



# Genetic Screening of Deficiency of Uridine Monophosphate Synthase (Dumps), Bovine Citrullinaemia (BC) and Factor Xi Deficiency (Fxid) in Hardhenu, Sahiwal and Haryana Bulls

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**Citation:** Magotra et al., 2020. A Genetic Screening of Deficiency of Uridine Monophosphate Synthase (Dumps), Bovine Citrullinaemia (BC) And Factor Xi Deficiency (Fxid) in Hardhenu, Sahiwal and Haryana. *International Journal of Bio-resource and Stress Management* 2020, 11(1), 035-039. [HTTPS://DOI.ORG/10.23910/IJBSM/2020.11.1.2071a](https://doi.org/10.23910/IJBSM/2020.11.1.2071a).

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

**Funding:** The research was conducted with the kind and supports from Rastriya Krishi VikasYojna

**Conflict of interests:** The authors have declared that no conflict of interest exists.

**Acknowledgement:** Author(s) acknowledge the assistance provided by Farm Staff is highly appreciated.

## Abstract

Genetic disorder is an inborn genetic abnormalities in animals that is due to mutation in genes which are quite rare and recessive in nature. Propagation of mutated alleles constitute a danger whose negative effect often become evident only after several generations of breeding. Most commonly reported genetic diseases like Deficiency of uridine monophosphate synthase (DUMPS), bovine citrullinaemia (BC) and factor XI deficiency (FXID) affecting significantly on economics of dairy farming. The present study involved screening of 50 animals viz. Hardhenu (n=25) Sahiwal (n=15) and Haryana (n=10) cattle for autosomal recessive genetic disorders such as Deficiency of Uridine Monophosphate Synthase (DUMPS), Bovine Citrullinemia (BC) and Factor XI Deficiency (FXID) using PCR based techniques. Blood samples were collected from the jugular vein into EDTA containing tubes and stored at -20 °C until genomic DNA extraction, which was carried out using Phenol Chloroform method. PCR-RFLP and allele specific PCR was used for screening. The amplified PCR products were digested with *Ava*I and *Ava*II restriction enzymes for DUMPS and BC respectively. Animals were screened for FXID based on PCR conformation. All the animals under study were found to be free from DUMPS, BC and FXID as the amplified PCR products upon RE digestion revealed fragments for normal animals. The genetic screening revealed that none of the animal screened under study was carrier for any genetic disorder.

**Keywords:** Hardhenu, Sahiwal, allele specific, DUMPS, BC, FXID

## 1. Introduction

Genetic disorder is an inborn genetic abnormality in animals that is due to mutation in genes which are quite rare and recessive in nature (Gebreselassie et al., 2019). A genetic disorder may or may not be inherited from the carrier individual to their offspring but most frequently caused by new mutations or changes to the DNA. Selective breeding of dairy cattle in a small herd has led to undesirable effects and genetic anomalies. (Cole et al., 2016). In Cattle, around 200 different genetic defects have been reported that leads to poor production and reproduction performance, structural unsoundness, semi-lethal disease or lethal disease etc. (Gholap et al., 2014). Globally, artificial insemination is widely used reproductive assisted technology in cattle breeding, to exploit and disseminate superior germplasm. Carriers of genetic diseases are likely present within the population of breeding sires. It is suggested

## Article History

RECEIVED in 06<sup>th</sup> February 2020

RECEIVED in revised form 19<sup>th</sup> February 2020

ACCEPTED in final form 25<sup>th</sup> February 2020



to screen breeding sires for genetic diseases in order to avoid an unnecessary spread within the population (Meydan et al., 2010). There are several genetic disorders reported in Holstein and Holstein crosses that affect performance traits, physiological functions and the survivability of calves (Avanus and Altinel, 2017). These include bovine leukocyte adhesion deficiency (BLAD), factor XI deficiency (FXID), bovine citrullinemia (BC) and deficiency of uridine monophosphate synthase (DUMPS) that leads to significant economic losses (Gholap et al., 2014; Morkuniene et al., 2019; Marron et al., 2004; Ramesha et al., 2017b). DUMPS is a hereditary genetic disease that causes early embryonic death during the implantation period of pregnancy. DUMPS is caused by a single point mutation (C→T) inside exon 5 of UMP synthase gene, mapped at bovine chromosome 1 (q31–36) (Citek and Blahova., 2004). BC is a recessive genetic disorder that inhibit synthesis of arginine succinate synthase (ASS). ASS is an enzyme crucial to the urea cycle that catalyzes the conversion of aspartate and citrulline to ASS. Since affected calves have a lack of ASS, ammonia accumulates in blood and tissue. As a result, new born calves develop an unsteady gait, aimless wandering, apparent blindness, convulsions and death within a week (Grupe et al., 1996) The bovine citrullinemia gene is located on chromosome 11 and its mutation is characterized in exon 5 of arginine succinate synthase (ASS). FXID is a blood coagulation defect which is due to an insertion of 76 base pairs (bp) in exon 12 of FXI gene, which leads to premature stop codon (Virgen-Méndez et al., 2019) is associated with excessive bleeding and reproductive problems in Holstein cattle (KorkmazAgaoglu et al., 2015). In affected animals, level of estradiol at the time of ovulation is very low due to smaller size follicles. FXI deficiency may also result in prolonged bleeding and anemia. In calves continued bleeding from the umbilical cord was reported in some cases. Prolonged oozing of blood following dehorning or castration may also be observed. Affected cows frequently have pink-colored colostrum. (Jorgensen et al., 1993; Mondal et al., 2016). In addition, animals are predisposed to mastitis, metritis and pneumonia (Gentry et al., 1996). The total economic loss due to above mentioned disease was due to mortality loss, reduction in milk yield losses due to reproductive failure, loss in animal draught power, cost of treatment of affected animals and additional labor costs. All these autosomal recessive genetic disorders are lethal in nature and directly affect economy of the farmers. Phenotypically, it's not possible to identify these autosomal genetic disorders in heterozygous carrier. Genetic screening of diseases that are controlled by single genes is easily possible for identification of bulls or semen samples by molecular techniques (PCR-based tests e.g.) to ensure the inclusion of bulls free from genetic disorders in artificial insemination programs (Meydan., 2017; Ramesha et al., 2017a). And therefore, prevent economic losses to farmers. Hence, the present study was designed to genetically screen Hardhenu crossbred, Sahiwal and Hariana animals for DUMPS,

BC, FXID by PCR-RFLP and AS-PCR.

## 2. Materials and Methods

Blood samples were collected during the period (2018-19) from 50 animals i.e Hardhenu crossbred cattle (Holstein Friesian Cross) (25), Sahiwal (15), and Hariana (10) maintained at Cattle Breeding Farm, LalaLajpatRai University of Veterinary and Animal Science (LUVAS), Hisar, India. Whole blood was collected from the jugular vein into a tube containing 5% EDTA. The samples were transported to the Animal Genomics laboratory (Department of Animal Genetics and Breeding, LUVAS, Hisar) in an icebox and stored at -20°C till further processing. DNAs from respective samples were isolated by standard phenol-chloroform method (Sambrook and Russel, 1989). Genomic DNA was stored at 4 °C until analysis. The quality and quantity of DNA were determined by using UV spectrophotometer.

### 2.1. PCR amplification

In order to characterize and to detect the mutation responsible for DUMPS and BC simple polymerase chain reaction followed by enzymatic restriction was performed. The genotypes of FXID were detected by PCR methods only. A 25 µL reaction mixture was made for amplification. The primers, PCR product sizes and restriction enzymes used for identification of DUMPS and BC and primers for FXID are shown in Table 1. DNA was amplified by initial denaturation at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 1 min, annealing (temperatures for each primer pair as shown in Table 1) for 1 min, extension at 72 °C for 1 min, with final extension 72 °C for 5 min. Amplified PCR product was resolved by electrophoresis on ethidium bromide stained 1.5% agarose gel.

### 2.2. SNP screening

10 µl of PCR product was digested with particular restriction endonuclease. Restriction products were electrophoresed on 3% agarose gel and analysed by visualizing the gel under Gel doc system.

## 3. Results and Discussion

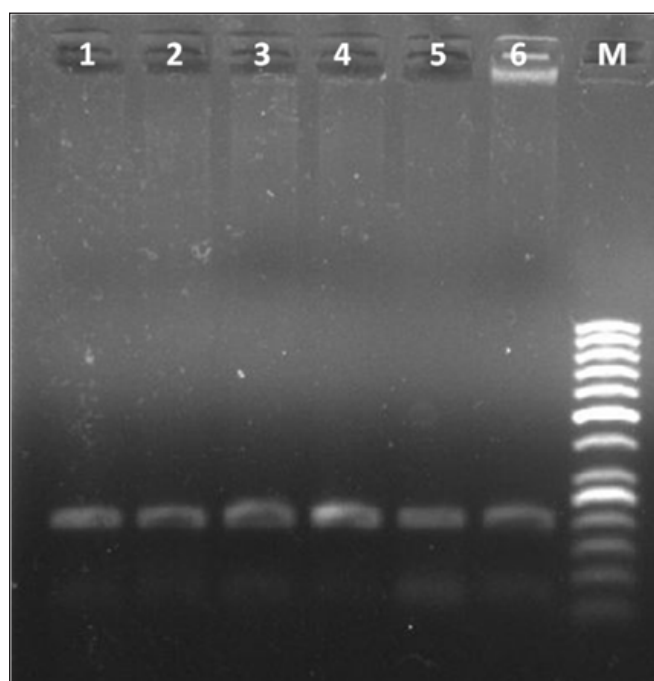
The samples optical density in between 1.7 to 2.0 were selected for PCR reactions checked on spectrophotometer (Analytica Jena). The PCR conditions were optimized with respect to parameters such as template concentration, primer concentration, MgCl<sub>2</sub> concentration and annealing temperature for all the primers with respected to targeted genes in our study. The primers listed in Table 1 successfully amplified the targeted regions of BC, UMP synthase and FXI gene. The PCR amplified product revealed 198 bp (Figure 1), 282bp (Figure 2), and 244 bp (Figure 3), bands of respective targeted genes in Hardhenu, Sahiwal and Hariana cattle.

The amplified PCR product of BC gene after digestion *Ava* II restriction enzyme respectively, revealed two fragments

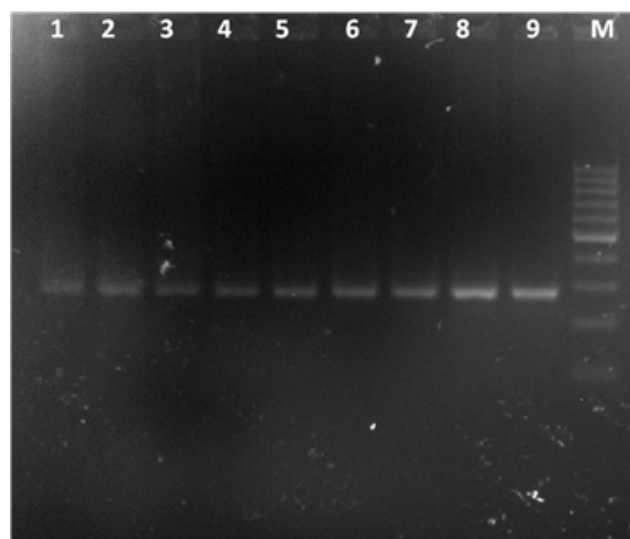


Table 1 : Primers used, PCR product size and restriction enzyme

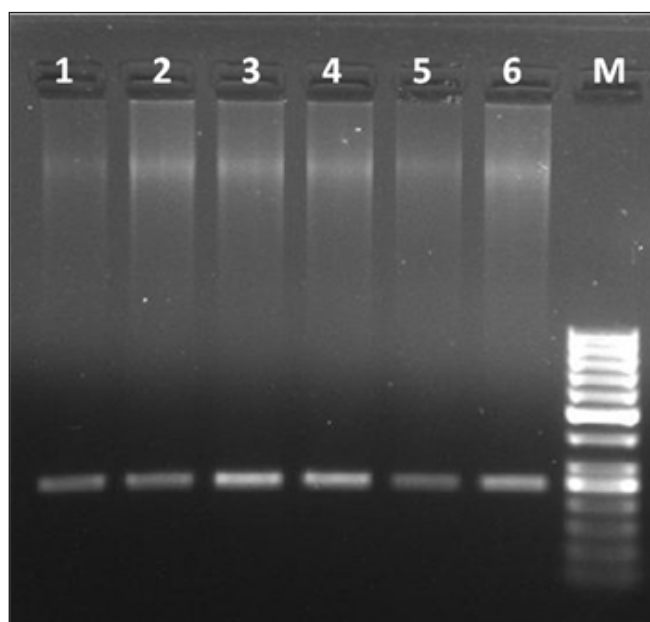
Genetic disorder	Primer Sequence	Annealing Temp.	PCR product	RE
Deficiency of uridine monophosphate synthase (DUMPS)	F: 5'-AGGGTCTTAGTGGAGCAGGT-3' R: 5'-GGCTTACCTCCTGCTTCTAACTG -3'	54 °C	282 bp	AvaI
Bovinecitrullinemia (BC)	F:5'GGCCAGGGACCGTGTTCATTGAGGA CATC 3' R: 5'TTCCTGGGACCCCGTGAGACA- CATACTTG3'	57 °C	198 bp	AvaII
Factor XI (FXID)	F: 5' CCCACTGGCTAGGAATCGTT 3' R: 5' CAAGGCAATGTGCATATCCAC 3'	55 °C	320 bp	-



Lane 1-6: PCR product 198 bp; M: 50 bp marker  
Figure 1: PCR amplified product of BC gene

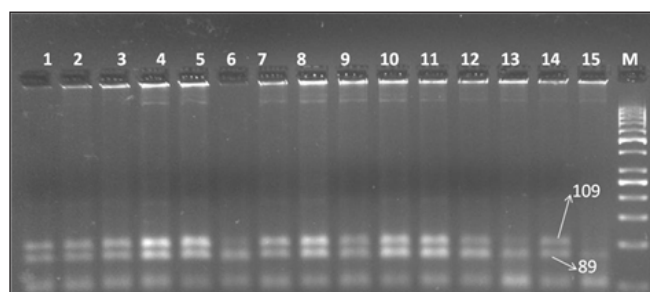


Lane 1-9: PCR product 282 bp; M: 100 bp marker  
Figure 2: PCR amplified product of UMP synthase gene



Lane 1-6: 244 bp; M: 50 bp marker  
Figure 3: PCR amplified product of FXI gene

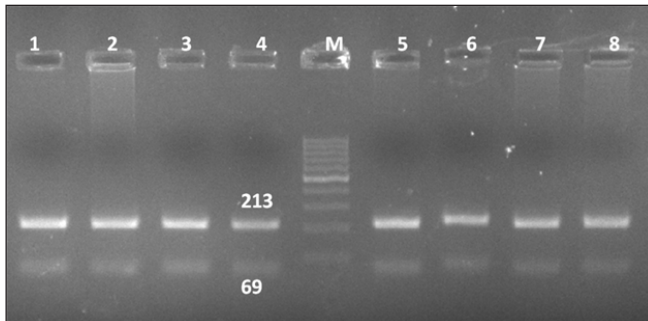
i.e 109 bp and 89 bp for BC (Figure 4) in all samples. Carrier animals produce three bands of 198, 109 and 89 bp and affected produce single band of 198 bp (Ramesha et al., 2017a). In case of UMP synthase gene after digestion *Ava* I restriction enzyme revealed two fragments 213 and 69



Lane 1-15: 109 bp and 89 bp; M: 50 bp marker  
Figure 4: PCR-RFLP genotyping using *Ava* II restriction enzyme

bp in all samples under study (Figure 5). Carrier animals produce three bands of (282 bp, 213 bp and 69 bp) and affected animals produce single band of 282 bp (Ramesha

et al., 2017a). After the AS-PCR for FXI gene, a single 244 bp (Figure 3) fragment was observed in animals under study. In homozygous affected animals, the fragment had a length of 320 bp and FXID carriers exhibited two fragments of 244 bp and 320 bp. Among the screened animals under study, none of the animals was found either carrier or affected with the DUMPS, BC and FXID. Results for DUMPS and BC carriers are similar as in Turkey (Karsli et al., 2011; Akyuz and Ertugrul, 2008) and in Gir cattle in Brazil, in Jersey cattle, Indian cattle (*Bos indicus*) breeds, *B. taurus* × *B. indicus* crossbreds and the river buffalo (*Bubalus bubalis*) cattle in India (Patel et al., 2007) and in Polish dairy cattle in Poland (Kaminski et al., 2005) in Brown Swiss bulls in Iran (Norouzy et al., 2005) and in Czech Republic (Citek et al., 2006). In case of BC and FXID also, all screened bulls have normal allele. Many researchers globally performed screening of BC and FXID carriers in Holstein populations. For example, Holsteins have been screened for BC in Germany (Grupe et al., 2006), Turkey (Akyuz et al., 2008)



Lane 1-8: 213 and 69 bp; M: 100 bp marker

Figure 5: PCR–RFLP genotyping using *Ava*I restriction enzyme

and the Czech Republic (Citek et al., 2006). None of these studies found any BC carriers. In contrast with these studies, the frequency of BC was found to be 0.16% in China (Kociba et al., 1969). As a result, the frequency of BC usually is very low or nonexistent in Holstein populations, which is consistent with the data in our study and in case of FXID Karsli et al., 2011 and Oner et al., 2010 stated the prevalence of the FXI carrier to be 0.4% and 1.2%, respectively with very low frequency. The frequency of bovine citrullinaemia was found to be high in Australia (Rajesh et al., 2006). In other countries, like the USA and Germany, the incidence of BC is very low (Robinson et al., 1993; Grupe et al., 1996).

#### 4. Conclusion

Recessive inherited disorders and abnormal karyotypes in cattle have very low frequency. The intensive use of artificial insemination and international trading of semen and breeding bulls has caused the spread to a large population of disease-associated recessive alleles. The DNA testing is currently available for some of the genetic diseases; however it is necessary to develop it for all the genetic diseases so that breeding sires can be effectively screened for undesirable alleles and culled to avoid further propagation in breeding population.

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