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Identification of Factor XI Deficiency in Khuzestan Buffalo Population of Iran

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Abstract: Factor XI (FXI) is a plasma protein that participates in the blood coagulation process. Factor XI deficiency is autosomal recessive hereditary disorder that may be associated with excess bleeding in cattle. In the present study overall 300 buffalo were genotyped to detect the mutation within exon 12 of the gene encoding for factor XI. The validity of identification was confirmed through sequencing of PCR products. The result of this study showed no mutation of Factor XI of Iranian buffalo. Although we did not observe any carrier but, widespread screening programs for detection of genetic disorders seems necessary.

Key words: Factor XI · Deficiency · Buffalo · PCR

INTRODUCTION

Iran country is one of most important countries for livestock diversity in Asia. Many indigenous breeds are distributed in different geographical regions of the country. About buffalo, statistics showed two main groups of this animal exist, the first group is centralized mainly in Mazandaran, Guilan and Azarbayejan and second group particularly are in Khuzestan province. Current investigation of agriculture ministry of Iranian showed that population of buffalo significantly reduced due to several reasons as inbreeding, substitution of high produced exotic cow with buffalo farming and mortality of animals due to common disease and disorders.

As one way for genetic conservation of buffalo, it is necessary to develop control test for identification of recessive defects such as BLAD, DUMPS, CVM, Citrullinaemia and FXI deficiency. It is important to accurately identify animals that may appear clinically normal, but carry the mutant allele.

Factor XI (FXI) is one of more than a dozen proteins involved in t he early blood coagulation cascade [1], FXI-deficiency syndrome being an autosomal recessive bleeding disorder first discovered in Holstein cattle in Ohio [2] but also reported in many other countries, such as the Canada [3], England [1] and Japan [4]. Bovines with FXI-deficiency may have lower calving and survival rates and increased susceptibility to infectious diseases [5]. The 3' end of the gene for bovine FXI is located on bovine chromosome 17 and was firstly characterized by Robinson *et al* . [6]. Marron *et al* [7], revealed that the molecular basis of coagulopathy in 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 [7]

In Iran, however, there has been no reported incidence of FXI-deficiency in buffalo. The objective of present study is identification of FXI deficiency in Iranian indigenous buffalo using PCR

MATERIALS AND METHODS

Animals: Overall 300 buffalo from two sexes was chosen from Khuzestan province and after registration of their ID and pedigree information blood sample was collected from tail vein using anti coagulate (EDTA 0.5M) and immediately samples are transfer in minus 4 flask and finally all samples was carried to biotechnology department of Animal Science Research Institute, Karaj for further analysis and storage in -20 freezer.

DNA Extraction: 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 $MgCl_2$

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and 1% t r iton 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 µ L of 20% (v/v) sodium dodecyl sulfate (80 mg) and 25 µL of 20 mg mL^{-1} (500 µ g) 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 under UV light.

PCR: Primer sequence and identification method was followed based on Marron *et al* [7]. Primers sequences for this experiment are: 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). Twenty µl of each PCR reaction contained: 1X PCR buffer; 2mM MgCl2; 0.25µM primers; 200 µM dNTPs; 1 unit of Taq polymerase (Metabion Company, Germany).; 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, 72°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 minutes. To confirm the FXI gene mutation, the PCR amplicon cloned into the pDK101-based T/A cloning vector, which was transformed in DH5 alpha competent cells. The clones were confirmed by plasmid DNA isolation followed by release of the insert (244 bp) using the *Nco I* restriction endonuclease. The single-strand of the entire clone (insert + vector) was sequenced using the M13U18 vector specific primer.

RESULTS

The primers were used to amplify 244 bp and 320 bp DNA fragment to detect studied population for FXI. After the PCR, the normal FXI allele in unaffected animals (homozygous wild type) produces a single 244 bp fragment (Fig. 1). Analysis of 300 buffalo reared in Khuzestan Province in Iran revealed that all buffalo possessed normal genotypes.

After screening for vector contamination, 247 bases were obtained 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. Our sequencing results of the mutant FXI allele were consistent with prior report of the FXI gene deficiency (Fig. 2).

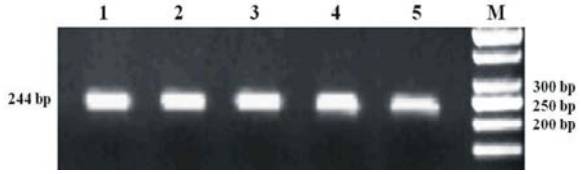

Fig. 1: Polymerase Chain Reaction (PCR) genotyping of FXI deficiency from all of the animals. Lane 1-5 were FXI deficiency free animals (homozygote genotypes) produced only one 244 bp fragment. Lane M is DNA Ladder (50 bp, Fermentase).

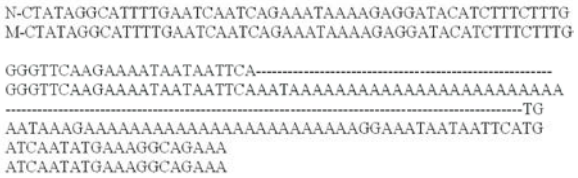

Fig. 2: Alignment of bovine FXI sequences from normal (top) and mutant (bottom) FXI allele

DISCUSSION

The molecular genetic techniques are now available to characterize genes responsible for inherited monogenic or oligogenic defects in buffalo. The number of inherited anomalies, which are identified on the molecular level, will be expected to increase in the next years. The mutation tests for genes responsible for inherited anomalies will be exploited in breeding programmes and are also useful for breeding animals on farms [8]. The genetic diagnosis of anomalies has great implications for breeders and breeding organizations because the origin of a deleterious gene may be traced back to widely used sires. Based on a precise genetic diagnosis, recommendations for the farmers and breeders can be given to improve eradication programmes for deleterious alleles [8].

It was previously hypothesized that FXI deficiency was due to the absence of the FXI protein [3]. In looking at over a decade of genotyping for FXI in the Holstein breed, it can be seen that different selection strategies define the decline rate of the mutation. A lethal recessive allele will normally be eliminated, given that homozygous recessives cannot mate [9]. This process alone is extremely inefficient for the elimination of a rare allele from a population. Therefore breeding programs are necessary to reduce recessive allele (q) in a reasonable time. If a DNA-based test is available to detect heterozygotes, a more efficient method to eliminate q is the testing of sires and exclusion of heterozygotes [10]. Using such a strategy would eliminate any qq individuals in the

following generation and the allele frequency would be halved in each generation. However, such strategies are influenced by the fact that several genes may have direct or indirect effects or are in linkage disequilibrium with economically important traits. In this case the prediction of the allele frequency is more complicated.

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%) [11], Indian Holstein cattle (0.2%) [12] and Czech Holstein and Simmental cattle (0.3%) [13]. 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 [11]. 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 [5].

FXI deficiency has never been observed in Iranian buffalo. 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 buffalo. This study provides a basis for further testing of Iranian buffalo for the FXI gene mutation.

CONCLUSION

The DNA-based test as PCR can detect the mutation responsible for FXI deficiency in buffalo in Iran. This is the 1st report on the FXI deficiency in buffalo in Iran. The bulls used for 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 buffalo population and selection program should be prepared to screen animals in order to eliminate the disorder.

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