

Incidence of BLAD and DUMPS carriers in Romanian cattle breeds

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Abstract

Bovine leukocyte adhesion deficiency (BLAD) is a genetic disease that affects the hematopoietic system by greatly reduced expression of the heterodimeric $\beta 2$ integrin, causing many defects in leukocyte function. It is known that BLAD deficiency is an autosomal recessive defect, caused by a point mutation in the gene encoding subunit CD18 for $\beta 2$ integrin adhesion molecule, which is lethal in the homozygous form [1].

DUMPS (Deficiency of Uridine Monophosphate Synthase) is a hereditary recessive disorder in Holstein cattle causing early embryo mortality during its implantation in the uterus. The only way to avoid the economic losses is early detection of DUMPS carriers.

A population from the Romanian Black Spotted cattle was used to investigate the incidence of these genetic diseases in the main dairy breed in Romania. The aim of our work was to optimize the PCR-RFLP analysis as a diagnostic test to identify BLAD and DUMPS carrier cattle.

Keywords: Romanian Black Spotted, BLAD, DUMPS, PCR, RFLP, genotype detection

Introduction

Bovine Leukocyte Adhesion Deficiency (BLAD) is a lethal autosomal recessive disease in Holstein cattle characterized by a greatly reduced level of expression of the $\beta 2$ heterodimeric integrin. Integrins are adhesion molecules that mediate the entry and passage of neutrophils across membranes to destroy invading pathogens [6; 9; 10].

The molecular basis of BLAD is a single point mutation (A-G) at position 383 in the cDNA of the CD18 gene. This mutation results in a substitution of a glycine for an aspartic acid at position 128 in the D128G protein [2; 5; 8; 11; 15]. Viana [16] and Shuster [15] also described the existence of the silent point mutation (C-T) at position 775 in the cDNA without phenotypic manifestation.

DNA studies with the use of restriction endonuclease *TaqI* or *HaeIII* have detected differences between healthy and affected calves. Gerardi [2] observed in regard to the method used that normal animals have fragments of 100, 200 and 300bp, and BLAD – calves have 200 and 400bp bands. Viana [16] described the length of fragments for normal animals (191, 152bp), for BLAD carriers (343, 191 and 152bp), and for affected animals (343bp).

Deficiency of Uridine – 5'-Monophosphate Synthase (DUMPS) is a genetic disorder, which interferes with pyrimidine biosynthesis and is inherited as a single, two-allele, autosomal locus [7; 14]. The enzyme uridine – 5'-monophosphate (UMP) synthase catalyses the conversion of orotic acid to UMP, the precursor of all other pyrimidine nucleotides and a normal constituent in the milk of cows and other ruminants [13; 14].

The genomic structure of the UMP synthase gene was determined and a PCR-based diagnostic test for carrier detection has been established. DUMPS is caused by point mutation (C-T) at codon 405 within exon 5 [16]. The UMP synthase gene was mapped to the bovine chromosome 1 (q31.36) [4].

A possible method of genotyping is given by Schwenger [12], Grzybowski [3]. A 108-bp product surrounding the mutation was amplified from genomic DNA. The PCR product was digested with *AvaI*; normal homozygote shows bands of 53, 36, and 19bp, heterozygote of 89, 53, 36, and 19bp, the recessive genotype is 89 and 19bp.

Material and methods

For this study we have used blood samples from 90 Romanian Black Spotted cattle (ICDB Balotești farm). White cells from fresh blood sample (300 μ l) were preserved in EDTA anticoagulant, and were hypotonically lysed. The isolation of genomic DNA was performed with Wizard Genomic DNA Extraction Kit (Promega). The total amount of isolated DNA was resuspended in sterile distilled water, measured spectrophotometrically and diluted to 50 ng for each reaction.

In order to characterize and to detect the mutation responsible for BLAD and DUMPS we performed a simple polymerase chain reaction (GeneAmp[®] PCR System 9700) followed by enzymatic restriction (Restriction Fragment Length Polymorphism – RFLP method). Extracted DNA was amplified for 45 cycles (95°C for 30 sec; 57°C (BLAD), and also 58°C (DUMPS) for 30 sec; 72°C for 1 min) in a 25 μ l reaction containing: PCR buffer, MgCl₂, dNTPs, AmpliTaq DNA Polymerase, sense primer (5'- CCT TCC GGA GGG CCA AGG GCT -3') and antisense primer (5'- CTC GGT GAT GCC ATT GAG GGC -3') for BLAD; in contrast, we used sense primer (5'- GCA AAT GGC TGA AGA ACA TTC TG -3') and antisense primer (5'- GCT TCT AAC TGA ACT CCT CGA GT -3') for DUMPS.

In both cases (BLAD and DUMPS), the first denaturation step was performed at 95°C (10 min) and the last extension was 30 minutes (72°C).

PCR products were digested with restriction endonuclease *Taq I* (BLAD) and *Ava I* (DUMPS) at 37°C for 3 h. Restricted products were analyzed by electrophoresis in 2% Agarose High Resolution gel stained with ethidium bromide.

Results and discussions

The identification of normal or carrier specimens for BLAD was made by PCR amplification of genomic DNA with specific primers designed for a region of 136bp followed by restriction with *Taq I* enzyme (figure 1). In contrast, the identification of normal animals or DUMPS carriers was made by PCR amplification of genomic DNA with specific primers designed for a region of 108bp followed by restriction with *Ava I* enzyme (figure 1).

For BLAD, normal homozygote should shown two bands of 108 and 28bp, carrier heterozygote three bands of 136, 108 and 28bp and affected homozygote only one band of 136bp. After endonuclease digestion two fragments of 108 and 28bp characteristics for the normal cattle were detected (figure 2).

Normal homozygote for DUMPS should shown three bands of 51, 36 and 21bp, carrier heterozygote four bands of 87, 51, 36 and 21bp and affected homozygote only two bands of 87 and 21bp. After endonuclease digestion three fragments of 51, 36 and 21bp, characteristics for the normal cattle were detected (figure 2).

Our results indicate that all tested cattle are normal, displaying normal genotypes.

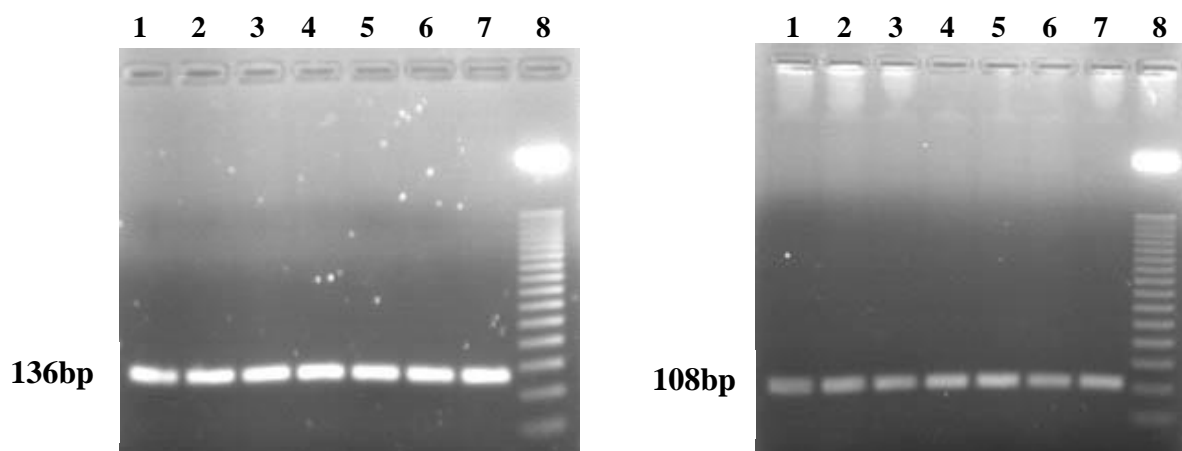


Figure 1. Amplified bovine genomic DNA with specific primers for BLAD and DUMPS detection separated by electrophoresis on 2% agarose gel. Line 1-7, fragments amplified for BLAD and also for DUMPS locus. Line 8, molecular size marker (50bp DNA Step Ladder).

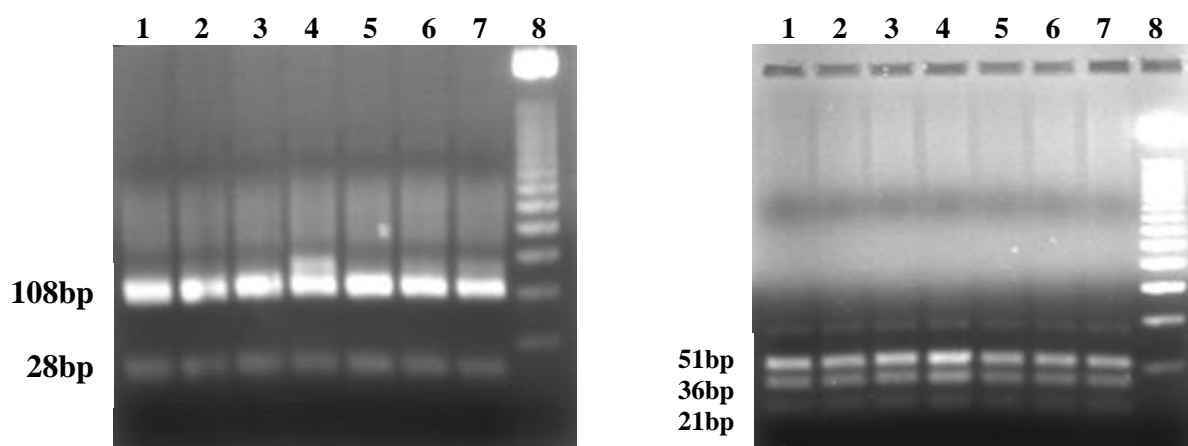


Figure 2. Electrophoresis pattern of amplified fragments after digestion with *Ava I* enzyme. *Lines 1-7, two fragments of 108 and 28bp characteristic for homozygous cattle (BLAD); lines 1-7, three fragments of 51, 36 and 21bp characteristic for homozygous cattle (DUMPS). Line 8, molecular size marker (50bp DNA Step Ladder).*

Conclusions

The detection method based on PCR amplification and RFLP analysis is a powerful tool for allelic diagnosis in BLAD and DUMPS disease. The primers used by us in this study successfully amplified the BLAD sequence (136bp in length) as well the DUMPS sequence (108bp in length).

The specific *Taq I* enzyme makes possible the identification of normal allele of D128G protein at BLAD locus by digestion of the amplified fragment. In the same manner, the specific *Ava I* enzyme makes the identification of normal allele of uridine monophosphate synthase at DUMPS locus by digestion of the amplified sample possible, showing fragments with similar size as literature data indicate.

This method is very important in animal breeding allowing a good and rapid identification of carriers.

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