Article AR1993

DOI: HTTPS://DOI.ORG/10.23910/IJBSM/2019.10.3.1993

International Journal of Bio-resource and Stress Management

June 2019

Crossref

Print ISSN 0976-3988

Online ISSN 0976-4038

IJBSM 2019, 10(3):319-322

Research Article

Natural Resource Management

Screening of Hardhenu Crossbred, Sahiwal and Hariana Bulls for Autosomal Recessive Genetic Disorder Bovine Leukocyte Adhesion Deficiency (BLAD)

Ankit Magotra[1]*, A. S. Yadav[2], B. S. Malik[3] and Asha Rani Garg[4]

Dept. of Animal Genetics and Breeding, LUVAS, Hisar, Haryana (125 001), India

Open Access

Corresponding Author

Ankit Magotra

e-mail: ankitoms@gmail.com

Citation: Magotra et al., 2019. Screening of Hardhenu Crossbred, Sahiwal and Hariana Bulls for Autosomal Recessive Genetic Disorder Bovine Leukocyte Adhesion Deficiency (BLAD). International Journal of Bio-resource and Stress Management 2019, 10(3):319-322. HTTPS://DOI.ORG/10.23910/IJBSM/2019.10.3.1993

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 no conflict of interests exist.

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

Abstract

The present study involved screening of Hardhenu crossbred, Sahiwal and Hariana bulls for autosomal recessive genetic disorder i.e Bovine Leukocyte Adhesion Deficiency (BLAD) using PCR based techniques. BLAD is a lethal autosomal recessive disease caused due to a point mutation (A→G) at nucleotide 383 in the CD18 gene which results in substitution of aspartic acid with glycine in the adhesion glycoprotein CD18. All animals under study were maintained at Cattle Breeding Farm, LalaLajpat Rai University of Veterinary and Animal Science (LUVAS), Hisar, India. About 5 ml blood was collected aseptically from jugular vein puncture into EDTA containing tubes, transported to the animal genomics laboratory and stored at -20 °C until genomic DNA extraction, which was carried out using Phenol Chloroform method. The targeted genomic area of interest was amplified by using simple PCR technique. The amplified PCR products were digested with TaqI restriction enzyme. The targeted amplicon of 357 bp after digestion of the PCR product, the normal allele in unaffected bull produced two fragments of 201 bp and 156 bp. All the animals under study were found to be free from Bovine Leukocyte Adhesion Deficiency. The molecular screening revealed that none of the bulls screened under study was carrier for BLAD. The present study suggests that the PCR and PCR-RFLP based technique could be employed as an efficient technique for screening of various genetic disorders for identification of carriers or affected animals before using them in breeding programs to minimize the spread of defective allele.

Keywords: Hardhenu, Sahiwal, BLAD, PCR

1. Introduction

India ranks first in the world in terms of its cattle population in the world but most of them are considered to be poor producers as compared to exotic animals. Cross breeding programs of non-descript Indian cattle with exotic germplasm of Holstein Jersey and Brown Swiss breeds have significantly increased milk yields in the crossbred progenies (Rajesh and Ashutosh, 2014) but with a trade off with quality by accumulating new diseases in cattle. Extensive use of Holstein semen along with lack of knowledge of their breed specific genetic disorders in past, various autosomal recessive genetic disorders have been reported in Holstein and their crossbred cattle in India.

Bovine leukocyte adhesion deficiency (BLAD), factor XI deficiency (FXID), bovine citrullinemia (BC), and deficiency of uridine monophosphate

Article History

RECEIVED in 01st May 2019 RECEIVED in revised form 27th June 2019 ACCEPTED in final form 30th June 2019

synthase (DUMPS) are such diseases which affect economics of dairy breeding significantly due to their effects on reproductivity and viability. BLAD is a lethal autosomal recessive disease in Holstein (Citek et al., 2006) caused due to a point mutation (A→G) at nucleotide 383 in the CD18 gene which results in substitution of aspartic acid with glycine in the adhesion glycoprotein CD18 (Nagahata, 2004) and decreased expression of the β2 hetero dimeric integrin (Shuster et al., 1992). Due to this mutation, neutrophil leucocytes are unable to pass through the endothelial layer and reach infected areas (Akyuz and Ertugrul, 2006). Clinically such individuals are immuno-deficient and more prone to recurrent and prolonged mucosal and epithelial infections. At certain stage of development, BLAD manifests itself in form of stunted growth in combination with hematological changes (Gholap et al., 2014) and a persistent progressive neutrophilia (often exceeding 100,000 μl^{-1} to as high as 300,000 μl^{-1}).

It is an autosomal recessive genetic disease (Shuster et al., 1992) that affects especially Holstein breed. The carrier frequency of BLAD among US Holstein cattle had once reached approximately 15% among active breeding bulls and 8% among cows. In Taiwan, Holstein is the only cattle breed for milk production where the percentage of BLAD carriers was estimated in more than 5% of total Holstein (Huang et al., 2000). Many cases were reported in Hungary, Korea, Lithuania, Uruguay , Iran, Poland , Japan, Czech ,China, Argentina , Denmark ,Brazil , Pakistan , Germany , USA etc. Besides, owing to the wide – spread use of top breeding HF bulls imported from USA, many countries reported a high incidence of BLAD carriers in their black and white population (Lubieniecki et al., 1999). In the US alone, 80% of the 10 million dairy cows are Holsteins. It is estimated that 16000 calves are born with BLAD each year. The average economic loss per calf is roughly 300 USD annually (Rajesh and Ashutosh, 2014).

In India, Holstein bulls and their semen doses are still maintained for extensive use in crossbreeding programs. BLAD carriers were first detected in two young Holstein bulls (1.33%) which were born through artificial insemination with the imported semen (Pedeeri et al., 1999). However, no mutation was detected in other cattle breeds including Jersey, zebu, and buffaloes. Since then, routine screening of BLAD in selected Holstein and their crossbreds has been in practice. Later, the blood samples and semen samples of 1250 phenotypically normal individuals, including 377 HF, 334 HF crossbred, 105 Jersey, 160 zebu cattle breeds and 274 water buffalo (*Bubalus bubalis)* belonging to various artificial insemination stations, bull mother farms (BMFs) and embryo transfer (ET) centres across the country were tested by PCR-RFLP technique to detect possible carriers for BLAD (Patel et al., 2007). The results indicate that out of 1250 cattle and buffaloes tested for BLAD, 13 HF purebreds out of 377 and 10 HF crossbreds out of 334 appear to be BLAD carriers. In the HF and HF crossbred population, the percentage of BLAD carriers was estimated as 3.23%. A study on a group of 55 Karan Fries that is crossbred devolved in India by using Holstein Friesian and Tharparkar breed (Mason, 1996), bull calves were tested for BLAD by PCR and RFLP analysis. Result indicated that out of 55 examined calves, 2 (3.64%) were BLAD carriers and 1 (1.82%) was BLAD affected (Yathish et al., 2010). In one more study, out of 42 HF and HF crossbred bulls, 2 bulls (4.76%) of HF were found to be heterozygous for BLAD (Patel et al., 2011).

2. Materials and Methods

Blood samples during the period (2017-2018) were taken randomly from 21 bull samples i.e Hardhenu crossbred cattle (Holstein Friesian Cross) (08), Sahiwal (05), and Hariana (08). All animals were maintained at Cattle Breeding Farm, LalaLajpat Rai University of Veterinary and Animal Science (LUVAS), Hisar, India. About 5 ml blood was collected aseptically from jugular vein puncture in a sterile vacutainer (Greiner bio-one vacuette containing 0.5% EDTA solution @10 µl ml^{-1} of blood). 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 Russsel, 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 BLAD, a simple polymerase chain reaction followed by enzymatic restriction was performed. A 25-µL reaction mixture was made for amplification. The primers, PCR product size and restriction enzyme used for identification of BLAD is 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 at 65 °C for 1 min, extension at 72 °C for 1 min, with final extension at 72 °C for 5 min. Amplified PCR product was resolved by electrophoresis on ethidium bromide stained 1.5% agarose gel.

2.2. SNP screening

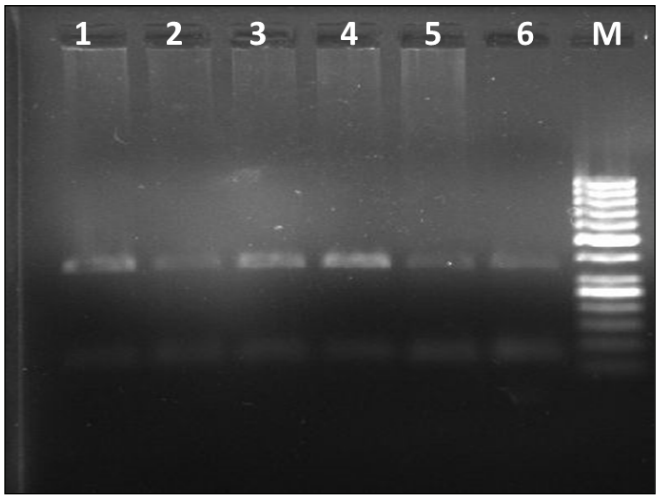
10 µl of PCR product was digested with fast digest restriction endonuclease: *Taq*I (Thermo Scientific, #FD0674) at 65 °C for 5 hrs. Restriction products were electrophoresed on 3% agarose gel and analyzed by visualizing the gel under Gel doc system.

3. Results and Discussion

Inherited disorders affect all kinds of farm animals. Functional and physiological defects arising from inherited disorders have negative impact on health and productivity of farm animals. Autosomal recessive disorders lead to economic loss in the dairy cattle industry which is kept on with Holstein cattle, due to difficulty in detection of carrier individuals. The primers listed in Table 1 successfully amplified the targeted amplicon of 357 bp (Figure 1) and after digestion of the PCR product in our resource population, the normal allele in unaffected bull

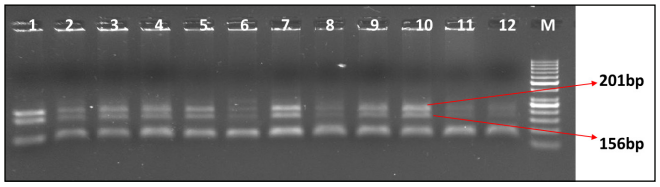
Table 1: Primers, PCR product sizes and restriction enzyme used for identification of BLAD

Primer Sequence	Annealing temperature	PCR product size bp	RE
F: 5' GAATAGGCATCCTGCATCATATCCACCA 3' R:5'CTTGGGGTTTCAGGGGAAGATGGAGTAG 3'	65 °C	357bp	*Taq*I



Lane 1-6 PCR product (357 bp)
M 50 bp Marker
Figure 1: PCR amplified product

produced two fragments of 201 bp and 156 bp (Figure 2). Among the screened bull under study, none of the animals was found either carrier or affected with BLAD. BLAD analysis revealed that of the 21 genotyped animals, all were found normal homozygous. All the screened bulls were found to



Lane 1-12 201 and 156 bp bands
M 50 bp Marker
Figure 2: PCR –RPLP genotyping using *Taq*I restricyion enzyme

be negative for BLAD. Similar results of very low or lack of incidence of BLAD were observed in earlier studies carried out for Holstein population (Nagahata et al., 1997; Ribeiro et al., 2000; Rahimi et al., 2006; Meydan et al., 2007; Arpita et al., 2012). Oner et al., 2010 did not find any carriers of BLAD. Debnath et al., (2016) were found negative for defective alleles for BLAD crossbred cow bulls. This difference may be due to the fact that populations from different regions were sampled. There is a wide variety in the results reported by various studies on BLAD carriers around the world. The frequency of BLAD carriers was found to be as high as 14.1% and the percentage of BLAD was estimated to be 0.2% at birth in 1992 (Shuster et al., 1992). In cattle, in very low frequency recessive inherited disorders and abnormal karyotypes were observed. The intensive use of artificial insemination and international trading of semen and breeding bulls leads to widespread of disease-associated recessive alleles to a large population. Therefore, there is a need for screening methods of various genetic disorders, to allow breeders to test their suspicious cows and all breeding bulls. So planned mating with known pedigree is very essential to prevent disease carrier animals in the dairy industry.

4. Conclusion

The genetic diseases cause huge economic losses due to poor animal performance in respect of calf viability. If genetic disease gets propagated from generation to generation continuously without being detected, it will increase the frequency of the undesirable genes in the breeding population. Molecular markers are identified as powerful tool which helps in diagnosis of genetic disorders at a very early stage. Hence it is required to establish the screening methods allowing breeders to test their animals to minimize the spread of disease in a population.

5. Acknowledgement

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

6. References

Akyuz, B., Ertugrul, O., 2006. Detection of bovine leukocyte adhesion deficiency (BLAD) in Turkish native and Holstein cattle. ActaVeterinariaHungarica 54, 173–178.

Arpita, R., Rajesh, K.P., Rosaiah, K., Radhika, A., Sanghamitra, K., 2012. New cases of Bovine Leukocyte adhesion deficiency (BLAD) carriers in Indian Holstein Cattle. International Journal of Veterinary Science 1, 80–82.

Citek, J., Rehout, V., Hajkova, J.,Pavkova, J., 2006. Monitoring of the genetic health of cattle in the Czech Republic. Veterinary Medicine 51, 333–339.

Debnath, A., Kumar, A., Maan, S., Kumar, V., Joshi, V.G., Nanda, T.,Sangwan, M.L., 2016. Molecular screening of crossbred cow bulls for important genetic disorders. Haryana Veterinarian 55(1), 93–96.

Gholap, P.N., Kale, D.S., Sirothia, A.R., 2014. Genetic Diseases in Cattle: A Review.Research Journal of Animal, Veterinary and Fishery Sciences 2(2), 24–33.

Huang, Y.C., Chang, H.L, Lin, D.Y., Liaw, R.B., 2000. Gene frequencies of Bovine Leukocyte Adhesion Deficiency of Holstein in Taiwan. Journal of Taiwan Livestock Research 33, 37–47.

Lubieniecki, K, Grzybowski, G., Lukaszewicz, M., Lubieniecka, J., 1999. Association between the presence of allele BL in the genome of dairy cows and their productivity.

Animal science papers and reports / Polish Academy of Sciences 17, 189–194.

Mason, I.L., 1996. A World Dictionary of Livestock Breeds, Types and Varieties Fourth Edition. C.A.B International. 273.

Meydan, H., Ozdil, F., Gedik, Y.,Yildiz, M.A., 2007. Detection of BLAD, DUMPS and factor XI by PCR-RFLP. In Proceedings ofthe 5thAnimal Science Congress, YuzuncuYil University, Van, Turkey.

Nagahata, H., 2004. Bovine leukocyte adhesion deficiency (BLAD): a review. The Journal of Veterinary Medical Science 66: 1475–1482.

Nagahata, H., Miura, T., Tagaki, K., Ohtaki, M., Noda, H., Yasuda, T.,Nioka, K., 1997. Prevalence and allele frequency estimation of bovine leukocyte adhesion deficiency(BLAD) in Holstein-Friesian cattle in Japan. The Journal of Veterinary Medical Science 59(4), 233–238.

Pedeeri, M., Khoda, V.K., Grupe, S., Mukhopadhya, P.N., Manfred, S., 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. Archiv Fur Tierzucht 42, 347–352.

Patel, R.K., Singh, K.M., Soni, K.J., Chauhan, J.B., Sambasiva Rao, K.S.R., 2007. Low incidence of Bovine Leukocyte Adhesion Deficiency (BLAD) carriers in Indian dairy cattle and buffalo breeds. Journal of Applied Genetics 48, 153–155.

Patel, M., Patel, R.K., Singh, K.M., Rank, D.N., Thakur, M.C., Khan, A., 2011.Detection of genetic polymorphism in CD18 gene in cattle by PCR-RFLP. Wayamba Journal of Animal Science, Article No. 1305108850.

Ribeiro, L.A., Baron, E.E., Martinez, M.L., Coutinho, L.L., 2000. PCR screening and allele frequency estimation of bovine leukocyte adhesion deficiency in Holstein and Gir cattle in Brazil. Genetics and Molecular Biology 23, 831–834

Rahimi, G., Nejati-Javaremi, A., Olek, K., 2006.Genotyping BLAD, DUMPS and CSN loci in Holstein young bulls of the National Animal Breeding Center of Iran. Pakistan Journal of Biological Sciences, 9(7), 1389–1392.

Rajesh, K.P., Ashutosh, K.P., 2014. Comparative review of recessive genetic disorders occurrence in Indian Cattle. Current Trends in Biotechnology and Pharmacy 8(3), 321–335.

Shuster, D.E., Kehrli, M.E., Ackerman, M.R., Gilbert, R.O., 1992. Identification and prevalence of a genetic defect that causes leukocyte adhesion deficiency in Holstein cattle. Proceedings of the National Academy of Sciences 89, 9225–9229.

Sambrook, J., Russell, D.W., 1989. Preparation and analysis of eukaryotic DNA. In: Molecular Cloning: A Laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press, New York: 6.1–6.62.

Oner, Y., Keskin, A., Elmaci, C., 2010. Identification of BLAD, DUMPS, citrullinaemia and factor XI deficiency in Holstein cattle in Turkey. Asian Journal of Animal and Veterinary Advances 5, 60–65.

Yathish, H.M., Sharma, A., Kumar, V., Jain, A., Chakraborty, D., Singh, A., Tantia, M.S., Joshi, B.K., 2010. Genetic Polymorphism of CD18 gene in Karan Fries Young bull Calves using PCR-RFLP Analysis. Current Trends in Biotechnology and Pharmacy 4, 900–907.