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# STUDYING THE GENETIC DEFECTS OF CVM IN HOLSTEIN COWS OF ALBORZ PROVINCE

**SABERI J[1], HEMATI B[*1] AND NOSHARY A[1]**

**1:** Department of Animal Sciences, Karaj branch, Islamic Azad University, Alborz, Iran

***Corresponding Author: E Mail: bzdhmt@gmail.com**

## ABSTRACT

In the present study the genetic defects of CVM (Complex Vertebral Malformations) in Holstein cow populations of Tehran and Alborz provinces has been studied. In this disease, the dead calve is born earlier with some disorders like a short neck, badly shaped legs, vertebral malformations, attached ribs and sometimes with cardiac abnormalities. Determination of genetic deficiency of CVM was performed by studying blood and sperm (semen) samples by now. This is the first time that this subject has been performed through milk somatic cells. In this research, milk samples of 120 cow populations, each of which was the agent of 100-400 milking cows, was evaluated in three stages separately. The samples, after collecting from the milk tank were transported to the research laboratory of agriculture college and sample DNAs were extracted. Polymerase chain reaction was performed using specific primer couples to reproduce the 233bp from the chromosome of 3 axons from 4 SLC35A3 genes. Enzymatic digestion was performed on SLC35A3 genes of polymerase chain reaction production using Eco T22 I enzyme to determine the genetic deficiency carriers of CVM and was electrophoresis on Agarose 1.5%. The results showed that there is a mutation of G to T in 599 genes of SLC35A3 in 26 cows of studied Holstein cows in Tehran and Alborz provinces. Then two herds containing 110 and 130 milking cows were randomly selected. Then after molecular test 8 cows were identified as carrier. In these two herds, a total of AA, AB genotypes were identified in 232, 8 cows in mentioned herd, which equals about 97% and 3% but due to abortion of recessive homozygote in the early growth stages, unavailability of mutant dead calves and lack of using sperms carrying the mutant gene, BB genotypes number were observed and recorded as 0%.

**Keywords: CVM, Holstein Cow, Genetic Deficiency, Somatic Cells of Milk**

## INTRODUCTION

All species of livestock are suffering from different kinds of genetic deficiencies. Spider syndrome and stress or mental stress syndrome are two outstanding examples in sheep, which have led to economic pressure in recent decades. Dwarf may be the most known example of genetic deficiencies in livestock industry in 20$^{th}$ century which in 1940-1950 was more prevalent in Herford and Angus cows. In addition, several other genetic deficiencies were found in different breed of beef and dairy calves in the past decades such as multi-fingered and adhesion of white blood cells. In very rare cases also some of cow breeders faced some cows which were born with several simultaneous deficiencies. Recently we face some new genetic deficiencies which is called CVM or complex vertebral malformations. This genetic deficiency leads to a fatal hereditary disease in Holstein cows. CVM inheritance is an autosomal recessive and is caused by a mutation from G to T nucleotide in 559chromosome of 3 axons of 4 SLC35A3 genes which the mutation makes Uri dine 5-diphosphate-en-steel glucosamine to change and results to the conversion of amino acid valine into phenylalanine **[1-3]**. SLC35A3 bovine gene has been completely sequenced and its full length is 22400 in open state (AY160 683 access number). Males that were identified as genetic deficiency carriers of CVM include Etazon Lord Lil, T Burma, Kol Nixon, and Carlin-M Ivanhoe Bell. In this disease, the dead calve is born earlier with some disorders such as shorter neck, badly shaped hands and legs, CVM, attached ribs, growth retardation and in some cases with heart disorders, i.e., junction of the main blood vessel to the heart is not in the right place and damage is observed in all bones except for external bones. Most of the time, the affected fetuses are aborted before day 260 of gestation **[4, 5]**. Soon after, this test was conducted in many countries such as America **[6]**; Germany **[7]** Czechoslovakia**;**; Japan **[8]**; Ireland; Slovenia **[9]** Italy **[2]** and China **[10]** and each of researchers found different frequencies in their herds with respects to CVM. The most economic impact of this gene is fetal loss and a long interval between calving. Non-Return rate to estrus (NRR) is often used as a measurement of fertility. According to a few reviews by **[10]**; there was seen no difference between healthy calves and vector ones with respects to NNR by day 28 gestation. As they reported, first difference were observed in the day 168 in NRR and according to their results, the only trait affected by CVM gene was the number of insemination in estrus cows. CVM reduces fertility rate in cows carrying the gene and these won't be

selected in the next insemination if they have abortions in two or three successive pregnancies and will be slaughtered **[4]**. Milk selection to extract DNA from present somatic cells is easier, as well as saving time and cost, to bleed and the cow suffers from no stress. Moreover, if the female cows are pregnant, bled is not permitted due to stress from breeder on the cow, so we fail to examine pregnant ones. Thus, milk samples were used in this research to evaluate female cows.

**MATERIALS AND METHODS**

120 dairy cows were randomly sampled in Tehran and Alborz provinces. All herds included about 100 to 400 milking cows. Most cows had complete recorded information and a family tree. Among all the selected herds, 30 ml milk sample was prepared, and then the samples were coded from 1 to 120 and was transported to laboratory in ice pack and stored in freezer at -20 temperature, 1ml of each milk sample was separately poured into the 1.5ml micro tube, then was placed in bath for 25m at 55℃ , after removing from bath it was centrifuged for 15m at 8000rpm then the floating material is evaluated and the remaining sediment is washed then DNA extraction was performed using extraction kit of Kiagene Company (Dnasy tissue). After genome DNA extraction, 1.5% Agarose gel was used to determine its quality. Designed primers by Kepenek, 2007, were used to reproduce the mutated region of CVM, the order of primers were as following:

F: 5'CACAATTTGTAGGTCTCACTG CA3'

R: 5'CGATGAAAAAGGAACCAAAAGGG3'

PCR kit from Kiagene Company (Hot star Taq PCR) was used to do polymerase chain reaction. The temperature planning of polymerase chain reaction including 30 reproduction cycles at 94℃ Initial denaturing temperature for 3m, 94c denaturing temperature for 45s, 55c junction temperature for 20s, 72c reproduction temperature for 40 s and final reproduction temperature for 10m. The production of polymerase chain reaction (PCR) was electrophorseized on 2% Agarose gel. It is worthy to note that in order to sequence and confirm gene, 8 samples of PCR production were sent to Kavosh Kosar Company and the result was compared and confirmed with NCBI.

Enzymatic digestion was performed using Eco T221 enzyme 9 Fermentas) on the production of PCR of SLC35A3gene to identify the cows carrying genetic deficiency of CVM and the samples were placed in thermo-cycler machine for 3h at 37 c in the rest of the operation 1.5 Agarose

gel was used to study and identify the genotype.

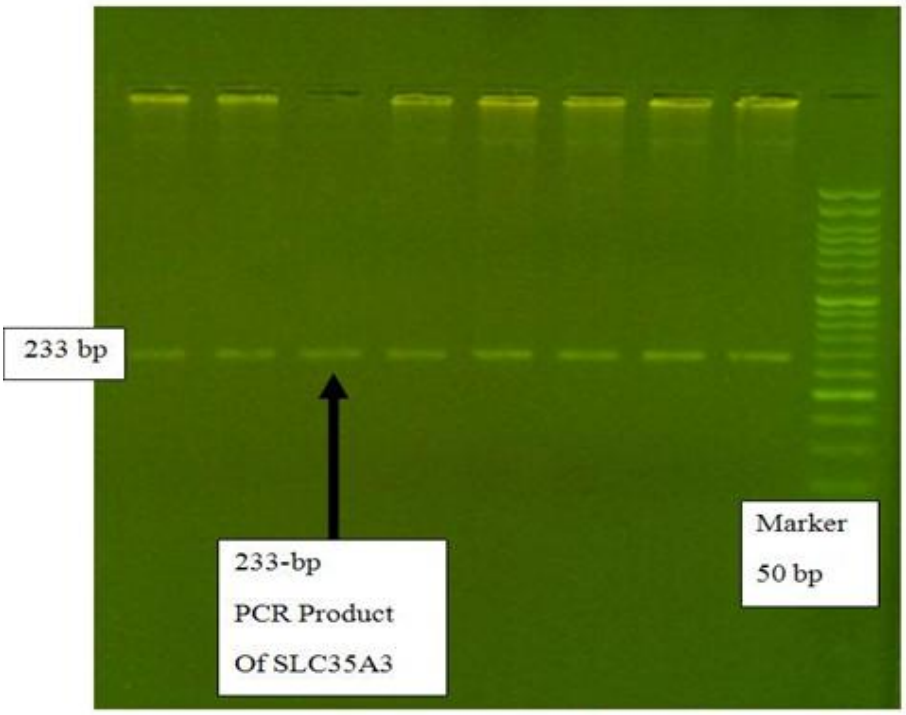
**RESULTS AND DISCUSSION**

The samples were loaded on 1.5% Agarose gel to determine the quality of DNA, and then were observed under ultraviolet light. The samples were free of any fractures and strain indicating its high quality and non-pollution and evaluating their quality showed a good quality for PCR. Then due to specificity of applied primers for reproducing axon4, chromosome of 3 SLC35A3 genes containing our desired mutation, thermo-cycler reproduced 233bp and was observed after loading on 1.5% Agarose gel with no non-specific bands. The production of PCR, after cutting by EcoT22 I, was taken on 1.5% Agarose gel to identify 120 genotypes of Holstein cows and determine genotype frequency.

Among 120 studied Holstein cows, genotype AB in 26 cows were identified showing the cows are carrier in CVM gene and just genotype AA was identified in 94 cows showing the healthiness of these cows regarding mutant allele of CVM gene.

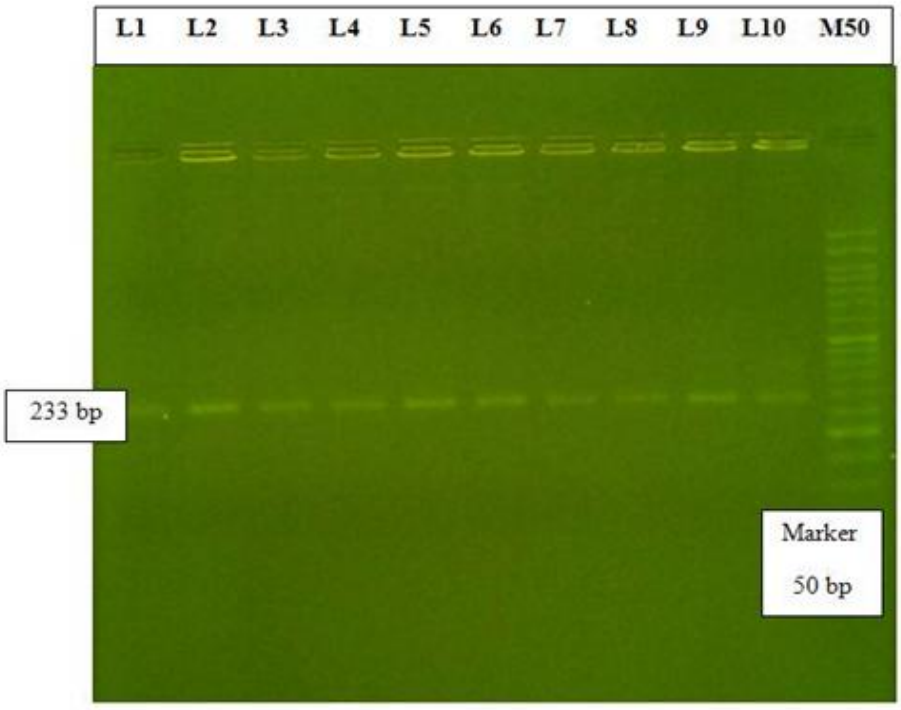
In the next stage two carrier cows were randomly selected to identify carrier cows. These cows including 110 and 13 milking cows, respectively which in the previous stage the mutant allele of CVM gene was observed as an agent in the tank of their milk samples and were recognized as the carrier of this deficiency.

Those with BB genotype die before the day 260 of pregnancy or are born dead. Therefore, lack of BB genotype in mentioned cows is normal. In these two herds the total of AA, AB genotypes were recognized as 232, 8 in the mentioned cows, respectively which almost equals 97% and 3%. But the number of BB genotypes were observed and recorded as 0% due to abortion in early stages of grow, unavailability of dead mutant calves or lack of using of sperms carrying mutant gene was significantly different from dominant homozygote cows which can be due to abortion in the early stages of grow. **[3]** observed no carrier cow in studied herds. **[4, 5, 8, 9, 11, 12]** have determined different frequencies in CVM carrier of their studied herds.




Research conducted by students in the college of Agriculture and Natural Resources Research Laboratory

**Figure 1**




L1-L10: PCR products of digestion by enzymes ECOT 221

M50: Indicate the range of 50 to 1500 bp

**Figure 2**

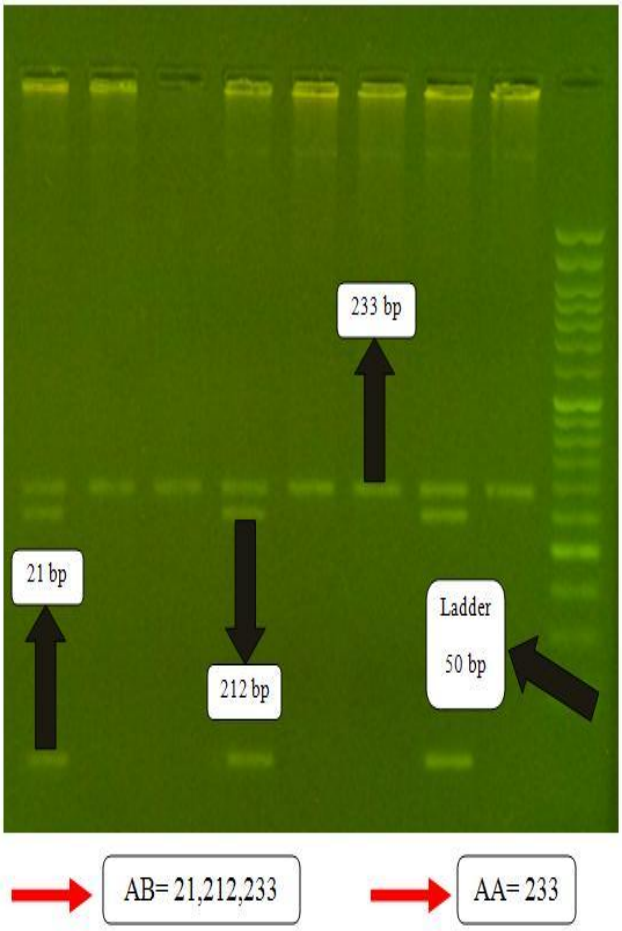



**Figure 3**

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