

## Study of Complex Vertebral Malformation Disorder in Iranian Holstein Bulls

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**Abstract:** The aim of this study was to identify the Complex Vertebral Malformation (CVM) in Iranian Holstein bulls. This disease is a hereditary recessive disorder in Holstein cattle. In total 144 blood samples were prepared from Holstein bulls from Abbas Abad Animal Breeding Center and Ferdowsi University of Mashhad's Dairy Farm in Khorasan state of Iran. Genomic Polymerase Chain Reaction-Single Stranded Conformation Polymorphism (PCR-SSCP) method was performed to amplify the polymorphic region of the bovine solute carrier family 35 member 3 (SLC35A3) gene at codon 559 from bovine Chromosome 3. PCR products from the polymorphic region of (SLC35A3) gene (177 bp) were analyzed by the SSCP method. The results of this study demonstrated that there was no carrier of CVM in Iranian bulls in these Centers. Although we did not observe any carrier but, widespread screening programs for detection of genetic disorders seems necessary.

**Key words:** CVM % PCR-SSCP % Iranian Holstein Bulls

### INTRODUCTION

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]. There are several autosomal recessive genetic diseases in cattle such as bovine leucocyte adhesion deficiency [3], bovine citrullinaemia [4], deficiency of uridine monophosphate synthase [5] and complex vertebral malformation [6].

The Complex Vertebral Malformation (CVM) is a hereditary lethal syndrome in Holstein calves and characterized by misshapen vertebrae in the cervical, thoracic and lumbar regions of the vertebral column, anomaly of sternum [7], abnormality of ribs [8], low body weight and lateral rotation of the fetlock joints [9]. Also, consideration of the heart reveals a right-sided hypertrophy and upper interventricular septal defect [10] and many CVM fetuses are aborted at gestation day 159, while other CVM calves are prematurely born or usually stillborn [11,12]. CVM was first described in Denmark in 2000 in Holstein calves [10] and the main ancestor of cattle carrying this mutation was Carlin-M Ivanhoe Bell.

It was an elite breeding bull of Holstein-Friesian (HF) breed born in 1974 [13]. The molecular cause of CVM is a substitution of guanine by thymine (G6T) in a solute carrier family 35 member 3 gene (SLC35A3), encoding a UDP-N-acetylglucosamine transporter (located on bovine chromosome BTA3). This transversion results in the substitution of valine by phenylalanine (V180F) at position 180 [14].

Because of Holstein semens extensively used for crossbreeding programmes with indigenous cattle, it has become necessary to screen all the crossbred animals, especially artificial insemination (AI) bulls, to minimize the risk of spreading genetic diseases [4].

Thus, the objective of the present study is identify the carrier of CVM from Abbas Abad Animal Breeding Centers and Ferdowsi University of Mashhad's Dairy Farm in Khorasan state of Iran.

### MATERIALS AND METHODS

In total 144 blood samples were prepared of Holstein bulls from Abbas Abad Animal Breeding Center (n = 129) and Ferdowsi's Dairy Farm (n = 15) in 2008. Genomic DNA was extracted from 100 µl of blood by Guanidium-Thiucianate Silica gel method [15]. PCR reaction was performed for amplification of polymorphic region of the

(SLC35A3) gene. The following primers were used for PCR for (SLC35A3) gene [16].

F: 5'-TCA GTG GCC CTC AGA TTC TC-3'  
R: 5'-CCA AGT TGA ATG TTT CTT ATC CA-3'

PCR was done in BiometraT-Personal Ver: 1.11 thermocycler by Gene pak PCR MasterMix Core Kit for amplification in a total volume of 20  $\mu$ l. The PCR mix contained: 2  $\mu$ l PCR buffer 10X, 1.5 mM MgCl<sub>2</sub>, 2 mM dNTPs, 3  $\mu$ l mix of primers (10 pM from each primer), 1u *Taq* DNA polymerase and 11.5  $\mu$ l ddH<sub>2</sub>O. PCR product from polymorphic region of (SLC35A3) gene (177 bp) was analyzed by electrophoresis in 1.5% agarose gel with ethidium bromide and M50 was used as ladder (Fig. 1).

For SSCP analysis, 4  $\mu$ l of PCR product with 14  $\mu$ l of sscp dye (95% formamide, 0.05% bromphenol blue and 0.05% xylene cyanol) were denatured for 5 min at 95°C and immediately plunged into ice, for 10 min. The dilution was loaded onto a 10% polyacrylamide gel for (SLC35A3) gene. The gel was run at a constant voltage of 250 V at 6°C for 6 hours and was visualized with silver staining. SSCP analysis of (SLC35A3) gene showed that two bands for normal animal and there was no carrier of CVM (CVM carriers show three bands) (Fig. 2).

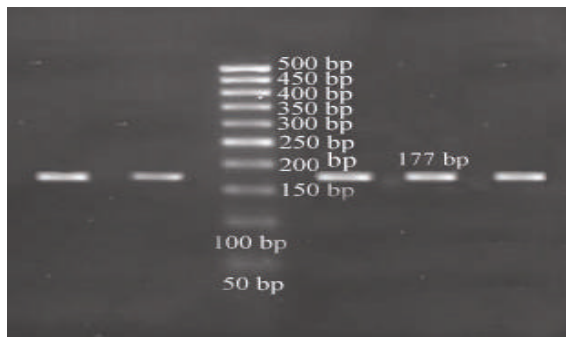


Fig. 1: Agarose gel electrophoresis of PCR products from (SLC35A3) gene revealed same band in all lanes (108 bp). DNA Ladder is M50



Fig. 2: SSCP analysis of (SLC35A3) gene showed that two bands for normal animal. The electrophoresis was performed in a 10% acrylamide gel without glycerol at 250 V and 6°C, for 6 h

## RESULTS AND DISCUSSION

Some studies were performed to prevent distribution of recessive alleles in dairy herds in Iran such as Bovine Leucocyte Adhesion Deficiency (BLAD), Citrolliemia and DUMPS. BLAD is a hereditary disease in Holstein dairy cattle. 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 [17]. Also Citrolliemia is a kind of hereditary disease in Holstein dairy cattle. For detection of Citrolliemia, 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 Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. In this population, there was no bovine Citrolliemia carrier [18]. 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. Samples were tested by (PCR-RFLP) method. In this study, all samples were free from DUMPS disease [19].

The Complex Vertebral Malformation (CVM) is an autosomal recessively inherited [20] and it causes intra-uterine mortality through the entire gestation period leading to repeat breeding and involuntary culling of cows and thereby economic losses. Effects of CVM on fertility was studied in Swedish holstein cattle. This study showed that, carriers of the CVM defect have a reduced reproductive performance compared with non-carriers [21]. Therefore, identification of this kind of genetic disorder at the DNA level seems necessary. 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 [13]. Polymerase chain reaction-primer introduced restriction analysis (PCR-PIRA) is a method that can be used for detecting a single nucleotide mutation in any gene without a restriction site around the mutation site. Kanae used PCR-PIRA method for discrimination between wild-type and CVM alleles of Holstein calves and showed that PCR-PIRA is a reliable and useful method for extensive screening for the CVM allele using DNA polymerase and PCR machines that are widely used in molecular diagnosis [22]. 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)[23]. Also, to determine the carrier frequency of the CVM-determining mutation in a population of Polish Holstein-Friesian (= Polish Black-and-White) cattle, 202 proven bulls and 403 unproven bulls (under evaluation for breeding value) were considered. Out of the 605 bulls examined, 150 heterozygotes were diagnosed [16].

In our study, there was no carrier of CVM in Iranian Holstein bulls from Abbas Abad Animal Breeding Center and Ferdowsi University of Mashhad's Dairy Farm in Khorasan state of Iran. 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 centers but, it does not follow that the disease does not exist in Iranian endemic cattle.

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