THE STUDY OF COMPLEX VERTEBRAL MALFORMATION GENETIC DEFECT IN A POPULATION OF SISTANI COWS

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ABSTRACT: In the present study, complex vertebral malformation (CVM) genetic defect was studied in a population of Sistani cows using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. The purpose of this study was to investigate carriers of CVM defect in a population of Sistani cows. In this genetic defect, dead calves would be born earlier than normal with disorders such as shorter neck, badly shaped legs, curved spines, connected ribs and heart disorders in some cases. Blood samples were collected by Venojects containing EDTA and then transferred to Research Laboratory of Islamic Azad University, Karaj branch. Polymerase Chain Reaction was performed for amplification of 233bp DNA fragments in Exon 4 of chromosome 3 of SLC35A3 gene using specific primers. Enzymatic digestion was performed on polymerase chain reaction products from SLC35A3 gene to identify carriers of CVM using Eco T221 enzyme and the restricted products were loaded on 1.5% Agarose gel. We didn’t find any affected cow with the mutant allele of SLC35A3 for CVM defect comparing with positive evidence. It seems that Sistani cows don’t have mutant alleles for this gene.

Key words: PCR-RFLP, complex vertebral malformations genetic defect, Sistani cows

INTRODUCTION

One of the main objectives of breeding experts is to identify, control and eliminate potential Autosomal recessive alleles in heterozygous individuals and prevent them from spreading in the community. In recent years over 5000 recessive genetic defects has been identified and reported in human using molecular genetic methods, however small number of genetic defects have been identified and studied in livestock [1]. Considering the important role of cow in providing meat and dairy products for world people, given these shortcomings and studying its properties in the genome is more noticeable [2]. Most genetic defects are inherited in a recessive manner. Considering that animals carrying genetic defects often have normal appearance, and identification of them is not possible by the symptoms. Therefore defective genes would be transferred to the next generation by livestock carriers, and are spread in the population. Thus finding accurate methods to detect genetic defects helps to the identification and elimination of carriers and to preserve the desired genetic resources. Based on the existing evidences, the history of investigating the genetic defects in the dairy industry refers to 20th century. Dwarfism was the most common genetic defect between 1940 to 1950 and has the most outbreaks in Herford and Angus cows. Moreover, in the past decades different genetic defects have been investigated and reported in the plumps and dairy breeds such as polydactyl, white blood cells adhesion etc... Recently, we have faced a new genetic defect in cows which is called CVM (complex Vertebral Malformation) [3]. CVM is a lethal and congenital anomaly in Holstein cows. Autosomal inheritance of CVM is recessive, due to a mutation in a single nucleotide of G to T in position 554, Exon 4 of SLC35A3 gene located on chromosome 3 and this mutation leads to a change in amino acid of valine to phenylalanine at position 180 and finally will cause defects in the action of Uridine 5 Diphosphate N-Acetyl Glucosamine. SLC35A3 gene has been completely investigated in sequence and its full length is 224kbp (access NO. AY 1606683) [4, 5, 6 and 7]. In this disease the calf would be born dead and earlier than normal with some disorders such as shorter neck, disfiguring limbs, curved vertebral, connected ribs, growth retardation and sometimes with heart disorders. Most of CVM-infected fetuses are aborted before day 260 of gestation [3, 8, 9 and 10]. The mutant allele of SLC35A3 gene is considered as a lethal genetic defect if both male and female are carriers of CVM. Most economic impact of CVM genetic defects is embryo loss, prolonged calving interval and elimination of carrier cows. Failure to return to estrus rate is usually used as a measure of fertility [8]. According to studies, no difference has been seen until 28 days of gestation between healthy animals and carriers in terms of NRR-(Non Return Rate) [11].
According to their report, first differences of NRR would be observed in day 168 of gestation and based on their results the only trait affected by CVM gene was the number of insemination times in estrous cows. CVM reduces fertility rate in carriers. Carrier cows suffered from abortion in two or three consecutive pregnancies are not selected for the next seminal and will be slaughtered. Most of male cattle with undesired allele of the gene also have numerous other genes, which have positive effect on milk production, milk composition percentage and other important traits. In other words the mutant allele has a positive correlation with genes responsible for production traits (amount of milk and fat percentage) [3 and 12].

MATERIALS AND METHODS
In this study 110 Sistani cows were randomly selected which 68 of them were bulls and 42 were cows. Blood samples were collected by venoject vacuum tubes containing EDTA. Samples were transferred to the Research Laboratory of Islamic Azad University, Karaj branch and were stored at -20°C until DNA extraction. DNA extraction from whole blood samples was performed using optimized and modified salt extraction method. We used 1% Agarose gel to determine quality of extracted DNA. We used special primers designed by Kopnek (2007) to amplify the mutant region of CVM. Sequences of primers were as follows:

Forward primer (Oligo Number: 30704B3-9642C07): 5' - CAC AAT TTG TAG GTC TCA CTG CA- 3'
Reverse primer (Oligo Number: 30704B3-9642D07): 5' - CGA TGA AAA AGG AAC CAA AAG GG- 3'

To perform polymerase chain reaction, after testing the different temperatures and programs (such as temperature changes and changes in Mgcl2 in PCR applications) the optimal PCR conditions with final volume of 25µl for the studied primers were obtained after suitable thermal program test. It is worth mentioning that several different thermal programs can cause low quality bands. Finally in order to optimize PCR reactions, thermal models were applied in thermal gradient method for amplification of target region and the most ideal conditions were selected for amplification. Temperature application of polymerase chain reaction includes 35 amplification cycles with initial denaturing temperature of 94°C for 3 minutes; denaturing temperature was 94°C for 45 seconds. Annealing temperature was 58°C for 60 seconds. Extention temperature was 72°C for 30 seconds and final extention temperature was 72°C foe 10 minutes. Then PCR products were electrophoresed on 1.5% agarose gel. Enzymatic digestion was performed on PCR SLC35A3 gene using EcoT22I enzyme to identify CVM carriers on the SLC35A3 gene. Then 1.5% Agarose gel was used to evaluate and identify the genotypes.

RESULTS AND DISCUSSION
In PCR considering the specific of the primers used for amplification of Exon 4, chromosome 3 from SLC35A3 gene containing our desired mutation, thermo-cycler device amplified the 233bp fragment and was observed after loading with no specific bands on a 1.5% Agarose gel (Figure 1).

Figure-1: PCR Product on 1.5% Agarose Gel

PCR product was used after cutting by EcoT22I enzymes to identify genotypes of Sistani cows and determine gene and genotype frequency. Since some calves are homozygous recessive according to CVM, are often aborted before day 260 of gestation or are born dead, so that's normal if there is no “aa” genotype in the herd, and genotypes “AA” or “Aa” are only expected to be visible in the herd.
Thus 233bp fragment was cut by EcoT22I enzyme and causes two fragments 212bp and 21bp if there is a mutation. Otherwise a fragment 233bp would be created representing failure of desired mutations. If there is mutation, it is called allele “a” otherwise in its absence it is called allele “A”. Genotype is identifiable by observing the numbers of bands in each lane, genotype is recognizable, if only one fragment of 233bp was observed, it is genotype AA and if three fragments 233bp, 212bp and 21bp were observed it is genotype Aa. The results from molecular analysis of 110 Sistani cows only genotype AA was identified showing that none of studied cow carry mutant gene and this means that there is no CVM carrier in our studied Sistani cows. A positive CVM sample was used to verify the accuracy of the detected genotypes. It seems that there are no carriers in Sistani breeds (Figure 2).

The results from this research on Sistani cattle showing that there has been seen no carrier in our studied herd.

REFERENCES