F XI Gene Deficiency in Sahiwal and Karan Fries cattle in India

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Summary
FXI gene deficiency is a 76-bp insertion of an imperfect poly-adenine (Poly-A) tract occurring in exon 12, this insertion introduces a stop codon that results in a FXI protein lacking the functional protease domain encoded by exons 13, 14 and 15. Since very little work has been done in India for FXI gene deficiency and it was very important to screen Indian cattle for presence of FXI gene deficiency. Blood samples for DNA extraction from 202 Sahiwal and 174 Karan Fries cattle were collected. DNA polymorphism using PCR technique was carried out to genotype the animals for FXI deficiency with reported primers to amplify exon 12 (244 and/or 320 bp PCR products) of FXI gene. PCR amplification was standardized at 55 °C. Genotyping of all the animals was carried out in 2.5% agarose gel at 100 volts for 40 minutes. No polymorphism was detected at FXI gene exon 12 of Sahiwal and Karan Fries cattle. Data on repeat breeding incidences were collected from reproduction records for Sahiwal and Karan Fries cattle. As no polymorphism of FXI exon 12 was observed, hence, it was not feasible to explore association with repeat breeding. It is concluded that Sahiwal and KF cattle included in the present study were free from FXI gene deficiency.

Keywords: author; guide- Coagulation Factor XI, Karan Fries, Sahiwal, PCR-RFLP

Introduction

Livestock plays an important role in Indian economy. About 20.5 million people depend upon livestock for their livelihood. Livestock contributed 16% to the income of small farm households as against an average of 14% for all rural households. Livestock provides livelihood to two-third of rural community. It also provides employment to about 8.8 % of the population in India. India has vast livestock resources. Livestock sector contributes 4.11% GDP and 25.6% of total Agriculture GDP.

India is
- World’s highest livestock owner at about 512.05 million
- First in the total buffalo population in the world - 105.3 million buffaloes
- Second in the population of cattle and goats - 140.5 million goats
- Second largest poultry market in the world - production of 63 billion eggs and 649 million poultry meat.
- Third in the population of sheep (72 millions)
- Fifth in the population of ducks and chicken
- Tenth in camel population in the world.
Disease acts as a negative influence on the livestock production system, thus setting off a cascading affect of low production, low income, and subsistent livelihood. The consequences of animal diseases in livestock can be complex and generally go well beyond the immediate effects on affected producers. These diseases have numerous impacts, including productivity losses for the livestock sector (production losses, cost of treatment, market disturbances), loss of income from activities using animal resources (energy, transportation, tourism), prevention or control costs (production costs, public expenditure) and suboptimal use of production potential (animal species, genetics, livestock practices). There are various types of diseases which cause huge economic losses in dairy sector. Among them one of the important diseases is repeat breeding. Repeat breeding may occur due to number of cause such as hormonal deficiency, nutrient deficiency, microbial infection etc. (McInerney, John (1996))

Repeat breeding syndrome is responsible for long service period and inter-calving interval thereby causing low milk and calf production resulting into greater economic loss to dairy industries. To curtail these losses, correct and early diagnosis of the exact etiology followed by timely veterinary interventions is a pre-requisite. The factors responsible for this malady are multiple viz. anatomical, hormonal, managerial and infectious, and vary from herd to herd, animal to animal and estrus to estrus. The incidence of repeat breeding in India has been reported from 5.5 to 33.33% in cattle and 6 to 30% in buffaloes (Saxena, 2004). In view of the fact that the incidence of repeat breeding syndrome has increased over decades, therefore, it becomes very important to have a check on incidence of repeat breeding and thereby reduce the economic loss to dairy sector. Prevention is better than the cure, so this prevention can be achieved by two different ways: either by immuno/chemoprophylaxis (vaccines) or by genetic improvement (selective breeding). Molecular and physiological markers of disease resistance or susceptibility are of particular interest because they allow identification and selection of disease-resistant animals in early stage of life.

Factor XI is the Zymogen form of factor Xla (FXIa), an enzyme of the coagulation cascade. Like many other coagulation factors, it is also a plasma serine protease. Factor XI (FXI) is produced by the liver and circulates as a homo-dimer in its inactive form. FXI gene of cattle is 19150 bp in length (17607721 - 17626871 nt). It has 15 exons and 14 introns and is located on BTA 27. Studies done at international level showed that FXI gene deficient cattle were highly susceptible to infectious diseases, showed increased frequencies of repeat breeding and lower rate of fetal and calf survival. (Gentry et al., 1993; Liptrap et al., 1995; Coomber et al., 1997)

The present study was carried out to detect the presence of coagulation factor XI (FXI) gene deficiency and to relate FXI gene deficiency with incidence of repeat breeding. Coagulation is a complex process by which blood forms clot. It is an important part of hemostasis. Coagulation is cessation of blood loss from a damaged vessel. A damaged blood vessel wall is covered by platelet and fibrin-containing clot to stop bleeding and begin repair of the damaged vessel. FXI is one of the 13 factors involved in the process of coagulation. Factor XI is the Zymogen form of factor Xla (FXIa), an enzyme of the coagulation cascade. FXIa and FIXa along with FVIIa are responsible for conversion of FX to its activated form FXa. The FXa converts prothrombin to thrombin which in turn results in the formation of insoluble fibrin clot. (Liptrap et al., 1995) found that FXI-deficient Canadian Holstein cattle have 50% greater prevalence of repeat breeding. Studies done at international level showed that FXI gene deficient cattle exhibited increased susceptibility to infectious diseases, increased frequencies of repeat
breeding and decreased rate of fetal and calf survival (Gentry et al., 1993; Coomber et al., 1997; Watanabe et al., 2006; Ohba et al., 2008). However, no comprehensive work has been done on FXI gene deficiency in indigenous cattle in India. So the present study was undertaken with following objectives:
1. To screen Sahiwal and Karan Fries cattle for the presence of Factor XI deficiency.
2. To find out association of Factor XI gene deficiency with repeat breeding.

Material and methods

Blood samples were collected from randomly selected 202 Sahiwal cattle (Bos indicus) and 174 Karan Fries cattle (HFxTP crossbred). DNA extraction was done using Phenol-chloroform method as described by (Sambrook et al. 1989) with few modifications and DNA isolation kit for mammalian blood (Roche Diagnostics GmbH). The quality of DNA obtained from both the methods was good, however, yield of DNA by phenol-chloroform extraction method was comparatively more than that of by Roche kit. Gene-specific oligonucleotide primers for bovine factor XI gene were synthesized as described by (Marron et al., 2004) to amplify exon 12 (244 and/or 320 bp) of FXI gene.

The PCR amplification was performed in programmed Thermal cycler (MJ research) in a final volume of 25 ul. 22 ul of PCR reaction mixture was mixed thoroughly by vortexing with 3 ul template DNA (50ng/ul) and PCR amplification was performed in a final volume of 25 ul. The PCR reaction mixture was incubated in thermal cycler initially at 94 °C for 2 minutes followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 1 minute, 72 °C for 1 minute and a final extension of 72 °C for 10 minutes to obtain PCR product of amplified DNA. The PCR products were checked by electrophoresis on 1.5% agarose gel at 100 volts for 30 minutes to verify the amplification of target region. Genotyping of animals was carried out by 2.5% Agarose gel horizontal electrophoresis at 100 volts for 40 minutes. The gels were stained with 1% ethidium bromide solution (@ 1µl/100 ml of gel) and scored for the presence of single and double bands for homozygous and heterozygous individual respectively. The gel photograph was taken by gel documentation system.

Result and Discussion

The genotyping of Sahiwal and Karan Fries cattle revealed only one band of 244 bp in all the animals as shown in Plate 1 and Plate 2 indicating that there is no 76-bp insertion in exon 12. The representative samples of Sahiwal and Karan Fries obtained by PCR analysis were custom sequenced. Sequence data was analyzed using ChromasVer.5,http://www.technelysium.com.au/chromas.html). Multiple sequence alignments were performed to study nucleotide and amino acid changes among Sahiwal, Karan fries and reference sequence of Bos taurus., indicating that there is no 76-bp insertion in exon 12. There was neither homozygous mutant nor heterozygous carrier individual, hence, it is concluded that there is no polymorphism of FXI gene in the studied animals of Sahiwal and Karan Fries breed of cattle. Only one nucleotide change from G to A at position 105 in Sahiwal and Karan Fries was observed when compared with Bos taurus reference sequence and similar substitution was also seen in chromatograph. This non synonymous mutation resulted into a change of amino acid from Arginine (R) to Glutamine (Q) in second codon ( CGA TO CAA) as revealed by in silico nucleotide translation and the alignments of Sahiwal, Karan fries and Bos taurus. The contigs
containing exon 12 of Factor XI gene were subjected to basic local alignment search (BLAST) to know the sequence similarity with the corresponding regions of other species. BLAST analysis of Factor XI gene for exon 12 regions of Sahiwal and Karan Fries cattle showed 96 to 98% sequence identity with buffalo and *Bos Taurus*.

**Plate 1**  
PCR patterns of FXI gene Exon 12 of Sahiwal Cattle

- **Lane 1-15**: 244 bp monomorphic
- **Lane M**: 100 bp ladder

**Plate 2**  
PCR patterns of FXI gene Exon 12 of Karan Fries Cattle
Lane 1-16 : 244 bp monomorphic
Lane M   : 100 bp ladder

Figure 1  Multiple sequence alignment of nucleotides of FXI Exon 12

Figure 2  Chromatogram of FXI Exon 12 showing nucleotide change
Figure 3  Multiple sequence alignment of amino acids of FXI Exon 12

References

REFERENCE


