Fundamentals for marker-assisted selection, transgenesis

UDC 636.2.034:636.082:575.162

doi: 10.15389/agrobiol.2016.6.775rus

doi: 10.15389/agrobiol.2016.6.775eng

ASSOCIATIONS OF *BoLA-DRB3* GENOTYPES WITH BREEDING VALUES FOR MILK PRODUCTION TRAITS IN RUSSIAN DAIRY CATTLE POPULATION

**A.A. SERMYAGIN¹, N.V. KOVALYUK², A.N. ERMILOV¹, I.N. YANCHUKOV¹,
V.F. SATSUK², A.V. DOTSEV¹, T.E. DENISKOVA¹, G. BREM^{1, 3}, N.A. ZINOVIEVA¹**

¹*L.K. Ernst All-Russian Research Institute of Animal Husbandry, Federal Agency of Scientific Organizations, 60, pos. Dubrovitsy, Podolsk District, Moscow Province, 142132 Russia, e-mail alex_sermyagin85@mail.ru;*

²*North-Caucasian Institute of Animal Production, Federal Agency of Scientific Organizations, 4, ul. Pervomayskaya, pos. Znamenskii, Krasnodar, 350055 Russia, e-mail nvk1972@yandex.ru;*

³*Institut für Tierzucht und Genetik, University of Veterinary Medicine (VMU), Veterinärplatz, A-1210, Vienna, Austria, e-mail gottfried.brem@agrobiogen.de*

Acknowledgements:

Supported by Federal Agency of Scientific Organizations and Ministry of Education and Science of the Russian Federation (project № 14.604.21.0062, RFMEFI60414X0062)

Received September 14, 2016

Abstract

Breeding for genetically resistant cattle is an attractive approach to eradicate infectious disease in livestock. Bovine leukaemia is one of the most common infectious diseases of dairy cattle, which causes significant economic losses. Numerous studies have demonstrated that polymorphisms in the *DRB3* gene at the bovine leukocyte antigen (*BoLA*) locus are associated with cow resistance to persistent lymphocytosis (PL), a clinical sign of leukaemia. Alleles associated with PL susceptibility are generally prevalent in dairy cattle populations and may reflect their association with milk productivity. Our objective was to evaluate the associations between genotypes at the *BoLA-DRB3* locus and milk production traits in dairy cattle in Russia. In total, 171 Holstein sires were genotyped for *DRB3* using the PCR-RFLP technique. Detected *BoLA-DRB3* alleles were assigned to three categories according to their PL susceptibility. The *DRB3.2*08*, *DRB3.2*16*, *DRB3.2*22* and *DRB3.2*24* alleles were defined as susceptible (S), whereas alleles *DRB3.2*11*, *DRB3.2*23* and *DRB3.2*28* were identified as resistant (R), and the remaining alleles were denoted as neutral (N). We calculated the estimated breeding values (EBV) for milk production traits, including milk yield (MY), fat yield (FY) and protein yield (PY), using genotype categories at the *BoLA-DRB3* locus as a fixed effect. We detected fifteen alleles, forty-nine genotypes and six genotype categories for the *BoLA-DRB3* gene. The PL-susceptibility alleles were prevalent in Russian dairy sires (ranging from 0.0877 for *BoLA-DRB3.2*08* to 0.2135 for *BoLA-DRB3.2*22*), assuming 0.6258. The bulls carrying the SS genotype category were characterized by significantly higher EBVs for MY and PY compared to the other genotype categories: 89.3 kg for MY ($p < 0.001$ vs. NN, NR, SN, $p < 0.01$ vs. SR, $p < 0.05$ vs. RR), 2.15 kg for FY ($p < 0.001$ vs. NN, NR, $p < 0.05$ vs. SN); 4.81 kg for PY ($p < 0.001$ vs. all other genotype categories). Our findings indicate that high frequencies of PL-susceptibility alleles in *BoLA-DRB3* may be a result of their association with milk production traits in dairy cattle in Russia. Therefore, to prevent the development of clinical forms of bovine leukaemia in dairy herds in Russia, the impact of the genotypes at the *BoLA-DRB3* gene locus on milk production traits must be considered.

Keywords: *BoLA-DRB3* genotypes, BLV susceptible alleles, milk production traits, genetically resistant cattle

Breeding to develop a livestock population genetically resistant to infection diseases is very important step toward the eradication of infection disease in farm animals and the sustainable food production. Bovine leukemia is an economically important infection disease of dairy cattle, which is caused by bovine leukemia virus (BLV). Economic losses due to BLV infection can come from reduced milk production, reduced reproductive efficiency, increased replacement costs, and increased veterinary costs [1]. High degree of BLV prevalence in dairy

herds worldwide has been shown [2]. In Russia, despite the combating this disease, its ration in the structure of infectious diseases of cattle is more than 50 % [3]. It has been reported, that highly polymorphic DRB3 locus, which is located in the Bovine leukocyte antigen (BoLA) class II region on BTA23 [4] is involved in susceptibility and resistance to clinical signs of leucosis in the form of persistent lymphocytosis (PL). Molecular basis for gene control of the resistance and susceptibility to PL is the presence of the amino acids Glu-Arg at putative Ag binding residues 70 and 71 of the BoLA-DRB3 [5]. It has been demonstrated that carriers of the *DRB3.2*11*, *DRB3.2*23* and *DRB3.2*28* alleles containing the Glu-Arg at residues 70-71 are resistant (R), i.e. they are not prone to the transition of leukemia to PL. Conversely, carriers of the *DRB3.2*08*, *DRB3.2*16*, *DRB3.2*22* and *DRB3.2*24* alleles are susceptible (S) to PL, i.e. they are more often affected by hematological diseases than other genotypes. The remaining alleles are neutral (N), i.e. they are not associated with either resistance or susceptibility to PL [5-9].

The intense studies to characterize the genetic variability at BoLA-DRB3 were performed in Holsteins [reviewed by 10] — the most widely used dairy cattle breed. Up to 29 alleles were identified in regional Holstein populations and S alleles were the most common in majority of them [11-15]. We hypothesized that prevalence of S alleles of *BoLA-DRB3.2* may reflect the effect of the selection pressure for milk production traits. There is experimental evidence that some of the *BoLA-DRB3* alleles are associated with milk productivity in cattle, but the associations are in some cases antagonistic [16-19]. No studies were performed to evaluate the effect of *BoLA-DRB3* genotypes assigned to categories according to susceptibility to PL on milk production traits.

Our objective was to study the effect of the genotype categories at *BoLA-DRB3* locus defined according their susceptibility to PL on estimated breeding values (EBVs) for milk production traits in dairy bulls in Russia.

Technique. The samples were collected from 171 sires having 62096 data records in 77 herds. Genomic DNA was extracted from semen samples, using Diatom Prep 100 kit (Izogen, Russia) according to recommendation by manufacture. The *BoLA-DRB3.2* genotypes were analyzed using PCR-RFLP technique as described by M.J.T. Van-Eijk et al. [20]. Genotype variants were identified by comparing the restriction patterns previously described in literature [20; BoLA nomenclature, ISAG]. Six categories of genotypes (SS, SR, SN, NR, NN, RR) were assigned according the presence of S, R and N alleles, whereas *DRB3.2*11*, *DRB3.2*23* and *DRB3.2*28* alleles are resistant (R), *DRB3.2*08*, *DRB3.2*16*, *DRB3.2*22* and *DRB3.2*24* alleles are susceptible (S) and the remaining alleles are neutral (N).

Association study was conducted using estimated breeding values (EBVs) for milk production traits including milk yield (MY), fat yield (FY) and protein yield (PY). EBVs were calculated by best linear unbiased estimator procedure (BLUP) written in the SAS IML software (SAS Inst., Inc., Cary, NC, USA). The heritability values calculated by ANOVA based on the estimated variance and covariance components were 0.390 for 305-d MY, 0.459 for FY and 0.316 for PY. To evaluate the association between the categories of genotypes (SS, SN, SR, NN, NR, RR) and EBVs for milk production traits the following mixed model was used:

$$Y_{ijkl} = \mu + HYS_i + \sum_k b_1 A_k + \sum_k b_2 DO_k + \sum_l \alpha_l BoLA_l + Sire_j + e_{ijkl},$$

where Y_{ijkl} is the dependent variable of production traits (MY, FY, and PY), μ is the population mean of the analyzed traits, HYS_i is the fixed effects of herd-year-season of the parity i ($i = 1, \dots, 2075$ subclasses), $b_{1,2}$ are the linear regres-

sion coefficients, A_k and DO_k is respectively the age of first calving and days open of the k_{th} cow, β_1 is the regression coefficient on the number of copies of the l_{th} BoLA-DRB3.2 allele (0, 1 or 2), $BoLA_l$ is the fixed effect of l_{th} BoLA-DRB3 genotype category ($l = 1, \dots, 6$ subclasses), $Sire_j$ is the random permanent sire effect of j_{th} bull assumed being distributed normally and independently with mean 0 and variance $A\sigma_a^2$, where A is the pedigree additive relationship matrix ($j = 1, \dots, 171$ sires) and finally e_{ijk} is the random residual effect with mean 0 and variance σ_e^2 .

Results. We identified fifteen *BoLA-DRB3* alleles in the investigated bull population. The *DRB3.2*08*, *DRB3.2*16*, *DRB3.2*22* and *DRB3.2*24* alleles, which are susceptible (S) to PL comprised 62.6 %. The frequency of resistant (R) to PL alleles (*DRB3.2*11*, *DRB3.2*23*) was 14.6 %. Nine alleles were neutral (N) to PL with the frequency ranged from 0.3% for *DRB3.2*06* to 9.1 % for *DRB3.2*03* assuming 22.8 % (Fig.).

Allele frequencies of BoLA-DRB3.2 in dairy bulls in Russia. The BoLA-DRB3.2 alleles are shown according their categories (S — susceptible, R — resistant and N — neutral) descending their frequencies within each of categories; alleles frequency of the each of alleles is indicated after the symbol of allele.

In total, we identified 49 genotype variants of *BoLA-DRB3.2* (*03/03 ... *24/27) in studied sires and assigned them to six genotype categories according to presence of S, N and R alleles: SS (9 genotypes), SN (17 genotypes), NN (6 genotypes), SR (8 genotypes), NR (8 genotypes) and RR (1 genotype). Results of association studies are summarized in Table.

Effects of *BoLA-DRB3.2* genotype categories on breeding values for milk production traits in dairy bulls in Russia

Bull genotype	Number of daughters	EBV _{MY} ±SEP	EBV _{FY} ±SEP	EBV _{PY} ±SEP
SS	25937	89.3±11.7	2.15±0.51	4.81±0.36
SR	12524	25.1±16.8**	1.62±0.74 ^{xx}	2.14±0.52 ^a
SN	11601	-0.7±17.5***	0.21±0.76 ^x	-0.31±0.54 ^a
NR	7273	-2.0±22.1***	-2.14±0.96 ^{xxx}	-0.18±0.69 ^a
NN	4145	-20.9±29.3***	-2.92±1.28 ^{xxx}	-1.18±0.91 ^{a, b}
RR	616	-90.7±75.9*	1.08±3.32	-5.28±2.36 ^{a, b, c}

Note. EBV — estimated breeding value (calculated using *BoLA-DRB3.2* genotype category as a fixed effect, 6 subclasses), SEP — standard error prediction.

*** $p < 0.001$ (SS vs. NN, SS vs. NR, SS vs. SN); ** $p < 0.01$ (SS vs. SR); * $p < 0.05$ (SS vs. RR); ^{xxx} $p < 0.001$ (SS vs. NN, SS vs. NR); ^{xx} $p < 0.01$ (SR vs. SN); ^x $p < 0.05$ (SS vs. SN, SN vs. NN); ^a $p < 0.001$ (SS vs. all other genotype categories); ^b $p < 0.01$ (SR vs. NN, SR vs. RR); ^c $p < 0.05$ (NR vs. RR).

The bulls carrying SS genotypes showed the highest EBVs for MY (89.3 kg), FY (2.15 kg) and PY (4.81 kg). The significant differences were observed be-

tween SS and all other genotype categories for MY (+64.2 kg vs. SR ($p < 0.01$), +90.0 kg vs. SN ($p < 0.001$), +91.3 kg vs. NR ($p < 0.001$), +110.2 kg vs. NN ($p < 0.001$) and +180.0 kg vs. RR ($p < 0.05$)) and for PY (+2.67 kg vs. SR ($p < 0.001$), +5.12 kg vs. SN ($p < 0.001$), +4.99 kg vs. NR ($p < 0.001$), +5.99 kg vs. NN ($p < 0.001$) and +10.09 kg vs. RR ($p < 0.001$)). Bulls carrying SS genotype category have significantly higher EBVs for FY comparing to three of the five remaining genotypes: +1.94 kg vs. SN ($p < 0.05$), +4.29 kg vs. NR ($p < 0.001$) and +5.07 kg vs. NN ($p < 0.001$).

The S alleles of *BoLA-DRB3* (*DRB3.2*08*, *DRB3.2*16*, *DRB3.2*22* and *DRB3.2*24*) have been shown to be prevalent in the majority of the Holstein population. Twenty-seven and fourteen alleles were identified in two studies of Canadian Holstein cows and S alleles were the most frequent assuming 62.2 and 59.3 %, respectively [11, 12]. S alleles were the most common (56.8 %) among sixteen alleles detected in Japanese Holstein cows [21]. Among 28 alleles identified in Iranian Holsteins 48.3 % were S alleles [13]. Twenty-nine *DRB3.2* alleles were identified in two herds of Polish Holstein-Frisian cattle and the frequencies of S alleles were 47.3 and 48.0 % [15]. S alleles comprised 44.7 % of all the alleles of *BoLA-DRB3.2* distributed in populations of American Holsteins [6]. The altered patterns of *BoLA-DRB3.2* allele distribution was observed in Chinese regional populations of Holsteins, that is probably the result of specific breeding strategy [22]. We observed the highest frequencies of S alleles in Russian dairy bulls (62.5 %) among all of the regional Holstein populations analyzed.

Numerous studies have been performed to find the association between *BoLA-DRB3* alleles and milk production traits. A significant positive effect of allele *DRB3.2*11* on MY and PY and negative effect of allele *DRB3.2*08* on MY, FY and PY were observed in American dairy cows [16]. In similar study, R. Rupp et al. [18] have also reported a positive effect of allele *DRB3.2*11* on MY, FY and PY in a Canadian dairy population. Conversely, S. Sharif et al. [17] reported a positive effect of allele *DRB3.2*08* and negative effect of *DRB3.2*22* on PY in Canadian cow population. Allele *DRB3.2*24* has been shown to be associated with increased FY in American dairy cows [16]. Cows of Black-and-White and Red Gorbato breeds, carrying homozygote genotypes for resistant alleles *DRB3.2*11*, *DRB3.2*23* и *DRB3.2*28*, were characterized by low milk yield comparing to cows carrying other genotypes [23]. In contrast, M. Pashimi et al. [13] observed a significant association of allele *DRB3.2*22* with an increase in PY. Using *BoLA-DRB3* genotype as a fixed effect we did not observed significant associations of alleles with EBVs for milk production trait in Russian dairy bulls, probably due the small number of animals carrying the certain genotypes (49 different *BoLA-DRB3* genotypes were detected in 171 sires) and high variability degree of milk production traits (data not shown). But association studies performed for *BoLA-DRB3* genotype categories assigned according their susceptibility to PL revealed significant association of SS genotypes (carrying ER motif at residues 70-71) with increased breeding values for MY and PY comparing to all other genotypes and for FY comparing to three of the five remaining genotypes. It became evident that the eradication programs for bovine leukemia based on the breeding genetically resistant cattle using *BoLA-DRB3* as a marker should be developed for one population of animals and not across populations and breeds, and presumable associations of this genetic marker with milk production traits should be previously evaluated.

Thus, based on our data, we conclude, that high frequencies of *BoLA-DRB3* alleles, which are susceptible to clinical signs of leucosis, observed in Russian dairy cattle, may reflect to their association with milk production traits. We detected significant higher breeding values for milk production traits in sires car-

rying two alleles of *BoLA-DRB3* susceptible to PL comparing to all other genotype categories for MY and PY and to three of five other genotype categories for PY. Thus, development of eradication programs for bovine leukemia based utilizing *BoLA-DRB3* locus as a genetic marker has to be taken into account the pleiotropic effects of this gene on milk production traits and has to be based on the balance between these important health- and economically related traits.

REFERENCES

1. Ott S.L., Johnson R., Wells S.J. Association between bovine-leukosis virus seroprevalence and herd-level productivity on US dairy farms. *Prev. Vet. Med.*, 2003, 61: 249-262 (doi: 10.1016/j.prevetmed.2003.08.003).
2. Nekouei O., Van Leeuwen J., Sanchez J., Kelton D., Tiwari A., Keefe G. Herd-level risk factors for infection with bovine leukemia virus in Canadian dairy herds. *Prev. Vet. Med.*, 2015, 119: 105-113 (doi: 10.1016/j.prevetmed.2015.02.025).
3. Ivanov O.V., Ivanova O.Yu., Fedotov V.P., Balandina M.V., Verkhovskii O.A., Fedorov Yu.N. Age dynamics in number of antibodies against leucosis virus in calves born from seropositive cows. *Agricultural Biology*, 2008, 6: 87-90 (in Engl.).
4. Sigurdardottir S., Lunden A., Andersson L. Restriction fragment length polymorphism of DQ and DR class II genes of the bovine major histocompatibility complex. *Anim. Genet.*, 1988, 19: 133-150 (doi: 10.1111/j.1365-2052.1988.tb00799.x).
5. Xu A.L., Vaneijk M.J.T., Park C., Lewin H.A. Polymorphism in *BoLA-DRB3* exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukemia virus. *J. Immunol.*, 1993, 151: 6977-6985.
6. Kelm S.C., Deitilleux J.C., Freeman A.E., Kehrli M.E. Jr., Dietz A.B., Fox L.K., Butler J.E., Kasckovics I., Kelley D.H. Genetic association between parameters of innate immunity and measures of mastitis in preparturient Holstein cattle. *J. Dairy Sci.*, 1997, 80: 1767-1775.
7. Lewin H.A., Russell G.C., Glass E.J. Comparative organization and function of the major histocompatibility complex of domesticated cattle. *Immunol. Rev.*, 1999, 167: 145-158 (doi: 10.1111/j.1600-065X.1999.tb01388.x).
8. Juliarena M.A., Poli M., Sala L., Ceriani C., Gutierrez S., Dolcini G., Rodríguez E.M., Marico B., Rodríguez-Dubra C., Esteban E.N. Association of BLV infection profiles with alleles of the *BoLA-DRB3.2* gene. *Anim. Genet.*, 2008, 39(4): 432-438 (doi: 10.1111/j.1365-2052.2008.01750.x).
9. Forletti A., Juliarena M.A., Ceriani C., Amadio A.F., Esteban E., Gutiérrez S.E. Identification of cattle carrying alleles associated with resistance and susceptibility to the Bovine Leukemia Virus progression by real-time PCR. *Res. Vet. Sci.*, 2013, 95(3): 991-995 (doi: 10.1016/j.rvsc.2013.07.018).
10. Behl J.D., Verma N.K., Tyagi N., Mishra P., Behl R., Joshi B.K. The major histocompatibility complex in bovines: a review. *ISRN Vet. Sci.*, 2012, Article ID 872710 (doi: 10.5402/2012/872710).
11. Sharif S., Mallard B.A., Wilkie B.N., Sargeant J.M., Scott H.M., Dekkers J.C., Leslie K.E. Associations of the bovine major histocompatibility complex *DRB3* (*BoLA-DRB3*) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. *Anim. Genet.*, 1998, 29: 157-160.
12. Ledwidge S.A., Mallard B.A., Gibson J.P., Jansen G.B., Jiang Z.H. Multi-primer target PCR for rapid identification of bovine *DRB3* alleles. *Anim. Genet.*, 2001, 32: 219-221.
13. Pashmi M., Qanbari S., Ghorashi S.A., Sharifi A.R., Simianer H. Analysis of relationship between bovine lymphocyte antigen *DRB3.2* alleles, somatic cell count and milk traits in Iranian Holstein population. *J. Anim. Breed. Genet.*, 2009, 126: 296-303 (doi: 10.1111/j.1439-0388.2008.00783.x).
14. Kovaljuk N.V., Satsuk V.F., Matviets A.V., Machulskaja E.V. Possible causes and consequences of the spread of individual allelic variants of the *BoLA-DRB3* locus in groups of Holstein and Ayrshire cattle. *Russ. J. Genet.*, 2010, 46(3): 379-382.
15. Oprządek J., Urtnowski P., Sender G., Pawlik A., Łukaszewicz M. Frequency of *BoLA-DRB3* alleles in Polish Holstein-Friesian cattle. *Anim. Sci. Paper Rep.*, 2012, 30(2): 91-101.
16. Starkenburg R.J., Hanses L.B., Kehrli Jr.M.E., Chester-Jones H. Frequencies and effects of alternative *DRB3.2* alleles of bovine lymphocyte antigen for Holsteins in milk selection and control lines. *J. Dairy Sci.*, 1997, 80: 3411-3419 (doi: 10.3168/jds.S0022-0302(97)76316-1).
17. Sharif S., Mallard B.A., Wilkie B.N., Sargeant J.M., Scott H.M., Dek-

- kers J.C., Leslie K.E. Associations of the bovine major histocompatibility complex DRB3 (*BoLA-DRB3*) with production traits in Canadian dairy cattle. *Anim. Genet.*, 1998, 30(2): 157-160 (doi: 10.1046/j.1365-2052.1999.00459.x).
18. Rupp R., Hernandez A., Mallard B.A. Association of bovine leukocyte antigen (BoLA) DRB3.2 with immune response, mastitis, and production and type traits in Canadian Holstein. *J. Dairy Sci.*, 2007, 90: 1029-1038 (doi: 10.3168/jds.S0022-0302(07)71589-8).
 19. Duangjinda M., Buayai D., Pattarajinda V., Phasuk Y., Katawatin T., Vongpralub T., Chaiyotvittayakul A. Detection of bovine leukocyte antigen *DRB3* alleles as candidate markers for clinical mastitis resistance in Holstein × Zebu. *J. Anim. Sci.*, 2009, 87: 469-476 (doi: 10.2527/jas.2007-0789).
 20. Van Eijk M.J.T., Stewart-Haynes J.A., Lewin H.A. Extensive polymorphism of the *BoLA-DRB3* gene distinguished by PCR-RFLP. *Anim. Genet.*, 1992, 23: 483-496 (doi: 10.1111/j.1365-2052.1992.tb00168.x).
 21. Yoshida T., Mukoyama H., Furuta H., Kondo Y., Takeshima S.N., Aida Y., Kosugiyama M., Tomogane H. Association of *BoLA-DRB3* alleles identified by a sequence-based typing method with mastitis pathogens in Japanese Holstein cows. *Anim. Sci. J.*, 2009, 80(5): 498-509 (doi: 10.1111/j.1740-0929.2009.00663.x).
 22. Wu X.X., Yang Z.P., Wang X.L., Mao Y.J., Li S.C., Shi X.K., Chen Y. Restriction fragment length polymorphism in the exon 2 of the *BoLA-DRB3* gene in Chinese Holstein of the south China. *J. Biomed. Sci. Eng.*, 2010, 3: 221-225 (doi: 10.4236/jbise.2010.32030).
 23. Gladyr' E.A., Zinov'eva N.A., Bykova A.S., Vinogradova I.V., Ernst L.K. *Dostizheniya nauki i tekhniki APK*, 2012, 8: 46-48 (in Russ.).