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Research Article

MOLECULAR SCREENING OF CROSSBRED COW BULLS FOR IMPORTANT GENETIC DISORDERS

ASHIS DEBNATH, AMAN KUMAR*, SUSHILA MAAN, VINAY KUMAR, VINAY G. JOSHI, TRILOK NANDA and M.L. SANGWAN

Department of Animal Biotechnology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, 125 004, India

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ABSTRACT

The present study was undertaken in crossbred cow bull with the objective to determine genotype of crossbred cow bull for Bovine Leucocyte Adhesion Deficiency (BLAD), Deficiency of Uridine Monophosphate Pathway (DUMPS) and Bovine citrullinamiae (BC) by using PCR-RFLP and Factor XI deficiency (FXID) by PCR. Blood samples were collected from crossbred cow bulls (n=50) from different organized farms in Hisar, Haryana. Genomic DNA was isolated from blood samples by phenol-chloroform isoamyl alcohol method. The PCR products of 357bp, 108bp, 198bp, 244bp size were obtained for BLAD, DUMPS, BC, and FXID, respectively using published set of primers. Restriction enzyme (RE) digestion of PCR amplified products was done for genetic analysis of BLAD, DUMPS and BC using *Taqα1*, *AvaI*, and *AvaII*, respectively. All the 50 animals were found to be free for all above genetic diseases as the amplified PCR products upon RE digestion revealed fragments for healthy (normal) animals. In the present investigation carrier frequency for BLAD, DUMPS, BC and FXID was found to be 0%.

Key words: BC, BLAD, DUMPS, FXID, PCR-RFLP, genetic disorders

Artificial insemination (AI) programme was adopted in India to augment milk production and it led to the development of large number of crossbred cattle in India. The cross breeding practice was implemented with the use of high pedigree exotic breed bull's frozen semen. The exotic breeds of cattle have been reported to be carrier of genetic disorders. Of these, autosomal recessive genetic disorders are most common which are expressed phenotipically in affected animals. There are more than 50 genetic disorders which have has been identified in exotic breeds. Of these, BLAD, BC, DUMPS, and FXID are the most prevalent disorder globally.

BLAD is a lethal autosomal recessive inherited congenital genetic disorder in Holstein Friesian cattle. It is due to mutation in integrin β2 (CD18) gene, forming defective β chain of integrin receptors involved in interaction with other cell and extracellular matrix. A single point mutation i.e. Adenine to Guanine (A→G) of nucleotide 383 in the CD18 gene located on the first chromosome of bovine is responsible for BLAD (Nagahata, 2004). Defective glycoprotein receptors lead to less or lack of migration of leukocytes at the infection site and it is associated with persistent marked neutrophilia in the affected homozygote calves. Due to immature immune mechanism, newly born calves are the most affected. Affected homozygous cattle die at an early age due to immature immunity and recurrent bacterial infection.

*Corresponding author: aman.abt@luvas.edu.in

DUMPS is a lethal hereditary autosomal recessive disorder in Holstein cattle breed. It ultimately leads to more services per calving as it causes early embryonic mortality and longer than normal calving intervals (Shanks and Robinson, 1989). DUMPS interfere with the nucleotide pyrimidine synthesis affecting vital cellular functions such as DNA and RNA synthesis and inherited as homozygous form (Brush *et al*., 1987). In affected homozygous recessive animal, growth is arrested leading to embryonic mortality around forty days of post conception (Eydivandi *et al*., 2011). Molecular basis of DUMPS is due to the single point mutation (C to T) occurs at codon 405 within exon five (5) of UMP synthase gene at bovine chromosome 1 (Meydan *et al*., 2010).

Bovine citrullinaemia is an autosomal recessive inherited genetic disorder caused by the transition of cytosine to thymine at codon 86 of the gene in chromosome 11 coding for arginosuccinate synthase leading to impaired urea cycle (Dennis *et al*., 1989) and was first observed in the Australian Holstein cattle population. This genetic disorder causes the synthesis of defective arginosuccinate synthetase enzyme (Ricci *et al*., 2011). This enzymatic dysfunction leads to edema in cerebral cortex, a common lesion in all affected calves due to elevation of citrulline concentration in body fluids. The condition is reported to be lethal (Healy *et al*., 1993).

Factor XI is one of the plasma serine protease involved in the early activation of the intrinsic blood

coagulation cascade. Congenital factor XI deficiency causes a mild-to-moderate bleeding disorder, which leads to haemorrhages, typically involves at mucosal surface like oral cavities, nasal cavities, urinary tract etc. (Smith and Galani, 2008). Affected animals appear to be more susceptible to disease such as pneumonia, mastitis, metritis etc. It leads to pink coloured colostrums, presence of blood in milk and reduced reproductive performance. In FXID, causative mutation consists of an insertion of 76bp segment into exon 12 of factor XI gene on bovine chromosome 27 and thus in PCR 320 bp of amplicon is generated in place of 244bp (Marron *et al*., 2004).

All these four autosomal genetic disorders directly affect economy of farm as they are lethal in nature. Inability to identify these autosomal genetic disorders phenotypically in heterozygous carrier calls for detailed genetic investigation of crossbred population. Hence, the present study was designed to screen representative samples of crossbred cattle for BLAD, DUMPS, BC, FXID using PCR, PCR-RFLP and sequencing.

MATERIALS AND METHODS

Blood samples were collected from fifty crossbred cow bull calves from Government Livestock Farm (n=32), Hisar and Cattle Breeding Farm (n=18), Department of Animal Genetics and Breeding, LUVAS, Hisar. Approx. 10 ml of venous blood sample was collected in 15 ml sterile polypropylene centrifuge tube containing 0.5 ml of 0.5 M EDTA as anticoagulant. Genomic DNA was isolated from blood samples as described by phenol chloroform method (Sambrook and Russel, 2001) with minor modifications. PCR-RFLP method was used for genotyping BLAD, DUMPS, BC, whereas PCR based genotyping was done for FXID. The primer pairs used in the study are given in Table 1. PCR reaction conditions are shown in Fig. 1.

PCR amplified products were analysed using 2% agarose gel electrophoresis. The restriction fragments were resolved by electrophoresis on 3% agarose gel having 0.5 µg/mL ethidium bromide (Sigma, USA) in 1X TAE buffer. As a DNA size marker, 100bp ready to use ladder (Thermo scientific) was used. The PCR products were purified for sequencing by BigDye terminator method. The gene specific primers (Forward/Reverse) were used for sequencing using an automated DNA sequencer (Applied Biosystem 3130 XL) in the department. Cycle sequencing PCR products were purified by BigDye Terminator v3.1Clean up (Tube Method). Genotypes were confirmed by sequencing.

RESULTS AND DISCUSSION

Amplification of BLAD, DUMPS, FXID and BC Gene Specific Target Sequences: The amplification of BLAD, BC, DUMPS and FXID gene specific target sequences were carried out using the published primers pair in an optimized PCR (Table 1). The molecular size of the PCR products was estimated to be 357bp, 198bp, 108bp, 244bp, respectively (Fig. 2, 4, 6, and 8, respectively). The PCR products of CD18 gene for BLAD, digested by *Taq*α1 at 65^0C overnight showed monomorphic band pattern of 201bp and 156bp in 3% agarose gel (Fig. 3). Monomorphic band pattern was obtained by the digestion of the PCR products of BC specific sequences by *Ava*II at 37^0C for overnight with pattern of 109bp and 89bp in 3% agarose gel (Fig. 5). Similarly by digesting DUMPS specific PCR products with *Ava*I at 37^0C overnight, showed monomorphic band pattern of 53bp and 36bp in 15% polyacrylamide gel electrophoresis (Fig. 7). The sequence data generated and percentage identity was analysed with the global sequences available in NCBI GenBank database using BLASTn for the conformation of amplified product. BLAST analysis of BLAD, BC, DUMPS and FXID specific generated sequences revealed 97-99% identity with *Bos taurus, Bos indicus* and *Bos frontalis* and no specific mutation was observed.

Presence of genetic disorders characterized by malformation leads to various morphological expressions like inflammatory responses in BLAD, dullness in BC, bleeding disorders in FXID, embryonic death in DUMPS

Table 1

The primer pairs used in the present study

Disease	Sequence (5'-3')	Amplicon size	References	Restriction enzyme
BLAD	F-GAATAGGCATCCTGCATCATATCCACCA R-CTTGGGGTTTCAGGGGAAGATGGAGTAG	357bp	Meydan *et al.* (2010)	*Taqα1*
DUMPS	F-GCAAATGGCTGAAGAACATTCTG R-GCTTCTAACTGAACTCCTCGAGT	108bp	Schwenger *et al.* (1994)	*AvaI*
BC	F-GGCCAGGGACCGTGTTCATTGAGGACATC R-TTCCTGGGACCCCGTGAGACACATACTTG	198bp	Grupe *et al.* (1996)	*AvaII*
FXID	F-CCCACTGGCTAGGAATCGTT R-CAAGGCAATGTCATATCCAC	244bp	Marron *et al.* (2004)	

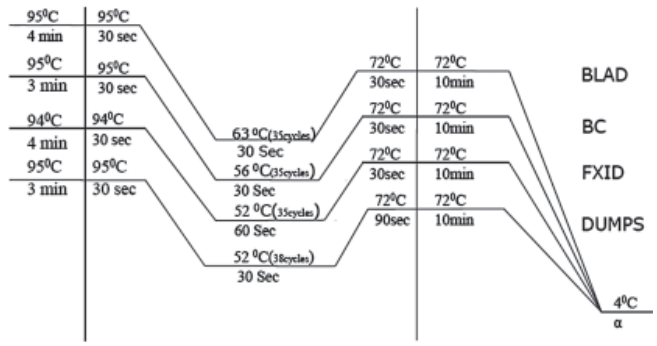

Fig 1. PCR reaction conditions used for amplification

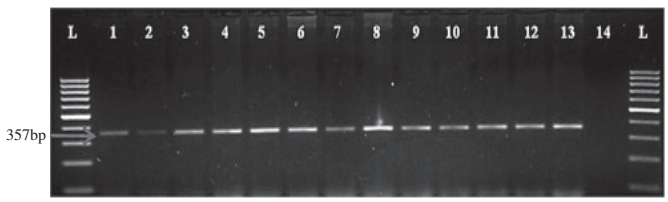

Fig 2. PCR amplified product of BLAD specific CD18 gene on 2% agarose gel electrophoresis [L=100bp ladder; Lanes 1-13=PCR product of BLAD gene (357 bp); Lane 14=Negative control]

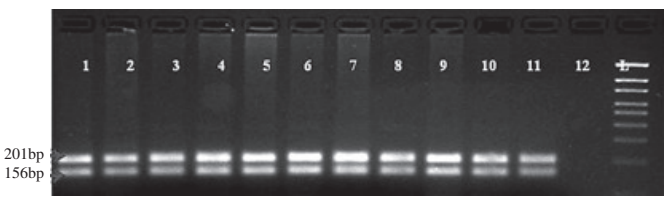

Fig 3. Resolution of RE digested product in 3% agarose gel electrophoresis [L- 100 bp ladder; Lanes 1-11=*Taqα1* RE digested products of BLAD (201 bp and 156 bp); Lane 12=Negative control]

Fig 4. PCR amplified product of BC specific gene on 2% agarose gel electrophoresis [L=100 bp ladder; Lanes 1-9=PCR product of BC gene (198 bp); Lane 10=Negative control]

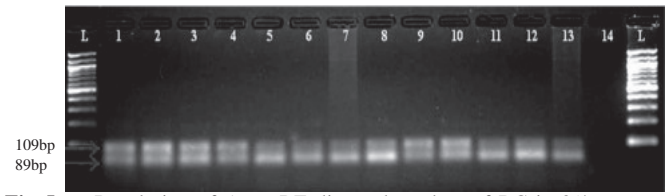

Fig 5. Resolution of *AvaII* RE digested product of BC in 3% agarose gel electrophoresis [L=100 bp ladder; Lanes 1-13=*AvaII* RE digested product of BC (109 bp and 89 bp); Lane 14=Negative control)]

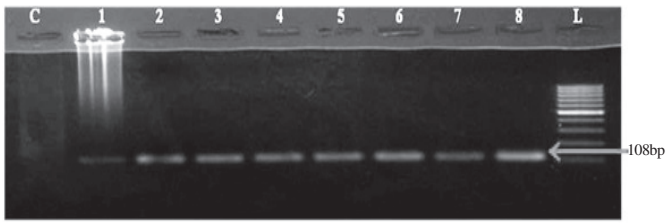

Fig 6. PCR amplified product of DUMPS specific gene (108 bp) on 3% agarose gel electrophoresis [L=100 bp ladder; Lanes 1-8=Amplified product of DUMPS specific gene; Lane C=Negative control)]

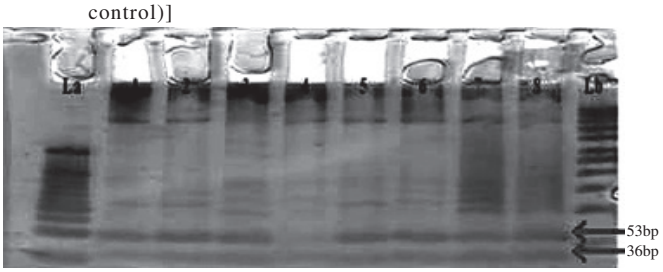

Fig 7. Resolution of *AvaI* digested fragments of DUMPS specific PCR products on 15% polyacrylamide gel electrophoresis [La=50 bp ladder; Lb=20 bp ladder; Lanes 1-8=*AvaI* digested fragments (53 bp and 36 bp)]

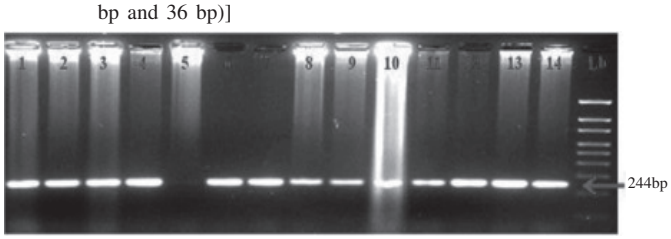

Fig 8. PCR amplified product of FXID in 2% agarose gel electrophoresis [Lb=100bp ladder; Lanes 1-14=Amplified products of FXID (244 bp)]

etc. and ultimately causes metabolic and physiological alterations, injuries or, more frequently secondary bacterial infection and death. One promising approach to reduce problems caused by genetic disorders is the selection of animals that are free from these genetic disorders. Out of different molecular screening method PCR-RFLP is ideal for quick and validated results formation. It can easily screen both homozygous and heterozygous animal. By reorganization of carrier heterozygous animal, we can avoid the spreading of the defective genes within the cattle population.

In this study PCR- RFLP analysis of target region of CD18 gene for BLAD using *Taqα1* revealed wild type normal. Hence, same pattern of digested fragments i.e., 201bp and 156bp (normal wild type) were observed instead of mutant type fragments i.e., 357bp, 201bp, 156bp as explained by Meydan *et al*. (2010). The prevalence of defective allele was found to be zero in the tested samples. The RFLP analysis of BC specific amplified PCR products using *Ava*II revealed single genotype of wild type. Hence, same pattern of digested fragments was observed as in wild type (109bp and 89bp). Further confirmation by sequencing revealed monomorphic wild type in all DNA samples. Patel *et al*. (2006) also reported nil occurrence of this disease in India.

The RFLP analysis of DUMPS specific amplified PCR products using *Ava*1 revealed single genotype of wild

type. Hence, pattern of digested fragments were 53bp and 36bp and 19bp (wild type) instead of 89bp, 53bp, 36bp and 19bp (mutant type). It was found to be monomorphic in all DNA samples of crossbred cattle. Thus, no carrier was found for DUMPS. However, Muraleedharan *et al.* (1999), Patel *et al*. (2006), Kumar (2009), Mahdi *et al*. (2010) and Yathish *et al*. (2010) have reported frequency of DUMPS as 1.33%, 3.23%, 21.82%, 7.31%, and 3.64%, respectively in Holstein animals. Sharma *et al.* (2009) reported absence of BLAD allele in water buffalo in India. Similarly none of the sample was found positive for mutant FXID gene in PCR-RFLP because, only wild type specific fragment (244bp) observed on gel after restriction digestion of amplified product. Earlier, Mondal *et al.* (2014) reported the presence of FXID carrier in Sahiwal bull.

In conclusion, the crossbred cow bulls under investigation were found negative for defective alleles for BLAD, DUMPS, BC and FXID by PCR-RFLP. Regular and obligatory screening of crossbred animals controlled the rapid spread of genetic disorders in several countries. Hence, the routine screening of these genetic disorders should be continued to eliminate the incidences among bovine population.

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REFERENCES

Brush, P.J., Anderson, P.H. and Gunning, R.F. (1987). Identification of factor XI deficiency in Holstein-Friesian cattle in Britain. *Vet Rec.* **121**: 14-17.

Dennis, J.A., Healy, P.J., Beaudet, A.L. and O'Brien, W.E. (1989). Molecular definition of bovine arginosuccinatesynthetase deficiency. *Proc. Natl. Acad. Sci.* **86**: 7947-7951.

Eydivandi, C., Seyedabadi, H.R. and Amirinia, C. (2011). Identification of BLAD, DUMPS and CVM in Kzuzestan Holstein Cattle Population of Iran. *Global Veterinaria*. **6(6)**: 519-524.

Grupe, S., Diet, G. and Schwerin, M. (1996). Population survey of citrullinemia on German Holsteins. *Livestock Prod. Sci.* **45**: 35-38.

Healy, P., Dennis, J., Rawlinson, R. and Andersson, L. (1993). Prenatal diagnosis of bovine citrullinaemia. *Res. Vet. Sci.* **55(3)**: 271-274.

Kumar, V. (2009). Genetic screening of KF animals for bovine leukocyte adhesion deficiency syndrome (BLAD) and complex vertebral malformation (CVM). M.V.Sc. thesis, NDRI, Karnal.

Mahdi, M., Sharma., A., Dubey, P.P., Singh, A., Tantia, M.S., Mishra, B., Kumar, V. and Joshi, B.K. (2010). PCR based identification of bovine leukocyte adhesin deficiency syndrome (BLAD) carriers in Karan Fries bulls. *Indian J. Anim. Sci.* **80(5)**: 433-35.

Marron, B.M., Robinson, J.L., Gentry, P.A. and Beever, J.E. (2004). Identification of a mutation associated with factor XI deficiency in Holstein cattle. *Anim. Gene.* **35**: 454-456.

Meydan, H., Yyldyz, M.A. and Agerholm, J.S. (2010). Screening for bovine leukocyte adhesion deficiency, deficiency of uridine monophosphate synthase, complex vertebral malformation, bovine citrullinaemia, and factor XI deficiency in Holstein cows reared in Turkey. *Acta. Vet. Sca.* **52(56)**: 1-8.

Mondal, K., Ghosh, A.K., Singh, C.V., Kumar, D., Barwal, R.S., Kumar, S. and Chakravarti, S. (2014). Identification of Queer mutation associated with factor XI deficiency in Sahiwal Breed. Compendium of XI National Symposium on Harmonizing phonemics and genomics for sustainable management of livestock for upliftment of rural masses.

Muraleedharan, P., Khoda, V.K., Grupe, S., Mukhopadhya, P.N., Manfred, S. and Mehta, H.H. (1999). Incidence of hereditary citrullinaemia and bovine leukocyte adhesion deficiency syndrome in Indain dairy cattle (*Bos Taurus*, *Bos indicus*) and buffalo (*Bubalus bubalis*) population. *Arch. Tierz. Dummerstorf.* **42(4)**: 347-352.

Nagahata, H. (2004). Bovine leukocyte adhesion deficiency (BLAD): A review. *J. Vet. Med. Sci.* **66**: 1475-1482.

Patel, R.K., Singh, K.M., Soni, K.J., Chauhan, J.B. and Sambasiva, R.K.R. (2006). Lack of carriers of citrullinaemia and DUMPS in Indian Holstein cattle. *J. Appl. Genet.* **47(3)**: 239-242.

Ricci, J.H., Laura, C.P. and Duane, C.E. (2011). Argininosuccinate synthase: at the center of arginine metabolism. *Int. J. Biochem. Mol. Biol.* **2(1)**: 8-23.

Sambrook, J. and Russel, D.W. (2001). Molecular Cloning. A Laboratory Manual. (3rd edn.). New York: Cold Spring Harbar Lab. Press.

Schwenger, B., Tammen, I. and Aurich, C. (1994). Detection of homozygous recessive genotype for deficiency of uridine monophosphate synthase by DNA typing among bovine embryos produced in vitro. *J. Reprod. Fertil.* **100**: 511-514.

Shanks, R.D. and Robinson, J.L. (1989). Embryonic mortality attributed to inherited deficiency of uridine monophosphate synthase. *J. Dairy Sci*. **72(11)**: 3035-3039.

Sharma, D., Kumar, S., Deb, S.M., Mitra, A., Niranjan, S.K., Naskar, S. and Sharma, A. (2009). Identification of novel allelic variants of integrin beta 2 (ITGB2) gene and screening for bubaline leukocyte adhesion deficiency syndrome in Indian Water buffaloes (*Bubalus bubalis*). *Anim. Biotechnol.* **20**: 3, 156-160.

Smith, S.B. and Galani, D. (2008). Update on the physiology and pathology of factor IX activation by factor Xia. *Expert Rev. Hematol*. **1(1)**: 87-98.

Yathish, H.M., Sharma, A., Kumar, V. Jain, A., Chakraborty, D., Singh, A., Tantia, M.S. and Joshi, B.K. (2010). Genetic polymorphism of CD18 gene in Karan Fries young bull calves using PCR-RFLP analysis. *Curr. Trend Biotechnol. Pharma.* **4(4)**: 900-907.