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RESEARCH PAPER

Investigation of Some Autosomal Recessive Inherited Diseases (BLAD, DUMPS, CVM, and FXID) in Holstein Cattle

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Abstract

Genetic disorders are often transmitted via autosomal recessive inheritance, which negatively affect health, welfare, and yield traits in farm animals such as cattle. In this study, a total of 80 animals belonging to Holstein Friesian (HF) reared in the dairy farm of Akdeniz University were investigated in terms of Bovine Leukocyte Adhesion Deficieny (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Factor XI Deficiency (FXID), and Complex Vertebral Malformation (CVM) via three molecular genotyping methods such as Polymerase Chain Reaction (PCR), PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), and Allele-Sprcific PCR (AS-PCR). BLAD and DUMPS were analysed by PCR-RFLP, while PCR and AS-PCR were utilized to investigate FXID and CVM disorders, respectively. Based on fragment patterns on agarose gel electrophoresis, animals were genotyped as normal, mutant, and carrier. In this study, no carrier or mutant animals were detected for BLAD, CVM, DUMPS, and FXID diseases in HF cattle, since these animals were previously imported from disease-free dairy farms located in different provinces of Türkiye. Nevertheless, this population should be periodically checked for autosomal genetic disorders, since inherited diseases may be observed in the next generations due to causative mutations in the related genomic regions.

Introduction

Being able to survive in almost all climatic zones of the world, cattle may convert some crops and industrial products, which cannot be directly utilized for human nutrition, into animal-derived protein resources. As reported by the Food and Agriculture Organisation of the United Nations (FAO), 84% and 30% of the world's milk and beef production are met exclusively by cattle (FAO 2020). In Türkiye, cattle play a more important role in agriculture, since according to Turkish Statistical Institute (TUIK), a higher part of the milk (92.1%) and beef (74.8%) production are obtained from cattle farming (TUIK 2022a; 2022b). Hence, cattle are of great importance for human nutrition and the economies of developing countries such as Türkiye. It is known that a large part of the cattle population are exotic breeds (59.4%) and their crosses (42.8%), whereas 7.8% of the total population is predicted to be represented by native Turkish cattle breeds. Among exotic cattle populations, Holstein Friesian (HF) is the most reared breed in Türkiye (Demir et al., 2023).

It is essential to increase the quantity and quality of economically important traits via selection, while health problems significantly decrease yield traits in livestock species. Therefore, the selection of healthy and tolerant animals is required to maintain the economic production systems. Inherited diseases negatively affect health and yield traits causing physical and functional anomalies in farm animals (Citek et al., 2006; 2007). Besides, as reported by Demir et al., (2021), genetic disorders are of negative effects on animal welfare.

Genetic disorders are mainly transmitted to the next generations via autosomal recessive inheritance in cattle (Windsor and Agerholm 2009). Using bulls with a high breeding value in artificial insemination increases the spreading risks of known and unknown genetic diseases in cattle breeding (Gentile and Testoni 2006; Citek and Blahova 2004). One of the most effective approaches to eliminating genetic diseases in a certain cattle population is to detect disease-carrier animals. However, due to the nature of the autosomal recessive inheritance, heterozygous animals, responsible for the occurrence of the diseases, show normal phenotype. This phenomenon requires accurate methods to detect

disease-carrier heterozygous animals. Thanks to molecular diagnosis methods, disease-carrier heterozygous animals could be detected even before birth in terms of numerous genetic disorders (Citek et al., 2006; 2007).

Bovine Leukocyte Adhesion Deficiency (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Complex Vertebral Malformation (CVM), and Factor XI Deficiency (FXID) are frequently observed genetic disorders in HF breed (Meydan et al., 2010). BLAD disease is caused by a point mutation (A→G) (Shuster et al., 1992), at position 383 of the gene encoding the CD18 glycoprotein on the bovine chromosome 1 (Nagahata et al., 2004) and DUMPS disease is caused by another point mutation (C→T transition at codon 405) occurring in the UMPS gene on the same chromosome (Avanus ve Altınel 2017). The molecular basis of CVM disease is the result of a point mutation in nucleotide position 559 of the SLC35A3 gene located on bovine chromosome 3, which causes guanine to turn into thymine (Eren et al., 2019). FXID is caused by a 76 bp insertion in exon 12 of the Factor XI gene (Ghanem et al., 2005). Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) may detect BLAD and DUMPS diseases, while FXID and CVM could be assessed via PCR and Allele -Specific PCR (AS-PCR), respectively (Karslı et al 2011; Şahin et al., 2013). Hence, this study aims to screen 80 animals belonging to HF breed reared in the dairy farm of Akdeniz University in terms of some genetic disorders.

Materials and Methods

Ethics Statement

This research was approved by the Akdeniz University Animal Experiments Ethics Committee, Antalya, Türkiye (Protocol No: 1393/2022.01.005)

Sample Collection and DNA Extraction

In 2022, a total of 80 animals belonging to HF breed were randomly chosen from the dairy farm of the Akdeniz University, Faculty of Agriculture located in Antalya province of Türkiye. Blood samples were taken from the jugular vein into vacuum tubes with K3EDTA as a coagulant. Blood samples were stored at -20°C until DNA extraction was performed. DNA was extracted via a salting-out method reported by Miller et al., (1988) following a spectrophotometer and 1% agarose gel electrophoresis in order to assess concentration and purity parameters.

Detection of BLAD, DUMPS, CVM, and FXID Genotypes

A total of three molecular methods such as PCR-RFLP (BLAD and DUMPS), AS-PCR (CVM), and traditional PCR (FXID) were utilized to genotype animals in terms of four genetic disorders. The methodology including oligonucleotide sequences as well as expected band sizes for normal, mutant, and carrier animals in terms of four genetic disorders was summarised in Table 1. Amplification was done in PCR reaction including 50 ng template DNA, 1.2 µL HQ buffer-GeneAll, 2 µL 10X buffer-GeneAll, 2.5 mM dNTPs, 10 pM primer, 2.5 U Taq DNA Polymerase, and 11.4 µL H2O with the following protocol: 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 52-62 °C for 45 seconds, and extension at 72 °C for 45 seconds, with an initial denaturation step at 95 °C for 5 minutes and a final extension step at 72 °C for 10 minutes. The annealing temperature was optimized at 57 ºC, 52 ºC, 55 ºC, and 62 ºC for BLAD, DUMPS, FXID, and CVM diseases, respectively. Since only one nucleotide differs in the primer sequence of the AS-PCR process, the annealing temperature was kept as high as possible to eliminate non-specific amplifications.

Results and Discussion

A 343 bp length PCR product cut by *Taq*I enzyme revealed two bands of 152 and 191 bp across all genotyped animals (Figure 1). No mutant or carrier was detected in the studied population in terms of BLAD disorder.

A total of three bands (53, 36, and 19 bp length) were observed in all animals in terms of DUMPS disease

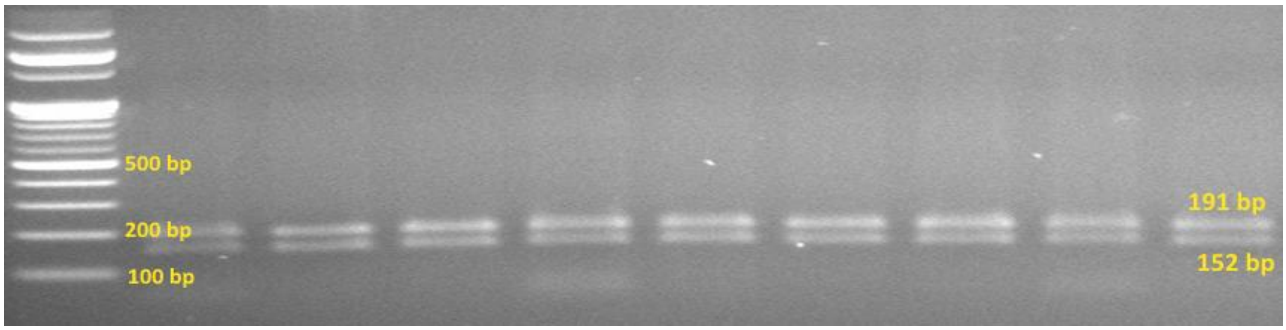

Figure 1. Image of a 2% agarose gel showing the band lengths obtained after cutting PCR products for BLAD disease via the TaqI restriction enzyme. (DNA Ladder: 100 bp, Thermo, Catalog number: 15628050)

Figure 2. 3% agarose gel image of the band sizes obtained as a result of PCR-RFLP with *AvaI* restriction enzyme of PCR products for DUMPS disease (DNA Ladder: 100 bp, Thermo, Catalog number: 10416014)

(Figure 2). Due to failure in the discrimination power of agarose gel electrophoresis, the band of 19 bp is not clearly observable by bare eyes in Figure 2.

In terms of CVM disorder, PCR products were successfully amplificated in all animals using normal primers, whereas no amplification was observed by using the mutant primer (Figure 3). This indicates that no CVM disease carrier is present in the studied cattle population.

All animals yielded a 244 bp length band in terms of FXID disorder, while the 230 bp length band was absent across the population. This finding indicates that there is no carrier or mutant animal in the studied cattle population.

Numerous studies showed that heterozygous carriers of the BLAD, DUMPS, CVM, and FXID disorders were present in HF breeds raised in different countries such as China (Zhang et al., 2010; Wang et al., 2011; Sun et al., 2011), Mexica (Virgen-Méndez et al., 2019), India (Patel et al., 2014), Poland (Gozdek et al., 2020). Similarly, Meydan et al., (2010) investigated the BLAD, DUMPS, CVM, Bovine Citrulinaemia (BC), and FXID diseases in 350 HF cattle reared in different regions of Türkiye in which a total of 14 and 12 animals were reported to be carriers of CVM and FXID disorders, respectively. On the contrary, no CVM carriers were reported in 150 heads of HF population raised in Kayseri province of Türkiye (Kulaklı and Akyüz 2011). Karsli et al., (2011) reported a low prevalence of FXID (0.4%), while no DUMPS carriers were present in a total of 504 animals belonging to HF breed reared in different districts of Antalya province of Türkiye. However, BLAD carriers were detected at the frequency of 2% and 4.8% in HF populations reared in Antalya and Kayseri provinces, respectively (Şahin et al., 2013; Akyüz et al., 2015). DUMPS and BC diseases were not reported in 219 Holstein cattle bred in Eskişehir, whereas two FXID and three BLAD carriers were present (Kaya et al., 2016). By using AS-PCR, Eren et al., (2019) confirmed that 7 out of 200 individuals analyzed were CVM carriers in HF population reared in Antalya. Screening a total of 48 animals in terms of BLAD and FXID disorders, Aksel et al., (2021) detected only one carrier animal for BLAD disorder.

Previous studies conducted on HF populations reared in Antalya province showed that the frequency of FXID carriers was 0.4%, while it was 2% for BLAD carriers, and 3.5% for CVM carriers (Karslı et al., 2011; Şahin et al., 2013). The frequency of FXID carriers in Holstein cattle reared in Burdur province was reported as 1.8% and the frequency of BLAD carriers as 2% (Ağaoğlu et al., 2015). Unlike previous researches, no carrier animals in terms of BLAD, FXID, and CVM disorders were detected in this study. It is thought that the main reason underlying this difference is the routine screening of artificial insemination bulls for these

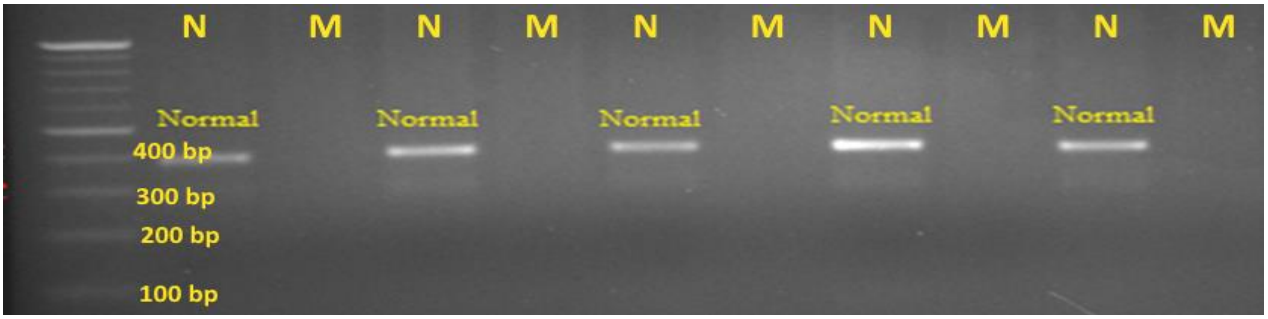

Figure 3. Image of band sizes obtained in AS-PCR for CVM disease on a 2% agarose gel. (DNA Ladder: 100 bp, Thermo, Catalog number: 15628050)

Table 1. The method used to identify the relevant mutations and some descriptive information about genotyping

Diseases		BLAD	DUMPS	CVM	FXID
Method		PCR-RFLP		AS-PCR	PCR
Primer Sequence	Forward	CCTGCATCATATCCACAG	GCAAATGGCTGAAGAACATTCTG	CACAATTTGTAGGTCTCATGGCG	CCCACTGGCTAGGAATCGTT
	Reverse	GTTTCAGGGGAAGATGGAG	GCTTCTAACTGAACTCCTCGAGT	GTTATACTACAGGAGTCACCTCT	CAAGGCAATGTCATATCCAC
	Mutant	-	-	CACAATTTGTAGGTCTCATGCAT	-
PCR lengths		343	108	395	244 or 320
Restriction enzymes		*Taq*I	*Ava*I	-	-
Genotypes	Mutant	343	89 and 19	395	320
	Carrier	191, 152, and 343	89, 53, 36, and 19	395	244 and 320
	Normal	152 and 191	53, 36, and 19	395	244
References		Şahin et al., (2013)	Karslı et al., (2011)	Eren et al., (2019)	Karslı et al., (2011)

diseases for a long time, from which semen is obtained both abroad and locally. Another reason may be considered to be that the sampling was made from a single enterprise. However, as reported by Demir and Balcioğlu (2019), HF population of the dairy farm of the Akdeniz University was re-established in 2017 by importing animals from several disease-free commercial enterprises located in ten different provinces of Türkiye. Therefore, the current population is thought to be a representative herd.

Conclusion

Genetic disorders in cattle, which are mostly transmitted through autosomal recessive inheritance, have negative impacts on animal health, welfare, and productivity. Molecular diagnosis methods are effective in detecting these disorders in carrier heterozygous animals, even before birth. In this study, no carrier animals in terms of BLAD, DUMPS, CVM, and FXID disorder were detected in HF population raised on the farm of the Akdeniz University due to the fact that these animals were imported from disease-free farms localized in ten provinces in Türkiye. This information can be used to improve breeding strategies, including the selection of animals with high breeding values and the prevention of the spreading of known and unknown genetic diseases in cattle breeding. Further studies are recommended to extend the screening to other cattle populations in different regions of Turkey with larger sample sizes to understand the distribution of these genetic disorders and develop strategies for the prevention and control of these diseases.

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Conflict of Interest

The authors declare no conflicts of interest.

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