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Kısa Bildiri ***Short Communication***

Identification of Allele Frequency of Factor XI Deficiency (FXID) in Holstein Cows Reared in Thrace Region of Turkey

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Abstract

Factor XI (FXI) is a protein that plays a key role in plasma coagulation. Factor XI Deficiency (FXID) is an autosomal recessive disease caused by an insertion into exon 12 of FXI gene. The aim of this study is to determine the allele frequency of Factor XI Deficiency (FXID) in Holstein cows reared in Thrace region of Turkey. Blood samples of 287 Holstein cows were used for DNA isolation. Amplification of FXI gene was followed by the evaluation of PCR products with visualization on 2% agarose gel electrophoresis. FXID mutant allele was not observed in any of the samples used in this study. In conclusion, none of the Holstein cows were neither affected nor carriers for FXID among all analysed Holstein cows reared in Thrace region of Turkey.

Özet

Türkiye'de Trakya Yöresinde Yetiştirilen Holştayn İneklerde Faktör XI Yetmezliği (FXID) Allel Frekansının Belirlenmesi

Faktör XI (FXI) plazma pıhtılaşmasında görev alan önemli bir proteindir. FXI eksikliği (FXID) FXI geninin 12. ekzonunda meydana gelen bir insersiyon sonucu oluşan otozomal resesif bir hastalıktır. Bu çalışmanın amacı Türkiye'nin Trakya bölgesinde yetiştirilen Holştayn ineklerde FXID allel frekansının belirlenmesidir. DNA izolasyonu 287 Holştayn ineğine ait kan örneklerinden yapılmıştır. FXI geninin polimeraz zincir reaksiyonu (PZR) ile çoğaltılmasını takiben oluşan ürünler %2'lik agaroz jel elektroforezi ile görüntülenerek değerlendirilmiştir. Örneklerin hiçbirinde FXID mutant alleli gözlenmemiştir. Sonuç olarak, Trakya bölgesinde taranan tüm Holştayn ineklerde FXID'den etkilenen veya taşıyıcısı olan inek tespit edilmemiştir.

Introduction

Factor XI (FXI), a plasma serine protease is one of the important components of the coagulation system. Coagulation is a balanced system with two important roles; forms fibrin after injuries so that limits any blood loss and enables blood coagulation in veins, thus gives prevention against deep vein thrombosis or myocardial infarction. This mechanism keeps the fluidity of the blood but leads to fibrin formation when it is necessary. When injury occurs in a blood vessel platelets adhere to the injury surface. Once platelets attach to the surface, they release chemicals that attract additional platelets to the damaged area. This system is the first response to stop bleeding. The action of the cascade is to stabilize the clot and form fibrin. In this cascade, factor XI and factor VIII are together responsible to activate factor X which initiates the conversion of prothrombin to thrombin (Brush et al., 1987).

Genetic improvement has been achieved in production characteristics of Holstein breed with intensive use of same elite sires by using artificial insemination. As the possible results of inbreeding, the genetic variation was decreased and homozygosity was increased, recessive genes became homozygous and some genetic defects such as FXID have appeared in Holstein population (Gentile and Testoni, 2006). FXID was diagnosed firstly in 1969 among the Holstein cattle population reared in USA (Kociba et al., 1969). The symptoms of this disease are; continuous bleeding (e.g. umbilical cord bleeding after birth, bleeding of wounds

after dehorning and castration), decreased resistance to pneumonia, mastitis, pink colostrum, blood in the milk, metritis and reduced reproductive performance in dairy cows (Brush et al., 1987; Liptrap et al., 1995). Reproductive performance in FXID carrier cows is affected by a smaller diameter of ovarian follicles, estrus cycle with slower luteolysis, and a lower peak of estradiol near the time of ovulation (Liptrap et al., 1995). Symptoms are variable; one or more can appear in each effected animal and can be observed severe in homozygous and milder in heterozygous animals (Gentry and Ross, 1993). Molecular diagnostic of recessive disorders is a great benefit for breeders and a step forward in the management of animal genetics. Definitive diagnosis can only be performed by identifying the mutant alleles of FXI gene that cause FXID. Mutation in FXI gene can be identified by using PCR based molecular techniques. The cause of the FXID in Holstein cattle is an insertion of 76 base pair adenine reach fragment [AT(A)28TAAAG(A)26GGAAATAATAATTCA] in exon 12 of FXI gene. This mutation introduces a premature stop codon and impairs the synthesis of functional protein. Therefore, the normal allele of FXI gene shows 244 bp, while the mutant allele exhibits 320 bp (Marron et al., 2004). Heterozygous animals are the carriers of this disease. Thrace region is one of the most important locations for dairy breeding in Turkey. The aim of this study was to determine the prevalence of FXID among the Holstein population in Thrace region which borders European Union.

Materials and Methods

This study was approved by Ethic Committee of the Istanbul University Veterinary Faculty (Approval number: 2015/04).

Holstein cows were randomly selected from different farms located in Edirne (n=49), Kırklareli (n=74), Tekirdağ (n=114) and Istanbul (n=50) provinces of Thrace region. Blood samples were collected from Vena jugularis and Vena coccigea into sterile EDTA tubes. Extraction of genomic DNA from blood samples was performed with automated nucleic acid extraction system (ExiPrep™ 16Plus, Bioneer). Amplification of exon 12 of FXI gene was carried out with primer pairs as described by Maroon et al. (2004). PCR was performed in a final volume of 50 µL. The reaction contained 25 µL of sterile ddH_20 (AccuGENE™, Lonza), 3 µL of gDNA (~100ng), 0,5 µL of 20 nM each primer (F 5'-CCCACTGGCTAGGAATCGTT-3' and R 5'-CAAGGCAATGTCATATCCAC-3'), 20 µL of HotStar Taq master mix kit (contains 2.5 U HotStarTaq DNA Polymerase, 1 x PCR Buffer with 1,5 mM $MgCl_2$, and 200 µM of each dNTP, Qiagen). Amplification conditions were started with initial denaturation at 95˚C for 15 min, followed by 34 cycles of 95˚C for 30 sec, 55˚C for 1 min 72˚C for 30 sec and end with final extension 72˚C for 10 min. After amplification, PCR products were visualized on 2 % agarose gel by ethidium bromide staining.

Results

A 244 bp product indicating the FXI gene was observed on gel electrophoresis. None of the PCR products were visualized as 320 bp for FXID (Figure 1). All samples analyzed for FXID were observed to have normal allele. The Holstein cows analysed in this study were neither affected nor carriers for FXID. They were all free from the mutant allele of FXID

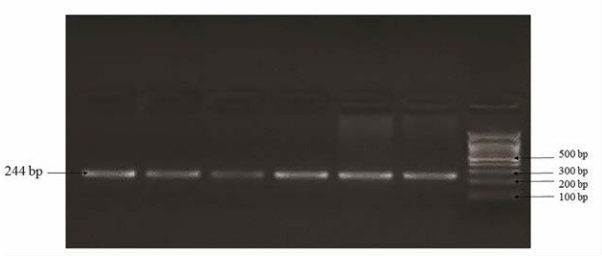

Figure 1. Visualization of Factor XI (FXI) allele (244 bp) on 2% agarose gel in Holstein cows reared in Thrace region of Turkey.

Şekil 1. Türkiye'de Trakya yöresinde yetiştirilen Holştayn ineklerde Faktör XI (FXI) alelinin (244 bp) %2'lik agaroz jel ile görüntülenmesi.

Discussion

Holstein is the most common cattle breed used in dairy farming. Among breeding methods, artificial insemination and inbreeding are being used to reach better production performance in cattle breeding. However, there are some undesirable defects that cannot be controlled with basic breeding methods; such as genetic disorders. One of the most important genetic disorders in Holstein breeding is factor XI deficiency (FXID) caused by autosomal recessive genes. Using the same elite sire may increase the homozygosis and frequency of recessive mutant alleles in related populations. If a genetic defect has finally been discovered due to clinical signs, the frequency of the recessive allele might have already reached high values in the population (Gentile and Testoni, 2006; Windsor and Agerholm, 2009).

FXID was reported firstly in Ohio USA (Kociba et al., 1969) and Marron et al. (2004) identified the mutant allele of FXI with molecular methods.

The prevalence of FXID were studied in Holstein cattle of various countries and reported as; 0,61% of 330

bulls in India (Patel et al., 2007), 1,2% of 419 cattle in USA (Marron et al., 2004), 1% of 500 cows in Japan (Ghanem and Nishibori, 2009), 0.36% of 279 cattle in Czech Republic (Čítek et al., 2008).

No carriers have been determined in 103 Polish Holstein cattle and among 28 repeat breeding cows only one was reported as heterozygous for the FXID allele (Gurgul et al., 2009). In India, any of 307 Holstein cattle was not identified as FXI deficient or a carrier (Mukhopadhyaya et al., 2006). Only one of 40 repeat breeding cows in Japan was reported as heterozygous for the FXID allele (Ghanem et al., 2005). However, in Canada the percentage of carriers was 11% (Gentry and Black, 1980). The percentage of FXID carriers were 3.4% and 3% among Canadian and British bulls respectively and one of the British bulls was identified as FXID (Gentry and Ross, 1993). Two carriers and a FXID animal of 576 Holstein cows were reported in China (Zhang et al., 2010).

FXID was firstly reported in Ankara province of Turkey and four animals were reported as carriers among 225 Holstein cows. Studies from Turkey reported the carrier prevalence and FXID mutant allele frequency as 1.7 % and 0.009 of 225 cows in Ankara (Meydan et al., 2009), 0,7 % and 0.004 of 150 cows in Kayseri (Yaşar and Akyüz, 2012), 0,4 % and 0.002 of 504 cows in Antalya (Karslı et al., 2011), 1,17% and 0.006 of 170 cows in Bursa (Oner et al., 2010) and 1,7 % and 0.009 of 69 bulls in Izmir and Ankara (Akyüz, 2013), 1,8% and 0.009 of 500 cattle in Burdur (Korkmaz Ağaoğlu et al., 2015) provinces of Turkey. In the present study that was conducted in Thrace region of Turkey, showed that among the 287 Holstein cows were neither affected nor carriers. Variations in results of carrier prevalence and FXID allele frequency may arise from the number, ancestor and gender of the animals used in these studies. Additionally not only in our study but also none of the FXID studies performed in Turkey were not consider repeat breeder syndrome in cows (Karslı et al., 2011; Korkmaz Ağaoğlu et al., 2015; Meydan et al., 2009; Oner et al., 2010; Yaşar and Akyüz, 2012). Therefore, if cows only with repeat breeder syndrome would be sampled for further studies, that might increase the detection rate of FXID allele among Holstein cattle raised in Turkey. Decrease in reproductive performance may lead to repeat breeder syndrome and FXID carrier cows cause important financial loss in dairy cattle breeding (Akyüz et al., 2012).

In conclusion, since FXID allele have not been identified in Thrace region which is the transition point of Turkey to Europe, can be interpreted as positively for breeding. In order to prevent emerge and prevalence increase in FXID, molecular tests should apply routinely on cows, imported semen samples and bulls. Animals and semen samples that identified as carriers should be excluded from breeding. Further investigations need to be carried out to identify the prevalence of FXID in other regions of Turkey.

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