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Full Length Research Paper

Study of citrullinaemia disorder in Khuzestan Holstein cattle population of Iran

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The present study investigated the occurrence of autosomal recessive genetic disease, citrullinaemia, in Khuzestan native cows and Iranian Holstein cattle. Genomic DNA was isolated from the blood of the cows (n = 330). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed to identify carriers of this disease. Then to determine existence of mutant citrullinaemia allele, all cows were confirmed by DNA sequencing. This study showed that none of the cows were carriers or mutants of citrullinaemia deficiency. More attention is necessary to check bulls related to possibility of being citrullinaemia carrier. This is important for economical reason and citrullinaemia mutation and its recessive hereditary disorder. However, no carrier was observed in this study, and for more detection of genetic disorders, it seems that a wide screening program is needed.

Key words: Citrullinaemia, sequencing, cattle, Iran.

INTROUDUCTION

Currently, 40 disorders and traits in cattle have been characterized in which the causative mutation has been identified at the DNA level (http://omia.angis.org.au). However, the routine analysis of only a few of these 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 mainly include, bovine citrullinaemia (Harper et al., 1986), factor XI deficiency syndrome (Brush et al., 1987), complex vertebral malformation (Steffen, 2001), bovine leukocyte adhesion deficiency (Kerhli et al., 1990) and deficiency of uridine monophosphate synthase (Robinson et al., 1993a). With the widespread use of artificial insemination and international trade of semen and breeding bulls, now, these genetic diseases in large population is distributed as an animal carrying the disease is a normal look. Effects of carrier bulls in-breeding programs are harmful, 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 he-terozygous carriers are mated, then 25% of their of-fspring will be affected with the disease, 50% will be car-riers and only 25% will be normal.

Citrullinaemia is a metabolic disorder that is due to lack of argininosuccinate synthetase (ASS) and is vital for urea cycle. This cycle is the biochemical process by which potentially toxic ammonia (a by-product of catabolism of proteins) is converted to urea, which is excreted in urine (Goodwin et al., 2004). It was first reported by Mcmurray et al. (1962) in humans. Calves with bovine citrullinaemia after birth are apparently normal. However, due to ammonia poisoning caused by this genetic defect two days after birth, they have low appetite and are lethargic. On the third day, calves were often seen wandering aimlessly abode with their head to the wall or fence to reduce the pressure. During the third to fifth day, the disease progressed rapidly, the calves became blind

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and then died (Healy et al., 1990).

The mutation responsible for this disorder has been characterized as a single-base substitution (C→T), converting the CGA codon that codes for arginine-86 to TGA, a translationtermination codon (Dennis et al., 1989). This conversion results in a truncated peptide product (85 long amino acids rather than the normal 412 amino acids) that lacks activity. There are few studies to determine the mutant alleles. Studies show that frequency of this mutant allele was high in Australia. Healy et al. (1991) found that 50% of Australian national HF herds and 30% of breeding bulls in AI centres were descendants of Linmack Kriss King (LMKK), which was a carrier of citrullinaemia. In other countries, like the USA and Germany, the incidence of the citrullinaemia is very low (Robinson et al., 1993b; Grupe et al., 1996). Among 367 Holstein cows tested in the United States of America, only one heterozygous was detected (Robinson et al.,1993b).

In the present study, 330 Holstein and native cattle, reared in Khuzestan Province in Iran, were screened for citrullinaemia. The aim of the this study was to estimate the incidence of citrullinaemia in these populations.

MATERIALS AND METHODS

Animals and sample collection

A total of one hundred Iranian Holstein and two hundred and thirty native cows were selected and tested for citrullinaemia mutation. Whole blood samples were collected from five different farms in Holstein populations and five regions for indigenous cattle in Khuzestan province, Iran. Jugular vein blood samples containing EDTA tube were collected and then transferred to the laboratory at 20°C until extraction of genomic DNA were kept.

DNA extraction

DNA using the salting-out technique, which was reported by Javanrouh et al. (2006) were extracted. Briefly, nuclei were isolated from blood 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 $MgCl_2$ (5 ml of 1M $MgCl_2$) 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 the precipitated protein pellet and then exactly two volumes of room temperature 100% ethanol was added and the tube was 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 tris EDTA (TE). DNA was dissolved at 37°C for 2 h.

PCR conditions

Genotyping was performed using primers according to Patel et al (2006) as follows: (5' GGC CAG GGA CCGTGT TCA TTG AGG ACA TC 3') and (5' TTC CTG GGA CCC CGT GAG ACA CATACT 3'). Twenty microliter of each PCR reaction contained: 1X PCR buffer; 2 mM $MgCl_2$; 0.25 µM primers; 200 µM dNTPs; 1 unit of Taq polymerase; 150 ng/reaction genomic DNA and ddH_{2o}.
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 were resolved by electrophoresis on 2% agarose gels followed by staining with ethidium bromide in TBE buffer for 40 min.

DNA sequencing

After the gel electrophoresis process, the amplicons of 185 bp to detect the point mutation in a gene coding for argininosuccinate synthase 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 sequencing using the version 3.3 software analysis sequences were analyzed (Applied Biosystems, Foster City, CA, U.S.A.).

RESULTS

The primers, used to detect the studied population for citrullinaemia, were used to amplify the gene responsible for citrullinaemia disease. After revealing the PCR mutation, a gene responsible for citrullinaemia disease produced two bands of 103 and 82 bp for normal animals (homozygous wild type) and none of the animals showed three bands of 185, 103 and 82 bp. So, no animal was found to be a carrier of citrullinaemia disease (Figure 1). Analysis of 330 Holstein and native cattle, reared in Khuzestan Province in Iran, revealed that all cows possessed normal genotypes. Also, partial sequencing was carried out in all cows in order to confirm whether these cattle were carriers or not. The study's sequencing results of the mutant citrullinaemia allele were consistent with prior report of the citrullinaemia gene deficiency (Figure 2).

DISCUSSION

The molecular genetic techniques are now available to characterize genes responsible for inherited monogenic or oligogenic defects in cattle. 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 in farms (Lin et al., 2001). 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

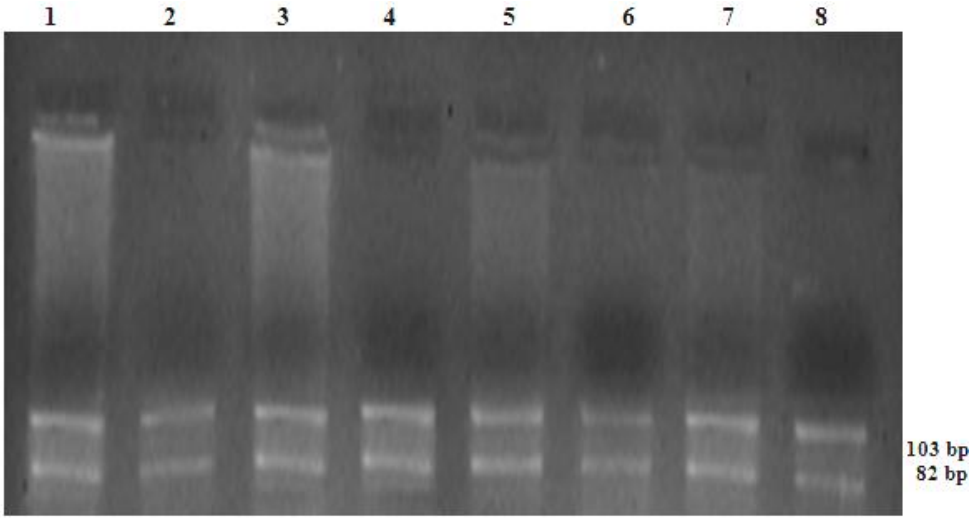

Figure 1. Polymerase chain reaction (PCR) genotyping of citrullinaemia deficiency from all of the animals. Lane 1-8: Citrullinaemia deficiency-free animals (homozygote genotypes) produced two 103 and 82 bp fragment.

Normal:Caaggagtttgtggaggagttcatctggccgccatccagtccagcgcact
Mutant:Caaggagtttgtggaggagttcatctggccgccatccagtccagcgcact

gtacgaggaccgatacctcctgggcacctctctcgccaggccctgcatcgccc
gtacgaggatcgatacctcctgggcacctctctcgccaggccctgcatcgccc

Figure 2. Alignment of bovine citrullinaemia sequences from normal (top) and mutant (bottom) citrullinaemia allele.

breeders can be given to improve eradication programmes for deleterious alleles (Lee et al., 2002)

Although some studies have reported that carrier animals of citrullinaemia among Holstein populations (Healy et al., 1991; Robinson et al., 1993; Grupe et al., 1996; Dennis et al., 1989), in our study are similar to the other results from the different countries for citrullinaemia (Meydan et al., 2010; Patel et al., 2006; Citek et al., 2006), and we did not find any carrier individual for this disease. When the genotyping of citrullinaemia, for over a decade, is taken into consideration in the Holstein breed, it can be seen that different selection strategies define the decline rate of the mutation. A lethal recessive allele will be eliminated, given that homozygous recessives cannot mate (Falconer and MacKay, 1996). 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 (Ronningen, 1973). 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.

Citrullinaemia 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 citrullinaemia 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 argininosuccinate synthase gene mutation.

Conclusion

The DNA-based test (PCR) described can detect the mutation responsible for citrullinaemia deficiency in Holstein and native cattle in Iran. This is the 1st report on the citrullinaemia deficiency in cattle in Iran. The bulls

used for artificial insemination should be screened to determine whether they are citrullinaemia deficien-cycarrier 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. Based on the results of this study, to review the situation with citrullinaemia disorder seems good. But the results prove the need for further analysis in cattle. This study showed that the frequency of carriers for genetic disorders among the studied populations is zero, but it can be said that citrullinaemia carrier cows in the population of Iran does exist.

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