Czech Journal of ANIMAL SCIENCE

Formerly ŽIVOČIŠNÁ VÝROBA

Volume 52 **2007**

Editorial Board

Chairman

František Jílek
Czech University of Life Sciences, Prague, Czech Republic

Editor-in-Chief

Věra Černíková
Institute of Agricultural and Food Information, Prague, Czech Republic

Members

Josef Bouška
Institute of Animal Science,
Prague, Czech Republic

Jiri Broz
Rheinfelden, Switzerland

Jozef Bulla
Research Institute of Animal Production,
Nitra, Slovak Republic

Josef Čeřovský
Institute of Animal Science, Prague,
Kostelec nad Orlicí Workplace, Czech Republic

Andrzej Filistowicz
Agricultural University, Wroclaw, Poland

Robert Mervyn Gous
University Kwa Zulu Natal,
Scottsville, South Africa

Karel Hala
Innsbruck Medical University,
Innsbruck, Austria

Oto Hanuš
Research Institute of Cattle Breeding, Ltd.,
Rapotín, Czech Republic

Pavel Jelínek
Mendel University of Agriculture and Forestry,
Brno, Czech Republic

Jan Kouřil
University of Southern Bohemia,
České Budějovice, Research Institute of Fish
Culture and Hydrobiology,
Vodňany, Czech Republic

Alojz Kúbek
Slovak University of Agriculture,
Nitra, Slovak Republic

Claus Leiding
Besamungsverein Neustadt a.d. Aisch e.V.,
Neustadt a.d. Aisch, Deutschland

Milan Margetín
Research Institute of Animal Production, Nitra,
Trenčianska Teplá Workplace, Slovak Republic

Václav Matoušek
University of Southern Bohemia,
České Budějovice, Czech Republic

Štefan Mihina
Research Institute of Animal Production,
Nitra, Slovak Republic

Dusan Nosal
Swiss Research Institute for Agricultural
Economics and Engineering,
Tanikon b. Aadorf, Switzerland

Jaroslav Petr
Institute of Animal Science,
Prague, Czech Republic

Antonín Stratil
Institute of Animal Physiology and Genetics,
Academy of Sciences of the Czech Republic,
Liběchov, Czech Republic

Eva Tůmová
Czech University of Life Sciences,
Prague, Czech Republic

Ladislav Zeman
Mendel University of Agriculture and Forestry,
Brno, Czech Republic

Czech Journal of ANIMAL SCIENCE

Volume 52, No. 3 **March 2007**

CONTENTS

ORIGINAL PAPERS

Study of SNP 775C>T polymorphism within the bovine *ITGB2* gene in Polish Black-and-White cattle and in local breeds of cattle

U. Czarnik, M. Galiński, T. Zabolewicz, Ch.S. Pareek

Department of Animal Genetics, University of Warmia and Mazury in Olsztyn, Olsztyn, Poland

ABSTRACT: The present study addresses the characteristics of the frequency and segregation of alleles determining the SNP 775C>T polymorphism within the bovine *ITGB2* gene in the Black-and-White cattle population as well as in two endemic breeds of Polish Red and Polish White-Back cattle population qualified to the international programme of genetic resource diversity preservation in farm animals. The SNP 775C>T polymorphism revealed three amplified restriction fragments of 31 bp, 77 bp and 108 bp, forming three genotypes *CC* (31 bp, 77 bp), *CT* (31 bp, 77 bp, 108 bp) and *TT* (108 bp). A group of randomly selected Black-and-White cows was characterized by a negligible percentage of homozygous genotypes *TT* (5.1%) and by a prevailing percentage of heterozygous *CT* (60.1%) and homozygous *CC* (34.8%). In contrast, the between breeds analysis revealed that cows involved in the programme of genetic diversity preservation had a high prevalence of homozygotes *CC* (Polish Red – 55.9%, Polish White-Back – 60.0%), and a low percentage of homozygotes *TT* (Polish Red – 8.8%, Polish White-Back – 5.7%) in comparison with the Black-and-White cattle population. The degree of homozygosity in groups of Polish White-Back cows (65.7%) and Polish Red cows (64.7%) was remarkably higher than that of the Black-and-White cows (39.9%).

Keywords: BLAD carriers; point mutation; lethal genes; SNP 775 C>T; polymorphism

Currently, advances in molecular diagnostics of DNA and bioinformatics afford a possibility of the complex analysis of genomes, including detection of point mutations as the cause of structural and functional variability of protein products, as well as gene expression in a wide range of physiological norms and pathology. A functional defect BLAD (Bovine Leukocyte Adhesion Deficiency) syndrome in cattle is an autosomal, recessive genopathy caused by leukocyte surface glycoproteins known as integrins and expressed as BL/BL homozygotes due to the susceptibility towards bacterial and fungal infections (Agerholm et al., 1993; Grzybowski et al., 1994), producing a lethal effect in animals before they achieve sexual maturity.

The first source of information on BLAD incidence in the US Holstein-Friesian cattle population was reported by Kehrli et al. (1990) and Shuster et al. (1992). A study described therein demonstrated that in a group of 2 025 bulls bred for reproductive purposes in the USA, 14.1% of individuals were carriers of BLAD, whereas in a group of the most valuable individuals recognized as a breeding elite, the percentage of carriers was higher and reached 17.1%, and in the cow population the frequency of BLAD carriers was estimated at a level of 5.8% (for a detailed review see Pareek and Kaminski, 1996; Nagahata, 2004). Worldwide many studies reported the incidence of BLAD affecting the Holstein breed, for example Hungarian Holstein (Janosa et al., 1999), Korean Holstein (Chung et al., 1997), Lithuanian Holstein (Miceikiene et al., 2000), Uruguayan Holstein-Friesian (Llambi et al., 2003), Iranian Holstein bulls (Esmaelizad et al., 2002), Argentinean Holstein (Poli et al., 1996), Danish Holstein (Jorgensen et al., 1993) and Brazilian Holstein (Ribeiro et al., 2000), Taiwan Holstein (Huang et al., 2000). However, few studies on BLAD incidence were reported in other breeds of cattle. The presented investigation was aimed to char-

acterize the frequency of alleles determining the SNP 775C>T polymorphism within the bovine *ITGB2* gene in Black-and-White cattle as well as in endemic populations of Polish Red and Polish White Back cattle involved in an international programme of preserving the sources of genetic variability in farm animals.

MATERIAL AND METHODS

Animals

The study was carried out on 49 Black-and-White bulls and randomly selected population of Black-and-White cows (n = 129), Polish Red cows (n = 34) and Polish White Back cows (n = 35).

Laboratory procedure

DNA was isolated from peripheral blood and sperm with the use of a Wizard genomic DNA purification kit (Promega, USA), following the producer's instructions. The number, purity, and quality of DNA preparations were controlled spectrophotometrically (GeneQuant, Pharmacia, USA) and electrophoretically on 1% agarose gel.

The SNP 775C>T polymorphism within the bovine *ITGB2* gene was determined by the PCR–RFLP method following the procedure elaborated by Czarnik et al. (2004). The amplification of a *CD* 18 gene fragment, 108 bp in length, was performed using 25 µl of a reaction mixture containing: 1.25 µl buffer 20 × Master Amp™ *Tfl* Buffer (Epicentre Technology) (500nM KCl, 100nM TRIS–HCl pH 9.0 1% TRITON X–100), 1.5 µl of nucleotide solution dNTP (dATP, dGTP, dCTP, dTTP), each at a concentration of 2mM, 1.0 µl primer solution at a concentration of 100 pM/µl: forward primer (5' GAG GAA ATC GGC TGG CGC AAT G 3'), reverse primer (5' GTC ATT GGG GGT GAG GAT G 3'), 2.0 µl of a 25mM solution of $MgCl_2$, 0.5U polymerase Tfl Master Amp™ (Epicentre Technology) at a concentration of 1 U/µl, 3.0µl 10 × Master Amp™ PCR enhancer, 1 µl DNA at a concentration of 100 ng/ml, H_2O up to a volume of 25 µl.

The PCR was carried out in a Rapidcycler, Idaho Technology, USA, in the "touch down" thermal programme (Don et al., 1991). The specificity of the PCR product was confirmed by sequencing performed at the DNA sequencing laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, using an automatic ABI PRISM™ 377 sequencer (Applied Biosystems). Positively verified PCR products were subjected to digestion with a restriction enzyme *Fnu* 4*HI* (BioLabs, UK). The polymorphism of restriction fragments was analyzed electrophoretically on 3% agarose gel (Cortified™ Low Range Ultra Agarose, Bio-Rad) against the molecular weight marker *Phi*X 174 digested with endonuclease *Hae* III. The results were recorded using the Fluor S™ Multimager system (BIO-RAD).

Statistics

The results were compiled taking into account the frequency of *CC*, *CT*, and *TT* genotypes for SNP 775C > T polymorphism in the examined population. Differences between the genotypes and allele frequencies, genetic equilibrium were verified using the chi-square test.

RESULTS AND DISCUSSIONS

The SNP 775C>T polymorphism within the bovine *ITGB2* gene was determined by subjecting a PCR product to the activity of endonuclease *Fnu* 4*HI* (BioLabs, UK). The substitution of cytosine with thymine at the position 775 cDNA resulted in a loss of recognition site for *Fnu* 4 *HI* restrictase (Figure 1).

In the analyzed population, three size variants of restriction fragments were identified, namely: 108 bp, 77 bp, and 31 bp. An analysis of the localization of migration bands of the restriction fragments enabled to identify three genotypes of "silent mutation C>T". The genotype *CC* represents the occurrence of two bands of 77 bp and 31 bp, genotype *CT* represents three restriction fragment bands of 108 bp, 77 bp and 31 bp, and genotype *TT* represents one band of 108 bp in length.

The results of SNP 775C>T polymorphism within the bovine *ITGB2* gene in the domestic population of Black-and-White cattle as well as herds of the Polish Red and Polish White-Back breeds included in the programme of genetic variability preservation are presented in Table 1.

The results indicate that the group of randomly examined Black-and-White cows had a negligible number of homozygous genotypes *TT* (0.051) and

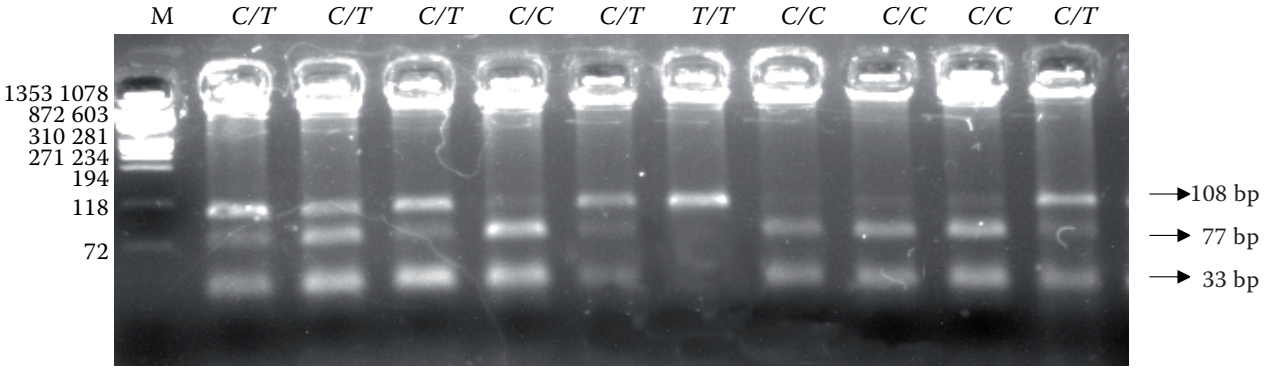

Figure 1. Agarose gel electrophoresis (3%) profile, showing the digestion of a PCR product with restriction enzyme *Fnu*-4*HI*

M – molecular weight marker *Phi*X 174 digested with endonuclease *Hae* III*; CC, CT* and *TT* – PCR-RFLP genotypes for SNP 775C>T

a prevailing number of heterozygous *CT* (0.601) and homozygous *CC* (0.348). The frequency of genotypes and alleles in these cows was similar to that reported previously on the macro-population scale for the Black-and-White breed (Czarnik et al., 2004). The dam population of Polish Red and White-Back cattle demonstrated a completely different genetic structure. In both cases, the most numerous group was that of homozygotes *CC* (0.559 in Polish Red cattle, 0.600 in Polish White-Back cattle), whereas intermediate values were reported for the group of heterozygotes (0.353 in Polish Red cattle, 0.343 in Polish White-Back cattle), and the lowest values for the group of homozygotes *TT* (0.088 in Polish Red cattle, 0.057 in Polish White-Back cattle). As a consequence, the degree of homozygosity appeared to be low in Black-and-White cattle (0.399) and remarkably higher in herds of Polish Red (0.647) and Polish White-Back cattle (0.657). The observed regularity is likely to result from the specificity of dam herd reproduction in preservation breeding, i.e. reproduction with a small number of bulls.

The differences in the frequency of genotypes and alleles of SNP 775C>T within the bovine *ITGB2* gene in bulls and cows of the Black-and-White breed are presented Table 2. The results revealed that the group of sires had a relatively high frequency of homozygotes *CC* (0.490), whereas the group of cows demonstrated considerable prevalence in the frequency of heterozygotes *CT* (0.651). In both male and female groups, there was a single case of the occurrence of homozygous *TT* animal (0.041 in bulls, 0.054 in cows). Consequently, the frequency of allele *C* in bulls (0.725) was higher compared to that of the cows (0.620).

Table 1. Frequency of genotypes and alleles of SNP 775C>T within the bovine *ITGB2* gene in Black-and-White, Polish Red, and Polish White-Back breeds

Animal group	Number of animals	Genotype frequency			Allele frequency	
		CC	*CT*	*TT*	*C*	*T*
Black-and-White	*n* = 178	0.348	0.601	0.051	0.649	0.351
Polish Red	*n* = 34	0.559	0.353	0.088	0.735	0.265
Polish White-Back	*n* = 35	0.600	0.343	0.057	0.771	0.229

Table 2. Frequency of genotypes and alleles of SNP 775C>T within the bovine *ITGB2* gene in bulls and cows of the Black-and-White breed

Animal group	Number of animals	Genotype frequency			Allele frequency	
		CC	*CT*	*TT*	*C*	*T*
Bulls	*n* = 49	0.490	0.469	0.041	0.725	0.275
Cows	*n* = 129	0.295	0.651	0.054	0.620	0.380

Table 3. Genetic equilibrium analysis for SNP 775C>T polymorphism within the bovine *ITGB2* gene in different breeds of cattle

Animal group	Number in genotype groups	Genotypes			χ^2
		CC	*CT*	*TT*	
Black-and-White	observed	62.00	107.00	9.00	18.138**
	expected	74.94	81.10	21.93	
Polish Red	observed	19.00	12.00	3.00	0.208
	expected	18.37	13.24	2.39	
Polish White-Back	observed	21.00	12.00	2.00	0.070
	expected	20.80	12.56	1.84	

**Statistically highly significant differences at $P < 0.01$

Table 4. Genetic equilibrium analysis for SNP 775C>T polymorphism within the bovine *ITGB2* gene in male and female Black and White cattle population

Animal group	Number in genotype groups	Genotypes			χ^2
		CC	*CT*	*TT*	
Bulls	observed	24.00	23.00	2.00	1.515
	expected	25.72	19.56	3.72	
Cows	observed	38.00	84.00	7.00	18.813**
	expected	49.62	60.77	18.61	

**Statistically highly significant differences at $P < 0.01$

The results of a genetic equilibrium analysis are illustrated in Table 3. The Black-and-White cattle had a deviation from the equilibrium, manifested by an increasing frequency of heterozygotes *CT* and a diminished frequency of homozygotes *TT* (a highly statistically significant difference), whereas the groups of cows representing the Polish Red and Polish White-Back breeds demonstrated an almost absolute conformity between the observed and expected numbers of genotype groups *CC, CT* and *TT*.

An analysis of the genetic equilibrium for SNP 775C>T polymorphism within the bovine *ITGB2* gene in the male and female breeding population of Black-and-White cattle (Table 4) indicates that the selected group of bulls had a statistically verified conformity of the observed and expected numbers of genotypic groups *CC, CT* and *TT*, whereas the randomly tested group of cows, representing a random population of Black-and-White cattle, demonstrated a distinct deviation from the state of equilibrium, manifested in an increased number of heterozygotes *CT* and a decreased number of homozygous genotypes (a highly statistically significant difference) for genotype *TT*.

The results indicate that the effect of breeding selection of male reproducers was not linked with the specificity of genetic equilibrium diversification, determined by the SNP 775C>T polymorphism within the bovine *ITGB2* gene. The higher number of heterozygous animals reported in the group of cows provides initial data confirming the selective prevalence of animals determined by a non-additive effect of natural selection.

CONCLUSIONS

SNP 775C>T polymorphism within the bovine *ITGB2* gene in randomly examined Black-and-White breed revealed the highest frequencies of *CT* genotypes, however, the Polish Red and White-Back breeds had the highest frequencies of *CC*. The genotype and allele frequencies of SNP 775C>T within the bovine *ITGB2* gene in the sire and dam population of Black-and-White cattle showed a high frequency of *C* allele in both groups, *CC* in sire population and *CT* in dam population. Furthermore, the SNP 775C>T polymorphism revealed genetic disequilibrium for Black-and-White cattle, as well as in both male and female population groups and genetic equilibrium for Polish Red and Polish White-Back breeds.

REFERENCES

Agerholm J.S., Houe H., Jorgensen C.B., Basse A. (1993): Bovine leukocyte adhesion deficiency in Danish Holstein Friesian cattle. Acta Vet. Scand., 34, 237–243.

Chung E.R., Kim S.K., Kim W.T. (1997): Early diagnosis of BLAD and DUMPS inherited diseases in Holstein dairy cattle using molecular genetic technique I. Screening and frequency of BLAD gene. Korean J. Dairy Sci., 19, 277–288.

Czarnik U., Zabolewicz T., Galiński M., Pareek C.S., Walawski K. (2004): Silent point mutation polymorphism of the bovine *CD*18 encoding gene. J. Appl. Genet., 45, 73–76.

Don R.H., Cox P.T., Wainwright B.T., Beker K., Mattick J.S. (1991): Touchdown PCR to circumvent spurious priming during gene amplification. Nucleic. Acids Res., 19, 4008.

Esmaelizad M., Asadzadeh N., Molasalehi M., Hashemnejad-Kh. (2002): Molecular characterization of *CD*18 gene and identification of BLAD carrier bulls in Iran. In: Proc. 7th World Congress on Genetics Applied to Livestock. Montepellier, France, 1–3.

Grzybowski G., Żurkowski M., Kurył J., Grodzicka M. (1994): Prevalence of D128G mutation in the Polish cattle population. Anim. Genet., 25, 61.

Huang Y.C., Chang H.L., Lin D.Y., Liaw R.B. et al. (2000): Gene frequencies of bovine leukocyte adhesion deficiency of Holstein in Taiwan. J. Taiwan Livest. Res., 33, 37–47.

Janosa A., Baranyai B., Dohy J. (1999): Comparison of milk production in daughters of BLAD carrier and healthy breeding bulls. Allattenyesztes-es-Tkarmayozas, 48, 315–322.

Jorgensen C.B., Agerholm J.S., Pedersen J., Thomsen P.D. (1993): Bovine leukocyte adhesion deficiency in Danish Holstein-Friesian cattle. I. PCR screening and allele frequency estimation. Acta Vet. Scand., 34, 231–236.

Kehrli M.E. Jr., Schmalstieg F.C., Anderson D.C., Van Der Maaten M.J., Hughes B.J., Ackerman M.R., Wilhelmsen C.L., Brown G.B., Stevens M.G., Whetstone C.A. (1990): Molecular definition of the bovine granulocytopathy syndrome: Identification of deficiency of the Mac-1 (*CD*11b/*CD*18) glycoprotein. Am. J. Vet. Res., 51, 1826–11836.

Llambi S., Guevara K., Rincon G., Zaffaroni R., Torres E-de., Barrera J., Arruga M.V., Rodriguez V., Postiglioni A., de-Torres E. (2003): Frequency of bovine leukocyte adhesion deficiency in a population of Holstein-Friesian cattle in Uruguay. Ars-Veterinaria, 19, 52–56

Miceikiene I., Kucinskiene J., Janusauskas K. (2000): PCR_RFLP screening for carriers of bovine leukocyte adhesion deficiency (BLAD). In: Proc. Conference of Lithuanian Society of Genetics. Lithuanian Vety Acad. Tilzes 18, LT-3022 Kaunas, Lithuanian.

Nagahata H. (2004): Bovine leukocyte adhesion deficiency (BLAD): J. Vet. Med. Sci., 66, 1475–1482.

Pareek S.Ch., Kaminski S. (1996): Bovine leukocyte adhesion deficiency (BLAD) and its worldwide prevalence. J. Appl. Genet., 37, 299–311.

Poli M.A., Deway R., Semorile L., Lozano M.E., Albarino C.G., Romanowski V., Grau O. (1996): PCR screening for carries of bovine leukocyte adhesion deficiency (BLAD) and uridine monophosphate synthase in Argentine Holstein cattle. Zentralbl Veterinaermed, A43, 163–168.

Ribeiro L., Baron E.E., Martinez M.L., Coutinho L.L. (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.

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

Received: 2006–01–03
Accepted after correction: 2007–01–26

Corresponding Author

Dr. hab. Chandra S. Pareek, Department of Animal Genetics, University of Warmia and Mazury in Olsztyn, 10 719 Olsztyn, Poland
Tel. +48 895 233 948, fax +48 895 233 948, e-mail: pareek@uwm.edu.pl