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Identification of BLAD, DUMPS, Citrullinamia and Factor XI Deficiency in Holstein Cattle in Turkey

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Abstract: In this study 170 Holstein cows reared in the Bursa Region of Turkey were screened in order to detect four autosomal recessive genetic diseases, i.e., BLAD, DUMPS, citrullinamia and FXI deficiency by Polymerase Chain Reaction (PCR) based methods. We found that none of the animals were carried BLAD, citrullinamia and DUMPS, but two cows were detected as carriers of FXI deficiency. After the PCR applications, FXI deficiency free individuals produced one bands were length of 244 bp and carriers individuals for FXI deficiency gave two bands length of 244 and 320 bp. The frequency of the FXI mutant allele and the FXI carrier prevalence were 0.06 and 1.17%, respectively. The results of present study showed that, the situation regarding to the investigated recessive disorders of BLAD, DUMPS, Citrullinamia and FXI deficiency seems to be good.

Key words: BLAD, DUMPS, citrullinamia, factor XI

INTRODUCTION

Inherited disorders affect all kinds of farm animals. Functional and physiological defects arising from inherited disorders have negative impact on health and productivity of farm animals. Autosomal recessive disorders lead to economic loss in the dairy cattle industry which is kept on with Holstein cattle, due to difficulty in detection of carrier individuals. BLAD (Bovine Leukocyte Adhesion Deficiency), DUMPS (Deficiency of Uridine Monophosphate Synthase), Citrullinamia and FXI (Factor XI) deficiency are four of Holstein-specific autosomal recessive disorders. Increased use of artificial insemination and worldwide use of service bull cause to widespread of this kind of disorders via carriers seem to be normal (Patel *et al*., 2006). Polymerase chain reaction-based techniques are very useful tools to detect autosomal recessive disorders and it can be used for eradication programme of these kinds of disease in dairy cattle herds.

BLAD is an autosomal recessive disease caused by reduced expression synthesis of C11/C18 protein family on the leucocytes surface (Kehrli *et al*., 1990; Shuster *et al*., 1992). It is known that BLAD is caused by the point mutation (A→G) at the position 383 of CD18 gene located on the first chromosome of bovine (Shuster *et al*., 1992). This disease leads to calf death in the early life stage (before 1 to 3 month) from chronic diarrhea, pneumonia, high fever and other infections (Kehrli *et al*., 1990; Nagahata *et al*., 1993). It is very important to

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detect carrier animals for this mutation in order to avoid spread of this disease across the dairy herds. The occurrence of the mutant allele leads to BLAD when homozygote situation has been reported in different countries. The existence of this mutation among Holstein populations is reported in previous studies (Nagahata *et al*., 1997; Ribeiro *et al*., 2000; Ertuğrul *et al*., 2004; Rahimi *et al*., 2006; Patel *et al*., 2007; Meydan *et al*., 2007).

Citrullinamia is a rare metabolic disorder due to lack of argininosuccinate synthetase (ASS) and is critical for urea cycle and was firstly reported by Mcmurray *et al*. (1962) in humans. This disorder is characterized by serious neurologic symptoms in newborn calves (Harper *et al*., 1986, 1989). The reason of the disorder is a C→T transition in the position of 86 on ASS gene (Dennis *et al*., 1989). There are few studies aimed to determination of existence this mutant allele. The allele frequency of the mutant allele was found to be high in Australia (Healy *et al*., 1991). Robinson *et al*. (1993) detected only one heterozygote among 367 Holstein bulls tested in USA. In other countries, such as Germany and India, the mutant allel didn't detect in studied populations (Grupe *et al*., 1996; Patel *et al*., 2006).

The UMPS is an enzyme which has a key role on the pyrimidine nucleotide synthesis which is essential for normal growth and development for several ruminant and nonruminant species (Healy and Shanks, 1987). Inactivation of this enzyme is caused by an autosomal-recessive heredity mutation which occurs in the gene of UMPS. The mutation (C→T) leads to the loss of the restriction site of *Ava*I site in codon 405 of the gene (Schwenger *et al*., 1993). This disorder is named as DUMPS in the Holstein cattle and characterized by lowered blood activity of enzyme UMPS (Healy and Shanks, 1987). DUMPS leads to embryonic death in early stage of pregnancy (Ghanem *et al*., 2006). So some serious reproductive problems take place in dairy herds. Several investigations were carried out in different countries. No carrier animals were found among Holstein populations in Poland (Kaminski *et al*., 2005), Iran (Rahimi *et al*., 2006), India (Patel *et al*., 2006) and Turkey (Meydan *et al*., 2006; Akyüz and Ertuğrul, 2008), but the mutant allele was detected in the studies carried out in U.S.A (Shanks *et al*., 1987) and Argentina (Patel *et al*., 2006).

FXI is a plasma serine protease acts in the transformation of insoluble fibrin clot from soluble fibrinogen (Brush *et al*., 1987). As other three disorders investigated with present study, FXI deficiency is inherited autosomal-recessive. It is caused by an insertion of 76 bp within the exon 12 of the gene of FXI (Marron *et al*., 2004). Heterozygote individuals for FXI deficiency have serious health problems (Brush *et al*., 1987). On the other hand both heterozygote and homozygote animals for this mutation have susceptibility to some diseases such as mastitis, metritis and pneumonia and also they have lower calving and survival rates (Liptrap *et al*., 1995). The frequency of mutant allele has been reported at very low in America and Japan (Marron *et al*., 2004; Ghanem *et al*., 2005; Citek *et al*., 2008; Meydan *et al*., 2009).

The aim of this study was genotyping of these four autosomal recessive disorders by using PCR-based techniques on Holstein cows reared in the Bursa district located on South Marmara region of Turkey and show applicability of these PCR-based techniques in the breeding systems for avoiding from autosomal recessive disorders.

MATERIALS AND METHODS

Whole blood samples were collected from one hundred seventy (170) Holstein cows which are reared in different animal farms from Bursa region (in the Northwest of Turkey) in 2008. Blood samples were collected from jugular vein into EDTA containing test tubes. DNA isolation was performed shortly after the collection of the blood samples. Total DNA was

Table 1: Primer sequences and restriction enzymes (R.E) were used for PCR and RFLP

Disease	Primer sequence (5'→3')	R.E	References
BLAD	CCTGCATCATATCCACAG GTTTCAGGGGAAGATGGAG	*Taq*I	(Kriegesmann *et al*., 1997)
Citrullinamia	GGCCAGGGACCGTGTTCATTGAGGACATC TTCCTGGGACCCCGTGAGACACATACTG	*Ava*II	(Grupe *et al*., 1996)
DUMPS	GCAAATGGCTGAAGAACATTCTG GCTTCTAACTGAACTCCTCGAGT	*Ava*I	(Schwenger *et al*., 1994)
FXI deficiency	CCCACTGGCTAGGAATCGTT CAAGGCAATGTCATATCCAC	-	(Marron *et al*., 2004)

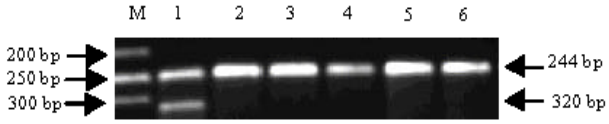

Fig. 1: Polymerase Chain Reaction(PCR) genotyping of FXI deficiency. Line 1 is FXI -carrier individuals(heterozygotes genotypes) has two fragments of 244 and 230 bp. Line 2-6 is FXI deficiency-free animals(homozygotes genotypes) produced only one 244 bp fragment. M is 50 bp DNA ladder (Fermentas, Lithuania)

extracted using a genomic DNA purification kit (K0512, Fermentas, Lithuania). Agarose gel electrophoresis and spectrophotometric methods were used to determine DNA quality, purity and quantity prior to PCR amplifications. The genomic DNA was stored at 4°C. While FXI deficiency was genotyped by polymerase chain reaction, BLAD, DUMPS and citrullinamia were genotyped by restriction fragment length polymorphism (PCR/RFLP). The sequences of primers for PCR and names of restriction enzymes used for RFLP application are shown in Table 1. PCR products and RFLP fragments were visualized on agarose gels stained with ethidium bromide (Fig. 1). The gene frequency of FXI locus was estimated according to Nei (1987) by counting the number of genes.

RESULTS

The primers were used to amplify 343 bp DNA fragment to detect studied population for BLAD. The *Tag*I restriction enzymes digested to PCR product into two fragments of 152 and 191 bp in normal homozygote animals. After the analyses all animals showed two fragments of 152 and 191 bp. For screening of possible mutation in a gene coding for UMP synthase as described by Schwenger *et al*. (1994), the 108 bp DNA fragment was amplified by PCR. The digestion of PCR product gave three bands of 53 , 36 and 19 bp for normal animals. Similarly, the 185 bp PCR products were digested by *Ava*II to reveal mutation in a gene responsible for citrullinemia disease produced two bands of 103 and 82 bp for normal animals. None of the animals showed three bands of 185, 103 and 82 bp, so no animals found to be as carrier of citrullinemia disease. As a result of electrophoretic analysis no carrier animal has been detected for BLAD, citrullinemia and DUMPS. It was found that two carrier cows were FXI deficiency carrier in the group of 170 animals. After the PCR applications, FXI deficiency free individuals produced one bands were length of 244 bp and carriers individuals for FXI deficiency gave two bands length of 244 and 320 bp (Fig. 1). The frequency of the FXI mutant allele and the FXI carrier prevalence were 0.06 and 1.17%, respectively.

DISCUSSION

Although, some studies have reported that carrier animals of BLAD among Holstein populations (Nagahata *et al*., 1997; Ribeiro *et al*., 2000; Ertuğrul *et al*., 2004; Rahimi *et al*., 2006; Meydan *et al*., 2007), in our study we did not find any carrier individual for this disease. In Turkey two studies were carried out in order to identify BLAD genotypes in Holstein populations (Ertuğrul *et al*., 2004; Meydan *et al*., 2007) and the frequency of mutant allele was found as 0.0084 (Ertuğrul *et al*., 2004) and 0.035 (Meydan *et al*., 2007).

We didn't find any carrier individuals of citrullinemia and DUMPS. In this respect results of present study are similar to the other results from the different countries for citrullinemia (Grupe *et al*., 1996; Patel *et al*., 2006) and DUMPS (Patel *et al*., 2006; Kaminski *et al*., 2005; Meydan *et al*., 2006; Akyüz and Ertuğrul, 2008). There are two studies of Holstein cows reared in Turkey for UMPS and no mutant allele was found leads to DUMPS (Meydan *et al*., 2006; Akyüz and Ertuğrul, 2008) in these studies. On the other hand, there have not been any studies carried out in order to identify genotypes of citrullinemia until now. So, results on citrullinemia were the first record in Turkey.

Undesirable allele of FXI deficiency has been determined in previous studies in Holstein cattle (Marron *et al*., 2004; Ghanem *et al*., 2005; Citek *et al*., 2008; Meydan *et al*., 2009). Kunieda *et al*. (2005) also reported another mutation located in exon 9 in Japanese black cattle and their results were confirmed by Citek *et al*. (2008). In Turkish Holstein cattle it was found four carrier animals in the group of 225 animals (Meydan *et al*., 2009). According to our results, mutant allele arising from an insertional mutation at the exon 12 of FXI has been found in Holstein cattle populations in Bursa district of Turkey at low frequency (0.06%). Large scale investigations are needed in order to determinate carrier animals for eliminate them from whole populations.

CONCLUSION

Based on the results of present study, the situation regarding to the investigated recessive disorders of BLAD, DUMPS, Citrullinamia and FXI deficiency seems to be good. But all of results need to be confirmed by further analyses in more cattle in Turkey. This study showed that the frequency of carrier individuals for this genetic disorder is very low among studied Turkish Holstein populations. At the same time, it is not possible to say there are no carrier individuals of BLAD, citrullinemia and DUMPS in Holstein population of Turkey.

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REFERENCES

Akyüz, B. and O. Ertuğrul, 2008. Detection of deficiency of uridine monophosphate synthase (DUMPS) in Holstein and native cattle in Turkey. Ankara Üniv. Vet. Fak. Derg, 55: 57-60.

Brush, P.J., P.H. Anderson and R.F. Gunning, 1987. Identification of factor XI deficiency in Holstein-Friesian cattle in Britain. Vet. Rec., 121: 14-17.

Citek. J., V. Øehout, L. Hanusová and P. Vrabcová, 2008. Sporadic incidence of factor XI deficiency in Holstein cattle. J. Sci. Food Agric., 88: 2069-2072.

Dennis, J.A., P.J. Healy, A.L. Beaudet and W.E. O'Brien, 1989. Molecular definition of bovine arginiiosuccinate synthetase deficiency. Proc. Natl. Acad. Sci. USA., 86: 7947-7951.

Ertuğrul, O., B. Akyüz and B. Çubuk, 2004. Detection of bovine leukocyte adhesion deficiency (BLAD) in Holstein cattle by restriction fragment length polymorphism (RFLP). TÜBİTAK-VHAG-Project Number 1918.

Ghanem, M.E., M. Nishibori, T. Nakao, K. Nakatani and M. Akita, 2005. Factor XI mutation in a Holstein cow with repeat breeding in Japan. J. Vet. Med. Sci., 67: 713-715.

Ghanem, M.E., T. Nakao and M. Nishibori, 2006. Deficiency of uridine monophosphate synthase (DUMPS) and X-chromosome deletion in fetal mummification in cattle. Anim. Reprod. Sci., 91: 45-54.

Grupe, S., G. Dietle and M. Schwerin, 1996. Population survey of Citrullinaemia on German Holsteins. Livestock Prod. Sci., 45: 35-38.

Harper, P.A.W., P.J. Healy, J.A. Dennis, J.J. O'Brien and H.D. Rayward, 1986. Citrullinaemia as a cause of severe neurological disease in neonatal Friesian calves. Aust. Vet. J., 63: 378-379.

Harper, P.A.W., P.J. Healy and J.A. Dennis, 1989. Animal model of human disease: Citrullinemia (argininosuccinate synthetase deficiency). Am. J. Pathol., 135: 1213-1215.

Healy, M.H. and R.D. Shanks, 1987. Performance of females heterozygous for deficiency of uridine monophosphate synthase. J. Dairy Sci., 70: 945-951.

Healy, P.J., J.A. Dennis, L.M. Camileri, J.L. Robinson, A.L. Stell and R.D. Shanks, 1991. Bovine citrullinaemia traced to the sire of Linmack Kriss King. Aust. Vet. J., 68: 155-155.

Kaminski, S., G. Grzybowski, B. Prusak and A. Rusc, 2005. No incidence of DUMPS carriers in polish dairy cattle J. Applied Genet., 46: 395-397.

Kehrli, M.E., F.C. Schmalstieg, D.C. Anderson, M.J. van Der Maaten and B.J. Hughes *et al*., 1990. Molecular definition of the bovine granulocytopathy syndrome: Identification of deficiency of the Mac-1 (CD11B/CD18) glycoprotein. Am. J. Vet. Res., 51: 1826-1836.

Kriegesmann, B., S. Jansen, B.G. Baumgartner and B. Brenig, 1997. Partial genomic structure of the bovine CD 18 gene and the refinement of test for bovine leukocyte adhesion deficiency. J. Dairy Sci., 80: 2547-2549.

Kunieda, M., T. Tsuji, A.R. Abbasi, M. Khalaj and M. Ikeda *et al*., 2005. An insertion mutation of the bovine F11 gene is responsible for factor XI deficiency in Japanese black cattle. Mamm. Genome., 16: 383-389.

Liptrap, R.M., P.A. Gentry, M.L. Ross and E. Cummings, 1995. Preliminary findings of altered follicular activity in Holstein cows with coagulation factor XI deficiency. Vet. Res. Commun., 19: 463-471.

Marron, B.M., J.L. Robinson, P.A. Gentry and J.E. Beever, 2004. Identification of a mutation associated with factor XI deficiency in Holstein cattle. Anim. Genet., 35: 454-456.

Mcmurray, W.C., F. Mohyuddin, R.J. Rossiter, J.C. Rathbun, G.H. Valentine, S.J. Koegler, D.E. Zarfas, 1962. Citrullinuria: A new aminoaciduria assocýated with mental retardation. Lancet, 279: 138-138.

Meydan, H., F. Ozdil and M.A. Yildiz, 2006. Identification of BLAD and DUMPS as genetic disorders using PCR-RFLP in Holstein bulls reared in Turkey. Proceedings of the 57th Annual Meeting of the European Association for Animal Production, Sept. 17-20, Antalya, Turkey, pp: 91-91.

Meydan, H., F. Özdil, Y. Gedik and M.A. Yıldız, 2007. Detection of BLAD, DUMPS and factor XI by PCR-RFLP. Proceedings of the 5th Animal Science Congress, October 5-8, Yuzuncu Yil University, Van, Turkey.

Meydan, H., M.A. Yildiz, F. Ozdil, Y. Gedik and C. Ozbeyaz, 2009. Identification of factor XI deficiency in Holstein cattle in Turkey. Acta Vet. Scand., 51: 5-5.

Nagahata, H., H. Nochi, K. Tamoto, H. Tamiyama and H. Noda *et al*., 1993. Bovine leukocyte adhesion deficiency in Holtein cattle Can. J. Vet. Res., 57: 255-261.

Nagahata, H., T. Miura, K. Tagaki, M. Ohtake, H. Noda, T. Yasuda and K. Nioka, 1997. Prevalence and allele frequency estimation of bovine leukocyte adhesion deficiency (BLAD) in Holstein-Friesian cattle in Japan J. Vet. Med. Sci., 59: 233-238.

Nei, M., 1987. Molecular Evolutionary Genetics. 1st Edn., Columbia University Press, New York.

Patel, R.K., K.M. Singh, K.J. Soni, J.B. Chauhan and K.R. Sambasiva Rao, 2006. Lack of carriers of citrullinaemia and DUMPS in Indian Holstein cattle. J. Applied Genet., 47: 239-242.

Patel, R.K., M.S. Krishna, J.S. Kalpesh, B.C. Jenabhai and R.S.S.R. Krothapalli, 2007. Low incidence of bovine leukocyte adhesion deficiency (BLAD) carriers in Indian cattle and buffalo breeds. J. Applied Genet., 48: 153-155.

Rahimi, G., A. Nejati-Javaremi and K. Olek, 2006. Genotyping BLAD, DUMPS and κ-CSN Loci in holstein young bulls of the national animal breeding center of Iran. Pak. J. Biol. Sci., 9: 1389-1392.

Ribeiro, L.A., E.E. Baron, M.L. Martinez and L.L. Coutinho, 2000. PCR screening and allele frequency estimation of bovine leukocyte adhesion deficiency in Holstein and Gir cattle in Brazil. Genet. Mol. Biol., 23: 831-834.

Robinson, J.L., J.L. Burns, C.E. Magura and R.D. Shanks, 1993. Low incidence of citrullinaemia carriers among dairy cattle of the United States. J. Dairy Sci., 76: 853-858.

Schwenger, B., S. Schober and D. Simon, 1993. DUMPS cattle carry a point mutation in the uridine monophosphate synthase gene. Genomics, 16: 241-244.

Schwenger, B., I. Tammen and C. Aurich, 1994. Detection of homozygous recessive genotype for deficiency of uridine monophosphate synthase by DNA typing among bovine embryos produced *in vitro*. J. Reprod. Fertil., 100: 511-514.

Shanks, R.D., D.B. Dombrowski, G.W. Harpestad and J.L. Robinson, 1987. Inheritance of UMP synthase in dairy cattle. J. Hered., 75: 337-340.

Shuster, D.E., M.E. Kehrli, M.R. Ackermann and R.O. Gilbert, 1992. Identification and prevalence of a genetic defect that causes leukocyte adhesion deficiency in Holstein cattle Proc. Natl. Acad. Sci., 89: 9225-9229.