

DETECTION OF BOVINE LEUKOCYTE ADHESION DEFICIENCY (BLAD) IN TURKISH NATIVE AND HOLSTEIN CATTLE

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The purpose of this work was to study whether the bovine leukocyte adhesion deficiency (BLAD) allele is present in native cattle breeds and the Holstein breed in Turkey. Blood samples were obtained from 120 Holstein, 20 Brown Swiss, 20 Anatolian Black, 20 Turkish Grey, 20 South Anatolian Red and 20 East Anatolian Red cattle. The isolated DNA materials were multiplied in PCR using the primer developed by Kriegesmann et al. (1997). In order to determine the area of mutation in PCR products, the PCR products were digested with *TaqI* endonuclease enzyme. The resulting fragments were analysed on 2% agarose gel for the absence of a *TaqI* restriction site. It was found that two of the Holstein cattle (a bull and a cow) were heterozygote BLAD carriers. There was no homozygote BLAD animal. The BLAD allele was not found in the other breeds used in the study. The mutant BLAD allele frequency in the 120 Holstein cattle calculations was 0.0084.

Key words: BLAD, Holstein, PCR, Turkey

Bovine leukocyte adhesion deficiency (BLAD) is a lethal, autosomal and recessive inherited disease (Healy, 1992; Kostetskii et al., 1996). The determining gene CD18 is located on the first chromosome and the disease is the result of a mutation changing guanine to adenine at the 383rd nucleotide (Kehrli et al., 1992).

The mutation prevents neutrophilic leukocytes to pass through the endothelial layer and reach the area of infection. In animals with BLAD, the

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CD11/CD18 complex is either not present or significantly decreased. Affected animals do not show specific clinical symptoms. Sick animals show oral ulcers, gingivitis, diarrhoea, pneumonia, bronchitis and bronchopneumonia right after birth (Kehrli et al., 1992; Ackermann et al., 1996; Gerardi, 1996). There is a steady increase in the leukocyte count (Gilbert et al., 1993), generally above 100,000 (Nagahata et al., 1997; Jánosa et al., 1999). Sick animals die at an early age. In some cases the death may be delayed until 2 to 3 years of age (Olchoway et al., 1994; Fésüs et al., 1999). Also, the surviving calves demonstrate retarded growth and weakness (Healy, 1992; Agerholm et al., 1993).

In the USA, the pedigree studies of sick calves revealed that the affected animals were the descendants of BLAD carrier Osborndale Ivanhoe, Penstate Ivanhoe Star and Carlin M Ivanhoe Bell, which were prominent Holstein sires in the USA (Shuster et al., 1992; Kriegesmann et al., 1997). Because of their very high milk production performances these bulls and also their male offspring were used extensively in inseminations. Thus BLAD spread not only in the USA, but also all over the world (Ryncarz et al., 1995; Jánosa et al., 1999). About 70% of dairy cows in the modern world are subjected to artificial insemination and some record bulls, such as the above-mentioned ones and their offspring, have been used repeatedly in many generations (Powell et al., 1996; Jánosa et al., 1999). This practice has increased the milk production but also the incidence of inherited diseases and abnormalities, such as BLAD, through increased homozygosity or inbreeding (Mirck et al., 1995).

Heterozygous animals do not show any clinical signs of BLAD. However, these animals pass the BLAD allele to 50% of their offspring. This reality makes imperative to eliminate carrier animals from the breeding herds. It has been reported that about 5.8% of Holstein animals all over the world were believed to be heterozygous for BLAD allele (Healy, 1992; Kriegesmann et al., 1997). For this reason BLAD is one of the worst inherited diseases, threatening the dairy industry of the USA and the whole world. BLAD cases have already been reported in many European countries, Australia, South and North America, and India (Gilbert et al., 1993; Zsolnai and Fésüs, 1996; Muraleedharan et al., 1999; Kijias et al., 2000).

The Holstein breed was introduced to Turkey from the USA in 1958 and importation has been continued in the form of animal, sperm and embryo imports from some of the European countries since that time. There has been no reported case of BLAD in Turkey. However, the possibility was suggested that animals carrying the BLAD allele existed in the Turkish Holstein population. The purpose of this study was to investigate the presence of the BLAD gene in the bulls and progeny testing bulls used by the Ministry of Agriculture and the Central Breeding Association in their joint progeny testing project in Turkey. The PCR-RFLP method was used in the determinations.

Materials and methods

The blood samples of 220 cattle were examined. The samples were collected from 120 Holstein cattle, and 20 cows from each of the Brown Swiss, East Anatolian Red, South Anatolian Red, Native Black and Gray Steppe Cattle breeds. The Holstein bulls were young animals to be used for progeny testing by the Holstein Breeders Association of Turkey. The locations of blood collections covered all the regions of Turkey. DNA was isolated by the phenol-chloroform method.

The PCR was performed using primers consisting of 19 bases each (5'-CCT GCA TCA TAT CCA CCA G-3' and 5'-GTT TCA GGG GAA GAT GGA G-3') as proposed by Kriegesmann et al. (1997). The PCR was made in 25 µl of final volume: 100 ng/ml sample DNA, 1 U *Taq* DNA polymerase. The PCR mixture was heated and kept at 95 °C for 5 min, then revolved in 33 cycles, each cycle consisting of 1 min of each of the temperature levels of 94, 57 and 72 °C. After the last cycle the samples were kept at 72 °C for 5 min and the PCR procedure was finished. After the PCR, a fragment of 343 bp length was amplified.

The PCR products were migrated on 2% agarose gel. On the gel the band of 343 bp length was studied in UV light and the fragment was cut with *TaqI* restriction enzyme. In evaluating the results of electrophoresis, two bands of 152 and 191 bp indicate homozygote normal individuals, a single band of 343 bp indicates homozygote sick individuals, and three bands of 152, 191 and 343 bp indicate heterozygote carrier individuals.

Results

The results indicated that all the breeds except Holstein were negative (–/–) for the BLAD gene. Among the 120 Holstein cattle no positive (+/+) sample was identified, but two cattle were found to be heterozygote (+/–) carriers for the BLAD gene. The frequency of the BLAD gene among the Holstein samples studied was estimated as 0.0084.

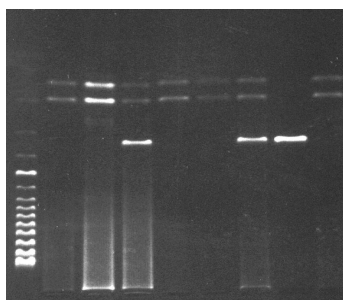


Fig. 1. Results of electrophoresis (from left to right: DNA ladder, East Anatolian Red, Brown Swiss, three Holstein samples and references of BLAD +/–, +/+, –/–)

Discussions

Shuster et al. (1992) were the first group to develop a method for identifying the BLAD allele. However, their method possessed two problematic points. Firstly, cutting the PCR product with *Hae*III and *Taq*I enzymes yields small RFLP fragments. These fragments would rapidly diffuse into the gel during electrophoresis, making it too difficult to be observed for identification. As a result, it could not be evaluated clearly whether or not a BLAD gene was present in the sample. Secondly, the method uses primers of 30 and 19 base pair lengths. Their annealing heats are not balanced. These factors would also reduce the reliability of the method. Additionally, an expensive NuSieve agarose is used in the method at 4% concentration, which makes the method rather expensive. On the basis of these considerations, the method developed by Kriegesmann et al. (1997) was used in this study.

After identification of the BLAD gene, Holstein breeders in the USA started BLAD control programs, and the frequency of this inherited disease has been markedly reduced. In Turkey, on the other hand, neither Holstein breeders nor veterinarians are aware of this problem. This situation makes it difficult to gather information on the extent of the problem and also to make an assessment on the economic losses caused by it in the livestock industry.

The frequency of the BLAD allele among 120 Holstein cows was found to be 0.84% in this study. This result indicates that the frequency of the BLAD allele is much lower in the Turkish Holstein population than in many other countries (Fésüs et al., 1999). This may be explained by the fact that the bulls used in Turkey are the descendants of American Holsteins which are subjected to a strict BLAD control program. However, there is no BLAD allele information in the pedigree records of sires imported from the USA and used in artificial insemination in Turkey. For this reason, a general screening and a sizeable collection of blood samples from the Turkish Holstein population are needed for obtaining a clear picture of the BLAD allele frequency. The Holstein population in Hungary is also mainly derived from American sires and dams. The BLAD allele frequency in Hungary is about 10% and similar to the frequency reported in the USA (Fésüs et al., 1999). It may be logical to expect a similar result when an intensive screening for BLAD allele is carried out in Turkey.

No BLAD alleles were found in native and Brown Swiss breeds. In fact, this allele has been reported only from Holstein cattle populations in the USA and Europe (Agerholm et al., 1993; Jánosa et al., 1999; Muraleedharan et al., 1999).

When the presence of the BLAD allele was identified in the Holstein population, a BLAD eradication program was initiated in each country, since BLAD is a serious inherited disease and causes vast economic losses to the country concerned (Powell et al., 1996).

There are about 271,000 Holstein cows in the pedigree herd book of the Breeders' Association in Turkey (Anonymous, 2004). Using the US estimate (Jánosa et al., 1999) as a reference, there may be about 543 Holstein calves born with the BLAD allele in Turkey each year. With the addition of these animals, the number of affected calves and their negative impact on the national economy would certainly become more significant. The losses would be listed as slower growth, reduced production, veterinary and drug expenses, and deaths.

With the present study, the presence of an inherited disease caused by the BLAD gene was identified in the Holstein population of Turkey. There has been no previous report on the existence of such a gene and its consequences. The frequency of the BLAD gene found in the present study is lower than that reported in other countries. However, it is believed that with the increase of the sample size the frequency of the BLAD gene would also increase. As a first measure to be taken, at least young breeding bulls should be tested for the BLAD gene, the carriers should be eliminated and the clean animals should be certified.

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