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Screening for bovine leukocyte adhesion deficiency, deficiency of uridine monophosphate synthase, bovine citrullinaemia and factor XI deficiency in Holstein cattle

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ABSTRACT

Holstein cattle reared in Eskisehir region were monitored with respect to the genetic disorders defined as bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS), bovine citrullinaemia (BC) and factor XI deficiency (FXID). To determine the presence of BLAD, DUMPS, BC and FXID genotypes, Holstein cattle (219) were sampled. Three BLAD and 2 FXID carriers were identified among the 219 Holstein cattle examined, while none of the cows carried DUMPS and BC. Current study demonstrated that carriers of BLAD and FXID are present in the Turkish Holstein population in Eskisehir region, although at a low frequency. Since, artificial insemination is widely used in these populations, determination of the carriers of hereditary disorders within the population of breeding sires has become essential. To prevent economic losses resulting from genetic disorders, it is advised to screen breeding sires for these genotypes. The aim of this study was to investigate existence and prevalence of BLAD, DUMPS, BC and FXID alleles in Holstein cattle reared in Eskisehir region by using PCR and PCR-RFLP methods.

Key words: BC, BLAD, DUMPS, FXID, Holstein cattle, PCR-RFLP

Genetic disorders have a major economic impact on livestock breeders directly by reduced production and indirectly by leading to reductions in the genetic diversity of animal population due to extensive culling of the carriers. Owing to their detrimental effects on animal production, the hereditary disorders need to be controlled by breeders and breeding associations through identifying and eliminating animals carrying mutant alleles. Currently, more than 90 genetic disorders and traits in cattle were characterized and responsible mutations were determined at the DNA level (OMIA 2014).

Bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS), bovine citrullinaemia (BC) and factor XI deficiency (FXID) are among the most important genetic disorders influencing Holstein cattle breed. A single point mutation (A→G) of nucleotide 383 in the *CD18* gene located bovine chromosome 1 is the molecular basis of BLAD (Schütz *et al.* 2008). DUMPS is triggered by a single point mutation (C→T) at codon 405 within exon 5 in the UMP synthase gene mapped to the bovine chromosome 1 (Schwenger *et al*. 1994). A nonsense mutation at codon 86 within exon 5 of the *ASS* gene mapped to the bovine chromosome 11 results in citrullinaemia by a C/T transition from (CGA/arginine) to (TGA/STOP) codon (Grupe *et al*. 1996). Marron *et al*. (2004) identified the mutation for FXID, consisting of a 76 bp segment insertion into exon 12 in bovine chromosome 27. Genetic disorders in cattle are mostly inherited, autosomal and recessive with a major concern worldwide, that the carriers or heterozygous cattle look normal. Ultimately, BLAD, DUMPS, BC and FXID are economically considerable diseases emphasizing the need for genetic screening to eliminate the mutant allele from cattle populations. Unfortunately, it is difficult to determine or diagnose losses due to genetic disorders since they may occur during early pregnancy or without an easily observable or accurate sign. An extensive screening of such disorders would possibly prevent the occurrence of infected calves and reduce the adverse effects on breeding. To identify carriers of cytogenetic anomalies would enable producers to cull these animals from breeding herds and consequently, maintain genetic health in the population.

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MATERIALS AND METHODS

Holstein cattle (219) were randomly selected and sampled from 4 herds in 3 different countries that have most of the Holstein population in Eskisehir. Genomic DNA was

extracted by using a salting-out method (Meydan *et al*. 2010) from whole blood samples and stored at 4ºC until analysis.

The genotypes for BLAD, DUMPS and BC were identified by using PCR-RFLP method. The FXID genotypes were detected by PCR method. PCR reaction was performed to amplify polymorphic region of the *CD18, ASS, UMPS* and *FXI* genes. The PCR reaction mixture (25µl) contained 50–100 ng DNA template, 10XTaq polymerase buffer, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 0.5U Taq DNA polymerase and 5 pmoles of each primer per reaction. The reaction mixture of 15 µl contained 10 µl of the PCR product and 5U of restriction enzyme (RE) were subjected to digestion followed by incubation for 6 h at related temperature (Table 1). All genotypes were determined by using agarose gel electrophoresis stained with RedSafe. The oligonucleotides, PCR profile and product lengths and RE for digestion of PCR amplicons for each of the genetic disorder are shown in table.

RESULTS AND DISCUSSION

The primers (Table 1) successfully amplified the DNA fragments. After the PCR amplification of FXID, unaffected (normal) animals produced a single 244 bp fragment, while FXID carriers produced 2 fragments of 320 and 244 bp (Fig. 1a). Restriction analysis of *CD18,* ASS and UMPS genes allowed discrimination between normal (201 and 156; 109 and 89; 53, 36 and 19 bp) and carrier (357, 201 and 156; 198, 109 and 89; 89, 53, 36 and 19 bp) animals, respectively, (Figs 2b-d).

Among 219 Holstein cattle reared in Eskisehir region, 3

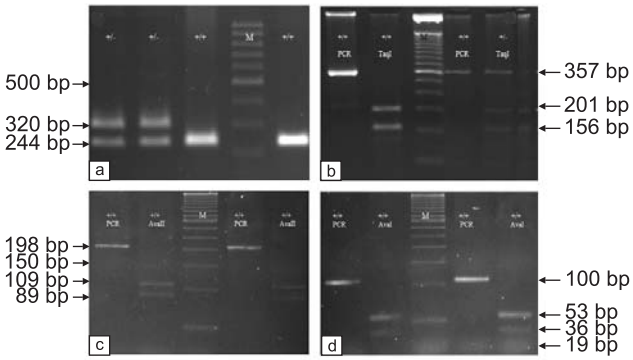

Fig. 1. Electrophoretic band pattern of (a) FXID, (b) BLAD, (c) BC, and (d) DUMPS genotypes (M, 100 bp GeneRuler DNA marker in a, 50 bp GeneRuler DNA marker in b, c, and d, +/+: unaffected and +/-, carrier genotype).

BLAD and 2 FXID carriers were determined while none of the animals were carriers for BC or DUMPS. The mutant allele frequency and prevalence of BLAD carriers was estimated as 0.7 and 1.4%, respectively, from the herds within the border of Eskisehir in this study. Carriers of BLAD were previously identified in Turkish Holstein cattle in a few studies with a prevalence of 1.6% (Akyüz and Ertugrul 2006), 2% (Sahin *et al*. 2013), 4% (Meydan *et al*. 2010) or no BLAD carrier as reported by Oner *et al.* (2010) in different provinces of Turkey. The reason for this discrepancy is probably sampling of different populations i.e. cattle in different regions and/or size of population studied, although it may also be influenced by the use of carrier sires over time. Average prevalence for BLAD is calculated as 2.12% by using data from 5 different studies

Table 1. The oligonucleotides, PCR product lengths and restriction enzymes (RE) and incubation temperature (IT) used for identification of BLAD, DUMPS, BC and FXID genotypes

Genetic disorder	Oligonucleotides	PCR				
		Profile			Product sizes	RE and IT
BLAD	F:5' GAATAGGCATCCTGCATCATATCCACCA 3'	94ºC	3 m	33 cycle	357 bp	*TaqI*
(Meydan *et al*. 2010)	R:5' CTTGGGGTTTCAGGGGAAGATGGAGTAG 3'	94ºC	30 s			65ºC
		65ºC	30 s			
		72ºC	30 s			
		72ºC	5 m			
DUMPS	F: 5' GCA AAT GGC TGA AGA ACA TTC TG 3'	94ºC	5 m	40 cycle	108 bp	*Ava*I
(Schwenger *et al*. 1994)	R: 5' GCT TCT AAC TGA ACT CCT CGA GT 3'	94ºC	60 s			37ºC
		58ºC	60 s			
		72ºC	90 s			
		72ºC	5 m			
BC (Grupe *et al*. 1996)	F: 5' GGC CAG GGA CCG TGT TCA TTG AGG ACA TC 3'	94ºC	3 m	35 cycle	198 bp	*Ava*II
	R: 5' TTC CTG GGA CCC CGT GAG ACA CAT ACT TG 3'	94ºC	30 s			37ºC
		57ºC	30 s			
		72ºC	30 s			
		72ºC	10 m			
FXID	F: 5' CCC ACT GGC TAG GAA TCG TT 3'	95ºC	10 m	34 cycle	320 bp	-
(Marron *et al*. 2004)	R: 5' CAA GGC AAT GTC ATA TCC AC 3'	95ºC	30 s			
		55ºC	60 s			
		72ºC	30 s			
		72ºC	10 m			

including the current study in Turkey. On the other hand, in different parts of the world, the prevalence of BLAD were reported as 2.8, 3 and 3.3%, in Brazil (Riberio *et al*. 2000), Poland (Czarnik *et al*. 2004), and Iran (Norouzy *et al*. 2005), respectively. By using molecular techniques, the allelic frequency of the BLAD in the Germany breeding population was reduced from 9.4% in 1997 to 0.3% in 2007 (Schütz *et al*. 2008) and BLAD was eliminated in USA utilizing screening program (Riojas-Valdéz *et al*. 2009).

The frequency of the mutant FXID allele and the prevalence of carriers were calculated as 0.45 and 0.9%, respectively. There are a few studies on FXID in Turkish Holstein cattle. In these studies, prevalence of carriers was estimated as 1.2, 0.4, 0.7, 1.24 and 1.2 % in Bursa (Oner *et al*. 2010), Antalya (Karsli *et al*. 2011), Kayseri region (Yasar and Akyüz 2012), the Middle Anatolian region of Turkey (Akyüzi *et al*. 2012) and Ankara and Sanliurfa together (Meydan *et al.* 2010), respectively. These findings showed that the mutant allele of FXID is present in Turkish Holstein cattle at low frequencies in different regions of Turkey. The prevalence of FXID carriers in Turkey calculated as an average of data from 6 studies including current study is 0.84%. This rate (0.84%) is comparable to or lowers than that determined in Czech Republic (0.36%; Citek *et al.* 2008), USA (1.19%; Marron *et al*. 2004) or Japan (2.5%; Ghanem *et al*. 2005). FXID might adversely affect reproductive performance such as repeat breeding and lower calving in homozygous and heterozygous cows. A repeat breeder cow with a higher prevalence of FXID costs $300 more than a normal fertile cow with a lower prevalence of FXID (Akyüz *et al*. 2012).

In this study, no carrier animals were found for DUMPS or BC. Similarly, no carriers were reported in previous studies conducted in Turkey (Karsli *et al.* 2011, Sahin *et al*. 2013). Current results confirming the previous studies show that mutant alleles of DUMPS and BC are absent in Turkish Holstein cattle breeds. Similarly, current results also correspond to findings in Poland (Kaminski *et al*. 2005), Czech Republic (Citek *et al*. 2006), Germany (Grupe *et al.* 1996), and Iran (Patel *et al*. 2006). The frequency of the mutant allele for DUMPS was estimated 1–2% in USA Holstein cattle (Shanks *et al.* 1990), 0.96% in Argentinian Holstein bulls and 0.11% in Argentinian Holstein cows (Poli *et al.* 1996) in studies performed during the 1990s. Similarly, carriers of BC were detected in USA (0.3%, Robinson *et al.* 1993) and China (0.16%, Jianbin *et al*. 2011). Overall from these data, it could be concluded that, frequency of mutant allele for DUMPS and BC is relatively low or not exist in different parts of the world.

Meydan *et al.* (2012) reported that no mutant alleles of BLAD, DUMPS, BC and FXID were found in native cattle breeds of Turkey, confirming that these mutant alleles were reported in only Holstein cattle throughout the world. It may be explained that the mutations causing these genetic disorders do not exist in Turkish native cattle breeds.The presence of mutant BLAD and FXID alleles in Turkish Holstein cattle can be explained that these mutant alleles could be transmitted through semen used for artificial inseminations because these cows, in most of the cases, are artificially inseminated and also there are no mutant alleles in Turkish native cattle. Identification of the carriers is principally more important for bulls considered as sires and to be used extensively in artificial insemination because of their potential to transmit the detrimental alleles to thousands of offsprings (Paiva *et al*. 2013). Screening of genetic disorders by using molecular techniques is a practical tool for quick screening of herds and to cull the carriers from breeding populations, thus decreasing the number of genetically disordered progeny. PCR and PCR-RFLP, strong and reliable methods to identify BLAD, DUMPS, BC and FXID as reported by Schütz *et al*. (2008) and Riojas-Valdéz *et al*. (2009), used successfully to screen the genetic disorders in the current study.

For the first time, this study revealed that carriers of BLAD and FXID are present in the Holstein populations reared in Eskisehir region, although at a low frequency. No carriers of BC and DUMPS were detected in the same population. If cattle has a mutant allele which remains undetected, then it will get propagated to next generation continuously, which will increase the occurrence of the undesirable genes in the breeding population affecting negatively on per animal productivity. Although, it is difficult and expensive to screen all cows in a country, it could be recommended to screen sample populations to determine prevalence of these mutant alleles and to get measures by culling cows or bulls to lower high mutant allele frequencies when determined. However, it could be strongly advised to screen bulls or semen samples by molecular techniques to ensure the utilization of bulls free from genetic disorders for especially artificial insemination programs to eliminate these genetic disorders, and therefore, prevent economic loses.

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