Nucleotide variants in the TLR5 gene and promoter methylation with a susceptibility to brucellosis in Chinese goats

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In this study, we aimed to identify genetic variants of the TLR5 gene and the promoter methylation level with a susceptibility to brucellosis in Chinese Saanen dairy goats (SN). We randomly collected 205 genetically unrelated animals from five Chinese goat breeds, including the Saanen dairy goat (SN, n = 67), Tibetan goat (TG, n = 35), Chuannan black goat (CN, n = 30), Meigu goat (MG, n = 37) and the Jianzhou big-ear goat (JZ, n = 36). A blood sample was collected from each individual and used for the detection of brucellosis, TLR5 polymorphism identification and the detection of promoter methylation. In total, twenty-four SNPs were identified in the five investigated breeds. The allele and genotype frequency of the four loci (g.435C>T, g.690G>T, g.978A>G and g.1832A>G) were correlated with a seroprevalence of brucellosis in the SN goats. An r² linkage disequilibrium (LD) was detected in SN, CN, TG and MG, but not in the JZ goats. Ten methylation sites were detected on the first CpG island with DNA methylation levels ranging from 0.2% to 5.2%, and twenty-two methylation sites were found on the second methylation island with a 2.3% to 12.1% methylation. The methylation levels of the two CpG islands in the healthy SN were significantly higher than those recorded in the brucella-infected SN (p <0.05). Our findings demonstrated that genetic variations and the promoter methylation of TLR5 were associated with a brucellosis risk in SN goats. These results will be helpful for further studies of disease resistance in ruminants.

Key words: Capra hircus, polymorphism, Brucella, seroprevalence, association.

Brucellosis is a widespread zoonosis caused by the genus Brucella, which has resulted in significant economic losses and poses a threat to public health (JIANG et al. 2020). In relation to livestock, brucellosis affects milk production and can cause infertility and miscarriage. Similarly, brucellosis causes influenza-like symptoms, as well as debilitating disease and pathological organ changes in humans (CRAIGHEAD et al. 2018). Although brucellosis is a localised disease, over 500,000 people are infected every year due to contact with infected animals like aborted foetuses, or by consuming unpasteurised products (SELEEM et al. 2010). Cattle, sheep, goats and pigs are the main reservoirs for human infection. People who are directly exposed to brucella-infected animals have a high risk of brucellosis infection, especially veterinarians, abattoir workers and herd keepers. Unfortunately, brucellosis has not been effectively
controlled or eradicated in China (Liang et al. 2020). Except for chronic asymptomatic infections, the seroprevalence of brucellosis in livestock constitutes a large percentage, reaching over 30% of livestock animals in Jilin Province, China (Ran et al. 2019).

In the literature, a number of studies have revealed that several candidate genes are involved in the resistance or susceptibility to brucellosis infection, such as IL-17, IFN-RI and TGF-β1 in humans (Rasouli et al. 2013; Fu et al. 2019; Naseri et al. 2019), as well as SLC11A1, TLR1, and TLR4 in cattle (Prakash et al. 2014). Toll-like receptors (TLRs) play an essential role in the immune response. To date, the single nucleotide polymorphisms (SNPs) in TLRs have been reported to confer some resistance to diverse bacterial diseases, including TLR1 to TLR6 for brucellosis resistance (Kesh et al. 2005; Carvalho et al. 2008; Grube et al. 2013; Fisher et al. 2017; Skonieczna et al. 2017), as well as TLR2, TLR6 and TLR9 for susceptibility to brucellosis (Xue et al. 2010; Alfano et al. 2014; Song et al. 2014; Zhao et al. 2017).

TLR5 primarily recognizes flagellin and stimulates inflammatory responses in the host species, with an adaptation of the TLR5 signalling and flagellin recognition (Tahoun et al. 2017). TLR5 is activated by flagellins from a wide variety of bacteria. The activated TLR5 can further activate the related signal transduction pathway in the NK cell, and then stimulates the production of cytokines by activating the nuclear transcription factor NF-κB, thereby mediating the innate immune response of the organism against pathogenic bacteria and stimulating the inflammatory response (Smith et al. 2003; Akira et al. 2006). In these studies, it was found that TLR5 inhibits viral replication when activated by flagellin, thereby avoiding or reducing viral-induced damage (Hosain et al. 2014; Zhang et al. 2014; Lee et al. 2016). Furthermore, TLR5 could protect the brains of mice from ischemic injury, which was considered to have a neuroprotective effect (Jeong et al. 2017). Studies have also shown that TLR5 has anti-radiation effects in mice; in particular, TLR5 was found to protect mice from lethal doses of ionising radiation (Burdelya et al. 2008; Zhang et al. 2017). In addition, genetic mutations of TLR5 are associated with many diseases, such as rheumatic heart disease and gastric cancer. For instance, it has been reported that the non-sense SNP (rs5744168) of TLR5 was associated with a breast cancer risk in relation to rheumatic heart disease and gastric cancer (Chen et al. 2017; Xu et al. 2017). By contrast, the effects of TLR5 on disease resistance are still little known with regard to livestock. Moreover, the determination of gene polymorphism is an essential element in farm animal breeding, in order to define the genotypes of animals and their associations with productive, reproductive and economic traits. Therefore, the objective of this study was to detect the polymorphic loci and promoter methylation in the TLR5 gene, and to assess their relationship with the occurrence of brucellosis in goats.

Materials and Methods

1. Ethics approval

The protocol for collecting the blood biopsies was approved by the Institutional Animal Care and Use Committee of the College of Animal Science and Technology of Sichuan Agricultural University, Sichuan, China, under Permit No. DKY-2020302113.

2. Sample collection, preparation and brucellosis testing

To investigate the polymorphisms in TLR5, 205 genetically unrelated animals from five Chinese goat breeds were randomly sampled. Saanen dairy goats (SN, n = 67) were collected from Heilongjiang Province (China), while Tibetan goats (TG, n = 35) were sampled from Abazhou in Sichuan Province (China). The Chuannan black goats (CN, n = 30), Meigu goats (MG, n = 37), and Jianzhou big-ear goats (JZ, n = 36) were all sourced from Sichuan Province (China). All of the goats included in the study had not been injected with the Brucella vaccine. A three-millilitre blood sample was collected from the jugular vein with acid citrate dextrose for each individual (stored at -80°C). In addition, another three-millilitre blood sample was collected in non-EDTA tubes for serum isolation with SN. The genomic DNA was extracted according to our previous protocol (Wang et al. 2015). The serum sample was separated by centrifuge at 5000 rpm for 5 min, and was analysed by the Rose Bengal Plate Test (RBPT) to detect the presence of Brucella antibodies (Saeed et al. 2019).

3. TLR5 gene amplification and polymorphism identification

Five primer pairs were designed, in order to scan for potential variations in a length of the 4187-bp region covering the promoter, coding region and 3′ untranslated region (3′-UTR, Table 1) of TLR5. A polymerase chain reaction (PCR) was performed in a 30 μl volume containing 50 ng of DNA, ten pmol/μl of each primer and 15 μl of Taq DNA M ix (Tiangen Bio-tech Co., Ltd., Beijing, China). The PCR conditions were an initial denaturing step for 5 min at 95°C, followed by 36 cycles of denaturing for 30 s at 94°C, annealing for 30 s at Tm, and extending for 60 s at 72°C, with a final extension step for 5 min at 72°C. The PCR products were checked by 2% agarose gel electrophoresis and were then sequenced on an ABI 3730XL DNA analyser (Applied Biosystems, Foster, CA, USA). Genetic variations of the goat TLR5 gene were
determined by an alignment analysis (Gene Codes Corporation, Ann Arbor, MI, USA). The protein sequence was predicted using the ExPASy online tool (https://web.expasy.org/translate/).

4. Detection of the TLR5 promoter methylation

The CpG islands in the promoter region (approximately 1000-bp upstream of the transcription start site, Acc. No. NC_030823.1) of the goat TLR5 was predicted by utilizing the online tool (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi), with the following criteria: the length of the CpG island was at least 100 bp, GC content was more than 50%, and the methylated island o/e was more than 0.6. Two pairs of nested primers (Table 1) were designed with the Methyl Primer Express v1.0 software. Six individuals were randomly selected from the TG, MG, CN and JZ breeds of goat. In the SN population, four Brucella ovis infected goats and four healthy individuals were used to quantify the DNA methylation level of the TLR5 promoter. The genomic DNA was treated with a bisulphite conversion with the EZ DNA Methylation™ Kit (Zymo Research), with one mM of each primer and 100 ng of bisulphite-treated DNA. The PCR conditions were as follows: at 94°C for 90 s, 30 cycles of 94°C for 20 s, 57.5°C for 20 s, 72°C for 36 s, and then at 72°C for 5 min. The obtained products were cloned into the pMD19T vector (Takara, Dalian, China), and at least 8-10 clones per sample were then sequenced.

5. Data analyses

The genotypic frequency, observed heterozygote (Ho), expected heterozygote (He) and the polymorphism information content (PIC) were calculated. The Hardy-Weinberg equilibrium was estimated using the DIYKtest. The haplotype was constructed with the PHASE 2.1.1 software, and the linkage disequilibrium (LD) was analysed with Haploview 4.2 (BARRETT et al. 2005). The methylation level of TLR5 was analysed using the QUantification tool (QUMA, http://quma.cdb.riken.jp/). The significance was determined with the SPSS 13.0 program (IBM, Chicago, IL, USA). In addition, the association between methylation and the susceptibility to brucellosis was performed using the Pearson’s correlation procedure in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).
Results

1. Detection of Brucella antibodies in SN goats

During the RBPT testing, a total of sixty-seven serum samples from the SN herd were tested. The results showed that twelve serum samples from 67 SN goats were positive for Brucella antibodies, while fifty-five serum samples were found to be negative. The seroprevalence of brucellosis was 17.91% in the SN goats.

2. Novel SNPs and the genetic parameters of goat TLR5

The goat TLR5 gene, which is located on chromosome 16, is comprised of only one exon encoding 871 amino acids (Figure 1). We obtained a length of the 3991-bp assembled fragment of TLR5, covering the coding region sequence (CDS, 2616 bp) and parts of the untranslated regions (5′-UTR, 645 bp; and 3′-UTR, 730 bp). 24 SNPs, which included 15 novel mutations, were identified in the five goat populations (Figure 1A). Among the 17 exonic SNPs, seven missense mutations (g.690G>T, g.1320G>C, g.1430C>G, g.1431A>G, g.1832A>G, g.2002G>A and g.2017G>T) were revealed, which resulted in amino acid substitutions (p.Lys230Asn, p.Leu440Phe, p.Pro477Arg, p.Asn611Ser, p.Val668Met and p.Val673Phe, Figure 1D). The wild allele was dominant at the two loci (g.1430C>G and g.1832A>G) in both the indigenous populations (TG, CN and MG) and the cultured breeds (SN and JZ). The observed heterozygosity (Ho) ranged from 0.000 to 0.647. A low polymorphism was found in g.1430C>G, g.1431A>G and g.1832A>T, while a medium polymorphism was revealed in g.690G>T, g.1320G>C, g.2002G>A and g.2017G>T. The result of the Chi-squared tests showed that only three loci (g.1320G>C and g.1832A>G in SN; g.2017G>T in CN) were not in the Hardy-Weinberg equilibrium (Table 2). The linkage disequilibrium (LD) plots showed a strong LD block in the cultured breed CB, which is specially bred for mutton. A part from JZ, existence of LD was also detected in the other three goat populations (SN, TG and MG) (Figure 2).
Table 2
Genetic parameters of the missense mutations of TLR5 in the investigated populations

| Breed | Mutation | Allele freq. (W/M) | Genotype freq. (WW/WM/MM) | Ho | He | PIC | $\chi^2$ |
|-------|----------|---------------------|--------------------------|----|----|-----|--------|
| SN (67) | g.690G>T | 0.358/0.642 | 0.164/0.388/0.448 | 0.388 | 0.463 | 0.354 | 1.63 |
| | g.1320G>C | 0.231/0.769 | 0.134/0.194/0.672 | 0.194 | 0.358 | 0.292 | 13.84 |
| | g.1430C>G | 0.948/0.052 | 0.896/0.060/0.045 | 0.104 | 0.100 | 0.094 | 21.6 |
| | g.1431A>G | 0.105/0.895 | 0.030/0.149/0.821 | 0.149 | 0.189 | 0.170 | 2.74 |
| | g.1832A>G | 0.910/0.090 | 0.866/0.090/0.044 | 0.030 | 0.164 | 0.150 | 13.62 |
| | g.2002G>A | 0.321/0.679 | 0.119/0.403/0.478 | 0.403 | 0.439 | 0.341 | 0.38 |
| | g.2017G>T | 0.888/0.112 | 0.791/0.194/0.015 | 0.194 | 0.200 | 0.179 | 0.04 |
| TG (35) | g.690G>T | 0.500/0.500 | 0.314/0.371/0.314 | 0.371 | 0.507 | 0.375 | 2.31 |
| | g.1320G>C | 0.500/0.500 | 0.314/0.371/0.314 | 0.371 | 0.507 | 0.375 | 2.31 |
| | g.1430C>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.1431A>G | 0.200/0.800 | 0.029/0.343/0.629 | 0.343 | 0.325 | 0.269 | 0.18 |
| | g.2002G>A | 0.514/0.486 | 0.314/0.400/0.286 | 0.400 | 0.507 | 0.375 | 1.39 |
| | g.2017G>T | 0.514/0.486 | 0.343/0.343/0.314 | 0.343 | 0.507 | 0.375 | 3.44 |
| CN (30) | g.690G>T | 0.211/0.789 | 0.105/0.211/0.684 | 0.211 | 0.341 | 0.277 | 2.55 |
| | g.1320G>C | 0.211/0.789 | 0.105/0.211/0.684 | 0.211 | 0.341 | 0.277 | 2.55 |
| | g.1430C>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.1431A>G | 0.132/0.868 | 0.053/0.158/0.789 | 0.158 | 0.235 | 0.202 | 1.82 |
| | g.2002G>A | 0.514/0.486 | 0.314/0.400/0.286 | 0.400 | 0.507 | 0.375 | 1.39 |
| | g.2017G>T | 0.737/0.263 | 0.623/0.211/0.158 | 0.211 | 0.398 | 0.313 | 3.97 |
| MG (37) | g.690G>T | 0.471/0.529 | 0.176/0.588/0.235 | 0.588 | 0.513 | 0.374 | 0.55 |
| | g.1320G>C | 0.471/0.529 | 0.176/0.588/0.235 | 0.588 | 0.513 | 0.374 | 0.55 |
| | g.1430C>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.1431A>G | 0.132/0.868 | 0.053/0.158/0.789 | 0.158 | 0.235 | 0.202 | 1.82 |
| | g.2002G>A | 0.471/0.529 | 0.176/0.588/0.235 | 0.588 | 0.513 | 0.374 | 0.55 |
| | g.2017G>T | 0.500/0.500 | 0.176/0.647/0.176 | 0.647 | 0.515 | 0.375 | 1.47 |
| JZ (36) | g.690G>T | 0.074/0.926 | 0.000/0.148/0.852 | 0.148 | 0.140 | 0.128 | 0.17 |
| | g.1320G>C | 0.093/0.907 | 0.000/0.185/0.815 | 0.185 | 0.171 | 0.154 | 0.28 |
| | g.1430C>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.1431A>G | 0.000/1.000 | 0.000/0.000/1.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.1832A>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.2002G>A | 0.111/0.889 | 0.037/0.148/0.815 | 0.148 | 0.201 | 0.178 | 1.69 |
| | g.2017G>T | 0.944/0.056 | 0.889/0.111/0.000 | 0.111 | 0.107 | 0.099 | 0.09 |

$\chi^2$ (HWE): Hardy–Weinberg equilibrium $\chi^2$ value, $\chi^2 = 3.81$, $p = 0.05$; $\chi^2 = 6.63$, $p = 0.01$. Goat breeds: SN – Saanen dairy goats, TG – Tibetan goats, CN – Chuannan black goats, MG – Meigu goats, JZ – Jianzhou big-ear goats.
3. **TLR5** gene association with brucellosis

The association between the **TLR5** SNPs and the seroprevalence of brucellosis in SN goats was analysed in the present study. The statistical analysis revealed that the loci of g.435C>T, g.690G>T and g.978A>G were correlated with the seroprevalence of brucellosis (Table 3). The allele frequency was significantly different between the seroprevalence group (SG) and the healthy group (HG, \( p<0.05 \)). Moreover, the genotype frequency of the three loci (g.435C>T, g.978G>A and g.1832A>G) was significantly different between the SG and HG goats (\( p<0.05 \)).

Two CpG islands were predicted in the promoter of **TLR5**, located at 660-088 bp upstream of the transcription start site (TSS). The first CpG island contained 10 CG dinucleotide pairs, while the second CpG island included 22 CG sites. The methylation

![Fig. 2. LD plots of the five breeds using the SNPs identified in the goat **TLR5**. Goat breeds: SN – Saanen dairy goats, TG – Tibetan goats, CN – Chuannan black goats, MG – Meigu goats, JZ – Jianzhou big-ear goats.](image-url)
rate of each CpG site and the methylation levels of the TLR5 promoter are presented in Figure 3. The methylation of the 1st CpG island was found to be significantly lower than that in the 2nd CpG island (p<0.05). However, in the CN goats, the methylation of the 1st CpG island was only around 2.7% less than that in the 2nd CpG island (p<0.05). In addition, the cultured breed JZ had the lowest methylation of all the studied breeds.

Four goats with a seroprevalence of brucellosis and four healthy individuals were randomly selected from among the SN goats to detect the relationship between the promoter methylation of TLR5 and brucellosis. The results showed that the total methylation level of the 1st CpG island was 6.9% in the four healthy samples and 0.4% in the infected samples (Figure 3 and Table 4). Meanwhile, the overall methylation level of the 2nd CpG island was 9.5% in the four healthy samples and 2.8% in the infected goats. The results of the T-test revealed that the overall methylation status of the first segment of the CpG island in the healthy goats was significantly higher than that in the goats with brucellosis.

### Discussion
The detection of Brucella is usually performed using the following three methods: RBPT, standard tube agglutination test (STAT) and I-ELISA. It has been found that RBPT has the highest sensitivity for detecting brucellosis, while I-ELISA has the lowest sensitivity (SADHU et al. 2015). As reported in the literature, the mixed prevalence of brucellosis in sheep and goats has increased from 1.00% to 3.20% in the past 20 years, with the seroprevalence rate of brucellosis in Eastern China being higher than in any other region. The overall seroprevalence rate of 315 sheep that were randomly tested in Shandong Province was found to be 27.6%, while the seroprevalence rate of 370 goats tested was only 11.1% (RAN et al. 2019). In addition, in 2059 goat serum samples from the Arabian Gulf, which were firstly determined by RBPT and then confirmed by I-ELISA, the results showed a seropositivity rate of only 0.78% (EBID et al. 2020). In our study, the seropositivity rate of brucellosis was recorded as up to 17.91% in the SN goats measured by RBPT. Therefore, compared with other studies, our positive rate was relatively high. Moreover, in one previous study it was reported that the prevalence of human brucellosis has undergone a significant geographic expansion in Northern China (LAI et al. 2017). The brucellosis expanded from the northern pasture areas in Heilongjiang Province to neighbouring grasslands and agricultural areas. In the present study, the serum samples of SN were collected from local farms in the Harbin City (Heilongjiang Province, China) and only detected by RBPT, so we can speculate that our result may be related to the region and the detection method.

| Loci   | SG allele freq. (W/M) | HG allele freq. (W/M) | p value | SG geno freq. (WW/WM/MM) | HG geno freq. (WW/WM/MM) | p value |
|--------|-----------------------|-----------------------|---------|--------------------------|--------------------------|---------|
| g.435CT | 0.583/0.417           | 0.891/0.109           | 0.000** | 0.500/0.167/0.333        | 0.800/0.182/0.018         | 0.001** |
| g.690GT | 0.542/0.458           | 0.318/0.682           | 0.039*  | 0.300/0.417/0.250        | 0.127/0.382/0.491         | 0.145   |
| g.705AC | 1/0                   | 0.964/0.036           | 0.343   | 1/0/0                    | 0.945/0.036/0.018         | 0.710   |
| g.978GA | 0.750/0.250           | 0.318/0.682           | 0.000** | 0.667/0.167/0.167        | 0.127/0.382/0.491         | 0.000** |
| g.1320GC | 0.333/0.667          | 0.209/0.791           | 0.300   | 0.167/0.333/0.500        | 0.127/0.164/0.709         | 0.330   |
| g.1430CG | 0.875/0.125          | 0.936/0.064           | 0.450   | 0.833/0.083/0.083        | 0.909/0.055/0.036         | 0.710   |
| g.1431AG | 0/1                   | 0.127/0.873           | 0.065   | 0/0/1                    | 0.036/0.128/0.782         | 0.203   |
| g.1671CT | 0.250/0.750          | 0.209/0.791           | 0.659   | 0.083/0.333/0.583        | 0.127/0.164/0.709         | 0.397   |
| g.1832AG | 0.875/0.125          | 0.909/0.091           | 0.609   | 0.750/0.250/0            | 0.891/0.036/0.073         | 0.028*  |
| g.1926TC | 0.250/0.750          | 0.218/0.782           | 0.735   | 0/0.500/0.500            | 0.018/0.400/0.582         | 0.752   |
| g.1969AG | 1/0                   | 0.973/0.027           | 0.413   | 1/0/0                    | 0.964/0.018/0.018         | 0.799   |
| g.2002GA | 0.375/0.625          | 0.309/0.691           | 0.531   | 0.083/0.583/0.333        | 0.127/0.364/0.509         | 0.372   |
| g.2017GT | 0.875/0.125          | 0.891/0.109           | 0.823   | 0.750/0.250/0            | 0.800/0.182/0.018         | 0.785   |

W – wild allele; M – mutant allele; SG – seroprevalence group; HG – healthy group; * p<0.05; ** p<0.01.
Fig. 3. A – Estimated CpG islands and designed primers in the promoter region of the goat TLR5. B – Methylation levels of CpG dinucleotide in the 1st CpG island tested by BSP in the five investigated breeds. A1-A10 represents a single methylated site in CpG1. C – Methylation status of CpG in the 2nd CpG island. B1-B22 represents a single site in CpG2. Promoter methylation status of TLR5 in Brucella-infected and healthy individuals (D and E).
The gene members of the TLRs family have been reported to harbour plenty of genetic variations in both humans and livestock (UENISHI & SHINKAI 2009). In a previous study, it was determined that the genetic mutations rs3804099 in TLR2 and rs352139 in TLR9 may be associated with the risk of pulmonary tuberculosis in the Moldavian population (VARZARI et al. 2019). SELVAM et al. (2017) found that the five TLRs genes (TLR3, TLR5, TLR6, TLR9 and TLR10) were highly polymorphic in the Kilakalsal and Vembur sheep breeds. Among the 25 SNPs, only the two loci (TLR3_1081_AC and TLR9_2036_CT) were monomorphic. Meanwhile, three SNPs within the TLR5, TLR7 and TLR8 genes were found to be polymorphic and were associated with parasite resistance characteristics in sheep (PERIASAMY et al. 2014). GOYAL et al. (2014) examined the genetic variations of TLR5 in 12 goat breeds and found that ten of the 16 SNPs were non-synonymous. In the present study, we detected seven non-synonymous SNPs of TLR5 in different breeds of Chinese goats. As was reported by E et al. (2015), CN has the largest observed heterozygosity (0.54±0.05) among the ten indigenous types of Chinese goats. Linkage disequilibrium (LD) plots showed that a strong LD block was observed in the cultured CN breed, which is specially bred especially for consumption as mutton.

TLR5 participates in the immune response. Many studies have found that polymorphisms of the TLRs genes are associated with resistance or susceptibility to diseases (FARIDI et al. 2017; BI et al. 2020; DVORNIKOVA et al. 2020). Furthermore, it has been reported that the TLR4 gene is significantly related to the occurrence of brucellosis in cattle (REZAZADEH et al. 2006). Associations between genetic variations of TLRs and infectious diseases have also been revealed in livestock. PRAKASH et al. found that the innate immunity genes (TLR1 and TLR4) were significantly associated with bovine brucellosis in a resource population of 83 cattle, with 38 cases of the disease.

Table 4
Association of methylation of the TLR5 promoter with the susceptibility to brucellosis in SN goats

| CpG sites | SG (%) | HG (%) | p    | CpG sites | SG (%) | HG (%) | p    |
|-----------|--------|--------|------|-----------|--------|--------|------|
| A1        | 0.0    | 31.0   | 0.001* | B1        | 8.7    | 3.8    | 0.453 |
| A2        | 0.0    | 6.9    | 0.264 | B2        | 0.0    | 0      | –    |
| A3        | 0.0    | 10.3   | 0.132 | B3        | 0.0    | 0      | –    |
| A4        | 0.0    | 3.4    | 0.518 | B4        | 4.3    | 0      | 0.469 |
| A5        | 0.0    | 13.8   | 0.065 | B5        | 4.3    | 3.8    | 0.724 |
| A6        | 0.0    | 3.4    | 0.518 | B6        | 0.0    | 3.8    | 0.531 |
| A7        | 0.0    | 0.0    | 0     | B7        | 4.3    | 3.8    | 0.724 |
| A8        | 3.7    | 0.0    | 0.482 | B8        | 8.7    | 3.8    | 0.435 |
| A9        | 0.0    | 0.0    | 0     | B9        | 0      | 3.8    | 0.531 |
| A10       | 0.0    | 0.0    | 0     | B10       | 8.7    | 0.0    | 0.215 |
|           |        |        |       | B11       | 4.3    | 15.4   | 0.215 |
|           |        |        |       | B12       | 4.3    | 11.5   | 0.353 |
|           |        |        |       | B13       | 0      | 30.8   | 0.003* |
|           |        |        |       | B14       | 0      | 15.4   | 0.071 |
|           |        |        |       | B15       | 0      | 11.5   | 0.141 |
|           |        |        |       | B16       | 0      | 15.4   | 0.071 |
|           |        |        |       | B17       | 4.3    | 7.7    | 0.547 |
|           |        |        |       | B18       | 4.3    | 23.1   | 0.069 |
|           |        |        |       | B19       | 4.3    | 11.5   | 0.353 |
|           |        |        |       | B20       | 0      | 23.1   | 0.016* |
|           |        |        |       | B21       | 0      | 11.5   | 0.141 |
|           |        |        |       | B22       | 0      | 7.7    | 0.276 |
| Total     | 0.4    | 6.9    | 0.000* | Total     | 2.8    | 9.4    | 0.000* |

SG – seroprevalence group; HG – healthy group; * p<0.05.
and 45 controls (PRAKASH et al. 2014). The SNPs in TLR5 were significantly associated with inflammatory bowel disease (IBD) in the German shepherd dog. In addition, the G22A SNP in TLR5 was strongly associated with IBD; but on the contrary, the remaining two SNPs were found to be significantly protective for IBD (KATHRANI et al. 2010). Muneta and colleagues confirmed that piglets with the T allele of swine TLR5 (C1205T) exhibit an impaired resistance to Salmonella typhimurium infection (MUNETA et al. 2018). YOON et al. (2012) found that residues of leucine (12th and 13th) in the TLR5 extracellular domain were the binding interfaces between flagellin and TLR5, which affected the transcriptional activity of the downstream NF-κB gene. It was also confirmed in their study that the polymorphisms of the TLR5 gene could influence the activity of 42-ring NF-κB, thereby affecting the occurrence of brucellosis. In addition, a recent study revealed that methylation of the TLR5 gene would lead to a decrease in the TLR5 gene expression and that piglets with a higher DNA methylation of TLR5 showed an increase in their resistance to F18 E.coli (DAI et al. 2019). Our results showed that the overall methylation of the first CpG islands in healthy SN goats was significantly higher than that of goats suffering from brucellosis. We therefore propose that the methylation of the TLR5 gene could improve the resistance to brucellosis in SN goats.

Conflict of Interest
The authors declare no conflict of interest.

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Research concept and design: T.Z., H.Z.; Collection and/or assembly of data: X.W., X.Z., T.L.; Data analysis and interpretation: X.Z., S.Z.; Writing the article: X.W., T.Z.; Critical revision of the article: L.N., L.W., S.Z., J.C., J.G., L.L.; Final approval of article: T.Z.

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