

Estimating allele frequencies of some hereditary diseases in Holstein cattle reared in Burdur Province, Turkey

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Abstract: The economics of dairy cattle breeding around the world has been significantly affected by the autosomal recessive hereditary diseases of bovine leukocyte adhesion deficiency (BLAD), factor XI deficiency (FXID), bovine citrullinemia (BC), and deficiency of uridine monophosphate synthase (DUMPS). The aim of this study was to determine BLAD, FXID, BC, and DUMPS alleles in Holstein cattle raised in Burdur Province in Turkey. This study involved taking blood samples randomly from 500 head of Holstein cattle at various dairy farms in different towns in Burdur Province. Polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis was employed to identify cows with these diseases. The results showed that the percentages of carrier animals for BLAD and FXID were 2% and 1.8%, respectively, while no DUMPS or BC was found in the study population. This is the first study on BLAD, FXID, BC, and DUMPS in Holstein cattle raised in Burdur Province in Turkey.

Key words: Cattle, BLAD, FXID, BC, DUMPS

1. Introduction

There are many genetic diseases that affect reproductive traits, the viability of calves, and any number of physiological functions in Holstein cows. These include bovine leukocyte adhesion deficiency (BLAD), factor XI deficiency (FXID), bovine citrullinemia (BC), and deficiency of uridine monophosphate synthase (DUMPS). These conditions cause significant economic losses (1). BLAD, FXID, BC, and DUMPS are breed-specific autosomal genetic diseases that have a significant impact on the breeding of Holstein cattle (2).

BLAD is a lethal autosomal recessive disease in Holstein cows (3) that is described by decreased expression of the β_2 heterodimeric integrin (4). The disease is caused by a point mutation (A→G) of nucleotide 383 in the CD18 gene that results in the substitution of aspartic acid with glycine in the adhesion glycoprotein CD18 (5). This mutation prevents neutrophil leucocytes from passing through the endothelial layer and reaching infected areas (6). Cattle affected by BLAD suffer from loss of teeth, recurrent mucosal infections, death at an early age, and delayed wound healing (5). BLAD has been studied by many researchers in a number of countries including China (7), Denmark (8), Japan (9), Turkey (2,6,10,11), and the USA (4). DUMPS is a hereditary disease that causes early embryonic death

during the implantation period of pregnancy. Furthermore, DUMPS is thought to be one of the causes of repeat breeder syndrome (12). DUMPS is caused by a single point mutation (C→T) inside exon 5. The UMP synthase gene is mapped to bovine chromosome 1 (q31–36) (13). DUMPS has been investigated in the Czech Republic (3), Poland (14), and Turkey (2). FXID is a blood coagulation defect that has been identified in cattle (15), humans (16), and dogs (17). FXI deficiency was described as an insertion of 76 base pairs (bp) in exon 12 of FXI, which resulted in a premature stop codon (1). Studies on reproductive performance have revealed that more than 50% of affected animals have repeat breeding syndrome. Follicular diameter is small in affected cows and such animals have a low estradiol level around the time of ovulation (18). In addition, cattle are predisposed to mastitis, metritis, and pneumonia (19). Correct identification of animals is an important part of controlling FXI deficiency because FXI-deficient animals may not exhibit any clinical symptoms (1). BC is a recessive genetic disease rarely found in Holstein cattle. The bovine citrullinemia gene is found on chromosome 11 (20) and its mutation is characterized in exon 5 of argininosuccinate synthase (ASS). ASS is an enzyme crucial to the urea cycle that catalyzes the conversion of aspartate and citrulline to ASS. Since affected calves have a lack of ASS, ammonia

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accumulates in blood and tissue. As a result, new born calves develop an unsteady gait, aimless wandering, apparent blindness, convulsions, and death within a week (20).

Holstein cattle are one of the most preferred cattle breeds by dairy breeders around the world; this is also the case in Turkey. Approximately 40% of cattle bred in Turkey are purebred Holsteins (21). Burdur is a province located in the West Mediterranean region of Anatolia that has a large population of Holstein cattle, numbering approximately 200,000 (21,22), being raised on approximately 22,000 farms (22). The number of Holstein cattle owned in Burdur province is 3.6% of the total number of purebred cattle in Turkey (21,22). The Holstein population in Burdur represents a significant portion of the total Holstein population in Turkey. As a result, detection of hereditary diseases is critical. Monitoring the genetic health of the cattle in Burdur in terms of certain autosomal genetic diseases is an important issue for dairy cattle owners there. To this end, the aim of this study was to determine diseases such as BLAD, FXID, BC, and DUMPS in Burdur's dairy cattle population.

2. Materials and methods

2.1. Sample collection and DNA extraction

Blood samples were collected randomly from 500 head of Holstein cattle belonging to 48 dairy farms at 11 towns in Burdur Province. Samples were taken from the tail vein into a tube including K3 EDTA and stored at -20°C . DNA was isolated with a DNA isolation kit (GeneJET Genomic DNA Purification kit). This study was approved by the Local Ethics Committee on Animal Experiments of Mehmet Akif Ersoy University (approval no: 03.12.2012/07).

2.2. Amplification

A 25- μL reaction mixture was made for amplification reaction of the CD18 gene that contained ~ 100 ng of genomic DNA, 2 mM MgCl_2 , 200 μM of each dNTP, 5 pmol CD18 primer, 1X PCR buffer, and 1 U Taq DNA polymerase

(Thermo Scientific). The amplification process for the CD18 gene was performed with an initial denaturation and polymerase activation at 95°C for 5 min followed by 33 cycles of 94°C for 60 s, 60.2°C for 60 s, and 72°C for 60 s. The final step of an elongation was at 72°C for 5 min.

PCR for the DUMPS and BC genes were performed in a 27- μL reaction mixture containing 5 pmol of each primer, 12.5 μL of 2X PCR Master Mix (Thermo Scientific, #K0171), and ~ 100 ng of genomic DNA template. For DUMPS an amplification reaction was performed with an initial denaturation step at 95°C for 3 min followed by 35 cycles of 95°C for 30 s, 60.1°C for 30 s, and 72°C for 30 s. The final step of an additional extension was at 72°C for 5 min. For the BC gene, thermocycling started with an initial step at 94°C for 3 min for denaturation. A total of 30 cycles of 94°C for 30 s, 55.2°C for 30 s, and 72°C for 30 s were followed by a final extension step at 72°C for 10 min. The PCR reaction was performed in a final volume of 25 μL for the FXID gene. The PCR mixture contained 1.5 mM MgCl_2 , 200 μM of each dNTP, 5 pmol of each primer, 1X PCR buffer, 1 U Taq DNA polymerase, and ~ 100 ng of genomic DNA template. The PCR amplification condition consisted of the initial denaturation step at 95°C for 10 min followed by 34 cycles of 95°C for 30 s, 55°C for 60 s, and 72°C for 30 s and the final extension step at 72°C for 10 min. The nucleotide sequences for the primers used are shown in the Table. All PCR amplification reactions were performed in a DNA Amplitronyx Series 6 thermal cycler. The amplified PCR products were electrophoresed on EtBr stained 2% agarose gels.

2.3. PCR-RFLP

The PCR products were digested with the following fast digest restriction endonucleases: *TaqI* (Thermo Scientific, #FD0674) for BLAD, *AvaI* (Thermo Scientific, #FD0384) for DUMPS, and *AvaII* (Thermo Scientific, #FD0314) for BC. Restriction products were electrophoresed on 3% agarose gel (BLAD) and 4% NuSieve agarose gel (DUMPS and BC), displayed under a UV-transilluminator.

Table. Nucleotide sequence of primers used in PCR and restriction endonucleases (RE).

Genetic disorder	Primer	PCR product size	RE
BLAD	F: 5'-CCTGCATCATATCCACCAG-3' R: 5'-GTTTCAGGGGAAGATGGAG-3'	343 bp	<i>TaqI</i>
DUMPS	F: 5'-GCAAATGGCTGAAGAACATTCTG-3' R: 5'-GCTTCTAACTGAACCTCTCGAGT-3'	108 bp	<i>AvaI</i>
FXID	F: 5'-CCCACTGGCTAGGAATCGTT-3' R: 5'-CAAGGCAATGTCATATCCAC-3'	320 bp	-
BC	F: 5'-GGCCAGGGACCGTGTTCATTGAGGACATC-3' R: 5'-TTCCTGGGACCCCGTGAGACACATACTTG-3'	185 bp	<i>AvaII</i>

3. Results

PCR amplification for BLAD, FXID, BC, and DUMPS was successful in all 500 samples that were analyzed. After the digestion of PCR product, two fragments were revealed that have been demonstrated in unaffected cattle: 152 bp and 191 bp. BLAD carriers demonstrate three fragments: 152 bp, 191 bp, and 343 bp. The normal DUMPS allele consists of three fragments in healthy cows: 19 bp, 36 bp, and 53 bp. Cattle of DUMPS carrier exhibited 4 fragments: 19 bp, 36 bp, 53 bp, and 89 bp. The unaffected allele of BC produced two fragments: 82 bp and 103 bp. The normal FXID allele in animals that were not affected exhibited a single 244 bp fragment. In FXID carriers demonstrated two fragments: 244 bp and 320 bp, while in homozygous animals, the fragment had a length of 320 bp. BLAD and FXID analysis revealed that of the 500 Holstein cows genotyped, 490 were identified as normal homozygous and 10 were identified as heterozygous for BLAD. Furthermore, 491 were identified as normal homozygous and 9 were identified as heterozygous for FXID. However, no carrier was identified for DUMPS or BC. As a result of this electrophoretic analysis, no mutant

animal was detected for BLAD, DUMPS, BC, or FXID (Figures 1a–1d).

4. Discussion

In Turkey, Akyuz and Ertugrul, (6), Meydan et al. (2), Akyuz et al. (23), and Sahin et al. (11) reported the prevalence of BLAD carriers in Holsteins to be 0.84%, 4.0%, 2.2%, and 2%, respectively. In contrast, Oner et al. (10) did not find any carriers of BLAD. This difference may be due to the fact that populations from different regions were sampled. In our study, the frequency of BLAD carriers among the Holstein samples evaluated here was estimated at 2%. 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 (4). Similarly, carrier cattle were found to be 21.5% and the frequency of affected animals was found to be 0.5% in Denmark (8). In contrast to these rates, BLAD carrier rates were found to be lower in Japan (9) and Poland (24) (5.4% and 0.8%, respectively). In studies conducted in

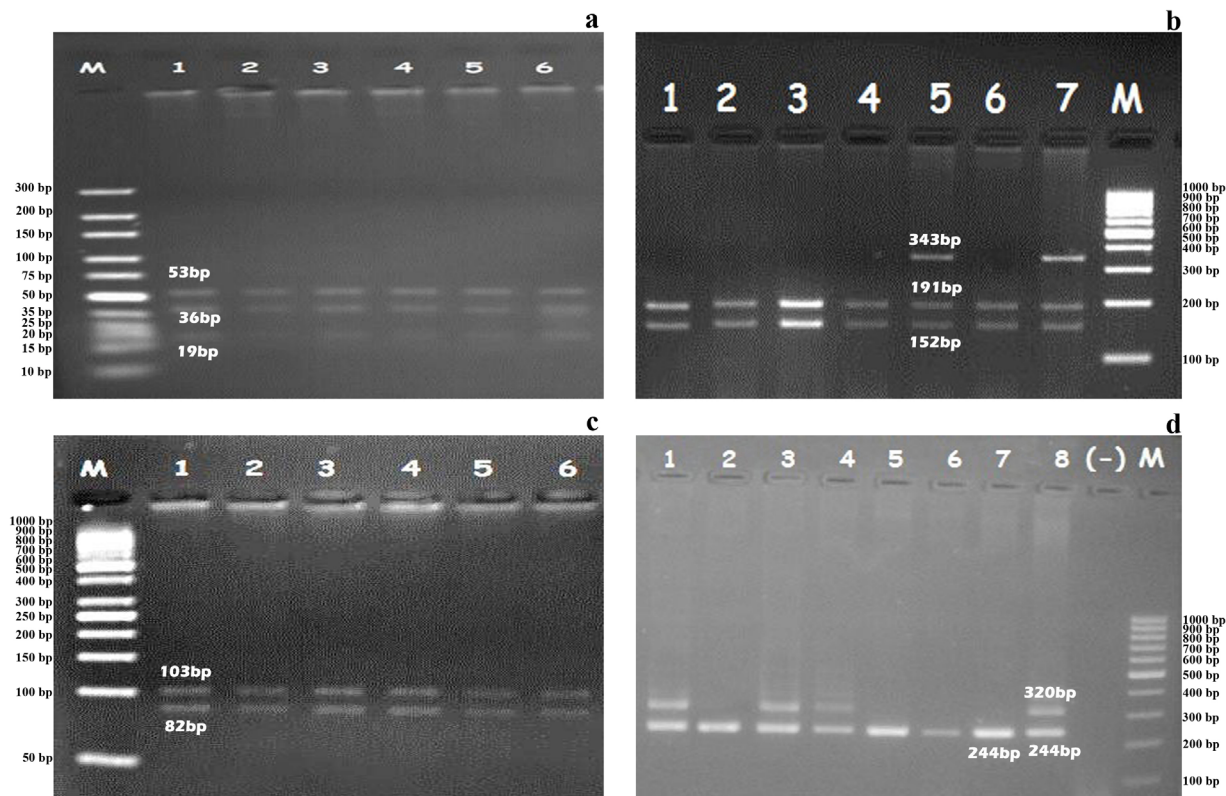


Figure 1. (a, left) Results of electrophoresis of DUMPS (from left to right: lanes 1–6: normal genotype for DUMPS, Lanes M markers (GeneRuler Ultra Low Range DNA Ladder; Thermo Scientific). (b, right) Results of electrophoresis of BLAD (from left to right: lanes 1–4, 6: normal genotype, lanes 5, 7: heterozygous carrier animal, Lanes M markers (100 bp). (c, left) Results of electrophoresis of BC from left to right: lanes 1–6: normal genotype for BC, Lanes M markers (50 bp). (d, right) Results of electrophoresis of FXID (from left to right: lanes 1, 3, 4, 8: heterozygous carrier animal, lanes 2, 5, 6, 7: normal genotype, and Lanes M markers (100 bp).

Turkey, Akyuz and Ertugrul (6) identified a carrier rate of 0.84%, while Oner et al. (10) found no carriers in 170 Holstein cows. In our study, the DUMPS allele was not detected. Our results for DUMPS are the same as those in other studies conducted in Turkey (2,25) and other countries such as the Czech Republic (3) and Poland (14). Factor XI (FXI) is one of many proteins involved in the coagulation process. It is an inherited disease that has been observed in humans, carnivores, and cattle (26). Factor XI deficiency has a significant impact on animal breeding in general and dairy farming in particular because affected cows are genetically predisposed to pneumonia and mastitis (18). The frequency of repeat breeders is higher in affected cows than in normal cows. Moreover, cows with FXI deficiency have pink colostrum and milk since blood mixes with their milk (27). It has been found that the mutation occurs by the insertion of a 76 bp segment of the FXI gene (1). FXID has been reported in many studies. In a study by Meydan et al. (2), four FXI carriers were identified among 350 Holstein cows evaluated in Ankara and Şanlıurfa provinces. Mutant allele frequencies and carrier prevalence were calculated at 0.006% and 1.2%, respectively. Akyuz (28) reported that the prevalence and frequencies of FXI carriers were 1.7% and 0.85, respectively, in 59 Holstein bulls. Karşlı et al. (25) and Oner et al. (10) stated the prevalence of the FXI carrier to be 0.4% and 1.2%, respectively. In our study, the frequency of FXI mutant alleles and carrier prevalence were calculated at 0.9% and 1.8%. Autosomal recessive genetic diseases such as FXID have a negative impact on animal breeding. It is difficult to detect heterozygous carrier cows in the population because they appear to be normal. As a result, recent years have seen increased use of molecular analysis of this genetic disease. Now that these techniques have begun to be used, it is possible to discover heterozygous carrier animals and cull them from the herd, thereby avoiding the risk of such diseases spreading through sperm used to artificially inseminate cows. A number of studies have been carried out to identify BC carriers in Holstein populations in various countries. For example, Holsteins have been screened for BC in Germany (29), Turkey (2,30), and the Czech Republic (3). None of these studies found any BC carriers. In contrast with these studies, the frequency of citrullinemia was found to be 0.16% in China

(7). As a result, the frequency of BC usually is very low or nonexistent in Holstein populations, which is consistent with the data in our study. In our study, no carriers of BC were found in the Holstein population of Burdur Province, which is consistent with the findings of other studies.

This study shows that FXID and BLAD carriers exist in the Turkish Holstein population, albeit at low rates. In contrast, no carriers of DUMPS and BC were exposed. This is the initial study on these diseases in Holstein cattle raised in Burdur Province. Genomic analyses were performed with PCR-RFLP analysis, which was found to be a powerful and reliable method for identifying these diseases in Holstein cattle. Holstein cows are being imported to Turkey as live cows or in the form of sperm, a method that is also used by other countries that are raising Holsteins. This situation creates a higher risk of these animals bringing certain hereditary diseases with them, depending on their country of origin. Furthermore, the breed's genetic pool is becoming narrower due to the fact that the sperm from a small number of breeding males is being used in many places around the world. This means that animals in the breed are becoming more and more closely related to each other, with the additional result that hereditary diseases are rapidly spreading to different continents around the world. In conclusion, if we are to make animal breeding more profitable, not only for farmers and ranchers on a small scale but also for the national economy on a large scale, it is imperative that breeding animals be screened for hereditary diseases and that animals found to be carriers are removed from the breeding process. Furthermore, in order to genetically improve the population of Holstein cows raised in Turkey and introduce more productive animals into Turkey's dairy industry, more comprehensive national projects similar to the study presented here need to be planned and implemented.

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