Full Length Article

Analysis of Single-Stranded Conformational Polymorphism at 3'UTR of *SLC11A1*Gene in Jersey Crossbred Cattle

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ABSTRACT

Intracellular bacterial diseases cause significant economic losses in livestock industries. Constraints in eradicating infectious diseases indicate the need for selection and breeding of animals based on natural disease resistance. Solute Carrier Family 11 Member 1(SLC11A1) gene expressed in macrophages has significant involvement in innate immune mechanism.Investigation of polymorphism of 3'untranslated region (3'UTR) of SLC11A1 gene was conducted and the Polymerase Chain Reaction - Single-Stranded Conformational Polymorphic (PCR-SSCP) pattern at 3'untranslated region (3'UTR) of SLC11A1 gene in Jersey crossbred cattle was analyzed. A fragment of 175bp encoding 3'UTR was amplified by PCR and the genotyping was done by using PCR-SSCP. SSCP pattern of 3'UTR revealed the presence of four genotypes, viz. AB,BB, CC and CB with a frequency of 0.29, 0.40, 0.17 and 0.13, respectively with allelic frequencies of A (0.144), B (0.615) and C (0.24). The result showed that the population was not in Hardy-Weinberg equilibrium for SLC11A1 gene. SLC11A1 gene was found to be highly polymorphic in the Jersey crossbred cattle population studied.

Key Words: Disease resistance, *SLC11A1* gene, PCR-SSCP, allele frequencies, Jersey crossbred cattle,

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INTRODUCTION

In India, diseases like Johne's disease (JD) is endemic and high prevalencehas been reported in domestic ruminants (Kennedy and Benedictus, 2001). Treatment of intracellular bacterial diseasesare expensive and also not successful (Sweeney*et al*., 2012). In these circumstances, genetic selection for disease resistance can be an effective strategy for the control of incurable and highly pathogenic diseases (Donagh*et al*., 2011).Earlier studies have

shown that considerable genetic variation in *SLC11A1* gene has an association with disease suscepatability. *SLC11A1* gene is also known as *NRAMP1,* primarily expressed in phagosomes. The gene product exhibits pleiotropic effects on iron transport, early innate immune response against intracellular bacterial growth through inducible nitric oxide synthase (iNOS) expression in mice (Alter-Koltunoff *et al*.,2008). Natural Resistance Associated Macrophage Protein 1 (*NRAMP1*/*SLC11A1*) is a candidate gene in regulating resistance and susceptibility towards a number of antigenically different microorganisms including *Mycobateriumbovis*, *Salmonellasp* and *Leishmaniasp* (Juste *et al*., 2005 and Reddacliff *et al*., 2005).Several reports shows that GT repeats in 3'UTR of *SLC11A1* gene are significantly associated with disease resistance and are also considered as disease resistance locus (Zhang *et al*.,2009). Allelic differences localized in the 3'UTR of the gene have been associated with macrophage function and resistance to disease in cattle and buffalo (Capparelli *et al*.,2007, Ganguly *et al*., 2008 and Kumar *et al*.,2011). In the present study, the allele frequency in 3'UTR of *SLC11A1* gene was detected by Polymerase Chain Reaction - Single-Stranded Conformational Polymorphism (PCR-SSCP) analysis in Jersey crossbred population.

MATERIALS AND METHODS

Population under study were 52 (40 from JD suspected Jersey crossbreds and 12 normal crossbreds) in and around Namakkal district. Animals were categorized as being suspected based on symptoms of JD that included diarrhea, weakness, weight loss. Genomic DNA was isolated fromblood samples using standard phenol chloroform extraction method by Montgomery and Sise (1990). Quality of DNA was checked by 1% agarose gel electrophoresis and concentration measured by Nanodrop, Thermoscientific. The ratio of optical density at 260/280nm of DNA for the above genetic group ranged from 1.8 to 1.89 respectively. Samples with DNA concentration above 50 ng/µl were taken for study. Serum samples were processed to identify the presence of MAP (*Mycobacterium avium* subspecies *paratuberculosis*) by employing the direct IS900 PCR as per the procedure described by Pillai and Jayarao (2002) using two primer sequence was 5'-CCGCTAATT GAGAGATGCGATTGG-3' and the 3' primner sequence was 5'-AATCAACTCCA GCAGCGCGGCCTCG-3' for detection of insertion sequence of 900 (IS900) of MAP which amplifies a 229 bp fragment PCR product.

Primers of 3'UTR forward 5'-AAGGCAGCAAGACAGACAGG-3' and 3'UTR reverse 5'-ATGGAACTCA CGTTGGCTG-3' were used for the amplifing the 3'untranslated region (3'-UTR) of *SLC11A1* gene as described by Zhang *et al.* (2009).Polymerase chain reaction (PCR) was conducted in a final volume of 25 µl reaction mixture containing 12.5 µl of amplicon redeye master mix, 10 pM of each forward and reverse primer and 50 ng of genomic template DNA. The Bio-Rad thermal cycling profile for the reaction include initial denaturation at 95°C for 3 min followed by 35 cycles of denaturation at 95° C for 30 sec, annealing at 64° C for 30 sec, extension at 72° C for 1 min and final extension at 72° C for 10min.

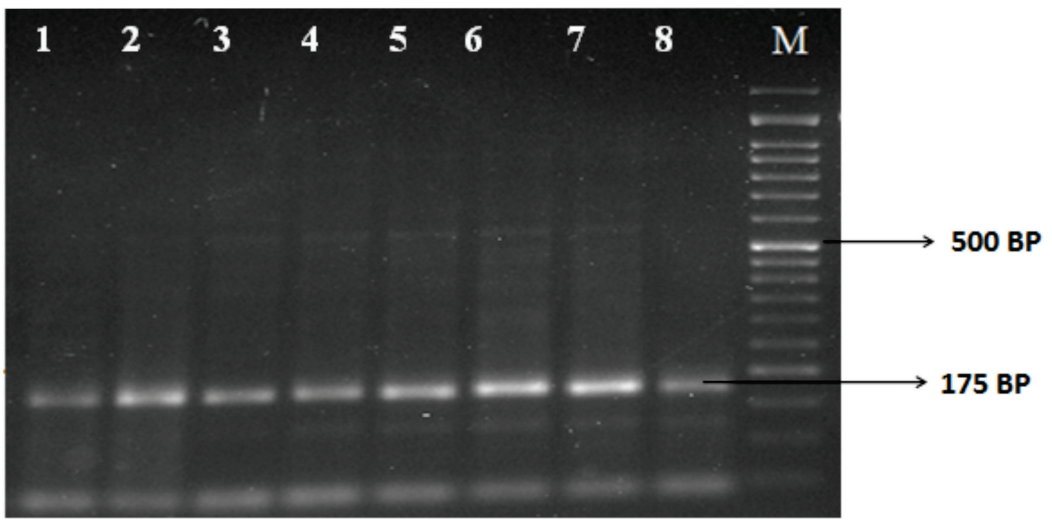
The PCR products were checked by 2 percent agarose gel electrophoresis using 50 bp ladder (Himedia) and visualized in UV transilluminator (Bio Rad, USA) after staining with ethidium bromide. The PCR products were further processed for SSCP by mixing the 5µl of PCR product with 15µl of SSCP dye. The mixture was subjected to denaturation at 95°C for 10 minutes, then immediately transfer to cool down at -20°C for 10 minutes.

To explore genetic polymorphism in 3'UTR of *SLC11A1* gene, after denaturation the PCR productswere subjected to 8% Polyacrylamide gel electrophoresis (29:1 Acrylamide: Bisacrylamide crosslinking). The pre run was given to Polyacrylamide gelat 100V for 30 minutes and then SSCP samples were loaded. Overnight Polyacrylamide gel Electrophoresis was carried out at 200V. After the run was completed, silver staining was carried out according to Bassam *et al*. (1991) with certain modifications to visualize the banding patterns. The allele and genotype frequencies were calculated and Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a Chi-square (χ^2)-test.

RESULT AND DISCUSSION

Johne's disease was confirmed as negative in all the suspected animals based on PCR technique and ELISA method. The PCR amplification yielded products at 175 bp (Figure 1) as expected for 3'UTR of *SLC11A1* gene. PCR amplicons were subjected to SSCP analysis to detect the polymorphic patterns of *SLC11A1*gene in Jersey crossbred cattle. PCR-SSCP analysis of *SLC11A1* gene (Figure 2) revealed AB, BB, CC and CB genotypes with predominance of BB genotype. The genotype frequencies of AB, BB, CC and

Figure-1. Resolution of SLC11A1 (3'UTR region) PCR product on 2 % agarose gel.



{Lane: 1 to 8 samples; Lane M: Marker (50 bp)

Figure-2. SSCP patterns of SLC11A1 gene (3'UTR region) in 8 % non-denaturing gels

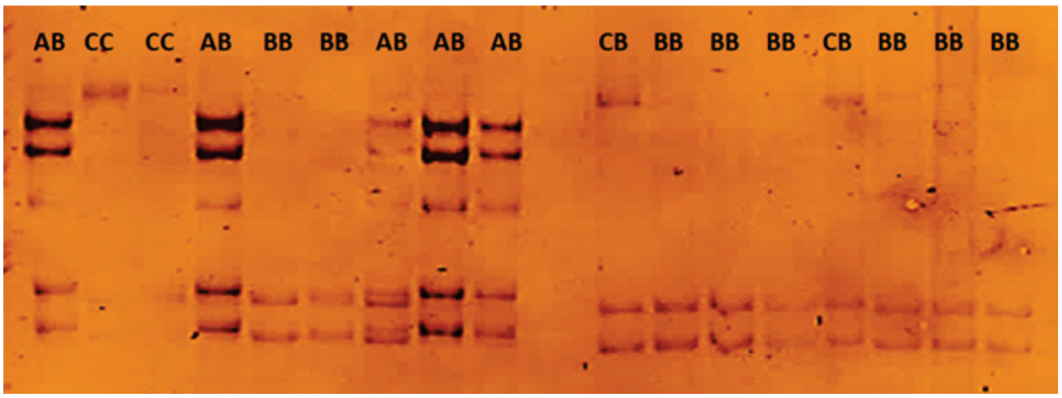


Table-1. Genotype and allele frequencies of SLC11A1 gene in Jersey crossbred cattle

Genotypic	Number of animals N=52	Observed Genotypic frequency	Allele frequency		Expected Genotype frequency	$\chi 2$ value (df = 2)
AA	0	0.00	**A**	0.14	0.021	21.32*
AB	15	0.288	**B**	0.615	0.177	
BB	21	0.404	**C**	0.24	0.378	
CC	9	0.173			0.058	
CB	7	0.135			0.295	

χ^2_{tab} value at 5% level and 2 df is 0.103. ($\chi^2_{cal} > \chi^2_{tab}$ value)

*Significant differences between observed and expected number of genotypes

CB were in the order of 0.29, 0.40, 0.17 and 0.14, respectively. The A, B and C allele frequencies were 0.14. 0.62 and 0.24 respectively (Table-1). The genotype AA was absent in the population studied. The present population was notin Hardy-Weinberg equilibrium for *SLC11A1*loci.

The variation of GT repeats in 3'UTR of *SLC11A1* gene have been reported to be associated with the resistance against Brucella and tuberculosis infection in cattle and buffalo (Ganguly *et al*. 2008, Capparelli *et al.,*2007), but natural resistance against mastitis could not be established (Zhang *et al*. 2009). Korou *et al.,* (2010) identified two polymorphic regions in the 3'UTR end of the *SLC11A1* gene and B_7 allele [AAGG$(GT)_7$GCACAC] was significantly associated with being ELISA negative for *Paratuberculosis* infection in goat.

Single-Stranded Conformational Analysis (SSCA) revealed a highly significant association of polymorphic $(GT)_n$ microsatellite identified in the 3'untranslated region (3'UTR) of bovine *NRAMP1* with natural resistance to *Brucellosis*(Adams and Templeton, 1998). The presence of polymorphism in 3'UTR

of this gene in the Jersey crossbred was reportedto be due to GT repeat sequence variation at targeted segment of *SLC11A1* gene(Adams and Templeton, 1998).

The PCR-SSCP analysisin Jersey crossbred cattle study revealed that *SLC11A1* gene was highly polymorphic and four PCR-SSCP patterns were observed. The highest *SLC11A1* genotypic frequency was observed for BB genotype (0.4). The allele frequency of B was 0.62. The detected polymorphic pattern can be used for identification of SNPs by sequencing the target region and association analysis with disease incidence caused by intracellular pathogens in larger population of Jersey crossbred cattle.

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