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The Prevalence of BLAD and Comparison of Some Production Parameters in Carrier and Non-Carrier Holstein Cows in Kayseri Province, Turkey

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Summary: In this study, randomly selected 262 Holstein cows reared in Kayseri province of Turkey were screened with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for bovine leukocyte adhesion deficiency (BLAD) and some production parameters such as age at first calving, calving interval and 305-day milk production of BLAD-carrier (BL) and BLAD non-carrier (TL) cows were compared. As a result, the prevalence of BL cows was found as 4.58%. There was not any difference between BL and TL cows for age at first calving and calving interval parameters ($p>0.05$). However, 305-day milk production of BL cows was higher than that of TL cows ($p<0.001$). Thus, the detection of BLAD carriers and properly planned mating are of great importance for preventing the spread of the disease in Holstein cows.

Key Words: BLAD, Holstein, Kayseri, PCR-RFLP

Kayseri İlinde BLAD Prevalansı ile Taşıyıcı ve Taşıyıcı Olmayan Holştayn İneklerinde Bazı Üretim Parametrelerinin Karşılaştırılması

Özet: Bu çalışmada, Kayseri ilinde yetiştirilen tesadüfî olarak seçilen 262 Holştayn inek, polimeraz zincir reaksiyonu-restiriksiyon fragment uzunluk polimorfizmi (PCR-RFLP) ile sığır lökosit bağlanma yetmezliği (BLAD) yönünden incelenmiş ve BLAD-taşıyıcısı (BL) ve BLAD-taşıyıcısı olmayan (TL) inekler ilkine buzağılama yaşı, buzağılama aralığı ve 305 günlük süt verimi gibi bazı üretim parametreleri bakımından karşılaştırılmıştır. BLAD taşıyıcı ineklerin prevalansı % 4.58 olarak bulunmuştur. BLAD taşıyıcı ve taşıyıcı olmayan inekler arasında ilkine buzağılama yaşı ve buzağılama aralığı parametreleri yönünden herhangi bir fark bulunmamıştır ($p>0.05$). Ancak, 305 günlük süt verimi BLAD taşıyıcı ineklerde daha yüksek bulunmuştur ($p<0.001$). Bu nedenle, Holştayn ineklerde bu hastalığın yayılmasını engellemek için BLAD-taşıyıcılarının belirlenmesi ve çiftleştirmelerin buna göre planlanması büyük önem taşımaktadır.

Anahtar Kelimeler: BLAD, Holştayn, Kayseri, PCR-RFLP

Introduction

Genetic improvement programs in cattle breeding have significantly contributed to large improvements in milk and meat production in the last 50 years (5). The worldwide use of genetic materials from progeny-tested top ranked studs resulted in the shallowing of the genetic pool in breed. On the other hand, increased use of artificial insemination (AI) in cattle breeding has led to spread of hereditary diseases. Inherited disorders affect all species of domestic animals, particularly cattle. In cattle breeding, genetic disorders are one of the most important diseases for farmers. Genetic disorders are hereditarily caused by physical, functional anomalies, and they reduce fertility, having negative impacts on farm animals (3,20). Therefore, these disorders are likely to result in economic losses in cattle breeding (20). It is important to note that the majority of inherited diseases in cattle are autosomal recessive for which efficient detecting of the carrier animals requires molecular techniques. During the last few decades, technological developments in the field of molecular genetics have enabled the identification of the genes responsible for a number of important genetic diseases such as bovine leukocyte adhesion deficiency (BLAD), complex vertebral malformation (CVM), deficiency of uridine monophosphate synthase (DUMPS) and factor XI deficiency (3,20).

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BLAD is a lethal autosomal recessive immunodeficiency disease of Holstein cattle, and it is caused by a deficiency on the leukocyte surface glycoproteins known as β_2 integrins (24,25). These glycoproteins are responsible for the cell-cell interactions that are necessary for neutrophils to adhere to endothelial cells, migrate to the sites of infection and tissue injury to eliminate invasive microorganisms in an immune response (1,4,15). In order to reach to infection sites from the leukocytes need adherence to endothelial cells for migration. This ability of adherence is dependent upon the coordination of integrins on leukocytes' surfaces with endothelial intercellular adhesion molecules (16). Without β_2 integrins, leukocytes especially neutrophils are unable to enter the tissues to destroy invading pathogens within vessel (3,17). A similar genetic disorder was previously described in humans (termed as leukocyte adhesion deficiency, LAD) and Irish setter dogs (termed as canine leukocyte adhesion deficiency, CLAD). In humans, LAD is clinically nearly identical to BLAD (7,17). BLAD is occurs as a single point mutation (A→G) in the bovine CD18 gene (11,13,25). The molecular basis of this mutation is, at position 383 of the bovine CD18 gene, caused by an aspartic acid to glycine substitution at amino acid in the glycoprotein (8).

Calves affected by BLAD are born from carrier x carrier mating. Calves homozygous for BLAD rapidly succumb to a general infection occurring in the herd. The fact that they are easily affected by opportunistic microorganisms ultimately leads to death (7,15). The symptoms in the affected calves are rather diverse (8). Affected calves may develop severe ulcers on oral mucous membranes, gingivitis, loss of teeth, impaired pus formation, delayed wound healing, recurrent diarrhea, chronic pneumonia, bronchitis, persistent neutrophilia, and bronchopneumonia right after birth (1,17,25). The symptoms often appear within two months after birth, and the majority of affected calves usually die before one year of age from chronic diarrhea, high fever and other infections (7). Some affected calves may survive in the first year of life; but they exhibit severely retarded growth and may suffer from various infections (7,8). The average Holstein calf mortality has been reported to be between 7.5 and 16.4% in Turkey (19). However, the definite etiology of these calf mortalities has not been reported. Therefore, there are not any data available on death calves due to BLAD in Turkey. Calves with BLAD are treated for diarrhea and pneumonia despite the fact that these symptoms are recurrent.

BLAD is also defined as the most important inherited disease that causes economic losses in Holstein breeding (23). Calf mortalities on dairy farms represent major economic losses. Additionally, calf treatment costs due to recurrent infections increase the total cost of farmers from BLAD.

The novelty of this study was to report the prevalence of BLAD and a comparison of some production parameters of BL and TL cows in Kayseri province of Turkey.

Materials and Methods

In this study, blood samples were collected from randomly selected 262 Holstein cows reared in Kayseri Province located in Middle Anatolian Region of Turkey. The samples were selected among not inbreed cows according to records of enterprise. The blood samples were collected from jugular vein into EDTA containing test tubes. The blood samples were collected after approved by Ethical Committee of the Erciyes University (No: 09/19). DNA was isolated by the phenol-chloroform method. The PCR assay was set up in sterile tubes containing 100 ng of genomic DNA template, 0.4 pM of both forward (5'-CCT GCA TCA TAT CCA CCA G-3') and reverse (5'- GTT TCA GGG GAA GAT GGA G-3') primers as proposed by Kriegesmann et al. (12).

The PCR was performed in 25 µl of final volume consisting of 1 U Taq DNA polymerase, 1 X PCR buffer and 400 µM of each dNTP. The PCR was carried out with an initial denaturation at 95 °C for 5 min followed by 33 cycles, each consisting of 1 min denaturation at 94 °C, 1 min annealing at 57 ° C, and 1 min extension at 72 °C; the final cycle products were extended for 5 min at 72 °C. After the PCR, a fragment of 343 bp length was obtained. Ten microliter aliquots of the amplification product were subjected to restriction in endonuclease digestion with 5 U *Taq*I. The digestion product was analyzed by 2% agarose gel electrophoresis. Genotyping of normal (two bands of 152 and 191 bp), carrier (three bands of 152, 191 and 343 bp) and affected (single band of 343 bp) animals was performed by the polymorphism in the length of restricted fragments.

Statistical analysis of the data was conducted by SPSS 11.0 version for Windows. The BL and TL were compared in terms of "age at first calving", "calving interval" and "305-day milk production" with Independent Sample T-Test. The data were expressed as means ± SEMs.

Results

Identification of normal and carrier animals for the CD18 locus causing BLAD was achieved by amplifying a 343 bp fragment (Figure 1) from isolated DNA of the selected animals. The restriction pattern of the amplified DNA product (343 bp) with *Taq*I resulted in two bands of 152 and 191 bp in normal animals. The carrier animals had three bands 152, 191 and 343 bp (Figure 1). In this study, 12 cows were detected as BLAD carrier. Therefore, the prevalence of BLAD carriers was found as 4.58%.

There was not any difference between BL and TL cows for "age at first calving" and "calving interval" parameters as shown in Table 1 ($p>0.05$). However, "305-day milk production" of BL cows was higher than that of TL cows ($p<0.001$).

Discussion

Identification of the causal mutations allows performing planned matings; helps prevent economic losses and exclude heterozygote carrier animals from animal breeding. At OMIA (Online Mendelian Inheritance in Animal) 180 Mendelian traits and disorders were identified in cattle and 87 of them are known to mutations (18). In cattle, the majority of the genetic disorders are recessive. Therefore, the detection of carrier cows and bulls is an important problem. New molecular genetic techniques have been used to overcome this problem. In domestic animals, breeders and their organizations focus on genetic disorders which have been monitored for the last two decades. Diagnosis and reporting of inherited diseases by the veterinary practitioner, farmer and animal breeding organizations are very important for preventing the economic losses resulting from

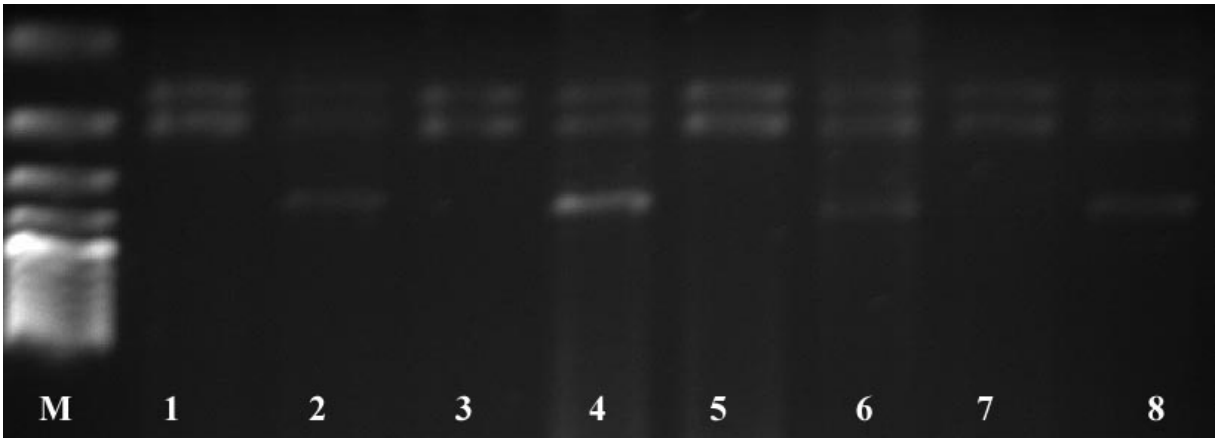

Fig. 1: Photograph of *Taq*I digestion products of BLAD gene on 2% agarose gel. Lane M: 100 bp marker, Lane 1, 3, 5 and 7: 152 and 191 bp bands of homozygous normal animals, Lane 2, 4, 6 and 8: 152, 191 and 343 bp bands of heterozygous BLAD carrier

Table 1: Effect of BLAD on some production traits of Turkish Holstein cows

Production traits	BL		TL		Statistically Significant
	n	$\overline{X} \pm S_{\bar{x}}$	n	$\overline{X} \pm S_{\bar{x}}$	
-Age at first calving	12	760.8±13.9	250	759.9±1.5	P>0.05
-Calving interval	34	406.2±14.5	203	422.3±5.9	P>0.05
-305-day milk production	40	9.214.5±321.4	453	7.635.9±74.9	P<0.001

$\overline{X} \pm S_{\bar{x}}$: Mean ± Standart Error of Mean (SEM)

inherited diseases. The genetic diagnosis of carrier animals is likely to improve the eradication programs for defective alleles.

BLAD is the most common inherited disease of Holstein population in the world. Recent reports on the BLAD allele frequency are available from different countries in Asia (1% in Pakistan, 1.9% in China, 3.2% in India, 3.3% in Iran, 5.8% in Taiwan and 8.1% in Japan); Europe (2.5% in Romania, 4.8% in Poland, 10.4% in Hungary and 13.4% in Denmark); and America (1.8% in Chile, 3.5% in Argentina, 5.7% in Brazil and 5.8-14.1% in USA) (10,11,15,16,17,21,22,24,26). These studies indicate that BLAD is prevalent in Holstein cattle all over the world.

The presence of BLAD allele in Turkey was first reported in 2006 when carrier prevalence was identified as 0.84% in Holstein bulls and candidate bulls (1). Further studies reported the prevalence of BLAD carrier cows as 2.2% and 4.0% in different regions of Turkey (2,14). In this study, the prevalence of BLAD carrier cows was found as 4.58%. This study showed that the BLAD is also prevalent in Turkey. The presence of BL cows may be due to not eliminating the carriers; the import of unknown sperm and cows for BLAD from the United States and European countries since 1958; and the use of uncontrolled bulls by farmers for service. In order to reduce the carrier prevalence, elimination of BLAD carrier cows and bulls can be suggested. In Poland, the results of elimination program showed a clear decreasing (from 7.9% to 0.8%) in BLAD carrier bulls (4).

On the other hand, the majority of the previous studies conducted in different countries are related to the presence and prevalence of BLAD. Studies based on the effects of BLAD on production traits are rare. The results of these studies vary from one another. For example; Janosa et al. (9) reported that milk yield of BL cows (7201 kg) was significantly lower than TL cows (7445 kg). In contrast, Fesüs et al. (7) did not find any statistical significance between 305-day lactation milk production of BL and TL cows (9854 and 9720 kg, respectively). In this study, similar to Dohy et al. (6) and Janosa and Dohy (9), 305-day lactation milk production of BL cows (9214.5 kg) was significantly higher than TL cows (7635.9). In contrast to this study, Powell et al. (23) reported that selection against BLAD does not exert a negative influence on production. In order to decrease the frequency of BLAD and economic losses, cows with high milk yield must be screened and must not be inseminated associated with sperms of BLAD carrier bulls.

There is not enough information about the share of calf mortalities due to BLAD in total calf mortalities in Turkey. However, BLAD might be the potential reason of some calf mortalities. Therefore, selection of BLAD carriers could prevent the spread of this defect and may decrease the rate of calf mortalities. The results of this study showed that the age at first calving and calving interval parameters were not affected by BLAD (P>0.05), which is in accordance with that reported by Fésüs et al. (7).

In Turkey, government and dairy organizations should encourage the farmers for screening Holstein bulls and candidate bulls for BLAD; and the mutation detected bulls should be reformed from breeding. Normal bulls should be certified by government and used for AI in the future. Otherwise, spreading of the disease and economic losses may have an increasing potential. The other challenge for farmers is that cross Holstein cattle with local breeds without controlling for BLAD and, therefore, there is a possibility of a transfer of this mutation to the local breeds. The detection of BLAD carriers and properly planned mating are of great importance for preventing the spread of the disease in Holstein cows and reducing the economic losses due to BLAD.

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