RESEARCH ARTICLE

Single nucleotide polymorphism at cluster of differentiation 14 (CD14) gene and its association with fertility traits in crossbred cattle of Kerala

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Abstract: Cluster of differentiation 14 (CD14) gene is an important molecule for innate immunity and found to be significantly associated with fertility traits in cattle. The objectives of present study were to detect the single nucleotide polymorphisms (SNPs) in exon 1 region of CD14 gene and to evaluate the association of genetic variants with fertility traits viz. Service period and Age at First Calving in crossbred cattle of Kerala. The study was conducted on two hundred and sixteen crossbred cattle maintained at various farms of Kerala Veterinary and Animal Sciences University. Genomic DNA was isolated and polymorphisms of gene were detected by Single Strand Confirmation Polymorphism. Two SNPs, c.445T>G and c.432C>T were detected in exon 1 coding region of CD14 gene. The frequencies of CC and CT genotypes were 0.82 and 0.18 in the population. In addition, a significant association between SNPs and service period was observed in crossbred cattle population under study. Crossbred cattle with CT diplo types showed lower values for service period than those with CC diplo types. The association of CD14 gene with these traits emphasizes the importance of bovine CD14 as a candidate gene for marker assisted selection for fertility traits in crossbred cattle

Keywords: Association, CD14, Crossbred cattle, Fertility, Polymorphism,

Introduction

India has the world's second largest bovine population with 192.49 million cattle, which is about 12.9% of the world's total cattle population. Crossbred cattle contribute around 24.45% to total milk and 54.89% to total cow milk production in the country (Anonymous, 2019). Kerala, one of the southern states of India, has a total cattle population of 1.33 million of which 94.20 % is crossbred cattle and only 5.80 % is indigenous (BAHS, 2019). Crossbred cattle of Kerala having exotic inheritance 50- 65 % arise due to crossing of low yielding *Bos indicus* with Holstein Friesian.

Fertility is economically important as it brings cattle into lactation, reduces reproductive disorders and maximizes the profitability by in time calf crop. Since milk production and fertility traits are negatively correlated, selection for enhanced milk performance may cause decline of cow fertility. Multitude of studies in dairy animals of developed countries have shown that selection for higher milk yield alone is associated with reduced health and fertility (Lucy, 2001; Van Raden et al. 2004; Cole and Van Raden, 2010; De Vries, 2019). Fertility can be measured by calving interval, calving rate, service per conception and age at first calving. Conventional selection relies upon phenotypic information only and causing slow genetic gain in the population. To augment the selection response for milk yield in dairy cattle, traditional selection methods could be complemented with gene assisted or marker assisted selection (MAS) using genomic variation of the traits (Wakchaure et al. 2015).

Cluster of differentiation 14 (CD14) gene is an important molecule for innate immunity that can act against a wide range of pathogens. CD molecule ranges from 1 to 166 with differential structure and functions of these CD14 is the most important molecule functions both as a cell membrane receptor and a soluble receptor for bacterial lipopolysaccharide (Goldsby et al. 2000; Pal et al. 2011). CD14 is found to be related to fertility traits in Holstein cattle by Cochran et al. (2013) and Ortega et al. (2017). Immune function is an important determinant of reproductive function. It might be due to involvement of immune function in the establishment of pregnancy (Hansen, 2011). Banos et al. (2013) identified significant phenotypic correlations of immune traits

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with reproductive performance traits. They found positive correlation of Cluster of differentiation (CD) cells within the peripheral blood mononuclear cell population and calving interval. Cochran et al. (2013) identified 10 genes containing SNP related to reproductive traits that were involved in immune function.

However there is no report regarding genetic variation of CD14 in crossbred cattle of Kerala state, India. Hence present study was carried out with the objective to identify genetic polymorphism in exon 1 of CD14 and to explore association of this region with fertility traits of crossbred cattle.

Material and Methods

Collection of blood and DNA isolation

Approximately, 5 ml of venous blood was collected from 216 crossbred cattle maintained at the University Livestock Farm, Mannuthy; Cattle Breeding Farm, Thumburmuzhy and different field centres of ICAR- Filed progeny testing scheme, Mannuthy. Samples were stored at -20 °C until isolation of DNA. Genomic DNA was isolated from the frozen blood samples using phenol-chloroform extraction method (Sambrook and Russel, 2001) and samples were checked for its quality, purity and concentration.

Polymerase chain reaction

Primers (forward 5'AGTGTGCTTGGGCAATGTTC 3', reverse 5'CGGGTACTCTCGTCTCAAGG 3') were designed using primers3 software from the published information available in Genbank (accession no: NC_037334.1) for the amplification of exon 1 region of CD14 and were custom synthesised (Sigma-Aldrich). PCR reactions were carried out in 25 µL volume using 50 ng of genomic DNA, 5 mL of 10X Buffer, 1 mL of 10mM dNTP, 10 pM each of forward and reverse primers, and 1 mL of JumpStart AccuTaq LA DNA Polymerase (2.5U/mL) with proofreading 5 Activity (Sigma-Aldrich) in a thermal cycler (Bio-Rad – My Cycler). The optimum PCR conditions was 95°C for 3 min, followed by 35 cycles (30 sec at 94°C, 45 sec at 61°C and 45 sec at 72°C), followed by 1 cycle at 72°C for 5 min, stopped at 4°C. Amplified PCR products were loaded into the wells of 1.5% agarose gel with a standard 50 bp DNA ladder (GenerRuler, MBI Fermentas, Germany) as a marker to check the size of the fragment. Electrophoresis was carried out at the rate 6 Volts/cm in 1X TBE buffer. Gels were stained with ethidium bromide and visualised under UV light and documented in a gel documentation system (Bio-Rad, USA).

Genotyping

Genotyping of the samples was done by Single Stand Confirmation Polymorphism (SSCP). The amplified fragments were mixed with SSCP loading buffer in the ratio of 1:3 (10 µL sample with 30µL dye), denatured at 95°C for 10 min and immediately snap chilled in ice. The products were run in 12% poly-acrylamide gel at 4°C

for 2 h 30 min at 200 V. The composition of poly-acrylamide gel was 30% acrylamide/bis-acrylamide (29:1)-12 mL, 10% Ammonium per sulphate-150µL, TEMED-30 µL, 1X TBE-3 mL and nuclease free water-14.82mL. Gels were stained with silver nitrate as per the procedure described by Byun et al. (2009) and SSCP fragments were visualised directly. Diplo types were detected directly by observing SSCP pattern of samples in the gels. The haplotype and diplo type frequencies were estimated by direct counting method (Falconar and Mackay, 1998). Representative samples from different diplo types were sequenced to find out the nucleotide differences between different haplotype by automated sequencer (ABI prism) using Sanger's dideoxy chain termination method.

Statistical analysis

Major fertility traits considered in the study were age at first calving (AFC) and service period (SP). Association analysis of genotypes with fertility traits and major non-genetic and genetic factors viz. season and period of calving, centre and sire, were analyzed using fixed General Linear Model (GLM) of SPSS V.21.

Results and Discussion

Fertility is a complex process, influenced by genetic, nutritional, environmental factors and genotype-environmental interactions. Fertility traits such as age at first calving and service period have important impact on the profitability of dairy industry. Therefore, selective breeding for optimal fertility traits is vital for improving genetic gain.

PCR-SSCP was performed to detect the SNPs in exon 1 of CD14. In the present investigation, 206 bp fragment were amplified and PCR-SSCP of this fragment, corresponding to the exon 1 region exhibited pattern with two bands were identified as CC and four bands as CT diplo type (Fig. 1). The sequencing analysis of different haplotypes reveals presence of two SNPs; one T to G transversion at 103th position and other C to T transition at 116th position of the 206 bp fragment (Fig 2). In comparison with available GenBank sequence (accession no: NC_037334.1), T to G transversion was located in nucleotide sequences at positions 445 in exon 1 of CD14 and at 149th position of ORF. Further analysis revealed c.445T>G, was a non-synonymous mutation and it resulted in an amino acid substitution due to a codon change of TCA to GCA. SNP (C>T transition) at 432 position of the exon 1 (144th position of ORF) was a synonymous SNP with codon change of CGC to CGT. Ibeagha-Awemu et al. (2008) detected five SNPs in 5' untranslated region (UTR) (g.C1291T), two in the coding regions (g.A1908G and g.A2318G) and two in the 3' UTR (g.A2601G and g.G2621T) of CD14 in Canadian Holsteins and Jersey cows. Pal et al. (2011) observed 27 SNPs in CD14 of crossbred cattle in which 18 SNPs are non-synonymous mutations. They also suggested that -synonymous substitutions exceeding synonymous substitutions indicate the evolution of

Fig 1. SSCP pattern of 206 bp fragment of CD14 gene (CC and CT genotype)

Fig 2. Sequence map for two Single Nucleotide Polymorphisms in the exon 1 of CD14 gene

this protein through positive selection among domestic animals. Selvan et al. (2014) screened Karan Fries (KF) cattle of India and reported six nucleotide changes in KF cows at positions T1117D, T1239G, T1291C, G1359C, G1361A and G1811A in a 832bp region (part of promoter, 5'UTR, exon 1, intron 1 and part of exon 2) of bovine CD14. However, Kumar et al. (2014) also reported these SNPs except T1117D in Sahiwal (*Bos indicus*) cows.

The diplotypic and allelic frequencies based on PCR-SSCP pattern are presented in the Table 1. CC diplo type frequency and the frequency of 'C' haplotypes were found to be predominant in studied population. In the present study homozygote, TT diplo type was not observed in the screened population and the 'C' haplotype was almost fixed in the population. Chi-square analysis showed non-significant differences between breeds with respect to CD14 locus. It revealed that the screened population is under Hardy Weinberg equilibrium ($\chi^2=2.13<3.841$).

In the association analysis of CD14 genotypes with fertility traits, it was revealed that SP was significantly influenced by genotype ($p < 0.05$). Significantly lower SP was observed for cattle with diplo type CT (98.85 days), compared to the heterozygous diplo type CC (131.393 days). AFC found to be significantly affected by centre and season ($p < 0.05$). Different genotypes of CD14 gene were not significantly associated with AFC. Ortega et al. (2017) evaluated 68 SNP in candidate genes associated with genetic merit for fertility traits such as predicted transmitting ability (PTA) for Daughter Pregnancy Rate and reported that SNP (rs109621328) in CD14 significantly associated with services per conception and days open. Cochran et al. (2013) genotyped 434 candidate SNPs in 550 Holstein breed and found that SNP (rs109621328) in CD14 is significantly associated with daughter pregnancy rate and heifer conception rate.

Table 1 Diplotype and haplotype frequencies of exon 1 (206 bp fragment) of CD14 gene based on SSCP pattern

Parameter	Total (216)
Diplotype Frequency	
CC	0.82 (177)
CT	0.18 (39)
Haplotype Frequency	
C	0.90 (393)
T	0.09 (39)
χ^2	2.13

Conclusions

PCR-SSCP analysis of exon 1 of CD14 established presence of a non-synonymous mutation (c.445T>G) and a synonymous mutation (c.432C>T) in crossbred cattle population of Kerala. In the present study two diplo types of CD14 in the screened animals were observed. Diplo types and fertility traits especially service period in the tested population of crossbred cattle showed significant association. Although CC diplo type was predominant in the population, CT was significantly associated with lower service period. Therefore, genetic variants of CD14 appear to be potential candidates for the selection of fertility trait improvement. However, SNP identified in the current study may be characterized by functional genomics studies, as well as in a large population for further validation.

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