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SHORT COMMUNICATION

Detection of Bovine Leukocyte Adhesion Deficiency (BLAD) Allele in Holstein Cows Reared in Kayseri Vicinity

Bilal AKYÜZ * Okan ERTUĞRUL ** Özgecan KORKMAZ AĞAOĞLU **

* Erciyes University, Faculty of Veterinary Medicine, Department of Animal Science, 38090 Kayseri - TURKEY
** Ankara University, Veterinary Faculty, Department of Genetics, 06110 Ankara - TURKEY

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Summary

The purpose of this work was to study whether the bovine leukocyte adhesion deficiency (BLAD) allele was present in the Holstein cows reared in Kayseri vicinity. Blood samples were obtained from 136 Holstein cows. In order to determine the area of mutation in PCR products, the PCR products were digested with TaqI endonuclease enzyme. It was found that three of the 136 Holstein cows were BLAD carriers. The mutant BLAD allele frequency in the 136 Holstein cows was calculated as 2.2%.

Keywords: *BLAD, Holstein cows, Hereditary disease, PCR*

Kayseri Civarında Yetiştirilen Holştayn İneklerde Sığır Lökosit Bağlanma Yetmezliği (BLAD) Allelinin Belirlenmesi

Özet

Bu çalışmanın amacı Kayseri civarında yetiştirilen Holştayn ineklerde sığır lökosit bağlanma yetmezliği (BLAD) allelinin varlığının araştırılmasıdır. Çalışmada 136 Holştayn inekten kan örneği toplanmıştır. Elde edilen PCR ürünlerinde mutasyon bölgesinin belirlenmesi için PCR ürünleri TaqI endonükleaz enzimi ile kesilmiştir. Çalışma sonunda incelenen 136 Holştayn ineğin üçünün BLAD taşıyıcısı oldukları belirlenmiştir. İncelenen 136 baş Holştayn inekte BLAD allelinin frekansı %2.2 olarak hesaplanmıştır.

Anahtar sözcükler: *BLAD, Holştayn inek, Kalıtsal hastalık, PCR*

INTRODUCTION

Bovine leukocyte adhesion deficiency (BLAD) is a lethal autosomal recessive disease in Holstein cattle [1]. The disease is caused by a point mutation that results an aspartic acid to glycine substitution in the adhesion glycoprotein CD18 [2,3]. The mutation prevents neutrofil leucocytes to pass through the endothelial layer and reach to the infection area [4]. BLAD affected cattle have recurrent mucosal infections, loss of teeth, delayed wound healing, persistent neutrophilia and death at an early age [5-7].

BLAD carriers were first reported by Shuster et al in US Holstein cattle population [2]. It is under table that expensive, international trading of sperm and bulls and also wide use of AI have made, this genetic disease easily spread to vast cattle populations around the world. BLAD cases have already been reported in many European countries, Australia, South and North America, Japan and India [3,6].

It is necessary to identify and eliminate BLAD carriers from Holstein breeding stock. In animal breeding it is difficult to select against BLAD since the alleles are present in the population in an invisible, heterozygous form. To detect BLAD allele a PCR-RFLP test was introduced by Shuster et al. in 1991 [7,8].

İletişim (Correspondence)
☎ +90 352 3380006/175
✉ bakyuz@erciyes.edu.tr

The prevalence of BLAD carriers in the Holstein bulls and candidate Holstein bulls was calculated 0.84% [4] and 0.6% [9] in Turkey. The prevalence of BLAD carriers in Turkish Holstein cows have not been reported in Turkey. The objectives of the study were to identify the cows with BLAD carriers in Holstein cow population in Kayseri vicinity and to collect the data regarding BLAD allele in Holstein cows in Turkey.

MATERIAL and METHODS

The materials of this study composed of 136 Holstein dairy cows aged between 3 and 6 years, which were reared in Kayseri vicinity. Blood samples were collected from the all cows into heparinised tube for DNA isolation. DNA's were isolated using phenol-chloroform method [10]. PCR was made in a 25 µl reaction mixture containing 100 ng/mL sample DNA, 1 U Taq polymerase, 0.2 mM dNTP, 20 pmol of each primer (forward 5'- CCT GCA TCA TAT CCA CCA G-3', reverse 5'- GTT TCA GGG GAA GAT GGA G-3'). DNA was amplified after initial denaturation at 95°C for 5 min, 33 cycles (94°C 1 min, 57°C 1 min, 72°C 1 min), after the last cycle the samples were kept at 72°C for 5 min and the PCR procedure was finished. The PCR products were analyzed by 2% agarose gel electrophoresis the 343 bp DNA fragment was amplified by PCR. Aliquots of 10 µl of PCR product were subjected to restriction digestion with TaqI in a 20 µl reaction volume using 1 µl TaqI restriction endonuclease and incubated at 65°C for 20 min. The digested product was visualized on 2% agarose gel. The amplified PCR product of 343 bp for the BLAD locus, upon digestion with the TaqI restriction enzyme, yielded two bands of 152 and 191 bp for normal animals, and three bands of 152, 191 and 343 bp for carrier animals.

RESULTS

Among 136 Holstein cattle no positive (+/+) sample was identified, but three cattle were found heterozygote (+/-) carriers for BLAD gene (Figure 1). The percentage of animals having the mutated gene was 2.2%.

DISCUSSION

In Turkey the BLAD problem was first reported in 2006 by Akyuz and Ertugrul [4]. The prevalence of BLAD carriers among Holstein bulls and bull candidates was found 0.84% [4]. The prevalence of BLAD carriers among 136 Holstein cows was found to be 2.2% in this study. This result indicated that the prevalence of BLAD carrier among Holstein cows in Turkish Holstein population is much higher than the prevalence of BLAD carrier among Holstein bulls and bull candidates in Turkey [4,9]. The prevalence of BLAD carriers for AI bulls and cows were much lower than the values reported in some other European countries [1-3] and Japan [7].

There are about 271.000 registered Holstein cows in the pedigree herd book of the cattle Breeders Association in Turkey [10]. Monitoring the prevalence of BLAD carriers in random selected cows may be helpful in judging the effectiveness of BLAD control program. This control program should be carried out with the cooperation of breeder associations.

In countries like Turkey, where Holstein bulls are extensively used for crossbreeding programmes, it becomes necessary to screen all Holstein bulls and their mothers, to minimize the risk of spreading this disease among future bulls and bull mothers. There was no previous report regarding the existence of BLAD gene in

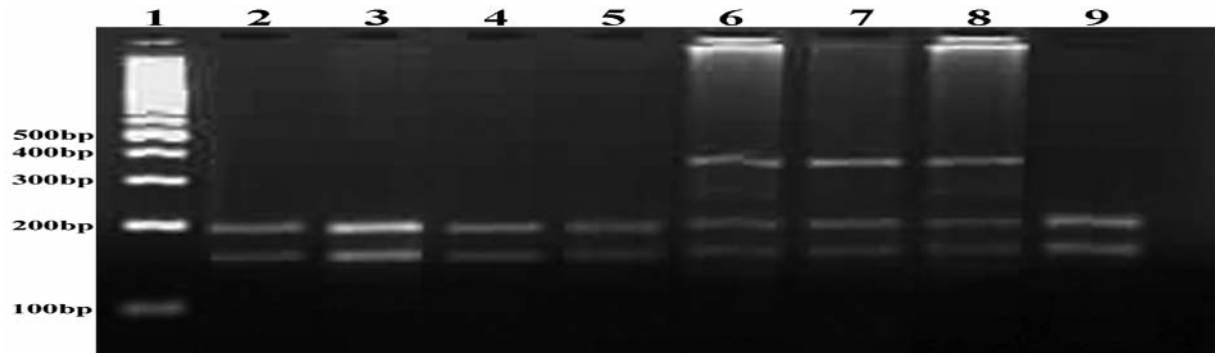

Fig 1. Photograph of TaqI digestion products of BLAD gene on agarose gel. Lane 1: 100 bp marker, Lane 2-5 and 9: 152 and 191 bp bands of homozygous normal animals, Lane 6-8: 152, 191 and 343 bp bands of heterozygous BLAD carrier

Şekil 1. BLAD geninin TaqI enzim kesim ürünlerinin agaroz jeldeki fotoğrafı. Hat 1: 100 bp'lik DNA belirteci, Hat 2-5 ve 9: 152 ve 191 bp'lik homozigot normal hayvanlara ait bantlar, Hat 6-8: 152, 191 ve 343 bp'lik BLAD alleli taşıyan heterozigot hayvanlara ait bantlar

Holstein cows in Turkey. In this study, BLAD allele frequency was found higher in Holstein cows than Holstein bulls. The results of this study that bull candidate and their mothers should also be investigated for eradication of BLAD allele in Turkish Holstein population.

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