, is an alternative strategy to control the disease (Fortes *et al.* 2013; Alim *et al.* 2016). Recently, genetic polymorphisms that were associated with susceptibility to, and severity of, haemonchosis have been identified. The detection and screening of single nucleotide polymorphism (SNP) associated with parasite resistance offers a means to identify disease-resistance animals. Kemper *et al*. (2011) discussed the distribution of SNP markers affecting fecal worm egg counts in sheep and the feasibility of using these markers to predict genetic differences in disease resistance.

Candidate genes including *TLR* and the *DRB* genes have been studied (Preston *et al.* 2014; Alim *et al.* 2016; Asif *et al.* 2016). However, there is little information about candidate genes which affect host resistance to parasite infection in goats. Associations of polymorphisms in the *NOD* and *NLRP* genes with autoimmune diseases in human, mice and pigs (Zhang *et al.* 2008; Castaño-Rodríguez *et al.* 2014; Shinkai *et al.* 2015) have been studied but associations with haemonchosis in goats remain unknown and need to be investigated. Therefore, this study was undertaken to (1) evaluate the susceptibility to haemonchosis in goat populations of China and Bangladesh under natural grazing conditions; (2) identify single nucleotide polymorphisms (SNPs) of *NOD1* and *NLRP9* genes; (3) then examine the associations of SNPs with disease resistance trait.

MATERIALS AND METHODS

Ethics statement: All animals used in this study were treated according to the guidelines for experimental animals established by the Animal Care Councils. All the experimental methodology and research on animals were conducted according to the regulations (No. 5 proclaim of the Standing Committee of Hubei People's Congress) approved by the Standing Committee of Hubei People's Congress, and the ethics committee of Huazhong Agricultural University, Wuhan, China (Permission number: 4200896859)

Experimental sites and animals: An in-depth field survey was conducted in experimental locations of Bangladesh and China to know the history of deworming practiced under natural grazing condition. Fecal samples were collected randomly from of 6 to 48 months old goats of both sexes and examined for the *Haemonchus contortus* infection. The experimental locations and goat breeds were finally selected based on the result of the survey. Three native goat breeds, viz. the Yichang White (YCW), Nanjiang Yellow (NJY), Enshi Black (ESB), and one crossbred population producing by crossing among Yichang White, Enshi Black, Nanjiang Yellow and Boar goats (Chinese crossbreds) from China and two populations of Black Bengal goats, viz. the Black Bengal Western (BBW) and Black Bengal Hilly (BBH) from Bangladesh were selected for this study. A total of 507 unrelated goats from these 6 different breeds/populations were selected. The numbers of samples collected from YCW, ESB, NJY, Chinese crossbred, BBW and BBH were 33, 36, 55, 155, 212 and 15, respectively.

All selected goats were kept on natural grazing lands under conditions of natural mixed parasite infection. In China, experimental animals were selected from two locations, namely the Enshi (N: 30°17′; E: 109°29′) and Yichang (N: 30°43′; E: 111°17′) cities located in Hubei province of southern China. In Bangladesh, experimental sites were located in Natore (N: 24°07.163′; E: 89°03.997′) and Bandarban Hill district (N: 24°47.523′; E: 091°43.893′).

Sample and data collection: Blood and fecal samples were collected from each goat before deworming at the end of rainy season in June through August, 2015 when parasitic infection become sever due to high moisture and ambient temperature (Omar *et al.* 2017). Peripheral blood samples were collected from the jugular vein into tubes coated with EDTA (ethylene diamine tetraacetic acid) as an anti-coagulant, carried in an ice box, and kept in the refrigerator at 4°C. Genomic DNA was extracted from whole blood using a modified phenol-chloroform-isoamyl protocol (MWer *et al.* 1988). Fecal samples were collected from the rectum of each animal directly by two fingering method. Numbers of eggs of *H. contortus* were determined by microscopic examination using the modified McMaster technique (Zajac and Conboy 2012). The numbers of eggs per gram of feces (epg) was calculated as:

$$Egg/gm = [no.of\ egg\ counted\ x\ (T/V)]/F$$

where T is the total volume of the mixture of feces and flotation solution, V is the total volume of solution examined on the slide, and F is the grams of feces used. The sensitivity of the assay was 50 eggs per gram of feces; each observed egg corresponded to 50 epg. The location, age, sex, and body weight of each animal was recorded.

Identification of polymorphisms: Genomic sequences containing the caprine *NOD1* and *NLRP9* genes were used to detect SNP in these genes, and primers were designed using the NCBI Primer-BLAST web Program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) from the CDS of the *NOD1* and *NLRP9* genes (NCBI, Gen Bank association number : NW_017189490 and NW_017189504 respectively). PCR was performed using a genomic pool DNA from goats belonging to all six populations (20 each, except for 15 BBH). The final PCR reaction volume was 50 µl, consisting of 50 ng of

genomic DNA in 2 µl, 1.5 µl of each primer, 20µl of double-distilled H_2O, and 25 µl of Taq premix (TaKaRa, Dalian. China). For all the primers, samples were incubated at 94ºC for 10 min then amplified for 35 cycles each consisting of denaturation at 95ºC for 30s; annealing at 58ºC for 30s; an extension at 72ºC for the 50s; and a final extension at 72ºC for 5 min. The PCR product was checked by electrophoresis on 1% agarose gel to confirm the amplification and sequenced using an ABI 3730XL genetic analyzer (Tsingke Biological Technology Co. Ltd., Wuhan, China). SNP were detected using SeqMen and BioEdit software and confirmed by manual inspection.

Genotyping of SNPs: The SNP loci were genotyped following Kompetitive allele-specific PCR (KASPar) based on FRET chemistry (KBiosciences, LGC Genomics, UK). Briefly, two forward primers (one specific to each allele) were designed with the respective proprietary tail sequence complementing the FAM or HEX fluorescence reporting system. A common reverse primer was designed for each genotyping assay. The primer sequence, thermal cycling parameters and recycling condition are presented in the Table 1a. Genotype calling was based on end-point measurements of fluorescent intensities recorded for each of the two alleles. The emission data of all the samples on the plate was plotted on their X and Y axis, respectively, for each allele and genotypes were called based on distinct clustering.

Touchdown PCR (TD-PCR) assays were used to amplify the variable regions of the different DNA sequences and allow genotyping of each of the target SNP loci. The TD-PCR protocol used 36 cycles of amplification, each including two steps of extension and annealing processes including pre-denaturing at 94ºC for 15 min followed by denaturing at 94ºC for 20s; annealing at (61ºC and 68ºC) for 60s, extension up to achieving the annealing temperature (55ºC and 62ºC) by10 cycles; and then extension and annealing at 55ºC and 62ºC for 60s for 26 cycles, totaling up to 36 cycles.

Table 1a. Primer pairs used for genotyping of caprine *NOD1* and *NLRP9* genes.

Gene	SNPs ID	Allele	Forward Primer (5'-3')	Reverse Primer (Common) (5'-3')	Touchdown Protocol
NOD1	146_A>G	X	CGGACGCCTGCACCTCA	GCATGGAGAAGAGCCTCTTTG	61-55 TD
		Y	CGGACGCCTGCACCTCG	TCTT	
	416_G>C	X	GGAAGATGATCCAGCAGAAGAGG	GATCAACTGGAGGCCAACCCC	61-55 TD
		Y	GGAAGATGATCCAGCAGAAGAGC	AA	
	552_C>T	X	CCGCGCAGGAGCGCCC	TCCCGCGACATCTCCTCTGGAA	68-62 TD
		Y	GCCGCGCAGGAGCGCCT		
NLRP9	43_A>G	X	CCTATGGAGGGACAGGTTCTCA	GGCCCGTGAGGAGGAAGACA	61-55 TD
		Y	CTATGGAGGGACAGGTTCTCG	AA	
	226_C>T	X	CCCAGTCTTCACAGAGGTCG	AGGAACTGAAGTTTGACTTGG	61-55 TD
		Y	CTCCCAGTCTTCACAGAGGTCA	ATTGCAA	
	457_A>G	X	TCCAGGAGAATGATGCATCCTCA	AGAGTGACCTCTTCTCCCTCAC	61-55 TD
		Y	CCAGGAGAATGATGCATCCTCG	AAA	

Statistical analysis

Descriptive statistics: The FEC data were not normally distributed, i.e. they were positively skewed. A logarithmic transformation therefore was applied before analysis (Rout *et al.* 2011; González-Garduño *et al.* 2013). Descriptive statistics (i.e., arithmetic means, variances, standard errors, etc.) were derived as described by (Chiejina *et al.* 2005) after data were transformed into $\log_{10}$ (n+25) where **n** is the number of egg per gram of feces. The analysis was performed using SAS (1998, version 9).

Hardy–Weinberg equilibrium (HWE) test: Correspondence of observed genotypic frequencies to expectations based on Hardy–Weinberg equilibrium (HWE) conditions was assessed using chi-square (x^2) tests. Genotype frequencies and allele frequencies were calculated by using Microsoft Excel (Version 2016) and tested to determine if the populations were in Hardy–Weinberg equilibrium using chi-square tests in the R statistical programming language (Version 3.2.5) (Ihaka & Gentleman 1996).

Association analysis: Relationship between SNP and fecal egg counts (FEC) were evaluated using a generalized linear model (GLM) and employing R programming (Version 3.2.5). The linear model for the analysis was:

$$y = \mu + B + V + G + e$$

where y is the observation of FEC trait; μ is the overall population mean; B and V are the fixed effect for breed and location, respectively, G is one of the total six SNP which were found to be associated with FEC, and e is the residual error. Multiple comparison analysis was made between genotypes of significant SNP and FEC in all

goat populations.

RESULTS

Infection status of *H. contortus* in Chinese and Bangladeshi goat population: Out of 507 goats, 237 were infected by *H. contortus*, giving an overall infection rate of 46.75%. The average infection rate was 68.9% and 19.4% for Chinese (n=280) and Bangladeshi goat breeds (n=227), respectively. Among the six breeds/populations, the highest and the lowest infection rate were found in ESB goats (88.89%) of China and the BBH population (0%) of Bangladesh, respectively (Table 1). Bangladeshi goats were less susceptible than Chinese goats to *H. contortus* infection. Regarding FEC, the highest transformed FEC was detected in Chinese crossbred goats while the lowest was recorded in the BBH goats (Table 1). In Bangladeshi goats, BBH had a lower FEC (1.40±0.15 epg) compared to BBW (1.59 ± 0.04 epg). Among the Chinese goats FEC was highest in Chinese crossbred goats (2.58±0.05 epg) but lowest in NJY (2.12 ± 0.08 epg) goats (Table 1).

Table 1. Descriptive statistics of infection status of *H. contortus* in different breeds/populations of China and Bangladeshi goats.

Breeds/ Populations	Total goats checked	Infection Rates (%)	FEC (epg) (mean ± SE) (Log_{10}+25)	Range of FEC (epg)
BBH	15	0	$1.40±0.15^{c}$	0 - 0
BBW	212	20.75	$1.59±0.04^{c}$	0 - 400
YCW	32	66.67	$2.31±0.11^{ab}$	0 - 2400
NJY	56	51.79	$2.12±0.08^{b}$	0 - 5400
ESB	37	88.89	$2.53±0.10^{a}$	0 - 3600
Chinese crossbreds	155	70.79	$2.58±0.05^{a}$	0 - 15600
Level of significance			**	

**Significant at P<0.01; figures in parentheses indicate the number of observations; a,b,c, lettering indicates significance levels between row; and raw data were converted to log_{10}(FEC+25).

Detection of SNPs in caprine *NOD1* and *NLRP9* genes: To associate disease resistance trait (FEC) with caprine *NOD1*and *NLRP9* genes through screening of amplified DNA sequence, a total of six polymorphisms (3 in *NOD1* and 3 in *NLRP9* genes) were detected within the CDS sequences of goats in China and Bangladesh. Among the six SNPs, we found 2 synonymous and 4 non-synonymous SNPs that was predicted to result in amino acid replacement from Lysine to Arginine at position 54366936 (K54366936R) in *NOD1* gene and Serine to Proline at position 63532970 (S43P), Threonine to Alanine at position 63533201 (T63533201A), Serine to Proline at position63533384 (S63533384P) in the *NLRP9* gene (Table 2).

Hardy-Weinberg equilibrium analysis: Frequencies of AA and AG genotypes were higher than that of the GG genotype in all population of Chinese and Bangladeshi goat for the *NOD1*_146_A>G locus. The only exception was for ESB goats where the AA genotype frequency (0.14) was low. No GG goats were found in the BBH population (Table 2). The frequency of the GG genotype was higher than those of AA and AG in all populations of goat for the *NLRP9*_43_A>G locus. The frequency of the AA genotype was very low in all populations of goats and was 0 for BBH. Frequencies of AG were moderate in all goat populations included in the present study.

Table 2. Amino acid replacement on SNPs in the *NOD1* and *NLRP9* genes.

SNP ID	Nucleotide		Chromosome No.	Amino acid	
	Genomic Position	Allele		Encode	Allele
*NOD1*_146_A>G	54366530	A/G	4	D=>D	synonymous
*NOD1*_416_G>C	54366800	G/C	4	P=>P	synonymous
*NOD1*_552_C>T	54366936	C/T	4	K=>R	non-synonymous
*NLRP9*_43_A>G	63532970	A/G	18	S=>P	non-synonymous
*NLRP9*_226_C>T	63533201	C/T	18	T=>A	non-synonymous
*NLRP9*_457_A>G	63533384	A/G	18	S=>P	non-synonymous

Note: Amino acid symbols; K=>Lysine, R=>Arginine, S=>Serine, P=>Proline, T=>Threonine, A=>Alanine

Table 2a. Tests of Hardy-Weinberg equilibrium for caprine *NOD1*_146_A>G and *NLRP9*_43_A>G genes in six goat breeds/population.

SNP ID	Breeds/ Populations	No. of goats	Genotype Frequency			Allele Frequency		Chi-square test	
			AA	AG	GG	A	G	x^2	P-value
*NOD1*_146_A>G	YCW	31	0.42	0.35	0.23	0.60	0.40	2.15	0.14
	NJY	54	0.43	0.35	0.22	0.60	0.40	3.81	0.05
	ESB	36	0.14	0.58	0.28	0.43	0.57	1.29	0.26
	Chinese crossbred	153	0.27	0.45	0.28	0.50	0.50	1.47	0.23
	BBH	15	0.93	0.07	0.00	0.97	0.03	0.03	0.86
	BBW	208	0.74	0.25	0.01	0.86	0.14	0.36	0.55
*NLRP9*_43_A>G	YCW	31	0.03	0.32	0.65	0.19	0.81	0.034	0.852
	NJY	55	0.09	0.29	0.62	0.24	0.76	2.073	0.149
	ESB	35	0.03	0.29	0.68	0.17	0.83	0.001	0.973
	Chinese Crossbred	154	0.11	0.34	0.55	0.28	0.72	3.511	0.061
	BBH	14	0.00	0.43	0.57	0.21	0.79	1.041	0.308
	BBW	204	0.03	0.37	0.6	0.22	0.78	2.087	0.149

Alleles A and G were present in both the *NOD1*_146_A>G and *NLRP9*_43_A>G genes (Table 2a). For the *NOD1*_146_A>G locus, the allele frequency of A was higher (0.97 and 0.86) than that of G (0.03 and 0.14) in both Bangladeshi goat populations. Allele frequencies of A and G were not different in the Chinese goat populations. For the *NLRP9*_43_A>G locus, the allele frequency for G was higher than that of allele A in all populations. The BBH and ESB population had the lowest allele frequencies of A (Table 2a). The chi-square test for departures from Hardy-Weinberg equilibrium was significant ($p<0.05$) for the NJY goat at *NOD1*_146_A>G locus, but was not significant for the *NLRP9*_43_A>G locus in any goat breed/population.

Population association analysis with FEC: Association analyses was carried out between SNP genotypes within the *NOD1* and *NLRP9* genes and disease resistance, as measured by FEC (Table 3). The result showed that two of the six polymorphisms, *NOD1*_146_A>G ($p=0.001$) and *NLRP9*_43_A>G ($p=0.003$), were significantly associated with FEC trait. In the total population, the number of AA genotypes (250) at the *NOD1*_146_A>G locus was higher than those for the AG (173) or GG (73) genotypes. Differences between the GG and AA genotypes ($p=0.008$) and the GG and AG genotypes ($p=0.002$) were significant for the *NOD1*_146_A>G locus. For the *NLRP9*_43_A>G locus, differences between the AG and AA genotypes ($p=0.009$) and the GG and AA genotypes ($p=0.003$) were significant.

Table 3. Association study and multiple comparison between of SNPs and FEC in different goats population.

Gene Name	SNP ID	Genotype frequency Number			Multiple comparison tests P-value			Association P-value
NOD1	146_A>G	AA	AG	GG	AG-AA	GG-AA	GG-AG	0.001^{***}
		250	173	74	0.665	0.008	0.002	
	416_G>C	CC	GC	GG	GC-CC	GG-CC	GG-GC	0.125
		52	218	231	0.998	0.469	0.164	
	552_C>T	CC	TC	TT	TC-CC	TT-CC	TT-TC	0.066
		150	217	137	0.079	0.681	0.456	
NLRP9	43_A>G	AA	AG	GG	AG-AA	GG-AA	GG-AG	0.003^{**}
		30	171	292	0.009	0.003	0.869	
	226_C>T	CC	TC	TT	TC-CC	TT-CC	TT-TC	0.647
		9	94	401	0.972	0.998	0.653	
	457_A>G	AA	AG	GG		GG-AG		0.607
		0	18	486		0.615		

** indicates $p<0.01$, and *** indicates $p<0.001$.

Descriptive statistics analysis of caprine *NOD1* and *NLRP9* genes: Results of least square analysis (mean ± SE) (Table 4) revealed that the means for FEC were higher for goats with the GG genotype at the *NOD1*_146_A>G locus (904.43 ± 140.65epg) and goats with the AA genotype at the *NLRP9*_43_A>G locus

(1129.57 ± 196.5epg). Means for FEC were lower for goats with the AG (353.19 ± 105.11epg) and AA (396.68 ± 67.22 epg) genotypes at the *NOD1*_146_A>G locus and with the GG (399.12 ± 106.4epg) and AG genotypes (462.99 ± 113.1 epg) at the *NLRP9*_43_A>G locus. In the overall population, numbers of GG genotypes (74) at the *NOD1*_146_A>G locus and AA genotypes (30) at the *NOD1*_146_A>G locus were lower than the numbers observed for the other two genotypes.

In the Chinese goat populations, average of FEC were much higher for the GG genotype at the *NOD1*_146_A>G locus and AA genotypes at the *NLRP9*_43_A>G locus. Numbers of goats with the GG genotype at the *NOD1*_146_A>G locus and the AA genotype at the *NLRP9*_43_A>G locus were 71 and 24, respectively. Number of goat with the other genotypes were higher, and corresponding means for FEC were moderate (Table 4). The average of FEC were also higher for goats with the GG genotype at the *NOD1*_146_A>G locus at with the AA genotype at the *NLRP9*_43_A>G locus compared to goats with the AG genotype at either locus. In Bangladeshi goats, means for FEC were very low for all genotypes at both the *NOD1*_146_A>G and *NLRP9*_43_A>G gene loci. The mean FEC was higher for goats with the AA genotype at the *NLRP9*_43_A>G locus, but the number of goats with the AA genotype was very small (Table 4).

Table 4. Results of least square analysis (mean ± SE) of caprine *NOD1*_146_A>G and *NLRP9*_43_A>G loci with fecal egg counts in Chinese and Bangladeshi goat populations.

SNP ID	Genotype	Overall		China		Bangladesh	
		No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE
*NOD1*_146_A>G	AA	250	396.68 ± 67.22	83	696.05±126.91	157	34.59±7.13
	AG	173	353.19 ± 105.11	120	620.67±156.08	53	45.34±14.19
	GG	74	904.43 ± 140.65	71	1216.16±231.09	13	58.99±25.77
*NLRP9*_43_A>G	AA	30	1129.57 ± 196.5	24	1619.07±263.6	6	97.52±25.80
	AG	171	462.99 ± 113.1	89	820.17±230.8	82	24.73±17.09
	GG	292	399.12 ± 106.4	162	674.98±214.6	130	44.51±21.62

Note: SE =Standard error, these analyses were based on untransformed Fecal Egg Counts.

DISCUSSION

In tropical and subtropical regions, and especially in Asia and Africa, goats play a significant role in rural development. Goats are easily reared by poor and marginal peoples in these regions, and millions of people receive benefits from this animal. One of the major factors that reduce goat productivity is haemonchosis caused by the gastrointestinal nematode *H. contortus.* The prevalence of *H. contortus* may become extreme during the rainy season, which usually extends from May to September and when humidity and temperature remain high (Omar *et al.* 2017). In the present study, the prevalence of *H. contortus* was recorded from goats reared under natural grazing system in subtropical regions of China and Bangladesh. Samples were collected from May to August, when humidity was relatively high and conditions were favorable for infection with *H. contortus* (Fakae *et al.*1990; Raza *et al.* 2009; Chiejina & Behnke 2011).

Fecal egg count, blood haemoglobin levels and packed cell volume are indicators of resistance to gastrointestinal nematodes in small ruminant species viz. sheep and goat. The FEC is generally considered to be the main indicator of resistance to gastrointestinal nematode (GIN) in small ruminants (Mandonnet *et al.* 2001; Bishop *et al.* 2011; Rodríguez *et al.* 2015). This study, therefore, focused mainly on FEC in goats in the study areas. In Bangladesh, this is the first comparative study to evaluate the susceptibility to *H. contortus* infection in different populations of Black Bengal goats. Moreover, this is the first comparative study to evaluate the susceptibility to *H. contortus* infection among different goat breeds of two countries in which goats were reared under similar conditions. The Chinese goat breeds had greater FEC compared to the Bangladeshi goat breeds. This differences might be attributed to genetic differences or to non-genetic factors such as topography, climate and animal management and were consistent with results of a previous study (Omar *et al.* 2017).

The discovery of genes related to immune function can have a vital role in understanding the physiology of parasite infection and be used to develop novel tools for control of parasitic diseases (Asif *et al.* 2016). Our study identified six SNP, two of which were significantly associated with FEC in goats. Four of these SNP were the result of missense mutations resulting in substitution of amino acid from Lysine to Arginine, Serine to Proline, Threonine to Alanine and Serine to Proline. These amino acid substitutions have potential to affect immunity to *H. contortus* infection. Proline plays important roles in molecular recognition, particularly in intracellular signaling whereas Lysine appears to have anti-anxiety action through its effects on serotonin

receptors in the intestinal tract. The function of the gene in body immunity system might be change due to the change in amino acid (Seyedhassani *et al.* 2011; Asif *et al.* 2016; Cao *et al.* 2017).

Exploration of genetic variation, either within specific regions of genome or in candidate genes involved in innate and adaptive immune pathways may help to identify DNA markers that are associated with GIN parasite resistance characteristics. In attempting to identify genes that are involved in control of disease resistance and susceptibility, candidate gene, microarray, and gene association studies have been conducted in several goat breeds (Alim *et al.* 2016). *NOD1* and *NLRP9* genes have an immune regulatory function and multiple studies of these genes have been carried out on humans and a few livestock and aquaculture species (Kim *et al.* 2004; Zhang *et al.* 2008; Castaño-Rodríguez *et al.* 2014; Nagyőszi *et al.* 2015; Poli *et al.* 2015; Shinkai *et al.* 2015). Previous studies reported that *NOD1* gene were associated with particular diseases like gastric cancer in human (Wang *et al.* 2012; Castaño-Rodríguez *et al.* 2014; Li *et al.* 2015; Li *et al.* 2016). Furthermore, several studies reported *NOD1* genes to be involved in intestinal anti-bacterial function in zebra fish (Laing *et al.* 2008; Oehlers *et al.* 2011). A group of researchers (Ponsuksili *et al.* 2006; Zhang *et al.* 2008; Tian *et al.* 2009; Nagyőszi *et al.* 2015; Poli *et al.* 2015) also reported a role in immune function for *NLRP9* genes in reproductive organs and tissues, pre-implantation embryos, and related diseases of humans and other mammalian species. Differential expression of *NLRP9* was reported in infected and normal pre-implantation human embryos, and its expression was lower at day 1 but higher at day 3 and 5 in abnormal embryos (Zhang *et al*. 2008). Recently, Shinkai *et al*. (2015) identified 12 synonymous and 9 non-synonymous SNP in the coding sequence of porcine *NOD1* of major commercial breeds. Among the non-synonymous SNP, two amino-acid alterations in the leucine-rich repeats region, from glycine to glutamic acid at position 641 (G641E) and from aspartic acid to asparagine at position 918 (D918N), impaired iE-DAP (γ-d-glutamyl-meso-diaminopimelic acid)-induced activation of NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells). They recommended that elimination of unfavorable alleles at *G641E* and *D918N* alleles from commercial pig populations would improve their disease resistance.

Despite the classic role of *NOD1* and *NLRP9* genes in the immune systems of humans and livestock species, there was no previous information on these two genes with regard to immune function in goats. Moreover, there were no reported associations of *NOD1* and *NLRP9* genes with resistance to gastrointestinal parasites such as *H. contortus* in goats. This is the first report of an association between these genes and *H. contortus* resistance in goats. The present association study revealed that two SNP polymorphisms viz., *NOD1*_146_A>G and *NLRP9*_43_A>G were significantly associated with FEC in all studied populations of Chinese and Bangladeshi goats implying their resistance to *H. contortus* infection. Further work should be done on these two genes to better understand their regulatory role in the goat immune system.

Conclusion: *Haemonchus contortus* infection was found to be lower in Bangladeshi goats than Chinese goats. SNP analysis reveals that of *NOD1* and *NLRP9* genes were associated with FEC in goats under conditions of natural mixed infection with GIN including, predominantly, *H. contortus*. Two polymorphisms (*NOD1*_146_A>G and *NLRP9*_43_A>G) had highly significant associations with FEC. Four non-synonymous SNP that changed the amino acid sequences of the resulting gene products were identified and might therefor change the function of those genes. Further studies on the immuno-genetics of the goat might assist in understanding the mechanisms associated with observed genotypic differences and developing disease-resistant goat breeds.

Conflict of Interest: We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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