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Polymorphisms in toll-like receptor (*TLR*) 1, 4, 9 and *SLC11A1* genes and their association with paratuberculosis susceptibility in Holstein and indigenous crossbred cattle in Turkey

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Abstract. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes major problem in a wide range of animal species. In ruminant livestock including cattle, it causes a chronic disease called Johne's disease, or paratuberculosis (pTB) which is currently considered as potential zoonosis, causing Crohn's disease in humans. MAP infection susceptibility is suspected to be controlled by host genetics. Thus, selecting individuals according to their genetic structure could help to obtain bovine populations that are increasingly resistant to MAP infection. The aim of the present work was to investigate the association between toll-like receptor (*TLR*) *1* (+1380 G/A), *TLR1* (+1446 C/A), *TLR4* (+10 C/T), *TLR9* (+1310 G/A) and solute carrier family 11 member 1 (*SLC11A1*) (+1066 C/G) mutations and MAP infection status in 813 cattle comprising East Anatolian Red crossbred, Anatolian Black crossbred and Holstein breed. *TLR1* (+1380 G/A) mutation showed an association with bovine MAP ($P < 0.05$). For the *TLR1* (+1380 G/A) locus, the odds ratio for AG and AA genotypes versus GG genotypes were 2.31 (1.24–4.30; 95% confidence interval (CI)) and $0 < 0.001$ (<0.001 to >999.999; 95% CI) which indicated that a proportion of AG homozygote was significantly higher in pTB-affected animals as compared with the control. General linear model analysis demonstrated higher MAP antibody response in *TLR1* (+1380 AG) genotype as compared with *TLR1* (+1380 GG) ($P < 0.0001$). Present findings suggest that selection against *TLR1* (+1380 G/A) may reduce the risk of pTB in bovine herds.

Keywords. Johne's disease; bovine; genetics; disease risk; anatolian indigenous cattle.

Introduction

Paratuberculosis (pTB), or Johne's disease is an intestinal infection caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (Chaubey *et al.* 2016). Animals are chronically infected, usually at a young age, and are generally believed to undergo an extended latent period (Marcé *et al.* 2010). The clinical signs of pTB in cattle are easily recognized by pipestream diarrhoea, weight loss and ultimately death (Fecteau 2018). Infections with MAP are difficult to control because of the long incubation periods

(1–7 years), absence of clinical signs until advanced stages of the disease and lack of completely reliable diagnostic methods in the preclinical stages of the disease (Fecteau 2018). MAP infection is known to result in lower milk production, decreased reproductive performance, early culling or latter fatal diarrhoea (Smith *et al.* 2017). Livestock disease costs have been estimated to be up to 20% and 35–50% of turnover within the livestock sector in developed and developing countries, respectively. In the US, MAP disease causes estimated losses of $200 million every year (Cho *et al.* 2012). Eradication has been

considered as the efficient way for controlling MAP. However, diagnosis of the disease could be problematic due to the lack of efficient diagnostic tools (Collins 2011). Therefore, the high costs involved in testing and culling approaches have shifted the focus to control strategies providing better benefit/cost ratios of more conservative strategies like vaccination (Juste 2012). Vaccination using killed inactivated vaccines has also been attempted for MAP control. The vaccines are said to afford protection by delaying the onset of clinical disease, but they does not reduce the number of infected animals (Wentink *et al.* 1994; Tewari *et al.* 2014).

Susceptibility to MAP infection can also be genetically influenced. Previous studies indicated that certain cattle breeds (Guernsey, Jersey and *Bos indicus* breeds) and family lines might have a genetic predisposition for MAP infections (Roussel *et al.* 2005; Elzo *et al.* 2006). Breeding for disease susceptibility is a central focus of livestock breeding programmes, as any successful variety must have the complete package of high yield, disease resistance, agronomic performance and end-use quality (Bishop and Woolliams 2014). Therefore, selection may be used as an additional tool for control of pTB (Bishop and Woolliams 2014; Bishop 2015). The use of genetic markers with the phenotypes in a process called marker-assisted selection (MAS) is mainly important and useful for phenotypes with low heritability (Meuwissen *et al.* 2013) such as trait-like antibody response to MAP which was estimated to be about 0.1 (Mortensen *et al.* 2004; Hinger *et al.* 2008). Recently, five single-nucleotide polymorphisms (SNPs) namely toll-like receptor (*TLR*) *1* (+1380 G/A), *TLR1* (+1446 C/A), *TLR4* (+10 C/T), *TLR9* (+1310 G/A) and solute carrier family 11 member 1 (*SLC11A1*) (+1066 C/G) have been found to be associated with bovine brucellosis (Prakash *et al.* 2014). TLRs are cell-surface receptors mediating activation of innate immunity that controls host defence against pathogens (Takeda and Akira 2005). TLRs target a range of microbial ligands, including lipopolysaccharide (TLR4), lipoproteins (TLR2) and unmethylated CpG motifs in DNA (TLR9) (Akira *et al.* 2001). Expression of TLRs on innate immune cells link microbial recognition to induction of antimicrobial mechanisms, such as production of reactive oxygen and nitrogen species and expression of antimicrobial peptides (AMPs) (Arpaia *et al.* 2011). Associations of different TLRs with various bovine diseases (Werling *et al.* 2009) including pTB infection (Fisher *et al.* 2011) were previously presented in the literature. *SLC11A1* (formerly *NRAMP1*) has many pleiotropic effects on immune cells including regulation of chemokines and interleukins, major-histocompatibility complex (MHC) class II molecules, tumour necrosis factor α (TNFα), as well as antimicrobial activity (Blackwell *et al.* 2001). The SLC11A1 protein prevents intracellular bacterial growth through transportation of divalent metal ions, for the cellular recycling of the latter (Ruiz-Larrañaga *et al.* 2010).

Microorganisms are mainly destroyed by the host tissues through processes that usually involve phagocytosis and lysosomal disruption (Trangoni *et al.* 2015). However, some microorganisms establish themselves within the cell and spend all or part of their life intracellularly such as genus *Mycobacterium* and *Brucella* bacterium (Trangoni *et al.* 2015) and genetics of host protection could be common for different genera of intracellular pathogens (Yap *et al.* 2006). It is hypothesized in this study that SNPs associated with bovine brucellosis caused by intracellular pathogen, may also show association with another intracellular pathogen (MAP)-borne bovine pTB. Therefore, the aim of this study was to investigate associations between *TLR4* (+10 C/T), *TLR9* (+1310 G/A), *TLR1* (+1380 G/A), *TLR1* (+1446 C/A) and *SLC11A1* (+1066 C/G) polymorphisms with bovine pTB in the Holstein population reared in Turkey and Turkish indigenous cattle crossbreds.

Materials and methods

Sample collection

All biological materials were collected from cattle slaughtered (showing clinical signs of pTB as cachexia and diarrhoea) at a local abattoir. Following the slaughter, blood samples were collected into vacutainer tubes (B.D. Bioscience, Germany) with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for genetic analysis and into vacutainer tubes (B.D. Bioscience, Germany) without an anticoagulant for serological analysis. All samples were delivered back to the laboratory in an ice box. Blood samples were collected from 2–3-year-old East Anatolian Red crossbred ($n = 288$), Anatolian Black crossbred ($n = 112$) and Holstein ($n = 418$) breeds from Kayseri province and vicinity, Turkey. Animals used in the present study were housed under similar environmental conditions and not vaccinated for pTB. Blood samples were used for the isolation of genomic DNA for restriction fragment length polymorphism (RFLP) genotyping of the cattle under study, whereas serum samples were used for detection of MAP antibodies by an enzyme-linked immunosorbent assay (ELISA). All experimental procedures were performed in accordance with the guidelines of the Local Ethics Committee for Animal Experiments at Erciyes University.

Genomic DNA isolation and genotyping

Genomic DNA was extracted from whole blood using the standard phenol–chloroform extraction procedure. DNA concentration of the samples was quantified by using a Nano Drop (NanoDrop, Thermo Fisher Scientific, Waltham, USA) and stored at −20°C until use. Primer pairs and restriction enzymes were obtained

from Prakash *et al.* (2014) (see table 1). Polymerase chain reaction (PCR) was carried out using 50 ng of genomic DNA in a total reaction volume of 25 μL containing 10× PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 10 pM of forward and reverse primers and 0.5 U of *Taq* DNA polymerase (Sigma-Aldrich, Darmstadt, Germany). Amplification reactions were performed with an initial denaturation step of 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 40 s of *x*°C (provided in table 1) and 30 s at 72°C with a final extension step of 7 min at 72°C. PCR products were screened by 1.5% agarose gel (Sigma-Aldrich) electrophoresis stained with ethidium bromide and visualized in a gel documentation system (Bio-Rad, Hercules, USA). These PCR fragments obtained with the primers were analysed by means of RFLP. Results from the PCR amplification were scored on agarose gel according to the band pattern of 100 base-pair ladder (Thermo Fisher Scientific). Suitable restriction enzymes (Thermo Fisher Scientific) were selected to identify RFLP patterns. All enzymes were allowed to react in a PCR machine at a temperature of 37°C for 6 h. Enzymes used for the PCR-RFLP are indicated in table 1. The digestion products were separated by horizontal electrophoresis in 3% agarose gel in 0.5× tris/borate/EDTA (135 V for 20 min) stained with ethidium bromide solution prior to visualization under ultraviolet light.

Detection of MAP-specific antibodies by ELISA

Serum samples ($n = 818$) were screened for detection of MAP-specific antibodies by using an ELISA kit (Idexx Paratuberculosis Screening, Maine, USA). Optical density (OD) values were measured at 450 nm using a microplate reader Elx800 (Bio-Tek Instruments, Winooski, USA). Positive and negative sera were included as controls. As per the manufacturer's instruction, serum samples with corrected sample/positive control ratio above 55% were considered as positive for pTB.

Statistical analysis

Data were analysed using SAS 9.2 software (SAS Institute, Cary, USA). Allele and genotype frequencies were calculated using the ALLELE procedure of SAS v9.2. The univariable analysis for logistic regression considered the infection status as categorical response variable (yes/no), and SNPs (*TLR4* and *TLR9* have two genotypes, *TLR1* and *SLC11A1* have three genotypes, therefore respective loci have two or three levels), breed (three groups, i.e. two indigenous crossbred and Holstein) and sex (male and female) were included as possible explanatory variables. Genotypes were considered as ordinal variables and as class variables with the major homozygous genotype deemed as baseline. Data were analysed using the PROC LOGISTIC procedure and odds ratio (OR)

Table 1. Selected SNPs and their primer conditions.

Gene	SNP location in gene	Primer sequence	T_a (°C)	RE	Amino acid change
TLR4 +10 C/T	C480 T, exon 1 (321–725)	F: 5'-CGTAACCCAGCACTGCTTTG-3' R: 5'-GCCTGTTAATGCCCTGTAACC-3'	59.2	*Bst*UI	Nonsyn R[Arg]⇒C[Cys]
TLR9 +1310 G/A	G2508A, exon 2 (2385–2745)	F: 5'-GTCTGCAGCTGAACTTCATC-3' R: 5'-ACGAACTGGGAGCCATTAAAC-3'	56.8	*Bse*RI	Nonsyn G[Gly]⇒E[Glu]
TLR1 +1380 G/A	G1409A, exon 1 (1311–1489)	F: 5'-TTTAGCAGCCTTTCCATACT-3' R: 5'-TCTACCACGTCACTGGATACT-3'	54.0	*Bsl*I	Syn
TLR1 +1446 C/A	C1475A, exon 1 (1311–1664)	F: 5'-TTTAGCAGCCTTTCCATACT-3' R: 5'-CAGATCCAGGTAGATACAGAG-3'	54.0	*Bst*XI	Syn
SLC11A1 +1066 C/G	C7401G, exon 11 (7111–7484)	F: 5'-ATCTCCTTCCTACTGCCCG-3' R: 5'-CACAAACTGTCCCGCGTAG-3'	54.0	*Pst*I	Nonsyn P[Pro]⇒A[Ala]

T_a, annealing temperature; RE, restriction enzyme; syn, synonymous; nonsyn, nonsynonymous; arg, arginine; cys, cysteine; gly, glycine; glu, glutamic acid; pro, proline; ala, alanine.
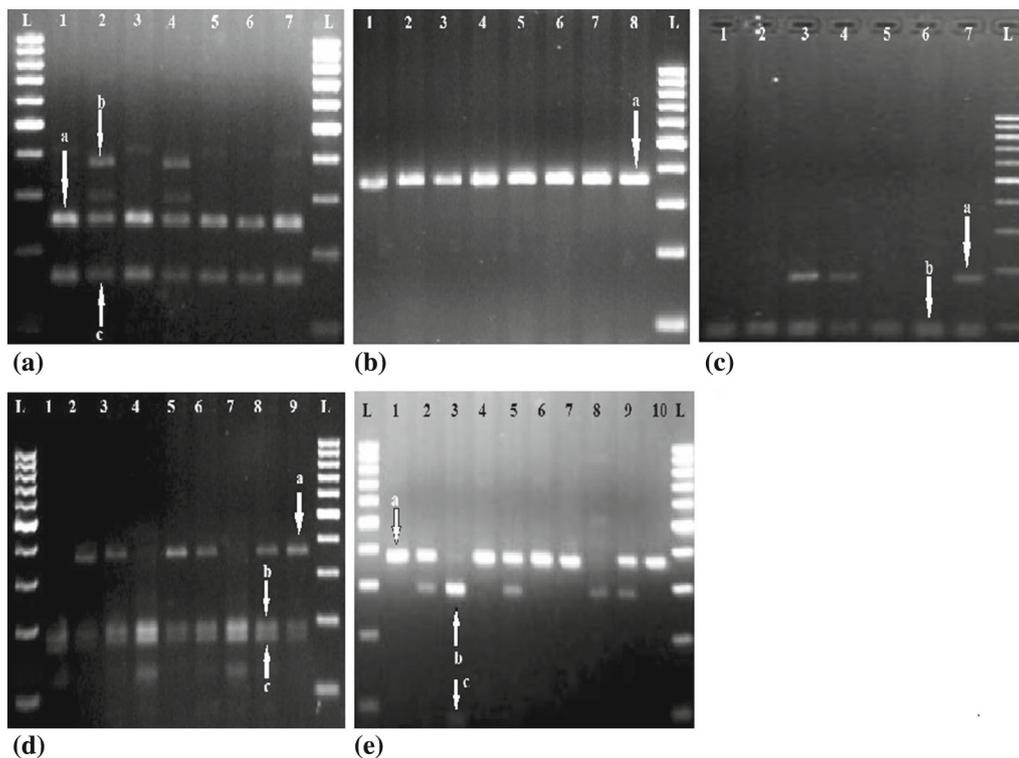
Figure 1. (a) Restriction digestion with the *Bst*UI enzyme of bovine *TLR4* (+10 C/T) SNP-amplified PCR products showing different genotypes in 3% agarose gel (a, 246 bp; b, 405 bp; c, 159 bp). (b) Restriction digestion with the *Bse*RI enzyme of bovine *TLR9* (+1310 G/A) SNP-amplified PCR products showing only a genotype in 3% agarose gel (only 392 bp). (c) Restriction digestion with the *Bsl*I enzyme of bovine *TLR1* (+1380 G/A) SNP-amplified PCR products showing different genotypes in 3% agarose gel (a, 179 bp; b, 93 bp; c, 86 bp). (d) Restriction digestion with the *Bst*XI enzyme of bovine *TLR1* (+1446 C/A) SNP-amplified PCR products showing different genotypes in 3% agarose gel (a, 354 bp; b, 187 bp; c, 167 bp). (e) Restriction digestion with the *Pst*I enzyme of bovine *SLC11A1* (+1066 C/G) SNP-amplified PCR products showing different genotypes in 3% agarose gel (a, 374 bp; b, 293 bp; c, 81 bp). A 100-bp ladder was used in all figures.

with 95% confidence interval (CI) were calculated. ELISA OD data were checked for normality before analyses with the PROC UNIVARIATE procedure of SAS v9.2 (SAS Institute). Associations of different genotypes and OD values were tested using PROC GLM. Genotypic comparisons were reported following Tukey–Kramer adjustment; $P \leq 0.05$ was considered significant.

Results

Five SNP loci detected by PCR-RFLP are shown in figure 1. Initially, a total of 818 individuals were subjected to RFLP analysis, however, 813 cattle for *TLR4* (+10 C/T), *TLR9* (+1310 G/A), *TLR1* (+1380 G/A), 817 animals for *TLR1* (+1446 C/A) and 815 animals for *SLC11A1* (+1066 C/G) were genotyped due to PCR or RFLP artefacts. As presented in table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/, the genotypic and allelic frequencies of all SNPs, except for *TLR9* (+1310 G/A) in Holstein cattle and for *SLC11A1* (+1066 C/G) in Anatolian Black crossbred cattle, exceeded threshold as

present in both the case and control groups, suggesting that these SNPs were significant polymorphic loci.

The presence of pTB in East Anatolian Red crossbred, Anatolian Black crossbred and Holstein cattle was tested by ELISA. The number of positive results were reported in table 2. The resulting ratio of cases to controls was 1:14.4 (53 cases versus 765 controls) in the cattle aged ≥ 2 years both for cases and controls. The seropositivity of pTB was 2.5, 3.7 and 11.1% for East Anatolian Red crossbred, Anatolian Black crossbred and Holstein cattle, respectively. The lowest was noticed in the East Anatolian Red crossbred and the highest in Holstein cattle. Among the animals with pTB, mean OD values were 1.96, 2.18 and 2.61, respectively, for East Anatolian Red crossbred, Anatolian Black crossbred and Holstein cattle (table 2).

Two analyses were applied to investigate association between SNPs and pTB status of cattle. Firstly, association analysis was applied in accordance to case (pTB) and control (disease free) status of animals. Secondly, 53 animals which have pTB according to ELISA OD values (≥ 1.4) were subjected to association analysis. The results from the univariate logistic regression for case–control and

Table 2. Descriptive data of pTB seropositive and seronegative animals.

pTB status	East anatolian red crossbred	Anatolian black crossbred	Holstein
Control (n)	281	108	376
Case (n)	7	4	42
OD values (≥ 1.4) (mean $\pm$ SE)	1.96 ± 0.44^a	$2.18 \pm 0.37^{a,c}$	$2.61 \pm 0.38^{b,c}$

SE, standard errors.

[a,b,c] Significance level was used $P < 0.05$.

Table 3. Genotype frequency distribution of innate immunity genes in cases and controls.

SNP	Genotype	Genotype frequency		P value	OR (95% CI)
		Cases	Control		
TLR4 (+10 C/T)	CC	53 (6.52)	752 (92.5)	0.98	1.00
	CT	0	8 (0.98)		<0.001 (<0.001 to >999.999)
TLR9 (+1310 G/A)	GG	53 (6.52)	755 (92.87)	0.99	1.00
	AG	0	5 (0.62)		<0.001 (<0.001 to >999.999)
TLR1 (+1380 G/A)	GG	15 (1.85)	354 (43.54)	0.03	1.00
	AG	38 (4.67)	403 (49.57)		2.31 (1.24 to 4.30)
	AA	0	3 (0.37)		<0.001 (<0.001 to >999.999)
TLR1 (+1446 C/A)	CC	1 (0.12)	56 (6.85)	0.49	1.00
	AC	52 (6.36)	697 (85.31)		0.23 (0.04 to 2.20)
	AA	0	11 (1.35)		>999.999 (<0.001 to >999.999)
SLC11A1 (+1066 C/G)	GG	3 (0.37)	72 (8.83)	0.38	1.00
	CC	7 (0.85)	136 (15.38)		1.86 (0.55 to 6.25)
	CG	58 (7.12)	538 (66.09)		0.93 (0.22 to 3.80)

generalized linear model (GLM) for OD values indicated a non-significant effect for sex and a significant effect for breed except for *TLR1* (+1380 G/A) SNP (table 3). Significant differences in genotype frequencies between two groups of individuals tested positive or negative for pTB infection were found only for *TLR1* (+1380 G/A) polymorphism ($P = 0.0042$, table 3). The estimated ORs for the univariable logistic regression models are presented in table 3. At the *TLR1* (+1380 G/A) locus, the OR of AG genotype versus GG genotype was 0.31 (0.16–0.62; 95% CI) in case (pTB) which revealed that relative proportion GG genotypes was significantly higher in case population than in control population, whereas AA genotype was exclusively not present in pTB positive animal and was present in three positive animals in the control population and its OR was towards infinity. Results of GLM are given in table 4. *TLR1* (+1380 G/A) was found to be associated with ELISA OD values ($P < 0.0001$, table 4). For the *TLR1* (+1380 G/A), locus genotype AG yielded higher antibody response as compared with genotype GG and none of the pTB animals showed genotype AA.

No significant differences in genotype frequencies between the cases and controls were found for the *TLR4* (+10 C/T), *TLR9* (+1310 G/A), *TLR1* (+1446 C/A) and *SLC11A1* (+1066 C/G) genotypes (table 3). Similarly, the GLM analysis did not show association between any of the polymorphisms analysed and ELISA OD values (table 4).

Discussion

pTB creates significant problem to the world dairy and beef industries and it is considered as a potential zoonotic disease (Pinedo *et al.* 2009). MAP has been incriminated as a triggering agent for Crohn's disease in genetically susceptible individuals in human (Sweeney *et al.* 2012). The role of genetic factors in association with susceptibility and resistance to mycobacterial infection including bovine pTB had been discussed in studies which are focussed on a candidate gene level (Mucha *et al.* 2009; Koets *et al.* 2010; Ruiz-Larrañaga *et al.* 2011; Pinedo *et al.* 2013) and estimations of heritability to MAP infection ranging from 0.01 to 0.183 have been reported in Holstein cattle (Kirkpatrick and Shook 2011). Currently, a few studies are involved in exploring association of host-candidate genes with susceptibility to MAP infection in cattle (Kirkpatrick and Shook 2011). Some of previous works reported positive association with MAP for the selected candidate genes, such as interferon gamma (*BoIFNG*), *TLR4* and *SLC11A1* (Mucha *et al.* 2009; Pinedo *et al.* 2009) which were also associated with bovine brucellosis (Prakash *et al.* 2014). However, in the present study, no associations were identified between genes including *TLR4* (+10 C/T), *TLR9* (+1310 G/A), *TLR1* (+1380 G/A) and *SLC11A1* (+1066 C/G) and MAP infections. This may have occurred due to two reasons. Firstly, none of the

Table 4. Least-squares means (LSM) and SE for OD values (≥ 1.4) across genotype of innate immune genes in Holstein and Turkish native crossbred cattle.

SNP	Genotype	OD			Fixed effects		
		LSM	SE	*P* value	$R^{2\#}$	Sex	Breed
TLR4 (+10 C/T)	CC (*n* = 53)	2.25	0.34	NA	0.12	NS	0.02
TLR9 (+1310 G/A)	GG (*n* = 53)	2.25	0.34	NA	0.12	NS	0.02
TLR1 (+1380 G/A)	GG (*n* = 15)	1.95	0.25	**<0.0001**	0.54	NS	NS
	AG (*n* = 38)	2.97	0.27				
TLR1 (+1446 C/A)	CC (*n* = 1)	3.73	0.88	0.07	0.18	NS	0.003
	AC (*n* = 52)	2.33	0.33				
SLC11A1 (+1066 C/G)	GG (*n* = 44)	2.28	0.50	0.85	0.05	NS	0.03
	CG (*n* = 6)	2.39	0.43				
	CC (*n* = 3)	2.22	0.35				

NS, not significant; NA, not applicable. #R^2 values derived from the GLM. *P* values showed for fixed effects in the table, derived from total variation explained by the fixed effects (R^2).

markers investigated in the current study may not be in linkage disequilibrium with genes affecting disease susceptibility. Secondly, genotype frequency did not show any difference between pTB-infected and noninfected animals. Unlike association mapping techniques that are attempting to detect linkage disequilibrium indirectly between marker alleles and phenotype, in case–control tests, this is done by comparing marker allele or genotype frequencies among affected individuals with their frequencies among unaffected individuals (Nielsen and Weir 2001). On the other hand, coinciding with our results, some experiments did not identify any association between investigated gene polymorphisms such as caspase recruitment domain 15 (*CARD15*) and *TLR4* and pTB (White *et al.* 2003; Taylor *et al.* 2006).

The results of this study, performed with the Turkish indigenous cattle crossbreds for the first time, suggest that *TLR1* (+1380 G/A) gene mutation might have a role in pTB susceptibility, but none of these have been previously highlighted by the other authors. Breed effect was reported as one of the factors that influence variation in susceptibility to pTB (Cetinkaya *et al.* 1997; Roussel *et al.* 2005; Elzo *et al.* 2006). Similarly, it was in the present study that breed had effects on pTB. The MAP-specific serum antibody level was significantly different between the investigated breeds of the present study (table 2). Holstein cattle breed showed the highest ELISA OD values, East Anatolian Red crossbred showed the lowest and Anatolian Black crossbred remained in the middle (table 2) which shows that Turkish native cattle breeds exhibit better resistance against MAP infections compared with Holstein cattle breed. The present findings are consistent with the findings of a previous experiment that investigated susceptibility to bovine tuberculosis in which native Zebu cattle were found more resistant to bovine tuberculosis than the exotic Holstein–Friesian cattle (Vordermeier *et al.* 2012). Roussel *et al.* (2005) also stated that cattle from *B. indicus*-based

herds were more than 17 times as likely to be seropositive as were cattle from *B. taurus*-based herds, and cattle from interspecies-based herds were 3.6 times as likely to be seropositive as were cattle from *B. taurus*-based herds. However, no study was devoted to compare MAP infection status of exotic cattle breeds with the native or native crossbred cattle breeds in Turkey.

TLR1, when dimerized with TLR2, is a cell-surface receptor that, upon recognition of bacterial lipoproteins, activates the innate immune system (Misch *et al.* 2008). In the present experiment, *TLR1* (+1380 G/A) SNP was found to be associated with MAP infections in cattle which are reared in Turkey (tables 3&4). TLR1 has been implicated as a coreceptor together with TLR2 in the recognition of bacterial triacyl lipopeptides which is an extracellular mycobacterial ligand (Wiersinga *et al.* 2007) and TLR1-deficient mice were defective in their response to bacterial lipopeptides that are triacylated (Takeda *et al.* 2002). Different *TLR1* variants were found to be associated with mycobacterial signalling including cattle MAP (Mucha *et al.* 2009; Ruiz-Larrañaga *et al.* 2011) and *M. bovis* in Holstein cattle (Sun *et al.* 2012). Additionally, two quantitative trait loci associated with susceptibility to MAP in Holstein cattle were identified in the same region where *TLR1* located on BTA6 (Kirkpatrick *et al.* 2011).

For the *TLR1* (+1380 G/A) locus, the OR for AG genotypes versus GG genotypes was 2.31 (1.24–4.30; 95% CI) which revealed that relative proportion AG genotypes was significantly higher in the case population than in the control population, whereas the AA genotype was not present in any pTB positive animals and its OR was low and near to zero in pTB-affected animals as compared with the control. It indicated that the AG genotype at the *TLR1* (+1380 G/A) locus was responsible for increasing the relative risk of bovine pTB and hence selection against the AG genotype may reduce seropositivity of pTB in cattle (table 3). Prakash *et al.* (2014) also investigated

association between *TLR1* (+1380 G/A) SNP and brucella susceptibility in indigenous Shri Mataji Goshala and Barsana cattle and found controversial to present results; proportion of GG homozygote was significantly higher in brucellosis-affected animals as compared with the control. Although, both pTB and brucella are intracellular bacterial pathogen caused diseases, susceptibility in terms of *TLR1* (+1380 G/A) SNP maintained with different genotypes among the cattle reared in Turkey and in India. This may be due to genetic origins of cattle breeds in Turkey and India. While cattle breeds in Turkey belong to humpless, the *B. taurus* lineage, humped Indian cattle breeds belong to *B. indicus* and clear phylogenetic distinction among these cattle breeds were shown genetically (Lin *et al.* 2010; Randhawa *et al.* 2016).

In conclusion, in the present study, genetic polymorphism was found in Holstein and two Turkish indigenous cattle crossbreds, and the association between genetic polymorphism of the synonymous *TLR1* (+1380 G/A) and risk of pTB infection. An ELISA antibody response showed that East Anatolian Red crossbred and Anatolian Black crossbred were more resistant to pTB as compared with Holsteins reared in Turkey. Additionally, selecting animals against *TLR1* +1380 AG genotype may decrease the risk of pTB in cattle which belongs to *B. taurus* species. Further analyses through expanded sampling in some breeds and detailed studies under various molecular levels are recommended to provide more consistent explanations for the molecular mechanisms of mutation-related risk of susceptibility.

Acknowledgement

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