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Identification of BLAD, Dumps and CVM Deficiency in Khuzestan Holstein Cattle Population of Iran

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Abstract: The present study investigated the occurrence of autosomal recessive genetic diseases, BLAD, DUMPs and CVM 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 BLAD, CVM and DUMPs diseases. Then to determine existence of mutant, all cows were confirmed by DNA sequencing. This study showed that none of the cows were carriers or mutants of BLAD, DUMPs and CVM deficiency. It is possible that with the mounting selection pressure, the international gene pool may diminish and consequently the risk of dissemination of inherited defects will increase. It is therefore recommended to screen breeding bulls for their breed-specific genetic diseases before they are inducted in artificial insemination programes, to minimize the risk.

Key words: BLAD · Dumps · CVM · Sequencing · Cattle

INTROUDUCTION

Elimination of animals and species affected by inherited defects is in the interest of all concerned with animal agriculture [1]. Understanding the molecular basis of a genetic defect renders it possible to detect carriers directly at the DNA level and, what is more important early in the animal's life and even in embryonic cells [2]. 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 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. With the widespread use of artificial insemination and international trade of semen and breeding bulls, now genetic diseases in large population is distributed as an animal carrying the disease is a normal look.

The most pressing problem in the genetics of health at present is the recessive and lethal Complex Vertebral Malformation (CVM) in the Holstein population. The defect can be traced back to the American elite sire Carlin-M Ivanhoe Bell. His father, Penstate Ivanhoe Star, born in 1963, was also found to be a carrier. Bell was formerly used extensively world wide, so the global impact on the mortality of Holstein calves is inevitable [3]. The affected calves are characterized by complex anomalies, including proportional dwarfism, symmetrical arthrogryposis of anterior and posterior limbs and multiple malformations of the cervical and thoracic parts of the vertebral column. A previous study revealed that the genetic mechanism of CVM is the single base mutation from G to T at the nucleotide position 559 of the bovine solute carrier family 35, member 3 gene (SLC35A3), which alters the amino acid sequence of uridine 59-diphosphate-N-acetylglucosamine transporter protein from a valine to a phenylalanine in position 180 [4]. Because DNA-based testing of CVM became available only in 2002, studies on the current prevalence of CVM are still rare. In a report by Rusc and Kaminski [5], 150 CVM carriers were identified out of 605 examined bulls in Polish Holstein cattle (24.8%). In a study performed with 200 Japanese Holstein cows, 26 animals were heterozygote CVM carriers (13%) [6]. Hence, in most Holstein populations the frequency of CVM carriers seems to be still high.

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Bovine Leukocyte Adhesion Deficiency (BLAD) is a lethal autosomal recessive disease in Holstein cattle characterized by a reduced level of expression of the â2 heterodimeric integrin. The defect was first identified in North American Holstein and was exported to other national Holstein populations. Animals with BLAD are characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, enteritis with bacterial overgrowth, periodontitis, loss of teeth, delayed wound healing, persistent neutrophilia and death at an early age [7]. BLAD is associated with the point mutation G383A of the bovine CD18 gene [8]. Recent reports on the BLAD allele frequency are available from Iran [9], China [10], Turkey [11], India [12], Canada [13] and Poland [14]. In these studies the frequency of BLAD carriers is currently approximately 0.8 to 3.45% of the tested cattle.

The deficiency of uridine monophosphate synthase is a hereditary recessive disorder in Holstein cattle causing early embryo mortality [15]. In mammalian cells, the last step of pyrimidine nucleotide synthesis involves the conversion of orotate to uridine monophosphate (UMP) and is catalysed by UMP synthase [16]. The mutation (C→T) in a gene for UMP_S at codon 405 leads to a premature stop codon, which subsequently produces a functionally impaired enzyme [17]. Embryos homozygous for $DUMP_S$ do not survive to birth and usually die early in gestation, so no homozygous recessive animal was detected so far. The embryos appear to be aborted or reabsorbed approximately 40 days after conception, leading to repeated breeding problems [18]. Occurrence of mutation in the UMPS gene was reported in Argentina [19], Hungary [20] and Taiwan [21]. Against these reports, there was no carrier of DUMPS in Holstein and native cattles of Turkey [22], Polish Holstein breed [15] and Holstein Friesian (HF) and HF crossbred cattle in India [23].

The major focus of this study has been to identify the normal homozygous, heterozygous carriers and affected homozygous cattle for CVM, BLAD and DUMPs syndrome in Khuzestan native cows and Iranian Holstein cattle and to implement a useful diagnosis methodology in order to assist veterinarians and breeders in controlling the disease and to report on the monitoring of the genetic health in Khuzestan native cows and Iranian Holstein cattle.

MATERIALS AND METHODS

Sample Collection: In total one hundred Iranian Holstein and two hundred thirty native cows were selected and tested for BLAD, DUMPs and CVM deficiency. Whole blood samples were collected from five different farms in Holstein populations and five regions for indigenous cattle Khuzestan province in Iran. Jugular vein blood samples containing EDTA tube were collected. Genomic DNA were extracted using salting-out method with some modifications [24]. Optimization inclues utilization of separate buffer instead of Buffy coat isolation. Chloroform for DNA phase isolation and achievment to purified DNA and sodium accetate for more concentrated DNA. The optimize porotocol would be more safe, simple, cheap and rapid.

PCR-RFLP: For detection of a mutation in a gene CD18 for BLAD desease, as described by Citek *et al.* [25], the 136-bp DNA fragment was amplified by polymerase chain reaction (PCR). PCR was set up under sterile conditions in PCR tubes containing 100 ng of genomic DNA template, 0.4 pM each of forward (5' CCT TCC GGA GGG CCA AGG GCT 3') and reverse (5' CTC GGT GAT GCC ATT GAG GGC 3') primers, 1X PCR buffer (10 Mm Tris-Cl: pH = 9.0, 50 mM KCl, 0.01% gelatine and 1.5 mM MgCl2), 200 μM each dNTP and 1 U of Taq DNA polymerase in a final reaction volume of 15 μL. The PCR was carried out in a thermal cycler (Biometra). Initial denaturation was achieved at 94°C for 5 min, followed by 45 cycles of 30 s for 94°C, annealing for 30 s at 57°C and extension for 30 s at 72°C, followed by final extension for 10 min at 72°C.

For detection of a possible mutation in a gene coding for UMP synthase, as described by Schwenger *et al.* [17] with minor modifications, the 108-bp DNA fragment was amplified by PCR. The PCR components are the same as in BLAD except forward (5'-GCA AAT GGC TGA AGA ACA TTC TG-3') and reverse (5'-GCT TCT AAC TGA ACT CCT CGA GT-3') primers. The amplification conditions include predenaturation at 94°C for 4 min, followed by 35 cycles of 60 s for 94°C, annealing of primers for 60 s at 61°C and extension for 30 s at 72°C, followed by final extension at 72°C for 10 min.

For detection of a mutation in a gene SLC35A3 for CVM desease, as described by Chu *et al.* [26], the 249-bp DNA fragment was amplified by PCR. The PCR components are the same as in BLAD except forward (5'-AGC TCT CCT CTG TAA TCC-3') and reverse (5'-TCT CAA AGT AAA CCC CAG-3') primers. The amplification conditions include predenaturation at 94°C for 5 min, followed by 35 cycles of 60 s for 94°C, annealing of primers for 60 s at 58°C and extension for 30 s at 72°C, followed by final extension at 72°C for 10 min.

The PCR products of expected size were analysed on 2.5% agarose gel, stained with ethidium bromide and visualized under UV-transilluminator. The PCR products were next digested overnight by using *Taq*I and *AvaI* restriction enzymes for BLAD and $DUMP_S$, respectively, in 1X reaction buffer at 37°C. The digested products were visualized on 4% agarose gel and documented on Gel Doc System (DNR imaging system).

DNA SEQUENCING: To determine existence of mutant, after the gel electrophoresis process, the PCR products, 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 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 AND DISCUSSION

BLAD: Our goal was to develop an easy and efficient method to be used for the accurate detection of the normal, carrier and affected cattle for BLAD disease. The identification of normal or carrier specimens was achieved via PCR amplification of genomic DNA with specific primers designed for a region of 136 bp followed by digestion with *TaqI* endonuclease. Normal homozygote should show two bands of 108 and 28 bp, carrier heterozygote three bands of 136, 108 and 28 bp and affected homozygote only one band of 136 bp. No BLAD carrier has been found. Our results then indicate that the population of Holstein and native cattle reared in Khuzestan Province in Iran is free from BLAD. The PCR-RFLP genotypes were verified by DNA sequencing (Figure. 1).

$DUMP_S$: The primers were used to amplify gene responsible for $DUMP_S$ disease to detect studied population for $DUMP_S$. After the PCR, to reveal mutation in a gene responsible for $DUMP_S$ disease produced three bands of 53, 36 and 19 bp for normal animals (homozygous wild type) and none of the animals showed four bands of 108, 53, 36 and 19 bp, so no animals found to be as carrier of $DUMP_S$ disease. PCR-RFLP genotypes were verified by DNA sequencing (Figure. 2).

CVM: PCR product from the polymorphic region of SLC35A3 gene 249 bp was analyzed by electrophoresis in 1.5% agarose gel. Figure 3 gives the sequences around the mutation position, indicating only G base for genotype AA at position 559.

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.

In looking at over a decade of genotyping for BLAD, DUMPs and CVM in the Holstein breed, it can be seen that different selection strategies define the decline rate of the mutation. 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 [27]. Some studies were performed to prevent distribution of recessive alleles in dairy herds in Iran such as BLAD, CVM, Citrolliemia and DUMPS. To identification of BLAD carriers in Holstein and Brown Swiss AI bulls in Iran, DNA samples from Holstein (n= 30) and Brown Swiss (n= 10) bulls from Abbas Abad AI center (Khorasan state, Iran) were analyzed. In this study, frequencies of BLAD carriers genotypes in Holstein and Brown Swiss bulls were 3.33 and 0%, respectively [9]. For detection of Citrolliemia and CVM, 26 blood and 4 semen samples were supplied from Iranian Holstein bulls used for AI from Abbas Abad Animal Breeding Center in Khorasan state of Iran. Samples were tested by PCR-RFLP method. In this population, there were no bovine Citrolliemia and CVM carriers [28,29]. In other study, Esmaelizadeh considered DUMPS in Razi Vaccine and Serum Research Institute in Iran. 130 samples of blood and sperm were supplied from Holstein bulls and sperm bank in AI center from Karaj Animal Breeding Center.

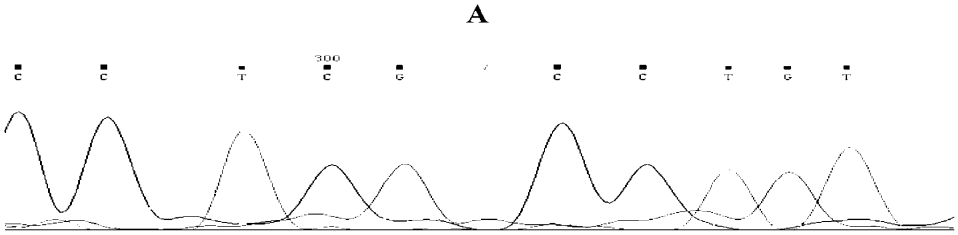

Fig. 1:The forward sequence of CD18 gene for normal cattle.

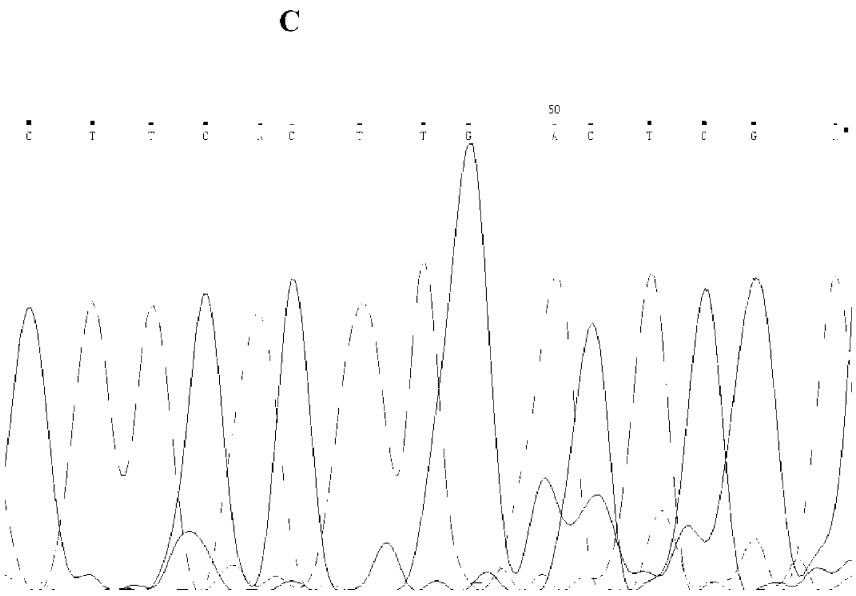

Fig. 2:The forward sequence of UMP_S gene for normal cattle.

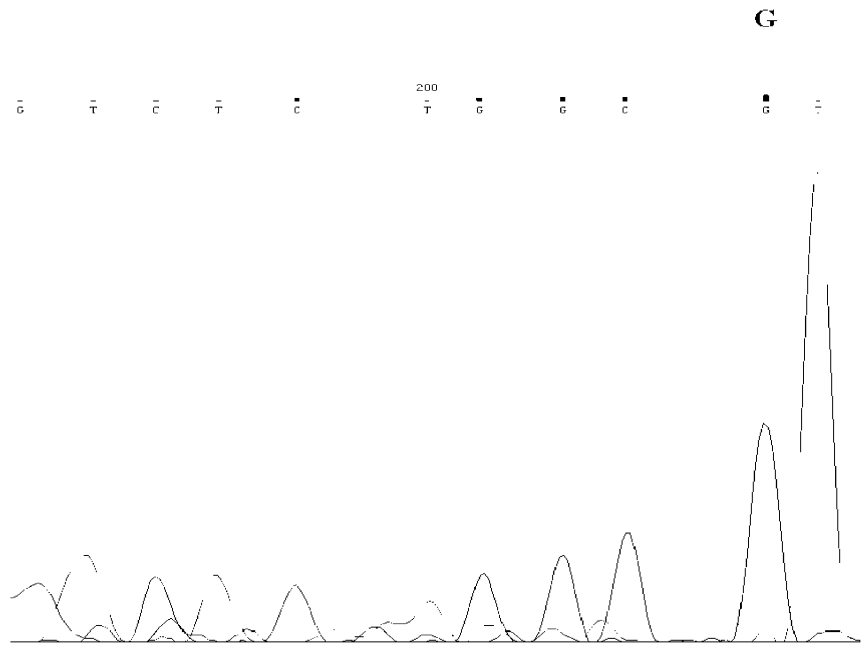

Fig. 3: The forward sequence of SLC35A3 gene for normal cattle.

Samples were tested by (PCR-RFLP) method. In this study, all samples were free from DUMPS disease [30]. To investigate the congenital CVM in Holstein calves, two breeding studies were performed including 625 Danish cows. Out of the 625 cows in this study, 26 CVM affected calf were found [31]. In other study, 111 females from Holstein population in the Czech Republic were studied, that 21 cases were found to be heterozygote (CVM carrier) and 90 cases were homozygous (non-carrier)[25]. Also, to determine the carrier frequency of the CVM-determining mutation in a population of Polish Holstein-Friesian cattle unproven bulls (under evaluation for breeding value) were considered. Out of the 605 bulls examined, 150 heterozygotes were diagnosed [5]. In our study, there were no carrier of CVM, BLAD and DUMPs in Khuzestan native cows and Iranian Holstein cattle. However, we did not observe any carrier for this genetic disease but, it is safe to say that the rate of infectious amount Iranian

endemic livestock is very low. Besides omitting all the infected bulls in the past few years at these centers has led to a non-carrier rate. We can state that this disease and its source are from industrial livestock, not from Iranian endemic breeds. Although, no carrier has been observed in these population but, it does not follow that the disease does not exists in Iranian endemic cattle

CONCLUSIONS

The primers used in our study for detection of normal and mutant CVM, BLAD and DUMPs alleles, successfully amplified and showing fragments with similar size as literature data indicates. Sequencing analysis used in the diagnosis method confirmed the sequences of amplified fragments from the normal CD18, UMPs and SLC35A3 gene. The diagnosis method based on PCR-RFLP test and sequencing analysis is a powerful tool for detecting the presence of these diseases, allowing a good and rapid identification of carrier cattle.

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