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Assessment of genetic resistance and abomasal tissues expression of Yichang white goat experimentally challenge with *Haemonchus contortus* infection

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Abstract

Our previous association study revealed the mutation in candidate immune genes (*NOD1* & *NLRP9*) was significantly associated with FEC of *Haemonchus contortus* infection in Yichang white goats, but the relative expression of mRNA of those genes associated with resistance to *H. contortus* was not investigated. Aim of the current experiment was to evaluate the susceptible and resistant individuals to nematode infection within the population of Yichang white goat (YWG) and assess the differential level of mRNA expression of those candidate genes in the abomasal tissues of susceptible and resistant goats. Fecal egg count (FEC) was determined using a modified McMaster technique, and the hematological parameter was measured by Mindray auto hematology analyzer. Phenotype data were collected and analyzed using a generalized linear model with SAS statistical program. Field investigate revealed that the prevalence (76%) with maxium parasite load (734.34±84.21epg) of *H. contortus* occurred in August within the experimental flock FEC in resistant group (103.38±1.20epg) and susceptible group (1180.25±43.53epg) group demonstrated the presence of two distinct goat populations within this breed. Four resistant and four susceptible goats were selected from each group. The parasite infection was established by artificially challenge with 5000 infective L_3 larvae of *H. contortus.* Abomasal tissues were collected from all experimental goats after 42 days of post-infection. FEC, Body weight, packed cell volume, and hemoglobin value were significantly different ($P < 0.01$) between resistant and susceptible group of goats. Quantitative real-time PCR in abomasal tissues revealed that the expression level of mRNA for *NOD1* ($P < 0.00001$), *IFNG* ($P < 0.0001$), *NLRP9, TLR8*, *IL32,* ($P < 0.001$) and *IGF1* ($P < 0.01$) was higher in resistant goat compared to susceptible, except *SFTPA1*. These findings revealed the presence of genetic resistant individuals to *H. contortus* within the goat breed and expression of *NOD1* and *NLRP9* genes proved the positive finding of our previous study. Presence of genetic resistant individuals in Yichang white goat YWG breed could be a good candidate for selective breeding and highly expressed genes related to resistant could be used as biomarkers to develop *H. contortus* resistant goat population.

Key words: Goat, *Haemonchus contortus,* challenge trail, gene expression, genetic resistance

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Introduction

Goat is one of the most valuable domestic animals among livestock species. It was domesticated about 11,000 years ago in the Asian region and is recognized as an important source of milk, meat, skin, fiber and great source of income for small farmers, and shepherds in marginal areas of developing countries (Chessa *et al.* 2009; Colli *et al.* 2015). Presently, goat production becomes a profitable business to the farmers especially the rural people who efficiently contribute to the economy of the country. However, goat production is threatened by the gastrointestinal parasitic diseases particularly in the tropical and subtropical regions of Asia and Africa. Among the gastrointestinal parasites, *Haemonchus contortus* is the predominant nematode that affected goat production severely under natural grazing condition (Yin *et al.* 2013; Ma *et al.* 2014; Omar *et al.* 2016).

H. contortus is a common blood-feeding nematode responsible for high morbidity and mortality in the flocks of high temperate and rainfall areas. Each adult parasite sucks around 0.05 to 0.07 ml of host blood daily thereby resulting in loss of around 250 ml blood infected by a total of 5000 parasites and can cause severe anemia and finally death (Clark *et al.* 1962; Rodríguez *et al.* 2015; Omar *et al.* 2016). Treatment with anthelmintic is a common practice for control of parasitic infestation in sheep and goats worldwide. This practice is leading to develop the widespread anthelmintic resistance to the nematode. In this situation, developing resistant goat breeds to gastrointestinal nematode could be the alternative strategy for controlling this problem (Getachew *et al.* 2015). Genetic variation for resistance to nematode infection varies among breeds and within breed even between individuals and this is controlled by several genes that have been recognized for sheep and goats (Mandonnet *et al.* 2001; Chauhan *et al.* 2003; Rout *et al.* 2011; Traoré *et al.* 2017). The proliferation and activation of mast cells, eosinophils, and globules leukocytes of the abomasal mucosa contributed to this genetic resistance against nematodes (Bambou *et al.* 2013). A number of studies described the genetic resistance to GIN of different sheep breeds, and it is mainly associated with the immune responses of hosts. However, few studies have been conducted on the immune response of goats to *H. contortus* infection (Baker *et al.* 2001; Chiejina & Behnke 2011; Omar *et al.* 2019; Kurukulasuriya *et al.* 2018). Several goats and sheep breeds are naturally resistant to gastrointestinal parasites infection and evaluating the relative resistance of goat breeds using artificial challenge trial should be considered as a way towards mapping of genes controlling internal parasite (Getachew *et al.* 2015). By the relative expression analysis, it has been proved that resistant animals express hundreds of genes involved in their immune response (Diez-Tascón *et al.* 2005). Subsequently, it was hypothesized that the expression of immune genes would increase the resistant capability to the parasite and would possibly be used as biomarkers for producing *H. contortus* resistant goat breeds.

China has many native goat breeds rearing for meat, milk and wool purpose scattered in the different agro-ecological postural zone and commonly infected by nematode species particularly *H. contortus* (Omar *et al.* 2019; Ma *et al.* 2014). Yichang white goat is the native goat breed of China and it has been observed a greater degree of resistance to *H. contortus* infection and great inconsistency of FEC within this breed (Omar *et al.* 2016; Omar *et al.* 2019; Alam *et al.* 2019) thus making it for perfect candidate for selective breeding program to improve the resistance to nematode infection. Our previous study (Omar *et al.* 2019) revealed some novel polymorphisms in nucleotide-binding oligomerization domain containing 1 (*NOD1)* and NLR family pyrin domain containing 9 (*NLRP9)* genes that were significantly associated with disease resistant trait, particularly *H. contortus* (Omar *et al.* 2019). However, several investigators also reported similar results for dopamine receptor binding 1 (*DRB-1),* insulin-like

growth factor 1 (*IGF-1),* interleukin 32 (*IL32),* interleukin 33 *(IL13)*, Surfactant protein A1 (*SFTPA1)* and interferon gamma *(IFNG)* genes with GIN resistance of goat (Bressani *et al.* 2014; Alim *et al.* 2016; Asif *et al.* 2016). But none of them reported the expression pattern of mRNA of those genes related to nematode infection that could be a good point of study to investigate their relationship with resistant or susceptibility to nematode infection. Therefore, the present study was conducted to assess the circumstances of resistance or susceptibility of Yichang white goat population to *H. contortus* infection within the breed and population for the selective breeding program and evaluate the differential expression of mRNA in our candidate genes for resistant or susceptible to *H. contortus* by artificially challenge with L_3 larvae of *H. contortus* in Yichang white goats population.

Materials and Methods

***Ethics statement*:** Designs of all experiments were accompanied in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006) and approved by the Standing Committee of Hubei People's Congress, and the ethics committee of Huazhong Agricultural University, Wuhan, China (Permission number: 4200896859).

***Experimental site*:** The present experiment was performed in a goat farm of Yichang, China (at 30° 48′N latitude, 111° 21′E longitude, and altitude of 58 m). Based on the meteorological data collected from 2000 to 2015 the annual rainfall of the area was about 1,140 mm, the rainy season occurs typically from April to August. During the experimental period, the minimum and maximum average temperatures and total rainfall were 26.8 °C, 36.7 °C, and 41.2 mm, respectively. The annual precipitation was about 70% in June, and the relative humidity was higher (84 to 80%) from the March to July 2017. Site selection was based on the fact that Yichang white goats (YWG) are commonly reared under natural grazing condition and semi-intensive system with comparable levels of exposure to gastrointestinal parasites.

***Screening the animals for parasitic load*:** For the evaluation of the parasitic load in the term of fecal egg count (FEC) expressed as eggs per gram of feces (epg), goats were selected from natural grazing condition and confirming no deworming history for the past one year within the flock. FEC was performed by the rapid modified Macmaster technique (Gordon & Whitlock 1939; Zajac & Conboy 2012) in every month from March 2016 to December 2016 to assess the parasitic load of *H. contortus* infection in the herd for goats age 6 month to above and to judge the resistance or susceptibility based on low and high responder of FEC of YWGs in our studied population. The numbers of epg was calculated as:

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

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.

An initial sample of 81 Yichang white goats of 9 to 12 month of age were selected from a flock of 450 goats and FEC, body weight, circulating hemoglobin levels (Hg), and pack cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) were determined to assess the susceptibility of parasites infection. Based on their FEC, goats were divided into two groups viz. resistant and susceptible group. Goats with High FEC group (FEC > 500) was considered as a high responder and assigned to be more susceptible group and goats with Low FEC (FEC < 500**)** was considered as a low responder and assigned to be the more resistant group to *H. contortus*. FEC, circulating hemoglobin (Hg), packed cell volume (PCV), Red Blood Cells (RBC), White Blood Cells (WBC) and animals body weight (BW) from resistant and susceptible goats were determined for selecting goats for the challenge experiment. Finally, 4 resistant goats (lowest number

of EPG count) and four susceptible goats (highest number of EPG count) with an average age of 305 ± 10 days were selected for the artificial challenge.

***Parasitological technique*:** To procure L_3 larvae for infection, adult female *H. contortus* was collected from the abomasum of the infected goats from the nearby abattoir. The female worms were washed and crushed using mortar and pestle to liberate eggs. Filtration was made to avoid debris, and eggs were collected by sedimentation process using slow centrifugation at 100 rpm for 2 min. Coprocultures were maintained under laboratory conditions (with 25^0C and 80% humidity) and moistened and aerated daily for eight days (Ojeda-Robertos *et al.* 2017). A culture of third-stage (infective stage) larvae (L_3) in vitro condition was recovered following the slandered Baerman technique (Hansen & Perry 1994; Ojeda-Robertos *et al.* 2017). Harvested larvae were counted in each of five 20ul aliquots, and larval density (larvae/ml) was estimated. After harvesting, larvae were stored in deionized water at 4^0C and used within one month to infect experiment animals.

***Nematode challenge trial*:** Eight goats (4 resistant and 4 susceptible) were selected to dry lot for challenge trial and treated with broad-spectrum anthelmintic viz., Ivermectin (0.25 mg/kg body weight) and 15 days later, with levamisole (8 mg/kg body weight) to ensure the animals were completely dewormed (Bambou *et al.* 2013; Kurukulasuriya *et al.* 2018). In dry lot, each goat were fed 30 g/kg BW of balance feed including GIN-free fresh grass per day on a dry-matter basis, and a concentrated mixture was provided twice per day at a rate of 1.5% of BW containing 18% crude protein and 10.6UJ/kg of metabolizable energy on a dry matter basis (Ojeda-Robertos *et al.* 2017). When all experimental goats achieved a FEC of zero ensured by microscopic examination, resistant and susceptible goats were inoculated with a single dose of 5,000 third-stage (L3) larvae of *H. contortus* (Notter *et al.* 2003; Getachew *et al.* 2015; Kurukulasuriya *et al.* 2018). The day of inoculation was considered to be day 0, and FEC, PCV, Hg, and BW were determined weekly for six weeks from the day of inoculation.

***Abomasal tissue preparation for RNA extraction*:** Animals were sacrificed following the proper procedure and by national humane euthanasia guidelines at 42 days post-infection. After slaughtering the animals, gastrointestinal tract and abomasum were removed from the digestive tract. The abomasal wall was then cut along the greater curvature and washed with phosphate buffer solution (PBS) at room temperature. A $2cm^2$ section of tissue, including the full thickness and 1-fold of the abomasal wall, was removed from the fundic region (MacKinnon *et al.* 2009). Approximately 100 gm samples were weighted, immediately frozen by liquid nitrogen and stored at -80^oC until extraction of total RNA.

***Candidate gene selection for mRNA expression*:** Candidate genes (*NOD1, NLRP9, TLR8, SFTPA1*, *IGF-1, IL32, IL33* and *IFNG*), that were significantly associated with FEC trait of resistance to *H. contortus* infection in goat, were selected based on our previous association study and reports of other investigators for such study (Bressani *et al.* 2014; Alim *et al.* 2016; Asif *et al.* 2016). Oligonucleotide primers were designed to see the mRNA expression for our selected genes using the coding sequences (CDS) of caprine mRNA following NCBI primer design web program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and optimized with the primer 7 software to avoid self-priming and primer-dimers. The primers used for Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) are presented in Table 1.

***RNA extraction and quantitative real-time PCR (qRT-PCR)*:** Total RNA was extracted using TRlzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommended procedure. Quantity of RNA was assessed using a Nano Drop ND2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and gel electrophoresis was used to ensure the quality of RNA (MacKinnon *et al.* 2009). Quantitative real-time PCR (qRT-PCR) was performed

to assess the expression of our selected candidate genes (Table 1) using cDNA generated from approximately one µg of RNA using a standard Prime Scipt™ RT reagent kit with gDNA eraser (Perfect Real Time, TAKARA Bio, Inc.). CFX-96 Bio-Rad thermal cycler with SYBR green real-time PCR master mix (Toyobo Co., Ltd., Osaka, Japan) was used for qRT-PCR. The qRT-PCR protocol was a single cycle of denaturation at 95 °C for 5 minutes, 45 cycles of denaturation at 95°C for 20 seconds, annealing at 58°C for 20 seconds, and extension at 72°C for 15 seconds. Beta-actin (*ACTB*) was used as a housekeeping gene to normalize the samples (Rebouças *et al.* 2013).

Table 1. Primers information for the measurement of mRNA expression by a quantitative reverse-transcriptase polymerase chain reaction.

Gene Name	Primer	Sequence 5' to 3'	Product length	Ref. Sequence no.
NOD1	F	CTCCCCAACCTCCGAGTTCC	105	XM_018047107.1
	R	GCACAGCGCTTGGTAGCC		
NLRP9	F	ACTTTGCATCGAATCTGGCA	109	XM_005692931.2
	R	GCATCCATATGGGAGAGGCT		
SFTPA1	F	TCCCTGGAGAACGTGGAGAA	126	NM_001009728.2
	R	CCTGTGACTGCAGGACTTGA		
TLR8	F	AAGGCTCTGGATTCAACC	135	XM_013976677.2
	R	ACATCGCAAGGATAGCTTC		
IL32	F	AAGTCACCGACTCTCAGGGA	139	XM_013974707.2
	R	GATGGAATCATCGAAGCCCCT		
IL33	F	CAGGGGAGAAATCAAAATAAGA	196	XM_013965943.2
	R	TTCCTGTTGTCCACACTTGG		
IGF1	F	TCTCAAGCCCACCAAGTCAG	196	XM_005680538.3
	R	GTAACTCGTGCAGAGCGAAG		
IFNG	F	AAGTTCTTGAACGGCAGCTCT	158	NM_001285682.1
	R	CTTCTCTTCCGCTTTCTGAGGT		
β- actin	F	GGACTTCGAGCAGGAGATGG	233	NM_001101
	R	GCACCGTGTTGGCGTAGAGG		

Note: F = Forward, R = Reversed

***Evaluation of gene expression in the artificial challenge trial*:** Differences in gene expression between resistant and susceptible goats in the challenge trial were tested using the $2^{-\Delta\Delta CT}$ method in SAS (Livak & Schmittgen 2001; Ling 2012). To compare gene expression in resistant and susceptible goats, Student's t-tests and a significance level of $P < 0.05$ were carried out using Graph Pad Software Prism7 (San Diego, CA USA).

***Statistical analysis*:** The data of FEC were not normally distributed among the samples and exhibited positive skewness. So, a logarithmic transformation [$\log_{10}$(FEC+25)] was therefore applied before analysis (Rout *et al.* 2011; González-Garduño *et al.* 2013) to minimize the heterogeneity of variance and increase normality of the FEC distribution. Analysis of FEC carried out using a repeated measures analysis of variance with the GLIMMIX Procedure of SAS version 9.2 (SAS Inst., Inc., Cary, NC, USA). Remaining

quantitative variables (RBC, WBC, PCV, and Hg) were assumed to be normally distributed and least-square means (LSM) and stander error (SE) for measured variables were analyzed with a one-way ANOVA and the means were compared with a Duncan test in the version of SAS 9.2 (Ojeda-Robertos *et al.* 2017). P-values lower than 0.05 were considered to be statistically significant.

Results

***Evaluation of fecal egg count*:** FEC of *H. contortus* infected goats from March to December 2017 demonstrated the natural stage of parasitic infection in YWG of our studied area (Figure 1A). The mean of FEC was much higher from July to August and slightly reduced in September. The mean of FEC was the highest in August whereas the lowest was recorded in March 2017. The prevalence of *H. contortus* infection was gradually higher from the March to July and reached the highest in August (76%) in the experimental herd of YWG (Figure 1B). Prevalence was also higher in July and October but it was comparatively lower in August 2017 (Figure 1B). A similar trend was observed for the prevalence of *H. contortus* infection in December (47%) and March (49%) 2017, respectively (Figure 1B).

***Descriptive statistic between resistant and susceptible group of goats*:** Resistant (low responder) and susceptible (high responder) group of goats were assessed based on their FEC. The mean of FEC for the resistant group of goats was 103.38±1.20epg with the maximum number of eggs for the individual goat was recorded as 450 epg. Similarly, the highest number of FEC was 3800 epg in susceptible goats with the average 1180.25±43.53 epg (Table 2). The mean of circular blood parameter like hemoglobin (Hg) and pack cell volume (PCV) significantly varied (P>0.05) between the resistant and susceptible group. Red Blood Cells (RBC) count and White Blood cells (WBC) count were almost similar between two groups of goats (Table 2). Mean of body weight (BW) was almost similar between two groups of a goat.

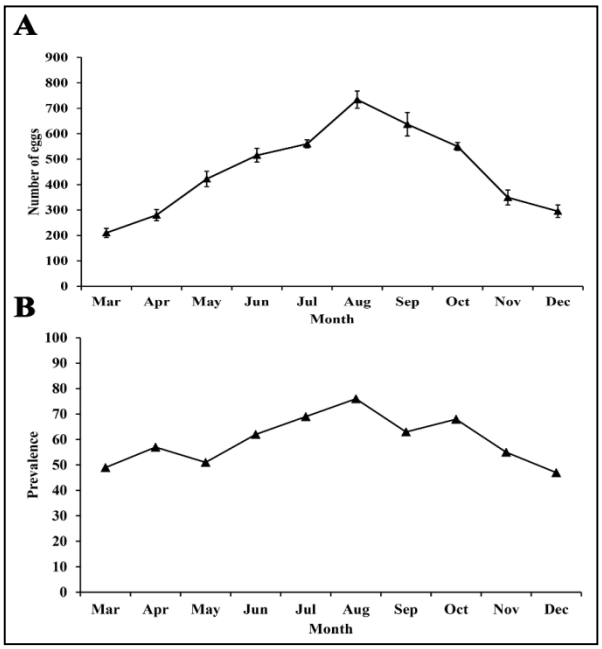

Figure 1. Monthly distribution of (A) mean of egg per gram (EPG) in feces and (B) prevalence of *Haemonchus contortus* infection in YWGs population under natural grazing condition.

***Artificial challenge trial*:** After the goats were classified as a resistant and susceptible group, artificial challenge trial was carried out with 5000 L3 larvae of *H. contortus,* and the comparison of parasitic infection between two groups are presented in Figure 2. The descriptive statistic for FEC confirms the expected right skewness of FEC distribution. During the challenge trial, FEC gradually increased from day 14 through day 42 and was higher in the susceptible group. In the resistant group, it rised very slowly than that of susceptible group during the experimental period (Figure 2A). The difference among FEC means at 28, 35, and 42 days after challenge was significant (P>0.01) between a resistant and susceptible group of YWG goat.

Moreover, the initial BW of resistance and susceptible goats were almost the same. Body weight of resistant goat was a little bit higher during the challenge trial and maintains their weight gain throughout the trial period after post infection. The average daily gain was

negative in susceptible goats, and it slightly declined after the day of 35 post-infection (Figure 2B).

Table 2. Fecal egg count, body weight and hematological parameters comparison between resistant and susceptible of YWG populations.

Parameters	Resistance group, n=33		Susceptible group, n=48	
	Low FEC <500		High FEC >500	
	Mean±SE	Range	Mean±SE	Range
Fecal Egg Count (epg)	103.38±1.20*	0 - 450	1180.25±43.53	600 - 3800
Body-weight (Kg)	19.04±1.37	11.0-33.0	18.03±1.69	10.0-34.0
Hemoglobin (Hg), (g/dl)	9.81±0.92*	9.0 - 13	7.51± 0.65	5.30 – 8.2
Packed Cell Volume (PCV), (%)	31.02±0.56*	27.0 – 35.0	25.83±0.69	18.0 – 29.0
Red Blood Cells (RBC), (10^{12}/L)	10.92±0.62	9.8- 11.3	9.98±0.32	9.10-10.80
White Blood Cells (WBC), (10^{9}/L)	10.65±0.27	10.5-10.9	10.95±0.27	10.72-11.23

*Significant ($p < 0.01$), based on assuming unequal variances Student's t-test.

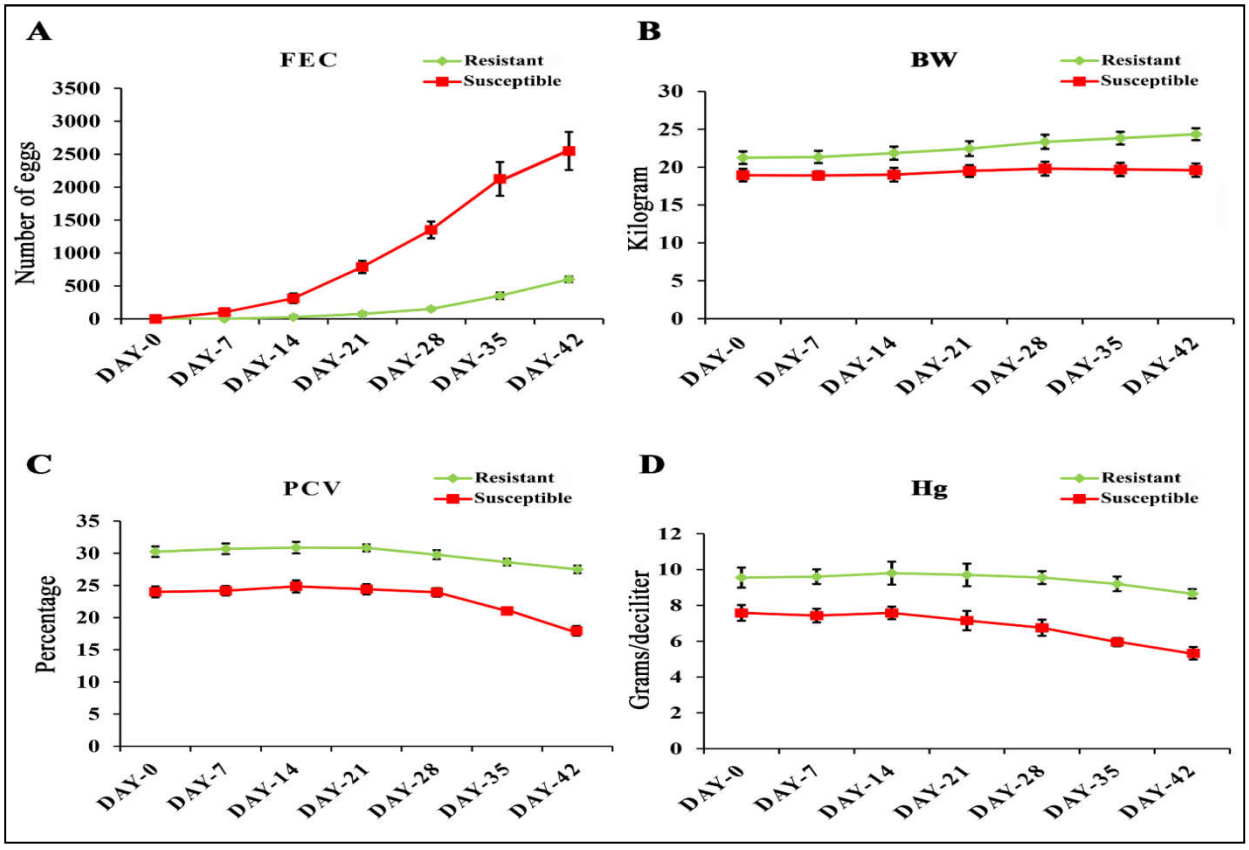

Figure 2. Means for (**A**) fecal egg count (FEC), (B) body weight (BWT), (**C**) packed cell volume (PCV), and (D) haemoglobin (Hg) level in resistant and susceptible groups of YWG at 0, 7, 14, 21, 28, 35 and 42 days after artificial challenge with 5000 infective L_3 larvae of *Haemonchus contortus* cultured under in vitro condition. Day 0 was the day of inoculation with the L_3 larvae.

The PCV was higher in the resistant group compared to that of the susceptible group and remained unchanged till day 21 of post-infection, but slightly declined from the day 28 to day 42. In the susceptible groups, PCV began to decline gradually at days 28 and continued to decline through day 42 indicating the high level of parasitic infection (Figure 2C). In susceptible goats, Hg value began to decline at days 21 and continued to decline up to the end of the experimental period showing the high responding to parasite infection. On the other hand, Hg values in resistant goats did not decline as rapidly as susceptible goats (Figure 2D).

***Differential gene expression analysis*:** The relative expression of *NOD1, NLRP9, TLR8*, *SFTPA1, IGF1, IFNG, IL32,* and *IL33* genes at the mRNA level are presented in Figure 3.

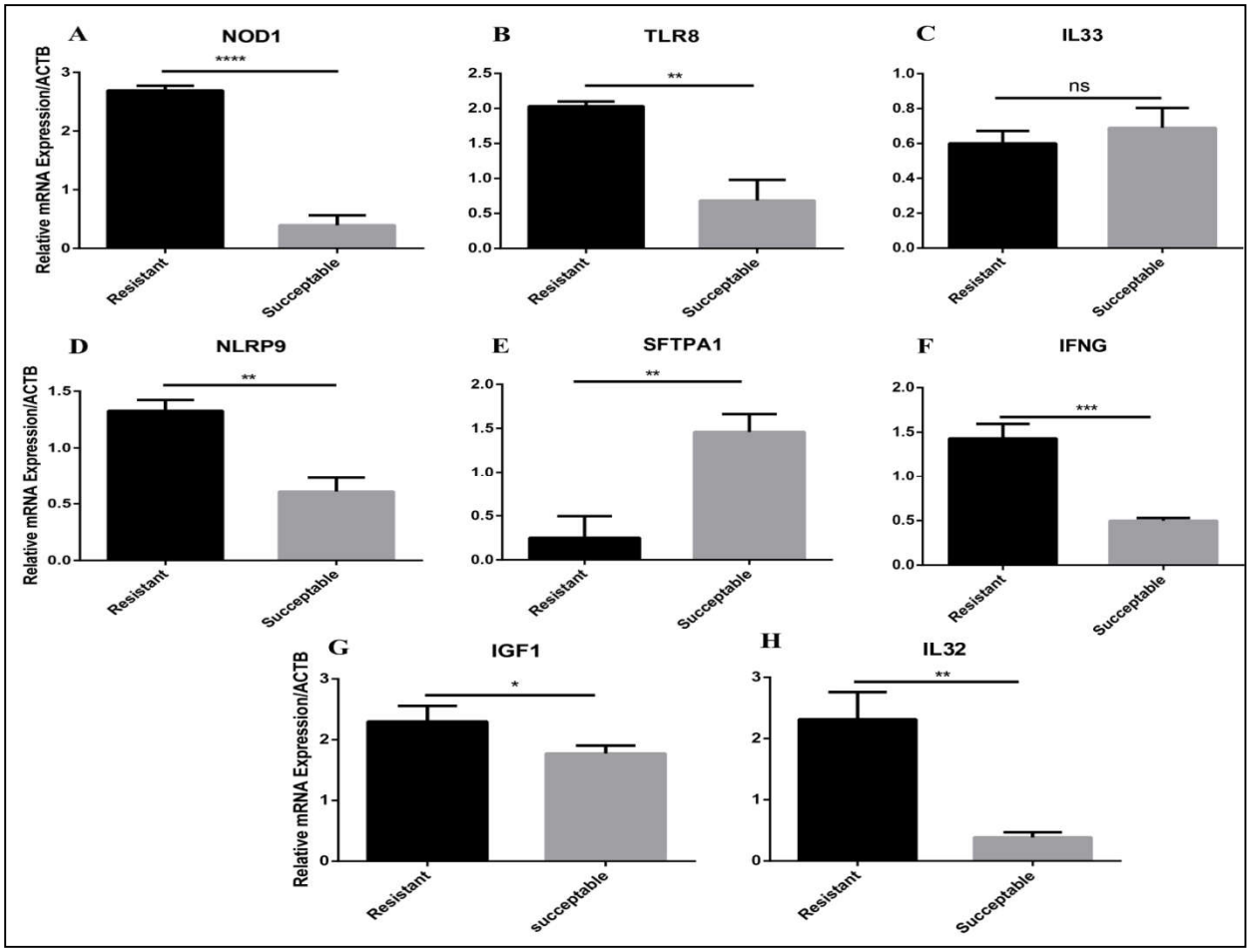

Figure 3. Relative expression of mRNA in abomasal tissue for (A) *NOD1* = Nucleotide binding oligomerization domain containing 1 (B) *TLR8* = Toll like receptor 8, (C) *IL33* = Interleukin 33, (D) *NLRP9* = NLR family pyrin domain containing 9, (E) *SFTPA1* = , (F) *IFNG* = Interferon gamma, (G) *IGF1* = Insulin like growth factor 1 and (H) *IL32* = Interleukin 32 genes measured by Quantitative Reverse-Transcriptase Polymerase Chain Reaction (qRT-PCR) in resistant and susceptible goats. Black and Gray color bars represent resistant and susceptible goats respectively. *($p < 0.01$) **($p < 0.001$), ***($p < 0.0001$), ****($p < 0.00001$) based on assuming unequal variances Student's t-test. Error bars represent standard error of the mean (SEM).

Relative expression of mRNA in resistant goats was higher than that of susceptible goats for *NOD1* ($P < 0.00001$) and very high for *IFNG* ($P < 0.0001$). Similarly, relative levels of mRNA expression for *NLRP9, TLR8*, *IL32,* (all $P < 0.001$) and for *IGF1* ($P < 0.01$) also significantly high in resistant goats. On the other hand, *SFTPA1* showed a higher expression in susceptible goat, but resistant and susceptible goats did not differ in expression for *IL33* (Figure 3).

Discussion

Gastrointestinal nematode (GIN) infection is the foremost constraints for the small ruminant production and resulting in economic losses especially in sheep and goats (McRae *et al.* 2014). The application of updated knowledge about the host genetic mechanism that is associated with resistance to GIN infection may help to improve parasite resistance or resilience of goat breed. To date, few studies have been conducted based on microarray-based gene expression to identify genes associated with resistance or susceptibility to GIN infection (Diez-Tascón *et al.* 2005; Keane *et al.* 2006). To our knowledge, this is the first study which directly compared YWG's susceptibility to *H. contortus* infection within breed and population through the gene expression profiles. Our selective immune genes were differentially expressed when goats were experimentally challenged with infective L_3 larvae of *H. contortus* in resistant and susceptible goats. The FEC is generally considered to be the main indicator of *H. contortus* infection in sheep and goats (Rodríguez *et al.* 2015; Omar *et al.* 2016; Kurukulasuriya *et al.* 2018). Previous reports revealed that resistant goats shed lower FEC and rapid recovery from initial elevations in FEC (Pralomkarn *et al.* 1997; Makun *et al.* 2008; Chiejina *et al.* 2015). This is the hypothesis that resistant goats are not fully free of the parasite but their worm burden is very low that may not affect their productivity with other relative advantages, due to their ability to cope with the internal parasite infection compared to susceptible goats.

The parasitic load can be expressed by the FEC, an effective way to the measurement for the parasite infection in goat. Host genetics significantly affect FEC, a vital phenotypic marker along with other parameters in goat that are resistant against *H. contortus* infection (Kim *et al.* 2015; Valilou *et al.* 2015). Variability of FEC gives a wide range of opportunity for selection and improvement of the *H. contortus* resistant goat breed within the population. The current study identified the variation for FEC within individuals of YWG population that might be caused by a genetic factor that was also supported by previous works (Omar *et al.* 2016; Omar *et al.* 2019). This is for the first time, the YWG goats have been evaluated for resistance to *H. contortus* using comparable experimental protocols and selection for artificial challenge trial on the basis of their FEC within the population followed by mRNA expression.

Our field investigation indicated the difference in parasite load between resistance and a susceptible group of YWG goats breed and identified resistant goats within this breed. This is in agreement to finding of Bhuiyan *et al.* (2017). This finding will help us to use this breed as a good candidate for exploring the genetic resistance by expression study to develop genetic markers for selective breeding program of nematode resistance goat population. Result of challenge trial with infective L_3 larvae of *H. contortus* showed that resistant goats had lower FEC compared to that of the susceptible. Although the mean dose of L_3 larvae was almost the same, approximately 239 and 265 larvae per kilogram of initial body weight for resistant and susceptible goat respectively. Susceptible goats showed approximately 60% higher FEC compared to that of resistant goats that were similar to previous finding (González *et al.* 2011). Additionally, the challenge trial also showed that resistant YWG had a tendency to delayed egg production and shed fewer eggs at day 28, 35, and 42 of post-infection. A similar result was also reported by several scientists on artificial challenge trial with infective L_3 larvae of *H. contortus* between resistant and susceptible goats

(González *et al.* 2008; Kurukulasuriya *et al.* 2018). The phenotypic of the resistance group in the term of FEC indicates an anti-fecundity effect of the immune response against parasitic infection.

Hematological parameters especially PCV and Hg also are considered as the sign of health and a good indicator to recognize the resistance or susceptibility of parasite infection. Our study demonstrated that PCV and Hg value had a distinct difference between the resistant and susceptible goats after challenge with L_3 larvae and the anemic condition was developed after five weeks of infection in the later group. Several investigators (Costa *et al.* 2000; Makun *et al.* 2008; Ameen *et al.* 2010; Kurukulasuriya *et al.* 2018) described that susceptible goats experienced progressively severe anemic condition during the experimental period. Similar pattern of change in the other hematological parameters such as PCV, RBC, and Hg after 5 weeks of infection with L_3 larvae of *H. contortus* also occurred. It has also been reported that hematological parameters especially PCV and Hg had strong negative correlations with FEC or worm burden during the natural or artificial infection by *H. contortus* (Blackburn *et al.* 1991; Fakae *et al.* 2004; Kaplan *et al.* 2004; Khobra *et al.* 2012; Chiejina *et al.* 2015). Increase in the BW could be a sign of good health and well-being in growing animals, and negative correlation has been observed between worm burden and BW gain in goat (Pralomkarn *et al.* 1997; Amarante *et al.* 2004; Burke & Miller 2004; Bishop 2012). Earlier studies implicated that increase the body weight, in contrast of EPG (egg per gram), has a significant effect to evaluate the status of resistance or susceptibility in sheep (Burke & Miller 2004; Mugambi *et al.* 2005). At the initial stage of infection, the body weight of our selected goats was almost similar for the resistant and susceptible group but the distinct difference of body weight was observed after four weeks of infection although all the goats of the resistant and susceptible group were treated with same doses of L_3 larvae. It can be suggested that a decline in the weight gain of susceptible goats were more sensitive to *H. contortus* than that of resistant goats.

Based on the results of our experimental trial on FEC, BW, PCV and Hg, it can be clearly stated that a well-distinguished infection status was present in resistant and susceptible goats in YWG population. The pattern of infection status, confirms the consistent variation in genetic resistance to *H. contortus* within this YWG population. Abomasal tissues were utilized for the assessment of the relative expression of our candidate genes for evaluation of the expression pattern of those genes with the YWG's, resistance to *H. contortus* infection. Several studies have been reported on our studied candidate genes with their expression in different tissues associated with human disease but very few reports are available on the goat (Tadaki *et al.* 2011; Castaño-Rodríguez *et al.* 2014; Lin *et al.* 2016; Naicy *et al.* 2017). This is for the first time we investigated those genes to understand their relative expression pattern in abomasal tissues of experimentally challenged goats with *H. contortus.* Polymorphisms of those genes have been established as candidate genes for resistant to *H. contortus* infection in goat breeds. Among the candidate genes, *NOD1, NLRP9, TLR8, IFNG, IGF1* and *IL32* showed significantly higher expression in resistant goats indicating the high potency of these genes to resistant proficiency to *H. contortus* infection. Positive expression of these genes in our current study alone with significant association results of previous reports to our studied candidate genes, resistant to *H. contortus* infection provides opportunity of developing biomarkers that are resistant to nematode infection particularly *H. contortus* in goats.

Additionally, *SFTPA1* and *IL33* showed a higher expression in susceptible goat and might be responsible for infection or promote susceptibility to Haemonchosis. Researchers reported that the patient with chronic obstructive pulmonary disease (COPD) showed decreased *SFTPA1* expression compared to non-COPD (Lin *et al.* 2016). That report is also in

favor of the present study. Our finding provides an important experimental basis of information for future research on the function of those genes and helpful for further investigation on those genes as biomarkers to develop the Haemonchosis resistant goat breeds.

Conclusion

In conclusion, lower FEC and a higher level of PCV and Hg value of resistant YWGs indicate the potential genetic resource for a selective breeding program to develop *H. contortus* resistant goat population. Also, the mRNA expression suggests that *NOD1, NLRP9, TLR8, IL32, IGF1,* and *TLR8 genes,* having a significant effect on nematode infection, are related to *H. contortus* resistance and could be used as biomarkers to develop *H. contortus* resistant goat breed in future.

Competing interests

The Authors have declared that they have no competing interests exist.

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Reference

Alim M, Fu Y, Wu Z, Zhao S, Cao J (2016). Single nucleotide polymorphisms of Toll-Like Receptors and association with *Haemonchus contortus* infection in goats. *Pakistan Veterinary Journal*, 36: 286-291.

Amarante Ad, Bricarello P, Rocha R, Gennari S (2004). Resistance of Santa Ines, Suffolk and Ile de France sheep to naturally acquired gastrointestinal nematode infections. *Veterinary Parasitology*, 120: 91-106.

Ameen S, Joshua R, Adedeji O, Ojedapo L, Amao S (2010). Experimental studies on gastro-intestinal nematode infection; The effects of age on clinical observations and haematological changes following *Haemonchus contortus* infection in West African Dwarf (WAD) Goats. *World Journal of Agricultural Sciences*, 6: 39-43.

Asif AR, Qadri S, Yuhua F, Alim M, Wu Z, Ijaz N, Cao J, Javed R, Ahmed S, Awais M (2016). Single nucleotide polymorphisms in DRB1, IGF1 and ILs associated with fecal egg count confers resistance against *Haemonchus contortus* infection in goats. *Pakistan Journal of Agricultural Sciences*, 53: 963-970.

Baker R, Audho J, Aduda E, Thorpe W (2001). Genetic resistance to gastrointestinal nematode parasites in Galla and Small East African goats in the sub-humid tropics. *Animal Science*, 73: 61-70.

Bambou JC, Larcher T, Cei W, Dumoulin PJ, Mandonnet N (2013). Effect of experimental infection with Haemonchus contortus on parasitological and local cellular responses in resistant and susceptible young Creole goats. *BioMed Research International*, 2013 (7): 902759; http://dx.doi.org/10.1155/2013/902759.

Bhuiyan A, Li J, Wu Z, Ni P, Adetula A, Wang H, Zhang C, Tang X, Bhuyan A, Zhao S, Du X (2017). Exploring the Genetic Resistance to Gastrointestinal Nematodes Infection in Goat Using RNA-Sequencing. *International Journal of Molecular Sciences*, 4:751 https://doi.org/10.3390/ijms18040751.

Bishop S (2012). Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. *Animal*, 6: 741-747.

Blackburn H, Rocha J, Figueiredo E, Berne M, Vieira L, Cavalcante A, Rosa J (1991). Interaction of parasitism and nutrition and their effects on production and clinical parameters in goats. *Veterinary Parasitology*, 40: 99-112.

Bressani F, Tizioto P, Giglioti R, Meirelles S, Coutinho R, Benvenuti C, Mudadu M, Vieira L, Zaros L, Carrilho E (2014). Single nucleotide polymorphisms in candidate genes associated with gastrointestinal nematode infection in goats. *Genetics and Molecular Research,* 13 (4); 8530-8536.

Burke J, Miller J (2004). Relative resistance to gastrointestinal nematode parasites in Dorper, Katahdin, and St. Croix lambs under conditions encountered in the southeastern region of the United States. Small Ruminant Research, 54: 43-51.

Castaño-Rodríguez N, Kaakoush NO, Goh KL, Fock KM, Mitchell HM (2014). The NOD-like receptor signalling pathway in Helicobacter pylori infection and related gastric cancer: a case-control study and gene expression analyses. *PloS One,* 9: doi:10.1371/journal.pone.009889998899.

Chauhan K, Rout P, Singh P, Mandal A, Singh S, Roy R (2003). Genetic resistance of Barbari and Jamunapari kids to natural infection with gastrointestinal nematodes. *Tropical Animal Health and Production*, 35: 397-408.

Chessa B, Pereira F, Arnaud F, Amorim A, Goyache F, Mainland I, Kao RR, Pemberton JM, Beraldi D, Stear MJ (2009). Revealing the history of sheep domestication using retrovirus integrations. *Science,* 324: 532-536.

Chiejina SN, Behnke JM (2011). The unique resistance and resilience of the Nigerian West African Dwarf goat to gastrointestinal nematode infections. *Parasites and Vectors*, 4:12; https://doi: 10.1186/1756-3305-4-12

Chiejina SN, Behnke JM, Fakae BB (2015). Haemonchotolerance in West African Dwarf goats: contribution to sustainable, anthelmintics-free helminth control in traditionally managed Nigerian dwarf goats. *Parasite*, 22; doi: 10.1051/parasite/2015006.

Clark C, Kiesel G, Goby C (1962). Measurements of blood loss caused by *Haemonchus contortus* infection in 177 sheep. *American Journal of Veterinary Research*, 23: 977-980.

Colli L, Lancioni H, Cardinali I, Olivieri A, Capodiferro MR, Pellecchia M, Rzepus M, Zamani W, Naderi S, Gandini F (2015). Whole mitochondrial genomes unveil the impact of domestication on goat matrilineal variability. *BMC Genomics*, 16: 1115; https://doi.org/10.1186/s12864-015-2342-2.

Costa C, Vieira LS, Berne M, Silva M, Guidoni A, Figueiredo E (2000). Variability of resistance in goats infected with *Haemonchus contortus* in Brazil. *Veterinary Parasitology*, 88: 153-158.

Diez-Tascón C, Keane OM, Wilson T, Zadissa A, Hyndman DL, Baird DB, McEwan JC, Crawford AM (2005). Microarray analysis of selection lines from outbred populations to identify genes involved with nematode parasite resistance in sheep. *Physiological Genomics,* 21: 59-69.

Fakae B, Musongong G, Chiejina S, Behnke J, Ngongeh L, Wakelin D (2004). Variability in the resistance of the Nigerian West African Dwarf goat to abbreviated escalating trickle and challenge infections with Haemonchus contortus. *Veterinary Parasitology*, 122: 51-65.

Getachew T, Alemu B, Sölkner J, Gizaw S, Haile A, Gosheme S, Notter DR (2015). Relative resistance of Menz and Washera sheep breeds to artificial infection with *Haemonchus contortus* in the highlands of Ethiopia. *Tropical Animal Health and Production*, 47: 961-968.

González-Garduño R, Mendoza-de Gives P, Torres-Hernández G (2013). Variability in the fecal egg count and the parasitic burden of hair sheep after grazing in nematode-infected

paddocks. *Pesquisa Veterinária Brasileira*, 33: 469-475.

González JF, Hernández Á, Meeusen EN, Rodríguez F, Molina JM, Jaber JR, Raadsma HW, Piedrafita D (2011). Fecundity in adult *Haemonchus contortus* parasites is correlated with abomasal tissue eosinophils and γδ T cells in resistant Canaria Hair Breed sheep. *Veterinary Parasitology*, 178: 286-292.

González JF, Hernández Á, Molina JM, Fernández A, Raadsma HW, Meeusen EN, Piedrafita D (2008). Comparative experimental *Haemonchus contortus* infection of two sheep breeds native to the Canary Islands. *Veterinary Parasitology*, 153: 374-378.

Gordon HM, Whitlock H (1939). A new technique for counting nematode eggs in sheep faeces. Journal of the Council for Scientific and Industrial Research, 12: 50-52.

Hansen J, Perry B (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants. A handbook. ILRAD.

Kaplan R, Burke J, Terrill T, Miller J, Getz W, Mobini S, Valencia E, Williams M, Williamson L, Larsen M (2004). Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. *Veterinary Parasitology*, 123: 105-120.

Keane OM, Zadissa A, Wilson T, Hyndman DL, Greer GJ, Baird DB, McCulloch AF, Crawford AM, McEwan JC (2006). Gene expression profiling of naive sheep genetically resistant and susceptible to gastrointestinal nematodes. *BMC Genomics*, 7: 42; https://doi.org/ 10.1186/1471-2164-7-42.

Khobra V, Gupta S, Gopal G, Narayanan K, Prasad A, Kumar P, Saravanan B, Sharma A, Rajesh V, Maurya P (2012). Major Histocompatibility class II DRB3 exon 2 polymorphism and resistance to gastrointestinal nematodes in Jamunapari goats. *Journal of Veterinary Parasitology,* 26: 108-111.

Kim ES, Sonstegard TS, da Silva MV, Gasbarre LC, Van Tassell CP (2015). Genome-wide scan of gastrointestinal nematode resistance in closed Angus population selected for minimized influence of MHC. *PloS One,* 10; https://doi.org/10.1371/journal.pone. 0119380.

Kurukulasuriya MS, Silva P, Dematawewa C, Ariyaratne H, Rajapakshe R, Wickramaratne S, Jayasooriya L, Munasinghe D, Lokugalappatti L, Notter D (2018). Responses of Sri Lankan indigenous goats and their Jamnapari crosses to artificial challenge with *Haemonchus contortus*. *Veterinary Parasitology: Regional Studies and Reports,* 11: 41-48.

Lin XF, Zhang L, Shi SY, Fan YC, Wu ZL, Zhang X, Sun DQ (2016). Expression of surfactant protein-A in exhaled breath condensate of patients with chronic obstructive pulmonary disease. *Molecular Medicine Reports*, 13: 1667-1672.

Ling D (2012). SASqPCR: robust and rapid analysis of RT-qPCR data in SAS. *PloS One*, 7: https://doi.org/10.1371/journal.pone.0029788.

Livak KJ, Schmittgen T (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402-408.

Ma J, He S, Li H, Guo Q, Pan W, Wang X, Zhang J, Liu L, Liu W, Liu Y (2014). First survey of helminths in adult goats in Hunan Province, China. *Tropical Biomedicine*, 31: 261-269.

MacKinnon K, Burton J, Zajac A, Notter D (2009). Microarray analysis reveals difference in gene expression profiles of hair and wool sheep infected with *Haemonchus contortus. Veterinary Immunology and Immunopathology*, 130: 210-120.

Makun H, Ajanusi J, Lakpini C, Ehoche O, Rekwot P (2008). Response of Red Sokoto and Sahelian goats to trickle *Haemonchus contortus* infection. *Journal of Biological Science,* 8: 753-759.

Mandonnet N, Aumont G, Fleury J, Arquet R, Varo H, Gruner L, Bouix J, Khang J (2001). Assessment of genetic variability of resistance to gastrointestinal nematode parasites in Creole goats in the humid tropics. *Journal of Animal Science,* 79: 1706-1712.

McRae KM, McEwan JC, Dodds KG, Gemmell NJ (2014). Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC Genomics*, 15: 637; https://doi.org/10.1186/1471-2164-15-637.

Mugambi J, Audho J, Baker R (2005). Evaluation of the phenotypic performance of a Red Maasai and Dorper double backcross resource population: natural pasture challenge with gastro-intestinal nematode parasites. *Small Ruminant Research,* 56: 239-251.

Naicy T, Venkatachalapathy T, Aravindakshan T, Raghavan KC, Mini M, Shyama K (2017). cDNA cloning, structural analysis, SNP detection and tissue expression profile of the IGF1 gene in Malabari and Attappady Black goats of India. *Journal of Genetics*, 96: 307-312.

Notter D, Andrew S, Zajac A (2003). Responses of hair and wool sheep to a single fixed dose of infective larvae of *Haemonchus contortus*. *Small Ruminant Research*, 47: 221-225.

Ojeda-Robertos N, Torres-Acosta J, González-Garduño R, Notter D (2017). Phenotypic expression of parasite susceptibility to *Haemonchus contortus* in Pelibuey sheep. *Veterinary Parasitology*, 239: 57-61.

Omar AI, Alam MBB, Faruque MO, Mondal MMH, Raihan M, Adetula A, Wu Z, Bhuiyan A, Soudy F, Cao J (2016). Variability in fecal egg count of *Haemonchus contortus* infection to native goat breeds of China and Bangladesh under natural grazing condition. *Progressive Agriculture,* 27: 473-481.

Omar AI, Yin L, Inayat S, Alam MBB, Faruque MO, Periasamy K, Samsuddin M, Wu Z, Zhao S, Du X, Liu L (2019). Assocoation of single necleotide polymorphism in NOD1 and NLRP9 genes with fecal egg count trait of *Haemonchus contortus* infection in Chinese and Bangladeshi goat breeds. *Journal of Animal and Plant Science,* 29: 370-378.

Pralomkarn W, Pandey V, Ngampongsai W, Choldumrongkul S, Saithanoo S, Rattaanachon L, Verhulst A (1997). Genetic resistance of three genotypes of goats to experimental infection with *Haemonchus contortus*. *Veterinary Parasitology*, 68: 79-90.

Rebouças EL, Costa JJN, Passos MJ, Passos JRS, Hurk RVD, Silva JRV (2013). Real time PCR and importance of housekeepings genes for normalization and quantification of mRNA expression in different tissues. *Brazilian Archives of Biology and Technology,* 56: 143-154.

Rodríguez A, Goldberg V, Viotti H, Ciappesoni G (2015). Early detection of *Haemonchus contortus* infection in sheep using three different faecal occult blood tests. *Open Veterinary Journal*, 5: 90-107.

Rout P, Chauhan K, Matika O, Bishop S (2011). Exploring the genetic resistance to natural gastrointestinal nematode infection in Indian goats. *Veterinary Parasitology*, 180: 315-322.

Tadaki H, Saitsu H, Nishimura-Tadaki A, Imagawa T, Kikuchi M, Hara R, Kaneko U, Kishi T, Miyamae T, Miyake N (2011). De novo 19q13. 42 duplications involving NLRP gene cluster in a patient with systemic-onset juvenile idiopathic arthritis. *Journal of Human Genetics,* 56: 343-347.

Traoré A, Notter DR, Soudre A, Kaboré A, Álvarez I, Fernández I, Sanou M, Shamshuddin M, Periasamy K, Tamboura HH (2017). Resistance to gastrointestinal parasite infection in Djallonké sheep. *Animal*, 11: 1354-1362.

Valilou RH, Rafat SA, Notter DR, Shojda D, Moghaddam G, Nematollahi A (2015). Fecal egg counts for gastrointestinal nematodes are associated with a polymorphism in the MHC-DRB1 gene in the Iranian Ghezel sheep breed. *Frontiers in Genetics*, 6: 105; https://doi.org/10.3389/fgene.2015.00105.

Yin F, Gasser RB, Li F, Bao M, Huang W, Zou F, Zhao G, Wang C, Yang X, Zhou Y (2013). Genetic variability within and among Haemonchus contortus isolates from goats and sheep in China. *Parasites and Vectors*, 6: 279-286.

Zajac AM, Conboy GA (2012). Veterinary clinical parasitology. John Wiley & Sons.