



## Carrier frequency of autosomal recessive disorders CVM and DUMPS in Sahiwal and Holstein Friesian cattle in Chhattisgarh

VINEET KUMAR<sup>1</sup>, ASIT JAIN<sup>2✉</sup>, TRIPTI JAIN<sup>2</sup>, KISHORE MUKHERJEE<sup>3</sup>,  
AAKANKSHA RATHORE<sup>4</sup>, KAISER PARVEEN<sup>3</sup> and FARHEEN ANSARI<sup>2</sup>

*Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya (DSVCKV), Anjora, Durg, Chhattisgarh 491 001 India*

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India holds the top position globally in cattle genetic resources, boasting 192.49 million cattle. Among these, 51.36 million are crossbred or exotic. Despite contributing approximately 26% and 1% of the total milk production (BAHS 2019), crossbred and exotic cattle are also introducing genetic disorders into the Indian herds, respectively (Yathish *et al.* 2010). According to the Government of India's Minimum Standard Protocol (MSP), breeding bulls and cows must be free from Complex Vertebral Malformation (CVM), Deficiency of Uridine Monophosphate Synthase (DUMPS) and other genetic disorders (Basic Animal Husbandry and Fisheries Statistics 2019).

Complex vertebral malformation is an autosomal recessive disorder found in Holstein cattle worldwide, typically manifesting during fetal development. This condition results in prenatal loss and a range of vertebral abnormalities (Berglund *et al.* 2004). DUMPS deficiency hampers the synthesis of UMP, vital for converting orotic acid into uridine monophosphate (UMP), a critical component of pyrimidine nucleotides essential for DNA replication during mitotic cell division. Homozygous embryos for the wild-type DUMPS do not survive beyond 40 days of conception, resulting in various reproductive complications like low conception rates and repeat breeding cycles (Patel *et al.* 2006).

It is imperative to screen all animals in organized dairy farms, especially breeding bulls, prospective bulls, and breeding cows, to prevent the spread of genetic defects within the population. Artificial insemination (AI) accelerates the dissemination of unwanted recessive genes. Molecular techniques enable the detection of carrier or

heterozygous animals, facilitating eradication programs for genetic defects. Consequently, this study aimed to determine the gene and genotype frequencies of CVM and DUMPS genetic disorders in Sahiwal and HF cattle populations and their crosses in Chhattisgarh, India.

*Experimental design, blood collection and DNA isolation:* All animal experiments were ethically approved. The study was carried out at the Genetic Screening Laboratory, Department of Animal Genetics and Breeding, College of Veterinary Science and A.H., Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya (DSVCKV), Anjora, Durg, Chhattisgarh, India. It involved 50 purebred Sahiwal cattle and 100 animals (HF and its crossbreeds). These animals were housed at various farms (BMEF, Yadav farm and ABIS), where blood samples (approximately 2.0 ml) were collected for DNA isolation via the jugular vein into pre-coated EDTA vacutainer tubes and promptly transported on ice to the laboratory. Genomic DNA extraction was performed using the HiPurA Blood Genomic DNA Miniprep Purification Kit (Hi-Media Cat # MB504) following the manufacturer's guidelines. Quality and quantity of the DNA were evaluated using 0.7% horizontal submarine agarose gel electrophoresis and NanoDrop spectrophotometer (NanoDrop Technologies, USA), respectively.

*PCR, sequencing and RFLP of SLC35A3 and UMPS genes:* PCR was conducted using a programmable thermal cycler (T-100, Bio-Rad) to amplify the SLC35A3 and UMPS genes. The detailed information of each primer is provided in Table 1. PCR reactions were carried out in a 25 µl volume, comprising 10 pM of each gene-specific primer, 5 µl of DNA (30 to 40 ng/µl), 12.5 µl PCR master mix (2×, Sigma, USA), and nuclease-free water. Cycling conditions included an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 30 s for SLC35A3 and at 59°C for UMPS gene, extension at 72°C for 60 s, and a final extension at 72°C for 10 min. After PCR amplification, 2% gel electrophoresis, containing SYBR safe DNA gel stain dye (Invitrogen, Cat# S33102), was used to

Present address: <sup>1</sup>Central Poultry Development Organisation, Northern Region, Chandigarh. <sup>2</sup>Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh. <sup>3</sup>Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg, Chhattisgarh. <sup>4</sup>Livestock Development Department, Government of Chhattisgarh. ✉Corresponding author email: [vetasit@gmail.com](mailto:vetasit@gmail.com)

Table 1. Details of primers used in the current study

Name of genetic disorders	Name of the genes and primers	Primer sequence (5' – 3')	Product length (bp)	Reference(s)
CVM	<i>SLC33A3</i> -F	CACAATTTGTAGGTCTCACTGCA	287	Kanae <i>et al.</i> 2005, Meydan <i>et al.</i> 2010,
	<i>SLC33A3</i> -R	CGATGAAAAAGGAACCAAAAAGGG		
DUMPS	<i>UMPS</i> -F	GCAAATGGCTGAAGAACATTCTG	108	Yathish <i>et al.</i> 2010
	<i>UMPS</i> -R	GCTTCTAACTGAACTCCTCGAGT		

visualize the products (Gel Doc, Bio-Rad), followed by purification using a Gel Purification Combo Kit (Himedia, Cat#MB563). Subsequently, representative samples were sent for sequencing at Redcliffe Life Diagnostics, India, to confirm or detect mutations in the genes.

For RFLP analysis, approximately 10 µl of PCR product was digested with *Pst*I for *SLC35A3* and *Ava*I for *UMPS* genes, following the manufacturer's instructions. Digested products were then examined by agarose gel electrophoresis as described above. Gene and genotype frequencies were determined using standard procedures.

In the current study, PCR analysis of the *SLC35A3* gene demonstrated a distinct 287 bp band (Fig. 1, lanes: 7-12). The RFLP pattern of the *SLC35A3* gene further elucidated the genetic status of the animals. While affected animals exhibited a single 287 bp fragment, carriers showed three fragments (287, 264, and 23 bp), and normal individuals displayed two fragments [264 and 23 bp (not visible due to very small size), Fig. 1]. Notably, the absence of the 287 bp band in the study animals confirmed their normal genotype for the *SLC35A3* gene, signifying a lack of susceptibility to CVM. These results were further supported by sequencing data, solidifying a gene frequency of 0.0 for CVM-affected genes and 1.0 for normal genes. Interestingly, while the current study reflects a lack of CVM carriers, previous research has indicated varying frequencies of carriers globally. Studies in Denmark (Thomsen *et al.* 2006), Sweden (Berglund *et al.* 2004), Turkey (Meydan *et al.* 2010), India (Mahdipour *et al.* 2010, Alyethodi *et al.* 2018) and other countries have reported considerable percentages of CVM carriers in cattle populations. However, in some studies in India and other countries, similar to the present findings, no

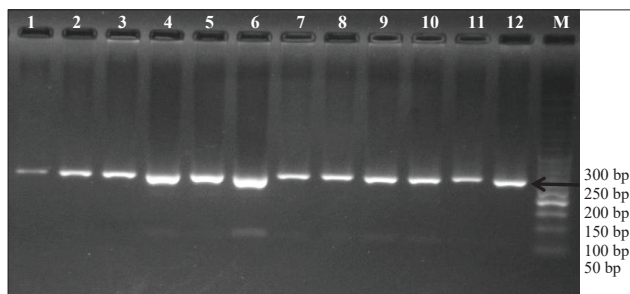


Fig. 1. Agarose gel electrophoresis (2.0%) of *SLC35A* gene for CVM after restriction enzyme digestion with *Pst*I. Lane M: 50 bp DNA ladder (Hi-Media); Lanes 12-7: represents the PCR product (287 bp, arrow mark) and Lanes 6-1: represents the corresponding RE digested PCR products of *SLC35A3* gene (264 bp and 23 bp) indicated CVM free animals.

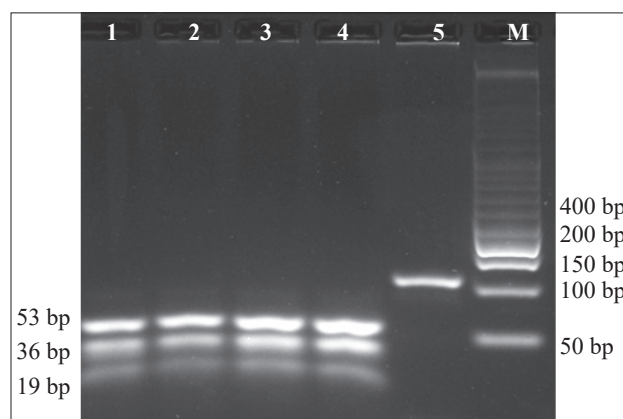


Fig. 2. Agarose gel electrophoresis (2.0%) of *UMPS* gene for DUMPS after restriction enzyme digestion with *Ava*I. Lane M: 50 bp DNA ladder (Hi-Media); Lane 5: represents the PCR product (108 bp) and Lanes 1-4: represents the corresponding RE digested PCR products of *UMPS* gene (53 bp, 36 bp and 19 bp) indicated DUMPS free animals.

or low frequencies of carriers have been reported in various cattle breeds (Yathish *et al.* 2010, Khade *et al.* 2014, Dagong *et al.* 2018). The mutation responsible for CVM, a guanine to thymine substitution in the *SLC35A3* gene, disrupts the function of the UDP-N-acetylglucosamine transporter, leading to structural abnormalities. These transporters are crucial for glycosylation processes essential for cellular functions (Thomsen *et al.* 2006).

In the present study, the PCR analysis targeting the *UMPS* gene exhibited a single and distinct band of 108 bp. PCR digestion displayed consistent patterns across all samples, showing fragments of 53, 36, and 19 bp, affirming the wild-type genotype in all animals. None of the subjects exhibited heterozygous genotypes (four fragments, i.e. 53, 36, 19 and 108 bp) or recessive homozygous/mutant genotypes (undigested products, 108 bp), as evidenced by the absence of the 108 bp fragment (Fig. 2). Validation of the PCR genotyping outcomes was achieved through directional sequencing. These analyses collectively indicated a gene and genotype frequency of 0% for DUMPS carriers or affected individuals, with a prevalence of 100% for normal animals and their alleles. Findings of the present study align with various studies conducted across different regions, including India (Patel *et al.* 2006, Ignietius *et al.* 2017, Magotra *et al.* 2020), Poland (Gozdek *et al.* 2020), Russia (Koschaev *et al.* 2018), etc. which also reported negligible frequencies or absence of DUMPS carriers. However, contrasting results have been documented in

studies from the Argentina and other countries (Poli *et al.* 1996) indicating the presence of DUMPS carriers in varying proportions. The genetic basis of DUMPS lies in a single point mutation (C to T) at codon 405 in exon 5 of the UMP synthase gene on bovine chromosome 1 (Harlizius *et al.* 1996).

While this study reports zero frequency for CVM and DUMPS carriers, this does not conclusively imply their absence in the state. Factors such as sample size limitations or pre-screening of HF breeds before herd entry may influence these results. Notably, the frequency of the CVM and DUMPS mutant alleles appear to have decreased over the past decade, possibly due to improved detection methods or selective breeding against the mutation(s). However, the widespread use of semen from elite Holstein bulls in crossbreeding programs poses a risk of transmitting defective alleles across lineages. Therefore, it is imperative to conduct genetic screening of breeding sires or their semen before introduction into herds.

In conclusion, the absence of CVM and DUMPS alleles in Chhattisgarh signifies a favourable environment for breeding. To curb the spread of these genetic defects, routine molecular testing of bulls, imported semen, and cows is recommended. Heterozygous/carrier animals should be excluded from breeding programs. Further research is necessary to assess the prevalence of these disorders in other regions of Chhattisgarh and neighbouring states.

#### SUMMARY

In the present study, genetic screening of Sahiwal and Holstein Friesian (HF) cattle and its crosses was conducted using the PCR-RFLP technique to detect the presence of CVM and DUMPS disorders. Genomic DNA was extracted from 50 Sahiwal and 100 HF animals and its crosses using commercially available kits. Gene-specific primers were utilized for *in vitro* amplification of *SLC35A3* and *UMPS* genes by PCR, followed by RFLP analysis using PstI and Aval restriction endonucleases for CVM and DUMPS, respectively. Subsequently, the PCR products were purified and sequenced to confirm any mutations in the DNA target sequences. Notably, no affected or carrier animals for CVM and DUMPS disorders were identified among the screened animals. Thus, the gene and genotype frequency of CVM and DUMPS carriers was zero, indicating a 100% frequency of normal animals. The study underscores the importance of continued screening for these genetic disorders, involving a larger sample size across the state, and advocates for mandatory screening before utilizing animals for breeding purposes, in adherence to minimum standard protocols.

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