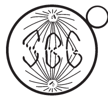
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Short Communication

Factor XI deficiency in Indian *Bos taurus*, *Bos indicus*, *Bos taurus x Bos indicus* crossbreds and *Bubalus bubalis*

Rajesh K. Patel[1], Kalpesh J. Soni[1], Jenabhai B. Chauhan[1], Krishna M. Singh[1] and Krothapalli R.S. Sambasiva Rao[2]

[1]*R&D-Biotechnology, National Dairy Development Board, Anand, India.*
[2]*Centre for Biotechnology, Acharya Nagarjuna University, Nagarjunanagar, India.*

Abstract

We investigated the occurrence of Factor XI (FXI) deficiency syndrome in the following Indian dairy animals: *Bos taurus* Holstein-Friesian and Jersey cattle, *Bos indicus* Indian cattle breeds, *B. taurus* x *B. indicus* crossbreds and the river buffalo *Bubalus bubalis*. Factor XI deficiency is an autosomal recessive bleeding disorder known to affect Holstein cattle worldwide. A total of 1001 dairy animals, mainly bulls, were genotyped to detect the mutation within exon 12 of the gene encoding for factor XI. Two Holstein bulls were detected as heterozygous (carrier) for FXI deficiency, giving a carrier frequency of 0.6% in Indian Holstein cattle. None of the other cattle or buffalo breeds was found to be a carrier for FXI. Sequence comparison between normal and heterozygous animals revealed that there is a 77 base pair insertion fragment (AT (A)29 TAAAG (A)27 GAATTATTAATTCT) within exon 12 of the *FXI* gene. Both sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank and assigned the accession numbers DQ438908 for normal Holstein Friesian animals and DQ438909 for heterozygous Holstein Friesian animals.

Key words: autosomal recessive disease, blood coagulation, buffaloes, Factor XI, Holstein-Friesian dairy cattle.

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Factor XI (FXI) is one of more than a dozen proteins involved in the early blood coagulation cascade (Brush *et al.*, 1987), FXI-deficiency syndrome being an autosomal recessive bleeding disorder first discovered in Holstein cattle in Ohio (Kociba *et al.*, 1969) but also reported in many other countries, including, but not only, Canada (Gentry *et al.*, 1975), England (Brush *et al.*, 1987) and Japan (Kunieda *et al.*, 2005). Bovines with FXI-deficiency may have lower calving and survival rates and increased susceptibility to infectious diseases (Liptrap *et al.*, 1975). The 3' end of the gene for bovine FXI is located on bovine chromosome 17 and was first characterized by Robinson *et al.*, (1997). Kunieda *et al.*, (2005) isolated and sequenced the entire 8910 bp coding region, comprising 15 exons and 14 introns, of the Japanese black cattle bovine FXI gene in bovines. In India, however, there has been no reported incidence of FXI-deficiency but since Holstein-Friesian *Bos taurus* cattle are extensively used for crossbreeding with many indigenous Indian *Bos indicus* cattle it is mandatory to screen breeding bulls, especially Holstein-Friesian and Holstein-Friesian crossbred bulls, for FXI-deficiency along with the ongoing screening for bovine leukocyte adhesion deficiency syndrome (BLAD), deficiency of uridine monophosphate synthase (DUMPS) and citrullinaemia in order to prevent the propagation of these genetic diseases in the Indian cattle population.

Send correspondence to R.K. Patel. National Dairy Development Board, 388 001 Anand, India. E-mail: rkpatel46@yahoo.com.

We examined a panel of 1001 dairy bulls made up of various breeds of exotic (B. taurus) cattle, native *B. indicus* zebu cattle, *B. taurus* x *B. indicus* crossbreds and water buffalo (*Bubalus bubalis*) is maintained at different semen production centers and breeding farms across India country (Table 1).

Blood samples (5 mL) were collected in heparinized tubes from each bovine by jugular veinipuncture. For genomic DNA extraction, blood samples were lysed, washed with 40 mL of lysing solution (0.32 M sucrose, 10 mM tris-HCl with pH 8.0, 5 mM $MgCl_2$ and 1% triton X-100) and pelleted by centrifugation. The leukocyte pellet was resuspended in 4 mL of nucleus lysis buffer (75 mM NaCl and 24 mM EDTA) plus 400 μL of 20% (v/v) sodium dodecyl sulfate (80 mg) and 25 μL of 20 mg mL^{-1} (500 μg) proteinase K (Genei, Banglore, India) and incubated overnight at 37 °C, after which the DNA was prepared by the organic extraction method described by Sambrook *et al.* (1989). The quantity of genomic DNA was determined spectrophotometrically and its quality by agarose

Table 1 - Details of the samples collected from different breeds of *Bos taurus, Bos indicus* and *B. taurus x B. indicus crossbred* cattle and the river buffalo *Bubalus bubalis*. (All the animals were bulls).

Breeds	Number of samples	Number of FXI mutants detected
Cattle		
B. taurus		
Holstein Friesian	330	2
Holstein Friesian x *B. indicus*	265	none
Jersey	105	none
Jersey x *B. indicus*	69	none
B. indicus		
Kankrej	23	none
Redsindhi	16	none
Khillari	13	none
Gir	11	none
Sahiwal	5	none
Haryana	4	none
Deoni	3	none
Red Khandhari	3	none
Dangi	1	none
River buffaloes		
Bubalus bubalis		
Murrah	56	none
Surti	46	none
Pandharpuri	34	none
Mehsana	15	none
Jaffarabadi	2	none
Total	1001	2

gel-electrophoresis, both by standard methods, prior to PCR amplification.

The insertion mutation within exon 12 was detected using the PCR assay described by Marron *et al.*, (2004) with minor modifications. The PCR assay was set up under sterile conditions in 200 μL capacity PCR tubes containing 100 ng of genomic DNA template, 0.4 pM each of forward (5'CCCACTGGCTAGGAATCGTT3') and reverse (5'CAAGGCAATGTCATATCCAC3') primers, 1X PCR buffer (10 mM Tris-Cl: pH - 9.0, 50 mM KCl, 0.01% gelatin and 1.5 mM $MgCl_2$), 400 μM of each dNTP and 1 unit of *Taq* DNA polymerase (Genei, Banglore, India) in a final reaction volume of 25 μL. The PCR was carried out using a Biometra T1 Thermocycler and the following protocol: initial denaturation at 94 °C for 3 min followed by 30 cycles of 94 °C for 1.5 min, annealing of primers at 55 °C for 1 min and extension at 72 °C for 2 min, followed by final extension at 72 °C for 10 min. The amplification products were separated in 2.5% (w/v) agarose gels, stained with ethidium bromide and viewed under UV light. The amplification products were commercially cloned and sequenced at Genei, Bangalore, India. A 320 bp amplification product, identified as the carrier for FXI-deficiency, and a 244 bp normal FXI product were purified using a PCR product purification kit (Genei, Bangalore, India) and cloned in a pDK101-based (a modified pGEM 2T) T/A cloning vector. The clones were confirmed by insert release using the Nco *I* restriction enzyme (Genei, Banglore, India). The cloned DNA was sequenced using a vector specific primer (M13U18) in an automated DNA sequencer with fluorescent dye terminator (ABI 3100, Applied Biosystems, California). The sequence obtained in our study was then compared with the GenBank database using the basic local alignment search tool (BLAST) program of the National Center for Biotechnology Information (NCBI).

Agarose gel-electrophoresis analysis of the amplified product from 999 of the bovines tested revealed only a 247 bp normal FXI fragment while two Holstein-Friesian animals exhibited both 247 bp and the 324 bp FXI-deficiency fragments (Figure 1), indicating that these animals both carried the Holstein-Friesian mutation in the FXI gene. The frequency of carrier bovines in Indian Holstein-Friesian cattle was, therefore, 0.6%.

To confirm the FXI gene mutation, the PCR amplicon was successfully cloned into the pDK101-based T/A cloning vector, which was transformed in DH_5 alpha competent cells. The clones were confirmed by plasmid DNA isolation followed by release of the insert (244 bp and 320 bp) using the *Nco I* restriction endonuclease (Genei, Banglore, India). The single-strand of the entire clone (insert + vector) was sequenced using the M13U18 vector specific primer (Genei, Banglore, India). After screening for vector contamination, 247 bases and 324 bases were obtained which and subjected to BLAST analysis to find the region of local similarity between sequences. The BLAST search revealed 98% homology with the factor XI gene of *Bos taurus* followed by *Bubalus bubalis.* The final 247 bp (Normal) and 324 bp (Carrier) bovine FXI gene sequences were submitted to NCBI and assigned the GenBank accession numbers DQ438908 and DQ438909 respectively. The complementary strand was not sequenced.

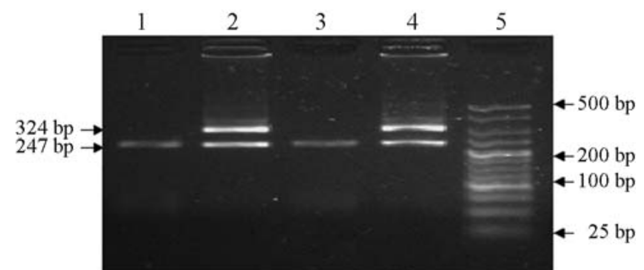

Figure 1 - Electrophoretogram of PCR product generated by amplification of genomic DNA using gene specific primers. Lane 1 and 3 # 247 bp fragment of normal animal, lane 2 and 4 # 324 bp and 247 bp fragments of carrier animals, lane 5# 25 bp DNA ladder.

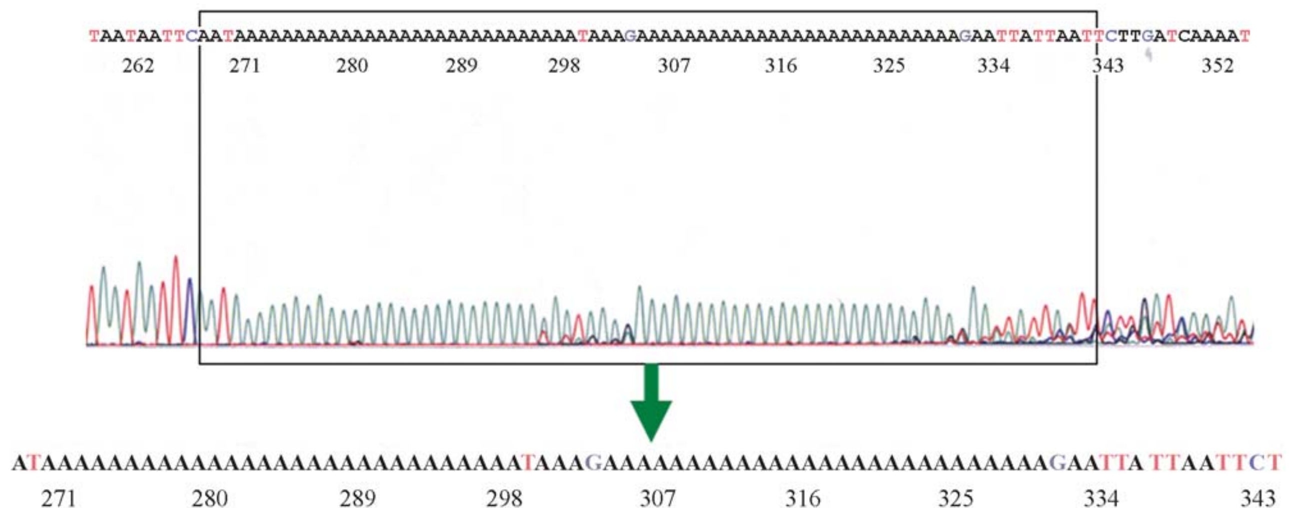

Figure 2 - Electropherogram showing fragment of 77 bases insertion mutation within exon 12 of the factor XI mutated allele from carrier Holstein-Friesian cattle.

In this study a total of 1001 dairy bulls were screened for FXI-deficiency but only two were found to carry the mutant FXI gene. One carrier was a three-year old Holstein-Friesian bull from a farm in southern India. To study the mechanism of FXI-deficiency inheritance we examined the pedigree of this bull and found that it was sired by artificial insemination using semen imported from Denmark, the dam exhibiting the normal FXI genotype. This suggests that the mutant allele could be transmitted from the sire. The other carrier was an 18-month old Holstein-Friesian bull from a farm in western India. A detailed inheritance pattern study revealed a normal sire genotype but the half-sib progeny of the FXI-carrier animal revealed that the possible mode of inheritance could be from the dam only. Unfortunately, the dam could not be genotyped because it had previously died. At 0.6% the frequency of FXI-deficiency carriers in the Holstein-Friesian population observed in our study is half that of the 1.2% reported in the Holstein sire population of the USA (Marron *et al.*, 2004). The mutant gene for factor XI deficiency in Indian Holstein-Friesian cattle was higher than citrullinaemia (Murleedharan *et al.*, 1999; Patel *et al.*, 2006) and very much lower than BLAD (Patel *et al.*, 2007), other autosomal recessive genetic diseases observed in Indian Holstein population. Because of the paucity of literature regarding the molecular diagnosis of FXI-deficiency, it is difficult to compare the carrier-frequency in the Indian Holstein-Friesian population to that found in other countries. In our study, sequence analysis of the single strand revealed a stop codon within the first four bases in the insertion followed by poly-adenine residues. The truncated protein could be missing the entire serine protease domain (Asakai, 1987). Sequencing of the PCR product followed by vector contamination revealed 247 bp and 324 bp fragments in normal and heterozygous carrier animals respectively, clearly suggesting that the PCR product size is not similar to the 244 and 320 bp bases reported by Marron *et al.*, (2004). One of the interesting findings in our study is that there was a 77 bp insertion (AT (A)29 TAAAG (A)27 GAATTATTAATTCT) in the exon 12 of the bovine FXI gene, the partial sequence of this insertion being shown in Figure 2). This contrasts with the findings of Maroon *et al.* (2004) who reported a 76 bp insertion in American Holstein cattle. Heterozygous bovines show varying symptoms and degree of reduced FXI activity (Gentry and Black, 1980), but this is difficult to distinguish because of the overlap in the normal and carrier activity ranges.

The occurrence of FXI-deficiency in two Indian Holstein-Friesian bulls is alarming and emphasizes the need for the regular genotyping of breeding bulls, especially Holstein-Friesian cattle and their crossbreds, for FXI-deficiency along with the ongoing screening for BLAD, citrullinaemia and DUMPS before induction of bulls into artificial insemination and crossbreeding programs.

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