

## Complex vertebral malformation: relationship between carrier status and milk yield in three holstein herds in western Mexico

Complejo de malformación vertebral: relación entre animales portadores y la producción de leche en tres hatos de la raza holstein en el occidente de México

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### Abstract

Complex vertebral malformation (CVM) is an autosomal recessive genetic syndrome present in Holstein cattle. The disease results in direct economic losses for cattle ranchers due to abortions and deaths of newborn calves. The purpose of the study was to estimate the allelic and genotypic frequencies of CVM syndrome in three Holstein cow herds in western Mexico and determine whether an improving effect between CVM genotype and milk production exists. A total of 308 cows were genotyped using the polymerase chain reaction–restriction fragment length polymorphism (PCR/RFLP) assays. The cow's genotypes and first- and second-lactation total milk yield adjusted to 305 days were compared using a mixed statistical model, and the genotypic frequencies were calculated. The total recessive allele frequency of the CVM was 0.06. Milk production by lactation did not differ between CVM carriers and normal cows. It is concluded that not a direct relationship was found between milk production and CVM carrier status in the Mexican herds sampled.

**Keywords:** Dairy production; genetic diseases; molecular markers; population genetics.

### Resumen

El complejo de malformación vertebral (CVM) es un síndrome genético autosómico recesivo presente en el ganado Holstein. La enfermedad causa pérdidas económicas directas para los ganaderos debido a los abortos y la muerte de becerros neonatos. El propósito del estudio fue estimar las frecuencias génicas y genotípicas del síndrome CVM en tres hatos de vacas Holstein en el occidente de México y determinar si existe un efecto mejorante entre el genotipo CVM y la producción de leche. Se genotiparon 308 vacas usando la técnica de reacción en cadena de la polimerasa con polimorfismos de longitud de fragmento de restricción (PCR/RFLP, por sus siglas en inglés). Se compararon los genotipos de las vacas y los promedios de producción de leche de primera y segunda lactancia ajustados a 305 días utilizando un modelo estadístico mixto, y se calcularon frecuencias génicas y genotípicas. La frecuencia total del alelo recesivo de CVM fue de 0.06. La producción de leche por lactancia no difirió entre los portadores CVM y las vacas normales. Se concluyó que no hay una relación directa entre la producción de leche y el genotipo de portador de CVM en los hatos mexicanos muestreados.

**Palabras clave:** Producción láctea; enfermedades genéticas; marcadores moleculares; genética de poblaciones.

**Recibido:** 15 de octubre de 2017

**Aceptado:** 26 de febrero de 2019

**Publicado:** 05 de septiembre de 2019

**Como citar:** Duifhuis-Rivera, T., Ayala-Valdovinos, M. A., Lemus-Flores, C., Galindo-García, J., & Sánchez-Chiprés, D. R. (2019). Complex vertebral malformation: relationship between carrier status and milk yield in three holstein herds in western Mexico. *Acta Universitaria* 29, e2110. doi: <http://doi.org/10.15174/au.2019.2110>

## Introduction

In recent decades, the complex vertebral malformation (CVM) syndrome has probably been one of the most frequently detected autosomal, recessive, hereditary defects in Holstein cattle (Patel & Patel, 2014; Zhang *et al.*, 2012). CVM is caused by a point mutation from Guanine (G) to Thymine (T) at nucleotide position 559 of the gene *SLC35A3* (bovine solute carrier family 35, member 3) in region BTA3 on chromosome 3 (Kanae, Endoh, Nagahata & Hayashi, 2005). The mutation provokes the substitution of valine to phenylalanine in amino acid 180 of the protein which inhibits the functioning of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), a nucleotide-sugar transporting protein residing in the Golgi apparatus. This peptide is essential for the control of vertebral formation in the mesoderm, and the defective molecule leads to vertebral malformation (Thomsen *et al.*, 2006). Approximately 80% of affected homozygous fetuses are aborted by the day 260 of gestation (Ghanem & Nishibori, 2008).

The defective allele for CVM was spread globally by the over-usage of a high-performance bull, Penstate Ivanhoe Star, and its offspring, *Carlin-M-Ivanhoe Bell*, which were both carriers of the deleterious allele (Agerholm, 2007). The propagation of recessive genetic defects in domestic animal populations negatively affects the economics of dairy farms and reduces the success of genetic improvement programs (Ghanem & Nishibori, 2008).

The objectives of this study were to identify cows carrying CVM in three Holstein herds in Western Mexico, to identify any differences in first- and second-lactation milk production between carrier cows and normal homozygotes, and to determine whether the carrier genotype has an improving effect on dairy production.

## Materials and Methods

This study included 308 Holstein cows with two or more lactations from three industrial dairies in the state of Jalisco, Western Mexico. The first, second, and third herds were comprised of 102, 100, and 106 cows, respectively. Total milk yield adjusted to 305 days in kilograms of milk production records for 2016, for the first and second lactations, in kilograms of milk for each cow, were obtained using DairyCOMP 305® version 5 software (DC305, 2013).

DNA was extracted from blood samples using the Quick-DNA™ Universal Kit (Zymo Research, Orange, CA, USA). For genotyping by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, the Thermo Scientific™ DreamTaq™ PCR kit (Takara Bio, Inc., Kusatsu, Shiga, JAP) was used.

The PCR for amplification of 233 bp fragments from cow's DNA was performed with reaction conditions as follows: 5 pmol of each forward and reverse primer, 0.5 mM dNTP, 1.25 mM MgCl<sub>2</sub>, 1.6 µl 10X DreamTaq™ Green Buffer, and 0.333U of DreamTaq™ polymerase in 12 µl reaction volumes. Cycling parameters included an initial 5 min denaturation at 94 °C followed by 35 cycles of 30 s denaturation at 94 °C, annealing for 30 s at 56 °C, and a 72 °C extension for 30 s. The cycling parameters were followed by a final extension at 72 °C for 5 min. The restriction enzyme *Pst*I from New England Biolabs™ (New England Biolabs, Inc., Ipswich, MA, USA) in a TECHNE™ TC-5000 (Techne Inc. Burlington, NJ, USA) thermal oscillator was based on methodologies described by Kanae *et al.* (2005) for gene *SLC35A3* to identify the CVM polymorphism. If the 233 bp amplified sequence belongs to the wild-type allele, the enzyme cut the fragment into two parts (21 pb and 212 bp); if it belongs to the mutant allele, there was no cutting site and the fragment will remain complete.

To calculate and analyze allelic and genotype frequencies, the POPGENE® software (version 1.32; POPGENE, 1997) was used. The SPSS software, version 20, 2011, was used to perform a mixed statistical model in each herd separately, in order to identify differences in first- and second-lactation milk production between carrier cows and wild type homozygotes of gene *SLC35A3*.

A fixed and random effects design was used considering the following mixed statistical model:

$$Y_{ij} = \mu + \text{genotype } SLC35A3_i + \text{animal}_j + e_{ij},$$

where  $Y_{ij}$  is a cow's first- and second-lactation milk production adjusted to two milkings at 305 days;  $\mu$  is the general average milk production; genotype *SLC35A3* refers to the genotypes for the *SLC35A3* gene in each animal (N/N and N/CVM);  $i$  is 1, 2; animal is the total random genetic component of each animal;  $j$  is 1, 2, ..., 308; and  $e_{ij}$  is the random error for each measurement.

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## Results

The genotyping of *SLC35A3* revealed 260 homozygotes cows for the wild allele and 40 carrier cows with CVM mutation. Table 1 shows the distribution and frequencies of *SLC35A3* genotypes in the three herds. The mutant homozygous genotype (CVM/CVM) was absent due to the sampling of only adult cows and the nature of the syndrome, as homozygous animals with this mutation survive for only a few hours after birth. Not a significant difference in milk production was observed between normal and heterozygous genotypes ( $p > 0.05$ ; table 2). Differences in milk production by lactation among herds, which can be explained by the different environmental and handling factors present in each daily farm, were found. Milk production did not differ between normal and heterozygous CVM genotypes.

**Table 1.** Genotype and allele frequencies of *SLC35A3* gene.

Herds	Genotypes		Allele frequencies	
	N/N	N/CM	N	CMV
1	85	17	0.92	0.08
2	87	13	0.94	0.06
3	96	10	0.95	0.05
Total	268	40	0.94	0.06

$p > 0.05$ . No difference in genotype or allelic frequencies was observed among herds.

N = wild allele; CVM = complex vertebral malformation mutant allele.

Source: Author's own elaboration.

**Table 2.** Average milk production (in kilograms) at 305 days per cow, two milking per day, per lactation by genotype and herd.

Herd	Lactation 1			Lactation 2		
	N/N	N/CVM	SE	N/N	N/CVM	SE
1	11,767	12,676	205	12,231	13,384	267
2	10,581	9,941	350	10,069	10,958	292
3	9,460	7,827	344	10,208	7,684	310

$p > 0.05$ . No significant difference in milk production was observed between wild homozygous allele and heterozygous carrier in both lactations. N/N = wild homozygous allele; N/CVM = heterozygous complex vertebral malformation carrier; SE = standard error.

Source: Author's own elaboration.

## Discussion

In the first decade of this millennium, high allelic frequencies of animals carrying CVM, such as 0.325 (Nagahata *et al.*, 2002) and 0.26 (Berglund, Persson & Stalhammar, 2004), were reported. However, in the second decade of this millennium, the frequency of the mutant allele of the CVM decreased. The genetic frequency of the CVM mutation in our study was 0.06, value which fits into the previously reported values ranging from 0.115 to 0.008 (Akyuz, Sariozkan & Bayram, 2015; Hemati, Gharaie-Fathabad, Fazeli, Namvar & Ranji, 2015; Mahdipour *et al.*, 2010; Meydan, Yildiz & Agerholm, 2010; Paiva *et al.*, 2013; Sun *et al.*, 2011; Wang *et al.*, 2011; Zhang *et al.*, 2012). This trend suggests gradual selection against the mutation since the year 2000. This trend suggests a gradual selection against the mutant allele; since the early reports in the first decade of this century, the frequency of the mutation has been progressively decreasing; in addition to that, the absence of the mutation in some herds has been reported since 2010 (Eydivandi, Sevedabai & Amirinia, 2011). The low frequency of CVM carriers in the present study does not imply that the frequencies of these diseases cannot be higher in other herds, but it is known that even the low frequencies of autosomal recessive genetic diseases have an economic impact on herds of dairy cattle (Uffo & Acosta, 2009); therefore, it is important to genotype the herds. Not a significant difference in milk production was observed between normal and heterozygous genotypes ( $p > 0.05$ ). A literature review revealed no previous study examining associations between dairy production parameters and the *SLC35A3* genotype causing CVM.

The CVM mutation was detected in three Mexican Holstein cow herds analyzed in this study, since a direct relationship between milk production and the CVM carrier genotype was not found. The use of molecular marker-assisted selection against CVM will not reduce herd productivity in Holstein-Friesian cattle in Mexico. The implementation of programs to monitor hereditary diseases among Holstein cattle ranches is necessary to avoid the spread and future presentation of genetic diseases.

## Acknowledgements

This study was fully supported financially by the Universidad de Guadalajara through project P3E-229355.

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