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Kaiho Kaisa

Research Scholars, Department of Animal Breeding and Genetics, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

N Shyamsana Singh

Associate Professor and Head, Department of Animal Breeding and Genetics, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

P Mayengbam

Associate Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

TC Tolengkomba

Assistant Professor (SG), Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

Corresponding Author:**TC Tolengkomba**

Assistant Professor (SG), Department of Animal Genetics and Breeding, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India

Exon 3 (A-3642C) of toll- like receptor 4 gene polymorphism in swamp buffalo of Manipur

Kaiho Kaisa, N Shyamsana Singh, P Mayengbam and TC Tolengkomba

Abstract

The study was conducted to study the polymorphism in bovine Toll-Like Receptor 4 gene in 50 swamp buffaloes of Manipur. Two different variants of SNP1 (A-3642C) of TLR4 gene Exon 3 viz. A and C were detected by PCR-RFLP using *AluI*. The frequency of C allele was predominant (0.95) among swamp buffalo population of Manipur. Among the genotypes, CC genotype was prevalent in very high frequency in the population. While the genotype AA was not observed in any of the sample evaluated. The population not conforming to equilibrium indicated exertion of sampling variation due to small population size in these buffalo population.

Keywords: indigenous cattle, North East India, toll- like receptor 4 gene, gene frequency

1. Introduction

Toll-like receptors 4 (TLR4) have been identified as crucial molecules for detection of invading pathogens and induction of host defence mechanism through recognition of pathogen-associated specific molecular patterns [1]. TLR4 gene have been found associated with mastitis in cattle and play a central role in innate immunity. It was reported that TLR2 and TLR4 genes played a role in the host response to inflammatory mastitis [2]. TLR-4 is able to recognize Gram-negative bacteria lipopolysaccharide (endotoxin) such as *Escherichia coli* and *Klebsiella*, cell wall components of other important bacteria and fungi such as *Mycobacterium tuberculosis*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans*, as well as cellular stress components, such as heat shock proteins, fibrinogen, among others [3]. TLR4 is critical in the immune response against Gram negative bacteria and virus [4]. Researchers have focused on identifying more informative genetic markers to allow faster and more accurate selection of bovine resistant to mastitis [5, 6, 7] and milk production [8].

The buffaloes of Northeast India inclusive of Manipur are mainly of swamp type. These swamp buffaloes are inhabitant of marshland or swamp areas, so, is their name derived where it wallows in mud, feeds on coarse marsh grass and comprised of 48 chromosomes. They are known for their adaptability and resistance to diseases, which need to be conserved as conservation and improvement of animal genetic resources available in different regions have been globally accepted.

The present work was therefore undertaken to study the genetic polymorphisms in SNP1 (A-3642C) position of TLR4 gene in the indigenous cattle of the three North East state of India vis-à-vis crossbred cattle by PCR-RFLP method.

2. Materials and Methods**2.1 Experimental animal and blood sampling**

The study was conducted on a total of 50 unrelated Swamp buffalo (*Bubalus bubalis*) of Manipur. These animals were randomly selected from field(s), private farm(s), institute(s) and organized herd(s) maintained in three districts of Manipur (viz. Imphal east, Imphal west and Senapati districts). Blood samples were collected aseptically from the jugular vein of the selected animals in vacutainer tubes containing EDTA. Cold chain was maintained during the transit of the sample from farm to laboratory and stored in deep freezer at -20 °C till further use.

2.2 Genomic DNA isolation

Genomic DNA was extracted using GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) according to the instruction manual.

The quantity and quality of DNA were checked with a NanoDrop MultiscanGo Spectrophotometer (Thermo

Scientific, USA). The primers and restriction enzyme used for PCR-RFLP analysis are given in Table 1.

Table 1: Gene location of locus, size of PCR product, primer sets, annealing temperature and restriction enzyme used for RFLF analysis

| Gene position (primer) | | Primer sequence (5' - 3') | RE | Product size (bp) | T ^A (°C) |
|------------------------|---|---------------------------|-------------|-------------------|---------------------|
| SNP1 (A-3642C) | F | TCAGGAATGCCACTTGTCTAG | <i>AluI</i> | 406 | 54 |
| | R | CAGGTCTGGGCAATCTCATA | | | |

2.3 PCR and RFLP

The PCR amplification was carried in a 25 µl of 10X PCR buffer, 2mM of MgCl₂, 200 µM of each dNTPs, 5 pM each of primers, 2 U Taq DNA polymerase and 60 ng genomic DNA. The following cycles were applied: at 95 °C for 5 min, followed by 35 cycles of – 94 °C for 30 sec, 54 °C for 45 sec, 72 °C for 30 sec and final synthesis at 72 °C for 10 min. The amplified DNA was digested with *AluI* enzyme by incubating at 37 °C for 3 hours. The digested products were separated in 2.5% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide and visualized under UV trans-illuminator and photograph were taken using Gel Doc system.

2.4 Statistical analysis

The allele and genotype frequency calculation as well as the chi-square test were carried out by using the Popgene32 software [9].

3. Results and Discussion

3.1 Genetic polymorphism in SNP1 (A-3642C) of TLR4 gene

The digestion of 406 bp PCR amplified fragment with *AluI* yielded two types of restriction patterns and accordingly two genotypes were identified. The genotype CC showed 248 bp and 158 bp fragments since there is restriction site for the enzyme in C allele. The AC genotype produced 406 bp, 248 bp and 158 bp fragments (Figure 1).

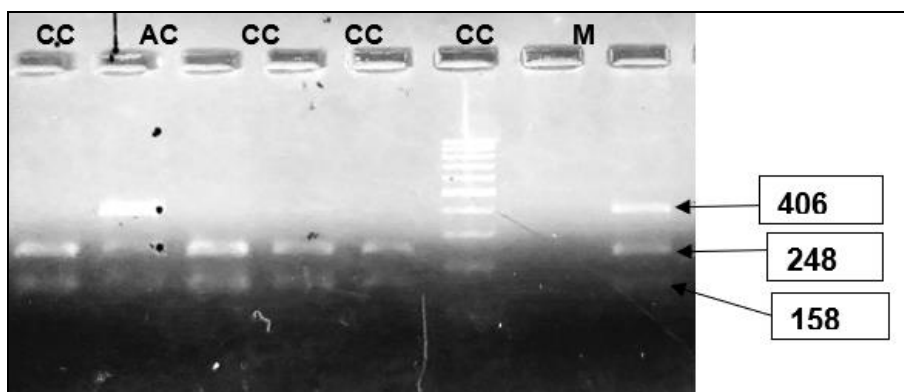


Fig 1: Genotype of SNP1 (A-3642C) of TLR4 gene digested with *AluI* in 2.5% agarose gel

The SNP1 (A-3642C) was found to be polymorphic with two alleles A and C in the swamp cattle population of Manipur. Similar polymorphism of this locus / position was also reported in Murrah buffalo [10].

3.2 Genotypic and Allelic frequencies in SNP1 (A-3642C)/*Alu I*

The genotypic frequencies of AC and CC genotypes were found to be 0.10 and 0.90, respectively in Swamp buffalo population of Manipur. The genotype AA was not observed in the population under study. In contrary to the present finding, all the three genotypes were reported to be observed in Murrah buffalo [10] with higher frequency of AC (0.62)

genotype.

In the present study, the A and C allele frequencies of SNP1 (A-3642C) were found to be 0.05 and 0.95, respectively. In previous study, Similar pattern with slightly lower distribution for C allele (0.604) was reported in Murrah buffalo [10]. In previous study, almost similar frequencies of A and C alleles (0.513 and 0.487) was reported in local cattle of Manipur [11]. The Chi square value revealed that the population was not conform to Hardy-Weinberg equilibrium. The reasons for absence of AA genotype and the population not following HWE may be attributed to the exertion of selection pressure towards a particular genotype or sampling error which may be ruled out by incorporating more samples.

Table 2: Genes and genotype frequencies of SNP1 (A-3642C) of TLR4 gene in the Swamp buffalo of Manipur

| Exon 4 (A-3642C) locus | | Frequency | χ^2 value |
|------------------------|----|-----------|----------------|
| Genotypes | AB | 0.10 (5) | 2412.58** |
| | BB | 0.90 (45) | |
| Alleles | A | 0.05 | |
| | C | 0.95 | |

** highly significant at $P > 0.01$; Values within the parentheses are the number of animals

3.3 Heterozygosity and PIC values

The present findings of high homozygosity and low heterozygosity in SNP1 (A-3642C) of TLR4 gene indicated low genetic variability within swamp buffalo population of

Manipur (Table 2). The Polymorphic Information Content (PIC) value for SNP1 (A-3642C) of TLR4 gene in swamp buffalo population of Manipur was low (0.091) which indicates less informativeness of the locus.

Table 3: Observed and expected homozygosities and Heterozygosities and PIC values of SNP1 (A-3642C) of TLR4 gene in the Swamp buffalo of Manipur

| Locus | Homozygosity | | Heterozygosity | | PIC |
|-----------------------------|--------------|----------|----------------|----------|-------|
| | Observed | Expected | Observed | Expected | |
| SNP1 (A-3642C) of TLR4 gene | 0.900 | 0.903 | 0.100 | 0.097 | 0.091 |

4. Conclusion

It can be concluded from above findings that SNP1 (A-3642C) of TLR4 gene showed polymorphism in the Swamp buffalo population of Manipur. Absence of a particular genotype reveals that selection pressure was imposed against the genotype. The information on variations in the locus may be useful in association studies of disease. Lower PIC values and heterozygosity suggested the scope for formulation of suitable breeding strategies to perpetuate the distribution of desirable alleles in swamp cattle population.

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