The present study investigated the occurrence of autosomal recessive genetic disease, factor XI (FXI), in Khuzestan native cows and Iranian Holstein cattle. Genomic DNA was isolated from the blood of the cows ($n = 330$). Exon 12 of the Factor XI gene of the cows was amplified by polymerase chain reaction (PCR). Additionally, all cows were confirmed by DNA sequencing to determine existence of mutant FXI allele. Normal cattle have only one DNA fragment of 244 bp while heterozygous cattle exhibited two DNA fragments of 320 and 244 bp for the FXI gene deficiency. The results of this study showed that none of the animals were carriers of FXI deficiency. Because of the economical significance of the FXI mutation and its recessive mode of inheritance, attention has to be paid to any case of a bull having in his or her origin any known FXI carrier. Although we did not observe any carrier, widespread screening programs for detection of genetic disorders seems necessary.

Key words: Factor XI, deficiency, sequencing, cattle, Iran.

INTRODUCTION

Currently, 40 disorders and traits in cattle have been characterized in which the causative mutation has been identified at the DNA level (Online Mendelian Inheritance in Animals; http://omia.angis.org.au). However, the routine analysis of only a few of those has entered breeding programs so far and is in some instances mandatory for animals that are used for breeding.

In cattle, the autosomal recessive genetic diseases are breed-specific. Some of them are Holstein-specific, which include mainly factor XI deficiency syndrome (Brush et al., 1987), complex vertebral malformation (Steffen, 2001), bovine leukocyte adhesion deficiency (Kerhli et al., 1990), bovine citrullinaemia (Harper et al., 1986) and deficiency of uridine monophosphate synthase (Robinson et al., 1993a). With the wide use of artificial insemination and international trading of semen and breeding bulls, these genetic diseases have already been spread to a large population, as animal carriers of the diseases look normal. Effects of carrier bulls in breeding programmers are deleterious, because if a bull is carrying one copy of the mutant gene (a heterozygote) and is mated with an unaffected cow, they will produce 50% heterozygous carriers in the population. If 2 heterozygous carriers are mated, then 25% of their offspring will be affected with the disease, 50% will be carriers and only 25% will be normal.

One of the protein factors involved in blood coagulation is a serine protease-factor XI (FXI), also known as plasma thromboplastin antecedent. It is synthesized in the liver as a zymogen, and after conversion to a proteolytic enzyme, it participates in intrinsic or contact activation of the process (Gentry and Downie, 1977). A rare hereditary disorder, known as FXI deficiency, has been
recognized in humans (Rosenthal et al., 1953), dogs (Dodds and Kull, 1971) and cattle (Kociba et al., 1969; Gentry et al., 1975). FXI deficiency is an autosomal recessive disorder, with partial deficiency of FXI coagulant activity in heterozygotes and considerable deficiency in homozygotes (Gentry and Ross, 1994). In cattle, FXI-deficient animals may be asymptomatic or display several symptoms, like prolonged bleeding, anemia, greater prevalence of repeat breeding (Gentry and Black, 1980; Brush et al., 1987; Liptrap et al., 1995), or even lower resistance to pneumonia, mastitis and metritis (Gentry et al., 1996). Affected animals can survive for years with no overt clinical signs, even though they appear to have a higher mortality and morbidity rate. 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 (Gentry et al., 1975). 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. Marron et al. (2004) revealed that the molecular basis of coagulopathy in Holstein cattle is an insertion of a 76-bp adenine-rich fragment in exon 12 of the FXI gene. This insertion, composed of an imperfect poly-adenine tract [AT(A)28TAAAG(A)26G] followed by a duplicated region of the normal coding sequence [GAAATAATAATTCA], introduces a premature stop codon, which impairs the synthesis of functional protein (Marron et al., 2004). By using biochemical or genetic tests, the FXI deficiency was shown in Holstein cattle in the USA, Great Britain, Canada and Japan (Brush et al., 1987; Gentry et al., 1996; Marron et al., 2004; Ghanem et al., 2005). According to the literature, FXI deficiency may affect reproductive traits and udder health in cattle. Because reproduction problems and udder inflammation may generate certain pecuniary losses in commercial farms, we decided to perform a preliminary screening of the Holstein and Native cattle reared in Khuzestan Province in Iran.

MATERIALS AND METHODS

Animals and sample collection

A total of one hundred Iranian Holstein and two hundred thirty native cows were selected and tested for FXI mutation. The blood samples were collected from five different Holstein farms and five different regions for native Khuzestan cattle in Iran. Blood samples were collected from the jugular vein into ethylenediaminetetraacetic acid (EDTA)-containing tubes and transported to the laboratory. They were stored at -20°C until the genomic DNA extraction was carried out.

DNA extraction

DNA was extracted by using salting-out method reported by Javanrouh et al. (2006). Briefly, nuclei were isolated from 1 to 2 tubes of blood and collected in EDTA tubes. After the addition of 9 volumes of buffer A (containing 0.32 M sucrose [109.5 g sucrose], 10 mM Tris HCl [10 ml of 1 M Tris-HCl, pH 7.6], 5 mM MgCl2 [5 ml of 1M MgCl2] and 1% Triton-100), they were properly mixed and kept on ice for 2 min. The solution was centrifuged at 1500 rpm at 4°C for 15 min. The nuclei pellet was re-suspended in 5 ml buffer B (containing 25 mM EDTA [50 ml EDTA, pH 8.0] and 75 mM NaCl [40 ml of 5 M NaCl]) and transferred to a 15 ml polypropylene centrifuge tube. Following the addition of 500 µl of 10% sodium dodecyl sulfate (SDS) and 55 µl proteinase K (10 mg/ml stock), it was incubated on a low-speed orbital shaker at 37°C overnight. Then, 1.4 ml saturated NaCl solution (approximately 6 M) was added to each tube and it was shaken vigorously for 15 s, followed by centrifugation at 2500 rpm in the low-speed centrifuge for 15 min. The supernatant was transferred into another 15 ml polypropylene tube, leaving behind the precipitated protein pellet and then exactly two volumes of room temperature 100% ethanol was added and the tube inverted several times until the DNA precipitate was visible. The DNA strands were removed with a pipette tip and transferred to an eppendorf tube containing 200 µl TE. DNA was dissolved at 37°C for 2 h.

Polymerase chain reaction (PCR) conditions

Genotyping was done using primers according to Marron et al. (2004) as follows: (5’ CCC ACT GGC TAG GAA TCG TT 3’) and (5’ CAA GGC AAT GTC ATA TCC AC 3’). Twenty µl of each PCR reaction contained: 1X PCR buffer; 2 mM MgCl2; 0.25 mM primers; 200 µM dNTPs; 1 unit of Taq polymerase; 150 ng/reaction genomic DNA and ddH2O. Thermal cycling included initial denaturation at 94°C for 3 min, 35 cycles of 94°C for 1 min, 55°C for 1 min and final extension at 72°C for 8 min. PCR products resolved by electrophoresis on 2% agarose gels following by staining with ethidium bromide in TBE buffer for 40 min.

DNA sequencing

After the gel electrophoresis process, the amplicons of 320 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 3130-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.).

RESULTS AND DISCUSSION

The primers were used to amplify 244 and 320 bp DNA fragment to detect studied population for FXI. After the PCR, the normal FXI allele in unaffected animals (homozygous wild type) produced a single 244 bp fragment (Figure 1). Analysis of 330 Holstein and native cattle reared in Khuzestan Province in Iran revealed that all cows possessed normal genotypes. 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 of the FXI gene deficiency (Marron et al., 2004) (Figure 2).

Although some studies have reported carrier animals of FXI among Holstein populations (Marron et al., 2004; Ghanem et al., 2005; Citek et al., 2008; Meydan et al.,...
Upon examination of the mutation in bovine FXI, it is most likely that the protein is not absent, but merely truncated prema Holstein cattle (2.5%) (Ghanem et al., 2005), Indian Holstein cattle (0.2%) (Rajesh et al., 2007) and Czech Holstein and Simmental cattle (0.3%) (Citek et al., 2008). The mutation that causes FXI deficiency introduces a premature stop codon. 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 (Ghanem et al., 2005). 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 (Liptrop et al., 1995).

FXI deficiency has never been observed in Holstein and native cattle in Iran. Large-scale screening of the population is needed to define a reliable frequency of the abnormal FXI allele and to estimate the potential risk of its spreading among Iranian cattle. This study provides a basis for further testing of Iranian cattle for the FXI gene mutation.
Conclusion

The DNA-based test (PCR) described can detect the mutation responsible for FXI deficiency in Holstein cattle in Iran. This is the first report on the FXI deficiency in Holstein cattle in Iran. 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 Iranian Holstein population and selection program should be prepared to screen animals in order to eliminate the disorder.

REFERENCES