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. SATSKU², A.V. DOTSEV¹, T.E. DENISKOVA¹, G. BREM¹, ³, N.A. ZINOVIEVA¹

¹L.K. Ernst All-Russia Research Institute of Animal Husbandry, Federal Agency of Scientific Organizations, 60, pos. Dubrovitsy, Podolsk Province, 142132 Russia, e-mail alex_sermyagin85@mail.ru;
²North-Caucasian Institute of Animal Production, Federal Agency of Scientific Organizations, 4, ul. Pervomayskaya, pos. Znamenskii, Krasnodar, 350055 Russia, e-mail nvk1972@yandex.ru;
³Institut für Tierzucht und Genetik, University of Veterinary Medicine (VMU), Veterinärplatz, A-1210, Vienna, Austria, e-mail gottfried.brem@agrobiogen.de

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Abstract

Breeding for genetically resistant cattle is an attractive approach to eradicate infectious disease in livestock. Bovine leukemia 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 leukemia. 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 FY 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 leukemia 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 infectious 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 manufacturer. 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-day 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:

\[
Y_{ijkl} = \mu + \text{HYS}_i + \sum_k b_{1,k} A_k + \sum_k b_{2,k} DO_k + \sum_l \alpha_l \text{BoLA}_l + \text{Sire}_j + e_{ijkl},
\]

where \(Y_{ijkl}\) is the dependent variable of production traits (MY, FY, and PY), \(\mu\) is the population mean of the analyzed traits, \(\text{HYS}_i\) is the fixed effects of herd-year-season of the parity \(i\) (\(i = 1, ..., 2075\) subclasses), \(b_{1,2}\) are the linear regres-
sion coefficients, $A_k$ and $D_{O,k}$ is respectively the age of fist calving and days open of the $k_{th}$ cow, $A_k$ 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 ($l = 1, ..., 6$ 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 ($j = 1, ..., 171$ sires) and finally $e_{ijk}$ is the random residual effect with mean 0 and variance $\sigma_e^2$.

**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.**

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

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); *** $p < 0.001$ (SS vs. NN, SS vs. NR); ** $p < 0.01$ (SR vs. NN); * $p < 0.05$ (SS vs. SN, SN vs. NN); # $p < 0.001$ (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 Gorbatov breeds, carrying homozygote genotypes for resistant alleles DRB3.2*11, DRB3.2*23 and 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.

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