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Effects of toll-like receptor 2 gene mutation on resistance to bovine brucellosis

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ABSTRACT

Toll-like receptor 2 (TLR2) is an important pattern recognition receptor that play a pivotal role in the innate and adaptive immune systems. TLR2 has been identified as susceptibility loci for various infectious diseases. The aim of the present study was to investigate whether the functional genetic variations in TLR2 gene (T385G, G398A and C1828T) confer susceptibility or resistance to bovine brucellosis. The variants of TLR2 gene were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 511 Chinese Holstein cows (143 infected with brucellosis, 368 non-infected). For SNP T385G, genotypic frequency of GT and GG were significantly difference between brucella-infected and non-infected animals [$p=0.0401$ for both GT and GG; GT Odds Ratio (OR)= 1.410 95% confidence interval (CI) (0.923–2.155); and GG OR=2.084 95% CI (1.116–3.893)]. Similarly, G allele showed significantly higher relative risk of brucellosis incidence than T allele [$p=0.0064$, OR=1.519 95% CI (1.123–2.053)]. The present study indicates that TLR2 gene could be a potential genetic marker for bovine brucellosis susceptibility.

1. Introduction

Bovine brucellosis is mainly caused by *Brucella abortus* (*B. abortus*), a gram negative bacteria and *brucella* has been classified as risk group III by the World Health Organization (WHO) laboratory biosafety manual. The disease can cause considerable damage to dairy farms, such as abortions during the last trimester of gestation, reduced milk production, increased calving intervals, the birth of weak calves, increased culling rate due to metritis following retention of the placenta and orchitis in bulls. Although some countries have been declared free from brucellosis, it is still epidemic, especially in developing countries. For example, in Khartoum State the brucellosis incidence rate is up to 22.6% (Salman and Nasri, 2012).

Studies have been conducted on the gene expression profile of *B. abortus*-infected monocyte-derived macrophages (MDMs) from native naturally resistant or susceptible cattle using a cDNA microarray technology (Rossetti et al., 2010). Heritability of natural resistance to brucellosis has long been recognized as a complex multigenic trait. Genes and their polymorphisms associated with resistance or susceptibility to this disease provide us a new tool for understanding the genetic basis of innate resistance (Adams and Schutta, 2010). Researchers have found that there was an association between *NRAMP1* and its gene polymorphisms on *in vitro* brucellosis phenotypes and resistance/susceptibility to brucellosis (Barthel et al., 2001; Martínez et al., 2010; Kumar et al., 2005). All these studies have been indicated that bovine brucellosis has a genetic component and it is feasible to conduct marker assisted selection for bovine brucellosis resistance study.

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Toll-like receptor 2 (TLR2), members of TLR family, can recongnize a variety of components derived from gram-positive bacteria, gram-negative bacteria, yeast, spirochetes, and fungi (Basith et al., 2011) and play an important role in innate immune responses and adaptive immune responses (Zhang et al., 2009). TLR2 has been shown to form homo- or heterodimers with TLR1 or TLR6 (Akira et al., 2006) and play important roles in inflammatory diseases. Numerous studies have been conducted on the association between TLR gene polymorphisms and disease resistance or susceptibility (Mikula and Mikula, 2011; Sun et al., 2012). TLR2, TLR4 and TLR9 have been implicated in host interactions with brucella (Oliveira et al., 2008) and TLR2 is critical for clearance of pathogenic Brucella strains (Surendran et al., 2012). Researchers have observed that *B. abortus* triggers TLR2 and TLR4 activation but only found the involvement of TLR4 in brucella resistance (Campos et al., 2004).

Taking into account both epidemiological studies and genomic analyses, there is no doubt that genetic variations influence the frequency and course of infectious diseases. To date, it is still not clear whether there are effects of TLR2 gene polymorphism on susceptibility to bovine brucellosis; therefore, the present study aimed to investigate the role of TLR2 gene polymorphisms in brucella infected and non-infected cattle.

2. Materials and methods

2.1. Samples and brucellosis detection

All procedures carried out on animals were approved by the Animal Care and Use Committee of Huazhong Agricultural University. A total of 511 Chinese Holstein cows aged 2–5 years were included in this study. The studied herd had the history of abortion or repeated abortion and no brucellosis vaccine were injected. The studied cows came from the same dairy farm located in Hubei Province were exposed to similar environmental and nutritional conditions to rule out their influence to brucellosis infection. Blood samples were collected from the jugular veins into the tubes which contains 1.5% EDTA for *B. abortus* detection and genomic DNA abstraction. Genomic DNA was isolated using the standard phenol–chloroform protocol, and then diluted to 50 ng/μl according to its concentration and stored at −20 °C for subsequent analysis.

Preliminary screening for brucellosis was carried out using Rose Bengal plate test (RBPT). As the interpretation of the RBPT results can be affected by personal experience (Cho et al., 2010), indirect enzyme-linked immunosorbent assays (I-ELISA) was adopted to complement the defect of RBPT, serological positive cows in I-ELISA were detected repeatedly. The detection was conducted according to the instructions of the bovine brucellosis ELISA kit.

For association study, animals were divided into brucella infected and non-infected groups. “Infected” groups are those serologically positive animals in these two tests accompany with abortion records. “Non-infected” or control groups are cows both serologically negative and no abortion records. Other situations are excluded from statistic analysis.

2.2. Primers synthesis and genotyping

TLR2 gene polymorphisms were first detected by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and different representative genotypes were sequenced in both directions by the Sangon Company. The nucleotide sequence variations of TLR2 gene exon2 were investigated using polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) and created restriction site PCR-RFLP. Primers were designed using primer 5.0 software. Detailed information on the primers is shown in Table 1.

PCR reaction was conducted in a final volume of 20 μL which contains 10 μL PCR master mix, 0.5 μL each primer, 1 μL genomic DNA as template, and 8 μL ultrapure water. The PCR amplification conditions were optimized in a Gene Amp PCR System 9600 (Applied Biosystems, Foster City, CA, USA). The cycling program consisted of an initial denaturation of 5 min at 94 °C, followed by 35 cycles of 40 s at 94 °C, 30 s at the annealing temperature (Table 1), 30 s at 72 °C, and a final extension of 10 min at 72 °C. For RFLP, the PCR products were digested with specific restriction enzyme in a reaction including 3 μL of the PCR product, 5 U of the restriction enzyme (NEB, Beijing, China), 1 μL of the corresponding 10 × reaction buffer, and then added water to make the final volume to 10 μL. The reaction was then incubated at the appropriate temperature for at least 4 h. Finally, the fragments were separated on either a 3% agarose gel or an 8% bisacrylamide gel according to the length of the fragments to be separated. To further confirm these results, different

Table 1
Primer sequences, information of the TLR2 gene and restriction enzymes used for RFLP analysis.

Location sites	Primer sequence	Annealing temperature (°C)	Product size (bp)	Product position	Restriction enzyme
TLR2-T385G	F: 5′-CTCTGTCTTGTGACCCAACT-3′ R: 5′-ACATAAAGGGACCTGAACC-3′	55	295	276–570	*Eco*RV
TLR2-G398A	F: 5′-CCCAACTGGTGTCTGCGATGG-3′ R: 5′-ACCTCTGCAGGTCTCTGTGGC-3′	54	130	289–419	*Hae*III
TLR2-C1828T	F: 5′-CAACTTGATTCTTTTCAGCAACTG-3′ R: 5′- AGTGCCTGCTGTCCCTGTTT-3′	57	101	1748–1848	*Dra*I

Note: F: Forward; R: Reverse; bp: base pairs. Shaded letters indicate mismatched base. GenBank number of TLR2 corresponding to NM_174197.2.