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Investigation of the genetic defects of Cholesterol deficiency and Brachyspina syndrome in Holstein breed cattle reared in Eskişehir

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Abstract: As a result of the intensive use of biotechnology in cattle breeding, the incidence of rare genetic defects in the population has started to increase. Studies have focused on identifying cattle with genetic defects using molecular methods. Identifying carrier cattle is crucial to reducing genetic defects in future generations. In a previous study conducted in Turkiye, Brachyspina Syndrome (BS) and Cholesterol Deficiency (CD) were detected in Holstein cattle. With regard to these two genetic defects, in the study conducted to investigate samples were taken from the Holstein cattle reared in Eskişehir by using PCR technique. 2 and 11 cattle were found to be carriers of the BS and CD, respectively, among 112 Holstein cattle. The possibility of the spread of genetic defects and economic damage can be prevented using molecular techniques. Some molecular methods can be used to detect genetic diseases. In this way, herds free of genetic defects can be produced.

Keywords: *ABOP*, Cholesterol Deficiency, *FANCI*, Brachyspina Syndrome, Holstein

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1 Introduction

Genetic diseases in cattle can result in substantial economic losses. Early detection of genetic defects is crucial for eliminating carriers from the breeding program. Recent technological advancements have led to the discovery of new genetic diseases and their detection. The recessive and genetic diseases Brachyspina Syndrome (BS) and Cholesterol Deficiency (CD) were identified in 2006 (Agerholm vd. 2006) and 2015 (Kipp vd. 2015) respectively.

Autosomal recessive diseases occur when functional changes happen in both pairs of genes at the same location. This type of inherited disease does not occur in every generation. Many genetic diseases in cattle are the result of autosomal recessively inherited genes. Autosomal recessive alleles spread unnoticed as they cannot be identified by external appearance in heterozygous inheritance (Agerholm 2007). OMIA reports that there are 630 diseases in cattle, of which 73 affect the Holstein breed (OMIA 2023).

BS was documented for the first time in 2006 (Agerholm et al. 2006). To date, it has been found in the Netherlands (Agerholm and Peperkamp 2007), Italy (Testoni et al. 2008), Germany (Buck et al. 2010), and Canada (Agerholm et al. 2010). BS is a rare inherited disease in Holstein cattle. The mutation that causes BS is located in the Fanconi anemia complementation group 1 (*FANCI*) gene (OMIA 2023). A deletion of 3329 bp in the *FANCI* on the 21st chromosome is the cause of the genetic disorder in cattle (Charlier et al. 2012). Due to mutation, the *FANCI* gene contains a prematüre stop codon in exon 28, resulting from a frameshift deletion from exon 25 to 27 of the original 37 exons (Li et al. 2016). As in the first case, these two stillborn calves had long legs and short bodies. Various abnormalities were also observed in the autopsy findings examined radiographically (Testoni et al. 2008). Apart from these cases, dead Holstein calves with identical phenotype and morphological signs have been reported in the Netherlands (Agerholm and Peperkamp 2007), Germany (Buck et al. 2010), and Canada (Agerholm et al. 2010). In Poland, in a study of 78 Holstein cattle by Ruść and Kamiński (2015), 8 out of 78 animals were carriers. In China, when 342 cattle born between 1996 and 2012 were screened by Li et al. (2016), 13 were found to be carriers. In 2021, a study was conducted on 250 Holstein cattle in Turkiye to test

for BS. One of the cattle was identified as a carrier (Bedir Dibıc, 2021).

The genetic CD defect was first detected in a bull named Mauglin Storm, born in 1991 (Menzi et al. 2016). As bulls descended from Mauglin Storm are used in artificial insemination, the frequency of the mutant allele causing the hereditary defect of CD has increased in many countries. Calves with homozygous inheritance of the defect showed signs of hypolipidemia and hypocholesterolemia. The CD leads to early death due to a 1.3 kb insertion in the 5th exon of the apolipoprotein B (*APOB*) gene on the 11th chromosome of BTA (*Bos taurus autosomal*), which affects lipid metabolism (Schutz et al. 2016). *APOB* is the structural chylomicron protein of low (LDL) and very low (VLDL) density lipoproteins (Kane et al. 1980). The resulting 1.3 kb insertion creates a stop codon in the open reading frame (ORF) of *APOB*. As a result, the protein is much shorter than its 140 amino acid length. In heterozygous animals may not show any symptoms, but the genetic defect may become apparent as they age. It has been observed that animals with a homozygous genotype for CD cannot excrete chylomicrons in the intestine at an early age. This indicates a problem in the cholesterol absorption stream, as shown in the study by Schütz et al. in 2016. After checking the Mauglin Storm pedigree, 27 Holstein cattle were selected in Poland, of which 9 were found to be carriers of the genetic disease CD. These results suggest that the mutation causing the genetic disease CD is also transmitted to Holstein cattle in Poland (Kamiński and Ruść 2016). A study was conducted in Switzerland with 254 Holstein cattle to investigate the effect of the *ABOP* genotype on cholesterol metabolism. The study revealed that none of the 254 Holstein cattle had a homozygous affected genotype. Out of the total, 36 were found to be carriers, while 218 had normal alleles. Of the 1,817 Russian cattle born in Russia between 2010 and 2017, a sample of 147 was randomly selected and found to carry the defect. A study was conducted in Turkiye in 2021 to test 250 Holstein cattle for carriers. Out of the 250 cattle tested, four were found to be carriers. The study found a low frequency of the genetic disease in Holstein cattle bred in Turkiye (Bedir Dibıc 2021).

The genetic defects of BS and CD, identified in cattle populations in different countries, were investigated in the Holstein cattle population reared in Eskişehir. PCR was employed to investigate two genetic defects in the Holstein breed to ensure healthier herds in Eskişehir.

2. Materials and Method

The study was conducted at the Molecular Genetics Laboratory, which is part of the Department of Agricultural Biotechnology, Faculty of Agriculture, Eskişehir Osmangazi University. The research involved the isolation of genomic DNA molecules from blood samples, PCR amplification of regions related to inherited defects, and interpretation of electrophoresis band patterns generated by PCR products in terms of genetic defects.

2.1 Material

Blood samples were collected from 112 cows from 4 different Holstein farms in Eskişehir. The study material consisted of 25 animals from each of the three farms, and 37 animals from the fourth farm. The age of the cows in the study was changing from 3 to 5 years old.

Genomic DNA was isolated from the blood samples using the commercial kit and stored at 4°C.

2.2 Methods

In the study, 112 blood samples were collected from animals and stored in 5 mL EDTA tubes at -20°C until DNA isolation. DNA was isolated from the blood using commercial kits (PureLink DNA Isolation Kit for Genomic DNA). After isolating the DNA, we checked the samples using agarose gel electrophoresis. The genotypes for BS and CD were determined using PCR.

The PCR reaction mixture (20 µl) contained 50–100 ng DNA template, 10X Taq polymerase buffer, 1.5 mM MgCl2, 2.5 mM dNTPs, 0.5 U Taq DNA polymerase and 5 pmoles of each primer (Table) per reaction. The PCR cycle profile was 94°C for 3 min; then 35 cycles of 94°C for 15 sec., 58°C (BS) and 65C (CD) for 20 sec., and 72°C for 30 sec; followed with 10 min at 72°C. PCR products were seperated by electrophoresis on 2% agarose gels. Primers and PCR product fragments for BS, ATP8 and CD are listed in table.

Table Primer sequences and PCR products of the BS, ATP8 and CD

Gene names	Primer sequence	Fragment size	Literature
BS	F:5' GCTCAAGTAGTTAGTTGCTCCACTG3'	409 bp	Li, Y., et al., 2016
	R: 5' ATAAATAAATAAAGCAGGATGCTGAAA3'		
ATP8	F-W: 5' TAAGTTAGAGATTGAGAGCC3'	269 bp	
	R-W: 5' GATAAGGGTTACGAGAGGGA3'		
CD	Forward-W: 5'GGTGACCATCCTCTCTCTCTGC3'	436 bp	Menzi, F., et al., 2016
	Reverse: 5'AGTGGAACCCAGCTCCATTA3'		
	Forward-M: 5'CACCTTCCGCTATTCGAGAG3'	249 bp	

3 Results

In the study, a PCR technique was used to determine BS and CD carrier animals in Eskişehir population. PCR products were loaded onto a 2% agarose gel to detect BS in cattle. Li et al. (2016) proposed the use of the ATP8 gene as a positive control for PCR accuracy. The PCR results indicated the presence of 269 bp in all healthy and affected cattle with the BS genetic defect. Normal animals produced a single 269 bp fragment, while BS carriers produced 2 fragments of 269 and 409 bp (Fig 1). The electrophoresis band pattern revealed the presence of 2 heterozygous individuals for the BS among the studied cattle.

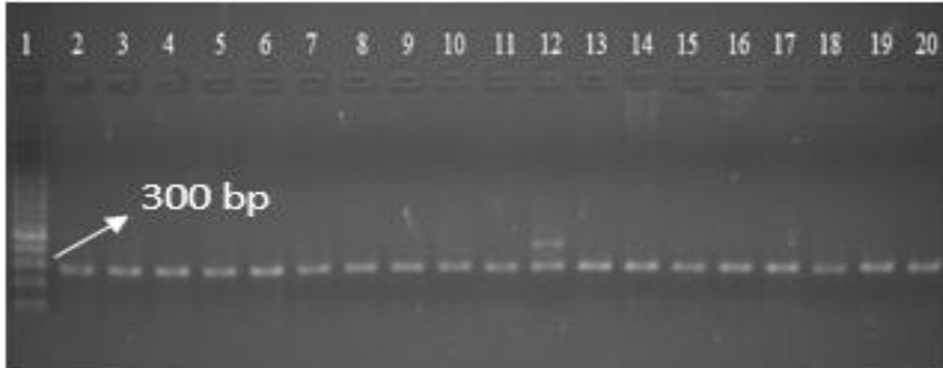

Fig 1. Electrophoretic band pattern for BS (M: 100 bp ladder; Fermentas® GeneRuler SMO241)

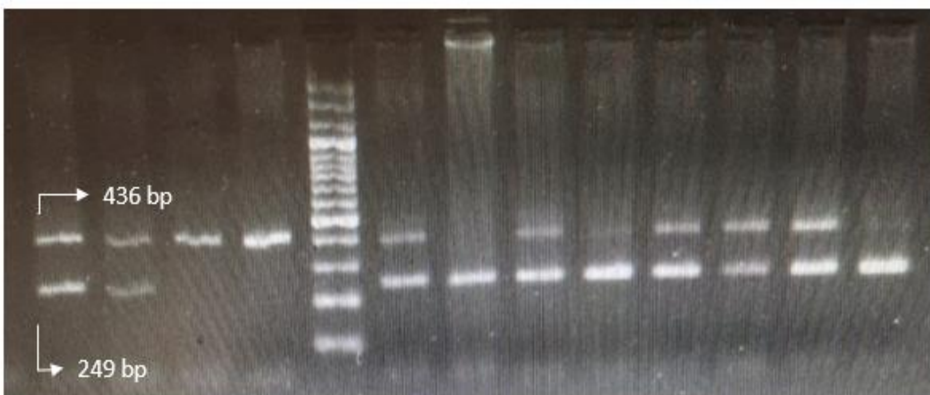

Fig 2. Electrophoretic band pattern for CD (L: 100 bp ladder; Fermentas® GeneRuler SMO241)

Menzi et al. (2016) developed and validated a PCR test using three primers to amplify 249 bp in healthy cattle, while 409 bp in CD-affected animals. CD carriers produced 2 fragments of 249 and 436 bp (Fig 2). During the visualization process, we identified 11 heterozygous individuals with CD among the studied cattle.

According to PCR studies carried out in Eskişehir, it was found that two out of the 112 Holstein cattle were affected by BS. Additionally, the deficiency allele for CD was present in eleven of them. The estimated frequency of the mutant and normal allele in CD and BS was 0.0491 and 0.0089, 0.9509 and 0.9911, respectively.

4 Conclusion

Predicting genetic disorders in animal husbandry and ensuring population health through controlled breeding are vital concerns. Identifying heterozygous animals is crucial for managing genetic diseases. Pre-breeding examination is necessary for cattle breeding to minimize risks. Molecular methods can be used to detect genetic diseases in animal populations. This approach will preserve the health of the future population and prevent any adverse impact on yield.

After the confirmed cases in Turkiye (Meydan et al., 2023), the cattle population in Eskişehir underwent a special examination for BS and CD genetic diseases, which resulted in the identification of the diseases.

The result of the study was determined that 2 and 11 Holstein cattle had the BS and CD mutant alleles, respectively, in the Eskişehir cattle population. Breeding selection should focus on reducing the frequency of genetic diseases in future populations through careful data analysis

Knowing the frequencies of these two genetic diseases that directly affect the yield in the population can identify the sires in the future population. This will prevent a direct decrease in yield by providing genetic disease control.

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