Identification of factor XI deficiency in Holstein cattle in Turkey
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Abstract

Background: Factor XI (FXI) is a plasma protein that participates in the formation of blood clots. Factor XI deficiency is autosomal recessive hereditary disorder that may be associated with excess bleeding in Holstein cattle.

Methods: In this study, 225 Holstein cows reared in Turkey were screened in order to identify FXI genotypes. DNA extractions were obtained from the fresh blood of the cows. Amplicons of FXI exon 12 were obtained by Polymerase Chain Reaction (PCR), and analyzed by 2% agarose gel electrophoresis stained with ethidium bromide. Additionally, all cows were confirmed by DNA sequencing to determine whether or not there was a mutant allele.

Results: Carriers of the FXI deficiency have two DNA fragments of 320 bp and 244 bp in size. The results of our study demonstrated that only four out of the 225 Holstein cows tested in Turkey carried the FXI deficiency. The frequency of the mutant FXI allele and the prevalence of heterozygous cows were found as 0.9% and 1.8%, respectively.

Conclusion: The DNA-based test determines all genotypes, regardless of phenotype or FXI activity. The mutation responsible for the FXI deficiency had not been detected in Holstein cattle in Turkey prior to this study. The frequency of the mutant FXI allele needs to be confirmed by carrying out further analyses on cattle in Turkey and the selection programs should be developed to eliminate this genetic disorder.

Background

Factor XI is one of more than a dozen proteins involved in blood clotting. FXI deficiency has been identified in several species of mammals, including humans, dogs and cattle [1-4]. In cattle, FXI deficiency has been described in Holstein cattle in Ohio [5] and later in Canadian cattle [6], while some cases of hemorrhagic problems in British cattle have been reported [7]. FXI deficiency may result in prolonged bleeding and anemia. Continued bleeding from the umbilical cord is sometimes seen in affected calves. Prolonged oozing of blood following dehorning or castration may also be observed.

Affected cows frequently have pink-colored colostrum. Blood in the milk led to the identification of the condition in a British dairy herd [8]. Additionally, FXI deficiency causes to reduced reproductive performance and affected animals appear to be more susceptible to diseases such as
pneumonia, mastitis and metritis. Therefore, the presence of this genetic defect may have a significant economic impact on the dairy industry [3,8-10]. Affected animals can survive for years with no overt clinical signs, even though they appear to have a higher mortality and morbidity rate.

Pedigree analysis indicates that FXI deficiency is an autosomal recessive disorder like BLAD (Bovine Leukocyte Adhesion Deficiency), DUMPS (Deficiency of Uridine Monophosphate Synthase), and CVM (Complex Vertebral Malformation). Accordingly, carriers (heterozygous) of the defective gene are outwardly normal, while affected animals (homozygous) have a mild hemophilia-like disorder; 25 percent of the offspring of a carrier bull and a carrier cow will be affected with a FXI deficiency [8]. Carrier cattle exhibit varying symptoms and degrees of reduced FXI activity. Current testing methods measure the activated partial thromboplastin time (APTT) to monitor FXI activity [6]. Although affected animals with FXI deficiency are relatively easy to classify, carriers of the disorder are often difficult to distinguish from normal individuals because of the overlap of activity ranges. To effectively control the spread of recessive defects such as BLAD, DUMPS, CVM, and FXI deficiency it is important to accurately identify animals that may appear clinically normal, but carry the mutant allele.

Marron et al. (2004) have identified the causative mutation for FXI deficiency. The authors found that the mutation consists of a 76 bp segment (AT(A)_{28}TAA AG(A)_{26}GGAATAATAATTCA) insertion into exon 12 of FXI on chromosome 27. The insertion consists of long strings of adenine (A) bases and contains a stop codon that prevents the full-length protein from being made [9,11].

The purpose of this study was to identify and calculate the frequency of the mutant FXI allele in Holstein cattle reared in Turkey.

Methods

Samples and DNA extraction

Two hundred twenty-five Holstein cows were sampled. The blood samples were collected from three different state farms managed by the TIGEM (General Directorate of Agricultural Enterprises) in Turkey. Blood samples were collected from the jugular vein into EDTA-containing tubes and transported to the laboratory. They were stored at -20 °C until the genomic DNA extraction which was carried out by using salting-out method [12]. The genomic DNA was stored at 4 °C until use.

PCR assay

The amplification reactions were prepared in a final volume of 20 μl containing as follows; 1 × PCR buffer, 0.2 mM dNTPs, 0.5 units Taq DNA polymerase, 1.5 mM MgCl₂, 20 nmol of forward (5’ CCC ACT GGC TAG GAA TCG TT 3’) and reverse (5’ CAA GGC AAT GTC ATA TCC AC 3’) primers (GenBank accession number, AY570504) as suggested by Marron et al. (2004) and 100 ng of genomic DNA. Amplification was performed using an initial denaturation of 10 minutes at 95°C, followed by 34 cycles of 30 seconds at 95°C, 60 seconds at 55°C and 30 seconds at 72°C and a final extension of 10 minutes at 72°C. PCR products resolved by electrophoresis on 2% agarose gels following by staining with ethidium bromide in TBE buffer for 40 minutes.

DNA sequencing

After the gel electrophoresis process, the amplicons of 320 bp and 244 bp were purified using a Qiamp Mini Kit (QIAGEN, Valencia, CA, U.S.A.). The purified samples were sequenced by a Big dye terminator chemistry on an ABI 3100-Avant DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The DNA sequences were analyzed using the Sequencing Analysis Software Version 3.3 (Applied Biosystems, Foster City, CA, U.S.A.).

The gene frequency of the FXI locus was estimated by counting the number of genes [13].

Results and Discussion

After the PCR, the normal FXI allele in unaffected animals (homozygous wild type) produces a single 244 bp fragment. In homozygous affected animals, the fragment has a length of 320 bp and the heterozygous (or carrier) cattle exhibit two fragments of 244 bp and 320 bp (Fig. 1.).

Analysis of 225 Holstein cows reared in Turkey revealed that four cows were FXI deficiency carriers. All other cows possessed normal genotypes. The frequency of the mutant FXI allele and the prevalence of the carriers were calculated as 0.9% and 1.8%, respectively.

We also carried out partial sequencing in all cows in order to confirm whether these cattle were carriers or not. Our sequencing results of the mutant FXI allele were consistent with prior report [3] of the FXI gene deficiency. These results for the mutant allele revealed a mutation consisted of a 76 bp insertion containing poly Adenine sequences with a stop codon (TAA) (Fig. 2.).

It was previously hypothesized that FXI deficiency was due to the absence of the FXI protein [2]. Upon examination of the mutation in bovine FXI, it is most likely that the protein is not absent, but merely truncated prema-
turely because of the presence of a stop codon that was introduced by the insertion. Because of the truncation, the mature protein would be lacking the entire serine protease domain encoded by exons 13–15 [3].

FXI deficiency in cattle has been reported in many different countries, such as the USA [5], Canada [6], Britain [7], Japan [9], and the Czech Republic [4]. However, no case of FXI deficiency was previously reported in Holstein cattle reared in Turkey. As a first attempt, this study found that the mutant FXI allele frequency in Holstein cows in Turkey is 0.9% and the prevalence of carrier cattle is 1.8%. The prevalence ratio found in this study is similar to those reports in Canadian Holstein cattle (1.2%) [3], Japanese

| Normal | GCACTGTATCTCAAGTTGGGAATTACTTGCTAACGGGAATTCTCTTT |
|--------|---------------------------------------------------|
| Mutant | GCACTGTATCTCAAGTTGGGAATTACTTGCTAACGGGAATTCTCTTT |

**Figure 1**

**Demonstration of all FXI genotypes on 2% TBE agarose gel.** Lane 1 is homozygous-unaffected producing a single 244 bp fragment, lane 2 is heterozygous (carrier) exhibiting two fragments of 244 bp and 320 bp, lane 3 is carrier control, and lane 4 is homozygous-affected control producing a single 320 bp fragment. Lane M is DNA Ladder (50 bp, Fermentase®). Control samples were supplied by Dr. Jonathan E. Beever.

| Normal | TCCTCTCTTACTCCTTTCAGGTCTGACCAATGTGGTGCTGT |
|--------|------------------------------------------|
| Mutant | TCCTCTCTTACTCCTTTCAGGTCTGACCAATGTGGTGCTGT |

**Figure 2**

**Alignment of bovine FXI sequences from normal (top) and mutant (bottom) FXI allele.** The mutation consists of a 76 bp segment insertion into exon 12 of FXI. The insertion consists of long strings of adenine (A) bases and contains a stop codon. The box indicates the premature stop codon generated as a result of the insertion.
Holstein cattle (2.5%) [9], Indian Holstein cattle (0.2%) [14] and Czech Holstein and Simmental cattle (0.3%) [4].

The mutation that causes FXI deficiency introduces a premature stop codon. The FXI gene mutation in Holstein cattle reared in Turkey was confirmed by this study. Our results indicate that normal cattle have only one DNA fragment of 244 bp while heterozygous cattle exhibit two DNA fragments of 320 bp and 244 bp for the FXI gene deficiency.

FXI deficiency has been shown to adversely affect the reproductive performance of cattle; the follicular diameter of the affected cattle is small and is accompanied by lower peak estradiol concentrations in plasma near the time of ovulation. The oestrous cycle of the affected cows is characterized by reduced follicular development and a slow process of luteolysis. Reproductive performance in cattle can be affected by metritis or mastitis, since neutrophil function appears to differ in cells that were isolated from normal cattle and those that came from FXI deficient cattle. Additionally, it has been suggested that both homozygous and heterozygous cattle might exhibit lower calving and survival rates. Therefore, the presence of this genetic defect may have a significant economic impact on the dairy industry [9,15].

Conclusion
The DNA-based test (PCR) described can detect the mutation responsible for FXI deficiency in Holstein cattle in Turkey. This is the 1st report on the FXI deficiency in Holstein cattle in Turkey. The bulls used for artificial insemination should be screened to determine whether they are FXI deficiency carriers or not. This is useful to decrease the frequency of the mutant allele in Turkish Holstein population, and selection program should be prepared to screen animals in order to eliminate the disorder.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HM participated in the design of the study, collected the blood samples, carried out the extraction of genomic DNA, PCR and DNA sequencing, performed the statistical analysis and participated in the writing of the manuscript. MAY conceived of the study, participated in its design and coordination, performed the statistical analysis and participated in the writing of the manuscript. FO and YG collected the blood samples, carried out the extraction of genomic DNA, PCR and DNA sequencing, drafted the manuscript. CO participated in designing the study and drafted the manuscript. All authors read and approved the final manuscript.

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References
1. Gentry PA, Black WD: Prevalence and inheritance of factor XI (Plasma Thromboplastin Antecedent) deficiency in cattle. J Dairy Sci 1980, 63(6):616-620.
2. Gentry PA: The relationship between factor XI coagulant and factor XI antigenic activity in cattle. Can J Comp Med 1984, 48:58-62.
3. Marron SM, Robinson JL, Gentry PA, Beever JE: Identification of a mutation associated with factor XI deficiency in Holstein cattle. Anim Genet 2004, 35:454-456.
4. Citek J, Rehout V, Hanusova L, Vrabcova P: Sporadic incidence of factor XI deficiency in Holstein cattle. J Sci Food Agric 2008, 88:2069-2072.
5. Kociba GD, Ratnoff OD, Loeb WF, Wall RL, Heider LE: Bovine Thromboplastin Antecedent (factor XI) deficiency. J Lab Clin Med 1969, 74:37-41.
6. Gentry PA, Crane S, Lotz F: Factor XI (Plasma Thromboplastin Antecedent) deficiency in cattle. Can Vet J 1975, 16:160-163.
7. Brush PJ, Anderson PH, Gunning RF: Identification of factor XI deficiency in Holstein-Friesian cattle in Britain. Vet Rec 1987, 121:14-17.
8. Haton BM, Beever JE, Robinson JL: Mutation that causes factor XI deficiency in Holstein cattle. Illini DairyNet Papers 2000.
9. Ghanem ME, Nishibori M, Nakao T, Nakazaki K, Akita M: Factor XI mutation in a Holstein cow with repeat breeding in Japan. J Vet Med Sci 2005, 67(7):713-715.
10. Mukhopadhyaya PN, Jha M, Muraleedharan P, Gupta RR, Rathod RN, Mehta HH, Khoda VK: Simulation of normal, carrier and affected controls for large-scale genotyping of cattle for factor XI deficiency. Genet Mol Res 2006, 5(2):323-332.
11. Kunieda M, Tsuji T, Abbasi AR, Khalaj M, Ikeda M, Miyadera K, Ogawa H, Kunieda T: An insertion mutation of the bovine F11 gene is responsible for factor XI deficiency in Japanese Black cattle. Mamm Genome 2005, 16:383-389.
12. Miller SA, Dykes DD, Polesk HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 1988, 16(3):1215.
13. Nei M: Molecular evolutionary genetics. Columbia University Press, NY; 1987.
14. Rajesh KP, Kalpesh JS, Jenahbai BC, Krishna MS, Krothapalli RS, Sambasiva R: Factor XI deficiency in Indian Bos taurus, Bos indicus, Bos taurus × Bos indicus crossbreds and Bubalus bubalis. Genet Mol Biol 2007, 30(3):580-583.
15. Liptrap RM, Gentry PA, Ross ML, Cummings E: Preliminary findings of altered follicular activity in Holstein cows with coagulation factor XI deficiency. Vet Res Commun 1995, 19:463-471.