e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 70 (2022), No 2
https://doi.org/10.3409/fb_70-2.07

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

Xinlu WANG[a], Xiaojie ZHANG[a], Taofeng LU, Lili NIU, Linjie WANG, Siyuan ZHAN, Jiazhong GUO, Jiaxue CAO, Li LI, Hongping ZHANG, and Tao ZHONG

*Accepted May 10, 2022* *Published online May 25, 2022* *Issue online June 30, 2022*

Original article

WANG X., ZHANG X., LU T., NIU L., WANG L., ZHAN S., GUO J., CAO J., LI L., ZHANG H., ZHONG T. 2022. Nucleotide variants in the *TLR5* gene and promoter methylation with a susceptibility to brucellosis in Chinese goats. Folia Biologica (Kraków) **70**: 55-66.

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 $r^2$ 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.

✉*Xinlu WANG, Xiaojie ZHANG, Lili NIU, Linjie WANG, Siyuan ZHAN, Jiazhong GUO, Jiaxue CAO, Li LI, Hongping ZHANG, and Tao ZHONG*✉, *Farm Animal Genetic Resources Exploration and Innovation Key Laboratory of Sichuan Province, College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, China.*
*E-mail: zhongtao@sicau.edu.cn*
*Taofeng LU, Institute for Laboratory Animal Research, Guizhou University of Traditional Chinese Medicine, Guiyang, China*

[a] *The above authors contributed equally to this work.*

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-R1* 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 recognises 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 (HOSSAIN *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 Mix (TIANGEN Biotech 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

Table 1

Primer sequences used in this study

| Primer name | Primer sequence (5′-3′) | Target region | Fragment (bp) | Tm (°C) |
|---|---|---|---|---|
| | SNPs detection (NC_030823.1) | | | |
| *TLR5*-1F | GAGAACATAGCGGACACC | 5′-UTR | 1053 | 59.0 |
| *TLR5*-11R | TTTATCTGACTTCCACCC | | | |
| *TLR5*-2F | TCCTGCTTCTTCAATGGT | Coding region | 901 | 55.3 |
| *TLR5*-2R | TGTTGTAGGCGAGGTTTA | | | |
| *TLR5*-2F | ATCTTCTCCCTGAACTCC | Coding region | 1003 | 55.3 |
| *TLR5*-3R | ATTCTTCTTCTTCGCAAC | | | |
| *TLR5*-4F | AATATCACAATATCTGGGTC | Coding region | 784 | 51.5 |
| *TLR5*-4R | AGGAGACGGTGGTTACAC | | | |
| *TLR5*-5F | CTTCAGTTACGCCCAGAG | 3′′-UTR | 1091 | 64.0 |
| *TLR5*-5R | TAGGGTTAGATCCAAGAGGT | | | |
| | Promoter methylation (NC_030823.1) | | | |
| *TLR5*-BSP1F | AGGATAGAAAGGGTTTGTTTTT | | 583 | 57.5 |
| *TLR5*-BSP1R | TCAACCCAAATAACCTCTTTAT | | | |
| *TLR5*-BSP2F | TTGGTTGAAATGTATTAAAATGTTT | | 228 | 57.5 |
| *TLR5*-BSP2R | ACCTCTATTAACTCCCAAAATATCC | | | |
| *TLR5*-BSP3F | GGAGTTAATAGAGGTTGTAGTTTTG | | 270 | 57.5 |
| *TLR5*-BSP3R | GTTGGTTAGAGAGAAAGAATAAAGA | | | |

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, Orange, CA, USA). To detect the methylation of the *TLR5* promoter, a nested PCR was carried out using BSP1F/1R as the outer primers (1st round PCR) and BSP2F/2R, BSP3F/3R (2nd round PCR) as the inner primers, respectively. The reaction was performed in a 50 μl volume containing 25 μl of Zymo-Taq™ PreMix (the enzyme was 4 U, 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 $\chi^2$ test. 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).

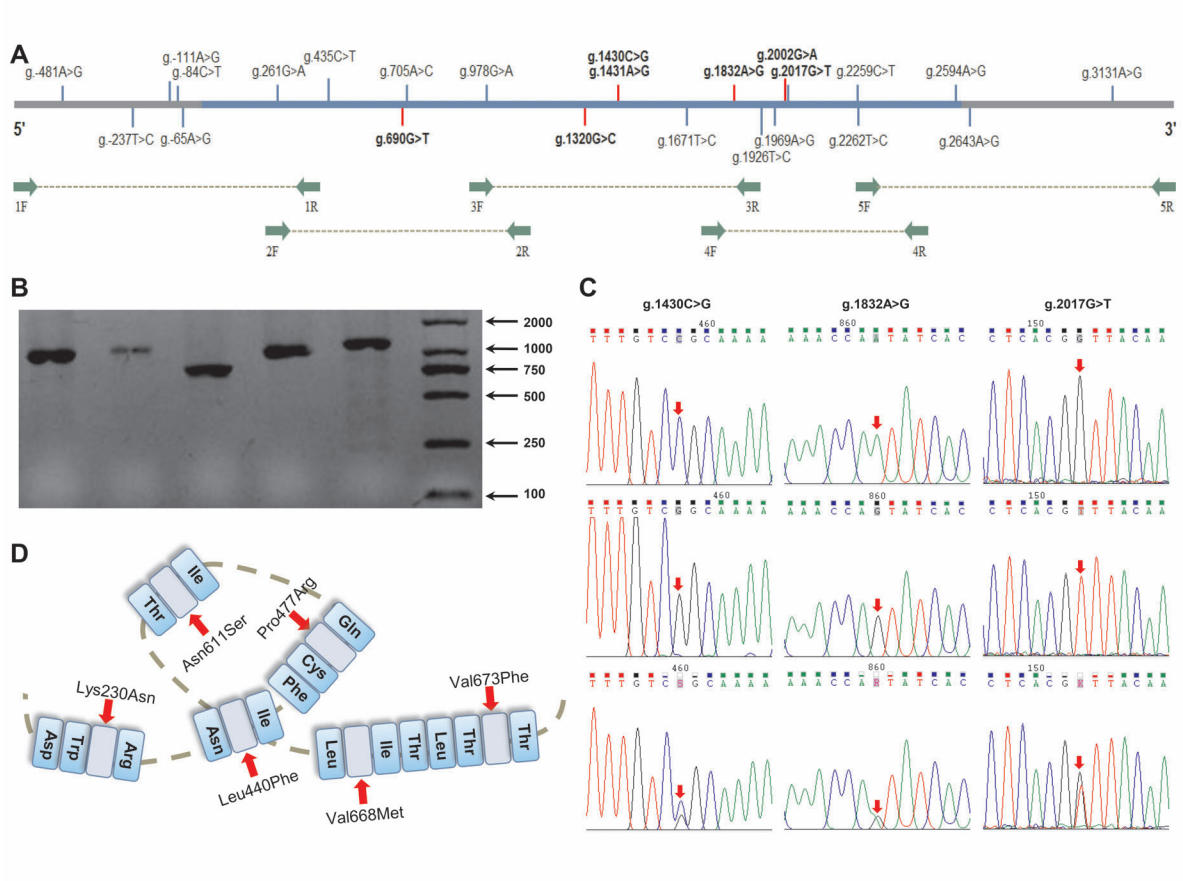


Fig. 1. A – Distributions of the identified mutations and the primers used in *TLR5*. B – Gel electrophoresis of the five overlapped PCR products. C – DNA sequences of the novel missense mutations that were found in this study. D – Amino acid changes in the goat *TLR5* induced by the missense mutations.

## 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. Apart 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.1832A>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | 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.1832A>G | 1.000/0.000 | 1.000/0.000/0.000 | 0.000 | 0.000 | 0.000 | NA |
| | g.2002G>A | 0.211/0.789 | 0.105/0.211/0.684 | 0.211 | 0.341 | 0.277 | 2.55 |
| | 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.1320GC | 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 | 0.000/1.000 | 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 | 0.471/0.529 | 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.

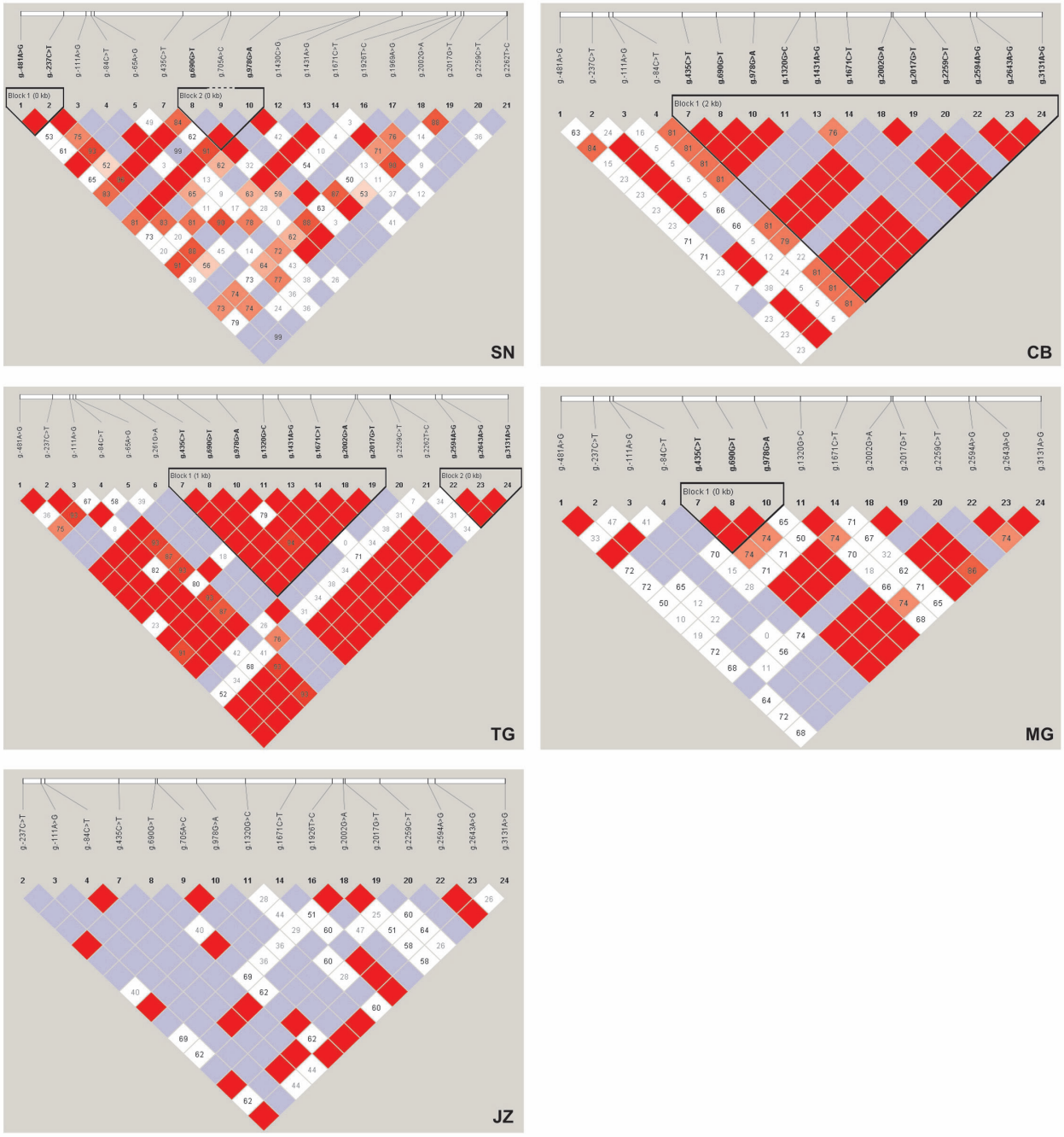


Fig. 2. LD plots of the five breeds using the SNPs identified in the goat *TLR5*. Goat breeds: SN – Saanen dairy goats, TG – Tibetan goats, CN – Chuannan black goats, MG – Meigu goats, JZ – Jianzhou big-ear goats.

### 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

Table 3

Correlations of allele and genotype frequency of *TLR5* SNPs and seroprevalence of brucellosis in SN goats

| 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 | 0.800/0.182/018 | 0.001** |
| g.690GT | 0.542/0.458 | 0.318/0.682 | 0.039* | 0.300/0.417/0.250 | 0.1270.382/0.491 | 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 | 0.891/0.036/0073 | 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; * $p<0.05$; ** $p<0.01$.

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 health 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 health 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.

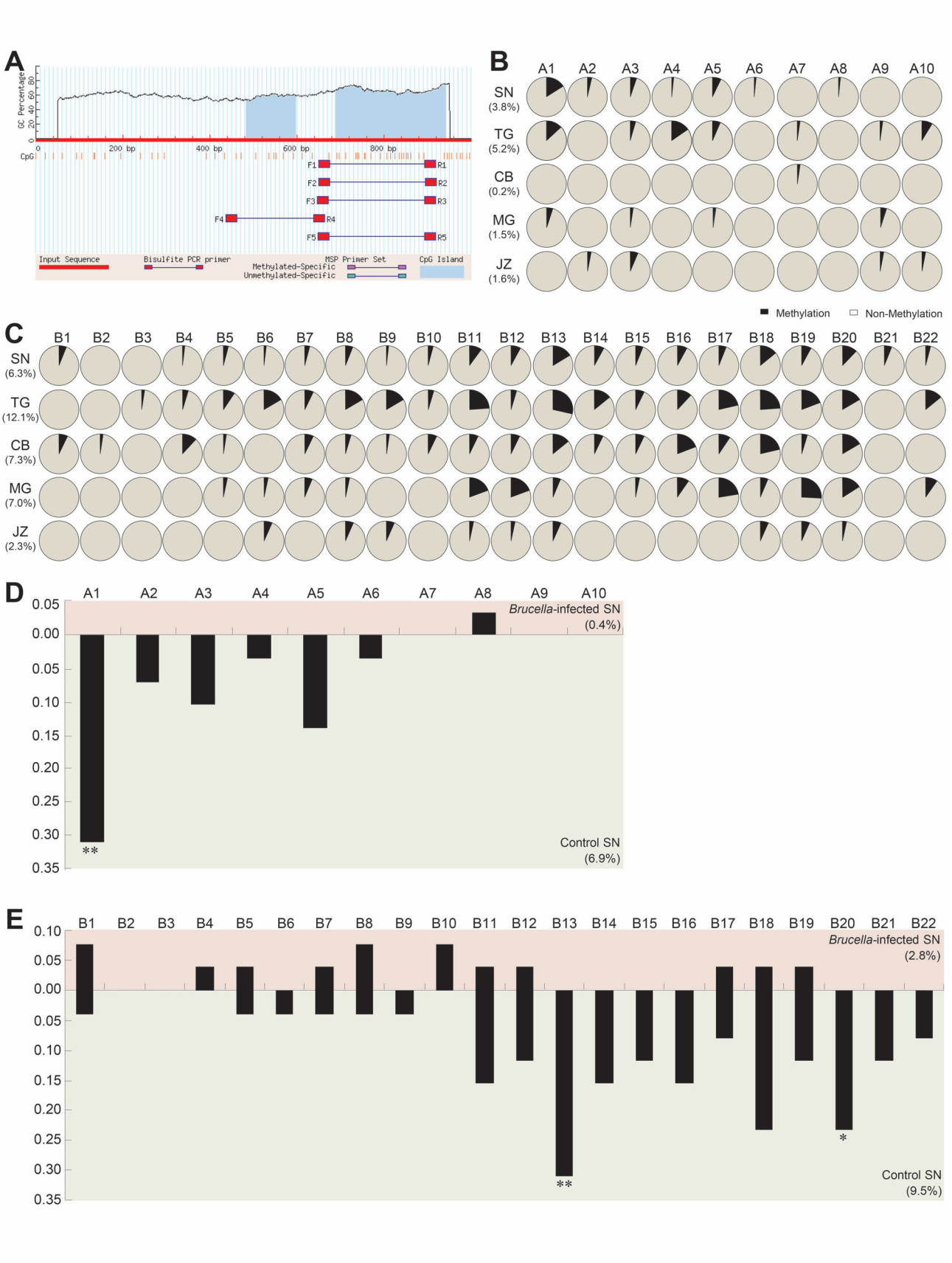


Fig. 3. A – Estimated CpG islands and designed primers in the promoter region of the goat *TLR5*. B – Methylation levels of CpG dinucleotide in the 1st CpG island tested by BSP in the five investigated breeds. A1-A10 represents a single methylated site in CpG1. C – Methylation status of CpG in the 2nd CpG island. B1-B22 represents a single site in CpG2. Promoter methylation status of *TLR5* in *Brucella*-infected and healthy individuals (D and E).

Table 4

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

| Ist CpG Island | | | | 2nd CpG Island | | | |
|---|---|---|---|---|---|---|---|
| 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 | – |
| | | | | B3 | 0.0 | 0 | – |
| A3 | 0.0 | 10.3 | 0.132 | B4 | 4.3 | 0 | 0.469 |
| A4 | 0.0 | 3.4 | 0.518 | B5 | 4.3 | 3.8 | 0.724 |
| A5 | 0.0 | 13.8 | 0.065 | B6 | 0.0 | 3.8 | 0.531 |
| A6 | 0.0 | 3.4 | 0.518 | B7 | 4.3 | 3.8 | 0.724 |
| A7 | 0.0 | 0.0 | – | B8 | 8.7 | 3.8 | 0.435 |
| A8 | 3.7 | 0.0 | 0.482 | B9 | 0 | 3.8 | 0.531 |
| A9 | 0.0 | 0.0 | – | B10 | 8.7 | 0.0 | 0.215 |
| A10 | 0.0 | 0.0 | – | 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; * $p<0.05$.

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 specially 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

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.

## Conclusion

In conclusion, we found that *TLR5* gene methylation may improve the resistance to *Brucella* disease in SN goats. Due to the limited sample size, further studies within a larger population are needed to validate the association between genetic variation and the promoter methylation status of the *TLR5* gene with brucellosis resistance.

## Acknowledgements

This work was supported by the Dual Project of Discipline Construction of Sichuan Agricultural University.

## Author Contributions

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

## Conflict of Interest

The authors declare no conflict of interest.

## References

AKIRA S., UEMATSU S., TAKEUCHI O. 2006. Pathogen recognition and innate immunity. Cell. **124**: 783-801. https://doi.org/10.1016/j.cell.2006.02.015

ALFANO F., PELETTO S., LUCIBELLI M.G., BORRIELLO G., URCIUOLO G., MANIACI M.G., DESIATO R., TARANTINO M., BARONE A., PASQUALI P., ACUTIS P.L., GALIERO G. 2014. Identification of single nucleotide polymorphisms in Toll-like receptor candidate genes associated with tuberculosis infection in water buffalo (*Bubalus bubalis*). BMC Genet. **15**: 139. https://doi.org/10.1186/s12863-014-0139-y

BARRETT J.C., FRY B., MALLER J., DALY M.J. 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. **21**: 263-265. https://doi.org/10.1093/bioinformatics/bth457

BI Y.N., DING Y.L., WU J.M., MIAO J.Q., WANG J.L., WANG F.L. 2020. *Staphylococcus aureus* induces mammary gland fibrosis through activating the TLR/NF-κB and TLR/AP-1 signaling pathways in mice. Microbial Pathogenesis **148**: 104427. https://doi.org/10.1016/j.micpath.2020.104427

BURDELYA L.G., KRIVOKRYSENKO V.I., TALLANT T.C., STROM E., GLEIBERMAN A.S., GUPTA D., KURNASOV O.V., FORT F.L., OSTERMAN A.L., DIDONATO J.A., FEINSTEIN E., GUDKOV A.V. 2008. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. **320**: 226-230. https://doi.org/10.1126/science.1154986

CARVALHO A., PASQUALOTTO A.C., PITZURRA L., ROMANI L., DENNING D.W., RODRIGUES F. 2008. Polymorphisms in toll-like receptor genes and susceptibility to pulmonary aspergillosis. J. Infect. Dis. **197**: 618-621. https://doi.org/10.1086/526500

CHEN S., YUAN W.G., FU Z.K., HUANG Y.K., YANG J.K., XUE J., LIU X.H., LI Y.; LI D.L. 2017. Toll-like receptor 5 gene polymorphism is associated with breast cancer susceptibility. Oncotarget. **8**: 88622-88629. https://doi.org/10.18632/oncotarget.20242

CRAIGHEAD L., MEYER A., CHENGAT B., MUSALLAM I., AKAKPO J., KONE P., GUITIAN J., HASLER B. 2018. Brucellosis in West and Central Africa: A review of the current situation in a changing landscape of dairy cattle systems. Acta. Trop. **179**: 96-108. https://doi.org/10.1016/j.actatropica.2017.12.026

DAI C.H., YANG L., JIN J., WANG H.F., WU S.L., BAO W.B. 2019. Regulation and Molecular Mechanism of TLR5 on Resistance to *Escherichia coli* F18 in Weaned Piglets. Animals (Basel). **9**: 735. https://doi.org/10.3390/ani9100735

DVORNIKOVA K.A., BYSTROVA E.Y., PLATONOVA O.N., CHURILOV L.P. 2020. Polymorphism of toll-like receptor genes and autoimmune endocrine diseases. Autoimmun Rev. **19**: 102496. https://doi.org/10.1016/j.autrev.2020.102496

E G.X., HUANG Y.F., ZHAO Y.J., MA Y.H., NA R.S., ZHANG J.H., GAO H.J., WU X. 2015. Genetic variability of ten Chinese indigenous goats using MHC-linked microsatellite markers. Vet. Immunol. Immunopathol. **167**: 196-199. https://doi.org/10.1016/j.vetimm.2015.07.013

EBID M., EL MOLA A., SALIB F. 2020. Seroprevalence of brucellosis in sheep and goats in the Arabian Gulf region. Vet. World. **13**: 1495-1509. https://doi.org/10.14202/vetworld.2020.1495-1509

FARIDI M.H., KHAN S.Q., ZHAO W., LEE H.W., ALTINTAS M.M., ZHANG K., KUMAR V., ARMSTRONG A.R., CARMONA-RIVERA C., DORSCHNER J.M., SCHNAITH A.M., LI X., GHODKE-PURANIK Y., MOORE E., PURMALEK M., IRIZARRY-CARO J., ZHANG T., DAY R., STOUB D., HOFFMANN V., KHALIQDINA S.J., BHARGAVA P., SANTANDER A.M., TORROELLA-KOURI M., ISSAC B., CIMBALUK D.J., ZLOZA A., PRABHAKAR R., DEEP S., JOLLY M.,

KOH K.H., REICHNER J.S., BRADSHAW E.M., CHEN J., MOITA L.F., YUEN P.S., LITSAI W., SINGH B., REISER J., NATH S.K., NIEWOLD T.B., VAZQUEZ-PADRON R.I., KAPLAN M.J., GUPTA V. 2017. CD11b activation suppresses TLR-dependent inflammation and autoimmunity in systemic lupus erythematosus. J. Clin. Invest. **127**: 1271-1283. https://doi.org/10.1172/JCI88442

FISHER C.E., HOHL T.M.., FAN W., STORER B.E., LEVINE D.M., ZHAO L.P., MARTIN P.J., WARREN E.H., BOECKH M., HANSEN J.A. 2017. Validation of single nucleotide polymorphisms in invasive aspergillosis following hematopoietic cell transplantation. Blood. **129**: 2693-2701. https://doi.org/10.1182/blood-2016-10-743294

FU J., HE H.Y., OJHA S.C., SHI H., SUN C.F., DENG C.L., SHENG Y.J. 2019. Association of IL-6, IL-10 and TGF-beta1 gene polymorphisms with brucellosis: A systematic review with meta-analysis. Microb. Pathog. **135**: 103640. https://doi.org/10.1016/j.micpath.2019.103640

GRUBE M., LOEFFLER J., MEZGER M., KRUGER B., ECHTENACHER B., HOFFMANN P., EDINGER M., EINSELE H., ANDREESEN R., HOLLER E. 2013. TLR5 stop codon polymorphism is associated with invasive aspergillosis after allogeneic stem cell transplantation. Med. Mycol. **51**: 818-825. https://doi.org/10.3109/13693786.2013.809630

GOYAL S.; DUBEY P.K.; SAHOO B.R.; MISHRA S.K.; NIRANJAN S.K.; SINGH S.; MAHAJAN R.; KATARIA R.S. 2014. Sequence based structural characterization and genetic diversity analysis across coding and promoter regions of goat Toll-like receptor 5 gene. Gene. **540**: 238-245. https://doi.org/10.1016/j.gene.2014.01.076

HOSSAIN M.S., RAMACHANDIRAN S., GEWIRTZ A.T., WALLER E.K. 2014. Recombinant TLR5 agonist CBLB502 promotes NK cell-mediated anti-CMV immunity in mice. PLoS One. **9**: e96165. https://doi.org/10.1371/journal.pone.0096165

JEONG J., KIM S., LIM D.S., KIM S.H., DOH H., KIM S.D., SONG Y.S. 2017. TLR5 Activation through NF-kappaB Is a Neuroprotective Mechanism of Postconditioning after Cerebral Ischemia in Mice. Exp. Neurobiol. **26**: 213-226. https://doi.org/10.5607/en.2017.26.4.213

JIANG H., O'CALLAGHAN D., DING J.B. 2020. Brucellosis in China: history, progress and challenge. Infect. Dis. Poverty **9**: 55. https://doi.org/10.1186/s40249-020-00673-8

KATHRANI A., HOUSE A., CATCHPOLE B., MURPHY A., GERMAN A., WERLING D., ALLENSPACH K. 2010. Polymorphisms in the TLR4 and TLR5 gene are significantly associated with inflammatory bowel disease in German shepherd dogs. PLoS One. **5**: e15740. https://doi.org/10.1371/journal.pone.0015740

KESH S., MENSAH N.Y., PETERLONGO P., JAFFE D., HSU K., VAN DEN BRINK M., O'REILLY R., PAMER E., SATAGOPAN J., PAPANICOLAOU G.A. 2005. TLR1 and TLR6 polymorphisms are associated with susceptibility to invasive aspergillosis after allogeneic stem cell transplantation. Ann. N. Y. Acad. Sci. **1062**: 95-103. https://doi.org/10.1196/annals.1358.012

LAI S.J., ZHOU H., XIONG W.Y., GILBERT M., HUANG Z.J., YU J.X., YIN W.W., WANG L.P., CHEN Q.L., LI Y., MU D., ZENG L.J., REN X., GENG M.J., ZHANG Z., CUI B.Y., LI TL., WANG D.F., LI Z.J., WARDROP N.A., TATEM A.J., YU H.J. 2017. Changing Epidemiology of Human Brucellosis, China, 1955-2014. Emerg. Infect. Dis. **23**: 184-194. https://doi.org/10.3201/eid2302.151710

LEE S.E., HONG S.H., VERMA V., LEE Y.S., DUONG T.N., JEONG K., UTHAMAN S., SUNG Y.C., LEE J.T., PARK I.K., MIN J.J., RHEE J.H. 2016. Flagellin is a strong vaginal adjuvant of a therapeutic vaccine for genital cancer. Oncoimmunology **5**: e1081328. https://doi.org/10.1080/2162402X.2015.1081328

LIANG P.F., ZHAO Y., ZHAO J.H., PAN D.F., GUO Z.Q. 2020. The spatiotemporal distribution of human brucellosis in mainland China from 2007-2016. BMC Infect. Dis. **20**: 249. https://doi.org/10.1186/s12879-020-4946-7

MUNETA Y., ARAI N., YAKABE Y., EGUCHI M., SHIBAHARA T., SAKUMA A., SHINKAI H., UENISHI H., HIROSE K., AKIBA M. 2018. *In vivo* effect of a TLR5 SNP (C1205T) on *Salmonella enterica* serovar Typhimurium infection in weaned, specific pathogen-free Landrace piglets. Microbiol. Immunol. **62**: 380-387. https://doi.org/10.1111/1348-0421.12591

NASERI Z., BAHMANI N., ALIKHANI M.Y., HASHEMI S.H., ROSHANAEI G. 2019. Polymorphisms in Promoter Region of the Interferon-Gamma Receptor-1 Gene and its Relation with Susceptibility to Brucellosis. Iran. J. Pathol. **14**: 206-211. https://doi.org/10.30699/ijp.2019.91536.1888

PERIASAMY K., PICHLER R., POLI M., CRISTEL S., CETRÁ B., MEDUS D., BASAR M., A K T., RAMASAMY S., ELLAHI M.B., MOHAMMED F., TENEVA A., SHAMSUDDIN M., PODESTA M.G., DIALLO A. 2014. Candidate gene approach for parasite resistance in sheep—variation in immune pathway genes and association with fecal egg count. PLoS One. **9**: e88337. https://doi.org/10.1371/journal.pone.0088337

PRAKASH O., KUMAR A., SONWANE A., RATHORE R., SINGH R.V., CHAUHAN A., KUMAR P., RENJITH R., YADAV R., BHALADHARE A., BAQIR M., SHARMA D. 2014. Polymorphism of cytokine and innate immunity genes associated with bovine brucellosis in cattle. Mol. Biol. Rep. **41**: 2815-2825. https://doi.org/10.1007/s11033-014-3136-3

RAN X.H., CHEN X.H., WANG M.M., CHENG J.J., NI H.B., ZHANG X.X., WEN X.B. 2019. Brucellosis seroprevalence in ovine and caprine flocks in China during 2000-2018: a systematic review and meta-analysis. BMC Vet. Res. **15**: 18. https://doi.org/10.1186/s12917-018-1749-9

RASOULI M., ASAEI S., KALANI M., KIANY S., MORAVEJ A. 2013. Interleukin-17A genetic variants can confer resistance to brucellosis in Iranian population. Cytokine **61**: 297-303. https://doi.org/10.1016/j.cyto.2012.10.012.

REZAZADEH M., HAJILOOI M., RAFIEI A., HAIDARI M., NIKOOPOUR E., KERAMMAT F., MAMANI M., RANJBAR M., HASHEMI H. 2006. TLR4 polymorphism in Iranian patients with brucellosis. J. Infect. **53**: 206-210. https://doi.org/10.1016/j.jinf.2005.10.018

SADHU D.B., PANCHASARA H.H., CHAUHAN H.C., SUTARIYA D.R., PARMAR V.L., PRAJAPATI H.B. 2015. Seroprevalence and comparison of different serological tests for brucellosis detection in small ruminants. Vet. World. **8**: 561-566. https://doi.org/10.14202/vetworld.2015.561-566

SAEED U., ALI S., KHAN T.M., EL-ADAWY H., MELZER F., KHAN A.U., IFTIKHAR A., NEUBAUER H. 2019. Seroepidemiology and the Molecular Detection of Animal Brucellosis in Punjab, Pakistan. Microorganisms **7**: 449. https://doi.org/10.3390/microorganisms7100449

SELEEM M.N., BOYLE S.M., SRIRANGANATHAN N. 2010. Brucellosis: a re-emerging zoonosis. Vet. Microbiol. **140**: 392-398. https://doi.org/10.1016/j.vetmic.2009.06.021

SELVAM R., MURALI N., THIRUVENKADAN A.K., SARAVANAKUMAR R., PONNUDURAI G., JAWAHAR T.P. 2017. Single-nucleotide polymorphism-based genetic diversity analysis of the Kilakarsal and Vembur sheep breeds. Vet. World. **10**: 549-555. https://doi.org/10.14202/vetworld.2017.549-555

SKONIECZNA K., STYCZYNSKI J., KRENSKA A., STAWIŃSKI P., PLOSKI R., DERWICH K., BADOWSKA W., WYSOCKI M., GRZYBOWSKI T. 2017. Massively parallel targeted resequencing reveals novel genetic variants associated with aspergillosis in paediatric patients with haematological malignancies. Pol. J. Pathol. **68**: 210-217. https://doi.org/10.5114/pjp.2017.71528

SMITH K.D., ANDERSEN-NISSEN E., HAYASHI F., STROBE K., BERGMAN M.A., BARRETT S.L., COOKSON B.T., ADEREM A. 2003. Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. Nat. Immunol. **4**: 1247-1253. https://doi.org/10.1038/ni1011

SONG Y.P., SUN L.P., GUO A.Z., YANG L.G. 2014. Toll-like receptor 6 gene polymorphisms increase the risk of bovine tuberculosis in Chinese Holstein cattle. Acta. Histochem. **116**: 1159-1162. https://doi.org/10.1016/j.acthis.2014.06.004

TAHOUN A., JENSEN K., CORRIPIO-MIYAR Y., MCATEER S., SMITH D.G.E., MCNEILLY T.N., GALLY D.L., GLASS E.J. 2017. Host species adaptation of TLR5 signalling and flagellin recognition. Sci Rep. **7**: 17677. https://doi.org/10.1038/s41598-017-17935-5

UENISHI H., SHINKAI H. 2009. Porcine Toll-like receptors: the front line of pathogen monitoring and possible implications for disease resistance. Dev. Comp. Immunol. **33**: 353-361. https://doi.org/10.1016/j.dci.2008.06.001

VARZARI A., DEYNEKO I.V., VLADEI I., GRALLERT H., SCHIECK M., TUDOR E., ILLIG T. 2019. Genetic variation in TLR pathway and the risk of pulmonary tuberculosis in a Moldavian population. Infect. Genet. Evol. **68**: 84-90. https://doi.org/10.1016/j.meegid.2018.12.005

WANG C., ZHANG H., NIU L.L., GUO J.Z., JIA X.J., WANG L.J., LI L., ZHANG H.P., ZHONG T. 2015. The novel SNPs of leptin gene and their associations with growth traits in Chinese Nanjiang Yellow goat. Gene **572**: 35-41. https://doi.org/10.1016/j.gene.2015.06.073

XU T.W., FU D.Q., REN Y., DAI Y.J., LIN J.G., TANG L.M., JI J. 2017. Genetic variations of TLR5 gene interacted with *Helicobacter pylori* infection among carcinogenesis of gastric cancer. Oncotarget **8**: 31016-31022. https://doi.org/10.18632/oncotarget.16050

XUE Y., ZHAO Z.Q., WANG H.J., JIN L., LIU C.P., WANG Y., LI J.C. 2010. Toll-like receptors 2 and 4 gene polymorphisms in a southeastern Chinese population with tuberculosis. Int. J. Immunogenet. **37**: 135-138. https://doi.org/10.1111/j.1744-313X.2009.00892.x

YOON S. I., KURNASOV O., NATARAJAN V., HONG M., GUDKOV A. V., OSTERMAN A. L., WILSON I. A. 2012. Structural basis of TLR5-flagellin recognition and signaling. Science **335**: 859–864. https://doi.org/10.1126/science.1215584

ZHANG B.Y., CHASSAING B., SHI Z.D., UCHIYAMA R., ZHANG Z., DENNING T.L., CRAWFORD S.E., PRUIJSSERS A.J., ISKARPATYOTI J.A., ESTES M.K., DERMODY T.S., OUYANG W., WILLIAMS I.R., VIJAY-KUMAR M., GEWIRTZ A.T. 2014. Viral infection. Prevention and cure of rotavirus infection via TLR5/NLRC4-mediated production of IL-22 and IL-18. Science **346**: 861-865. https://doi.org/10.1126/science.1256999

ZHANG B.Y., OYEWOLE-SAID D., ZOU J., WILLLIAMS I.R., GEWIRTZ A.T. 2017. TLR5 signaling in murine bone marrow induces hematopoietic progenitor cell proliferation and aids survival from radiation. Blood Adv. **1**: 1796-1806. https://doi.org/10.1182/bloodadvances.2017006981

ZHAO Z.Q., XUE Y., HU Z.G., ZHOU F., MA B.B., LONG T., XUE Q., LIU H.S. 2017. Toll-like receptor 2 gene polymorphisms in Chinese Holstein cattle and their associations with bovine tuberculosis. Vet. Immunol. Immunopathol. **186**: 51-54. https://doi.org/10.1016/j.vetimm.2017.02.007