UDC 575.113:63.27.082(477)

POLYMORPHISM OF *TLR1*, *TLR4,* AND *SLC11A1* GENES IN POPULATIONS OF DIFFERENT CATTLE BREEDS OF UKRAINIAN SELECTION

R. O. Kulibaba [1*], Yu. V. Liashenko [2], O. Yu. Ivashchenko [1]

[1] National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine,
15, Heroyiv Oborony Str., Kyiv, 03041
[2] Institute of Animal Science, NAAS of Ukraine, Kharkiv,
1A, Tvarynnykiv Str., Kharkiv, 61026

E-mail: romankx37@gmail.com; yurij2303@gmail.com; ivaschenko.oksana28@gmail.com*

Received September 16, 2021 / Received October 09, 2021 / Accepted November 19, 2021

Aim. To study the specificities of population genetic structure of Ukrainian Black-and-White dairy breed, Ukrainian Red-and-White dairy breed, and Ukrainian Grey cattle breed by polymorphism of *TLR1*, *TLR4*, and *SLC11A1* genes. **Methods.** The study was conducted using the method of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). **Results.** The results of the study demonstrated that in all the experimental populations, the locus *TLR4* by mutations 8732G>A, 8834G>C, and 2021C>T was monomorphic, while loci *TLR1* (1596G>A) and *SLC11A1* (7400C>G and 7808A>T) were polymorphic. For the *TLR4* gene by 8732G>A mutation, only individuals with genotype BB were found; by 8834G>C – with genotype GG; by 2021C>T – with genotype CC. By BclI-polymorphism in the first exon of *TLR1* (1596G>A) the A and G allelic variants were found in each experimental population. The deviation from the Hardy-Weinberg equilibrium state was revealed for the populations of Ukrainian Black-and-White and Ukrainian Grey cattle breeds. A considerable excess of heterozygous individuals was fixed in both cattle populations (31 and 39 % respectively). Considering PstI-polymorphism in exon 11 of *SLC11A1* gene, allelic variants C and G were found for SNP5 (7400C>G), as well as A and T for SNP6 (7808A>T) in all experimental populations. As for SNP5 (7400C>G), a considerable excess (from 15 to 30 %) of heterozygous individuals was determined for all experimental groups. Unlike other breeds, there was no excess (Fis = 0,04) of heterozygotes for SNP6 (7808A>T) in the population of Ukrainian Grey cattle. **Conclusions**. The parameters of genetic variability in the different cattle populations of Ukrainian selection by *TLR1*, *TLR4*, and *SLC11A1* loci were studied. The results of the study showed the impossibility of using *TLR4* locus by 8732G>A, 8834G>C and 2021C>T mutations in the programs of marker-associated selection of the investigated cattle breeds due to its monomorphic nature. The analysis of the allele and genotype distribution by *TLR1* and *SLC11A1* loci (presence of individuals with different genotypes in all experimental cattle breeds) indicates the possibility of using different allelic variants of these genes in the breeding programs for the studied cattle populations.

Key words: polymorphism, restriction, variability, allele, gene, population.

DOI: https://doi.org/10.15407/agrisp8.03.025

INTRODUCTION

High productivity indices of animals are incorporated into the main purpose of selection in animal breeding, the process of implementing which involves the use of modern DNA-technologies that form the foundation for marker-assisted selection (MAS) (Wakchaure R et al, 2015; Salisu IB et al, 2018). In addition to rather well-studied marker mutations and genes with extensive data not only about the population genetic structure of different cattle breeds, but also about the results of the analysis of their association with economically valuable traits of animals, which opens immediate wide perspectives for the application of MAS. There are also new promising trends, including the analysis of novel candidate genes and mutations (polymorphisms), previously not described for specific breeds (Bordbar F et al, 2020).

To date, there are almost no data on the genetic structure features of different cattle breeds of Ukrainian selection by gene polymorphism, associated with the parameters of animal resistance to various diseases, which leads to the impossibility of using MAS in breeding work on these populations. In this context, promising objects of study are the loci *TLR1*, *TLR4,* and *SLC11A1*, which will be explained below.

Toll-like receptors (TLR) are a family of receptor molecules (transmembrane proteins), expressed on leucocytes, T- and B-cells and some non-immune cells, whose main function is to activate the immune system in response to the impact of pathogenic microbes and their virulence factors (El-Zayat SR et al, 2019). In animal genetics, particular functional elements of the TLR family have long attracted attention in the context of studying the issues of resistance breeding (Novak K et al, 2019).

The *TLR1* gene is located on chromosome 4, contains 4 exons and 3 introns, the total size is about 8.5 kb. Among rather a large number of alternative alleles in the genetics of cattle, mutations (polymorphisms) have been determined with prominent phenotypic manifestation. For instance, the association was found between different allelic variants of *TLR1* and the susceptibility of cattle to bovine tuberculosis (the study was conducted using PCR-RFLP and SSCP-markers) (Sun L et al, 2012). Different mutations of *TLR1* were also found in the context of susceptibility to *Mycobacterium avium paratuberculosis* infection in cattle of Holstein and local Turkish breeds (Cinar MU et al, 2018). In addition to the abovementioned diseases, an association was found between different single nucleotide polymorphisms of locus *TLR1* (SNP-79T>G and +2463C>T) with clinical mastitis in Holstein Friesian cattle (Russell CD et al, 2012). Recently, along with the study on the resistance parameters of animals to diseases, there have also been studies, aimed at analysing the association of polymorphism in the loci of TLR family and the indices of reproductive parameters of animals, such as indicators of calving ease, production longevity and others (Bjelka M et al, 2020).

Gene *TLR4* is located on chromosome 8, contains 3 exons and 2 introns, the total size is about 11 kb. Similar to *TLR1*, this locus is actively studied in the context of the resistance of animals to diseases. For instance, significant association was found between haplotype C-T (–226G>C and 2021C>T) in *TLR4* gene with susceptibility to *Mycobacterium avium ssp. paratuberculosis* in Canadian Holsteins (Sharma BS et al, 2015). Furthermore, there are studies of an association between different allelic variants of *TLR4* and the resistance to mastitis and the content of somatic cells in milk (Li Z et al, 2014; Gupta PH et al, 2015; Razak R et al, 2015). Bilgen et al studied the polymorphism of *TLR4* by several marker systems using animals of both commercial and local cattle breeds in Anatolia, Turkey (Bilgen N et al, 2016). In addition to the study of the effect of different mutations in *TLR4* gene on the resistance/susceptibility to diseases, there are also recent investigations on their influence on economically valuable traits in cattle such as milk production, including milk quality, in different countries (Zhou H et al, 2017; El-Domany WB et al, 2019; Mišeikienė R et al, 2020).

Gene *SLC11A1* is located on chromosome 2, contains 15 exons and 14 introns, the total size is about 11 kb. Gene *SLC11A1* encodes protein NRAMP (Natural Resistance Associated Macrophage Protein 1), which plays a key role in the immune control of a wide spectrum of different infectious agents (Cheng Y et al, 2015; Holder A et al, 2020). Similar to *TLR1* and *TLR4* a relation between mutations in gene *SLC11A1* susceptibility to bovine tuberculosis has been established (Kadarmideen HN et al, 2011; Liu K et al, 2017; Okuni JB et al, 2021). There have been some recent articles from Spain and China, reporting the relation between *SLC11A1* alleles and economically valuable traits of cattle, including milk production traits (Canive M et al, 2020; Ma Y et al, 2021).

Unfortunately, the scientific literature practically lacks the data about the specificities of the population genetic structure of cattle, bred in Ukraine, regarding the abovementioned loci despite the perspectives of their further application in the programs of marker-assisted selection. Taking the abovementioned into consideration, this article is aimed of studying the population genetic structure of different cattle breeds of Ukrainian selection by *TLR1*, *TLR4*, and *SLC11A1* gene polymorphism.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Molecular, Genetic, Physiological, and Biochemical Research in Animal Science of the Institute of Animal Science, NAAS of Ukraine.

The populations of different cow breeds were used in the study – Ukrainian Black-and-White dairy breed (n = 100, state experimental farm “Hontarivka”), Ukrainian Red-and-White dairy breed (n = 100, state experimental farm “Hontarivka”) and Ukrainian Grey cattle (n = 30, State experimental farm “Polivanovka”).

Hair follicles were used as a source of biological material. DNA isolation was conducted using a commercial set of reagents DNA-Sorb-B according to the manufacturer's protocol (AmpliSense Biotechnologies, Russia).

The following variants were studied to determine the gene polymorphism: *TLR1* – Bc1I-polymorphism in exon 1 (1596G>A); *TLR4* – polymorphism in exon 3 (8732G>A, MspI; 8834G>C, RsaI; 2021C>T, BsiHKAI); *SLC11A1* – PstI-polymorphism in exon 11 (7400C>G and 7808A>T). The information about the primers, amplicon lengths and restriction endonucleases shown in the Table 1.

The amplification of the selected genome fragments was conducted using a programmed thermocycler "Tertsik" following the program: 1 cycle – denaturation at 94 °C for 3 min; 35 cycles – denaturation at 94 °C for 45 s, annealing for 45 s (64 °C – for *TLR1*; 59 °C – for *TLR4*; 60 °C – for *SLC11A1*), elongation at 72 °C for 45 s; 1 cycle – final elongation at 72 °C for 10 min. The volume of the reaction mixture was 20 μL, the concentration of primers – 0.2 μM for each case.

The genotyping of animals was done using PCR-RFLP method. The restriction procedure was conducted according to the manufacturer's protocols (Thermo Scientific).

Bc1I-polymorphism in exon 1 of *TLR1* gene – genotype AA (BclI+) is presented on the electrophoregram by fragments of 261, 72, and 21 bp; AG – 333, 261, 72, and 21 bp; GG (BclI–) – 333 and 21 bp.

In case of MspI-polymorphism in exon 3 of *TLR4* locus genotype BB is presented on the electrophoregram by fragments of 490, 110, and 82 bp; AB – 682, 490, 110, and 82 bp; AA – 682 bp. As for RsaI-polymorphism, genotype AA is presented on the electrophoregram by fragment of 664 bp; GG – 348, 212, and 104 bp; AG – 664, 348, 212, and 104 bp.

BsiHKAI-polymorphism in exon 3 of *TLR4* – genotype CC is presented with fragments of 485 and 94 bp; CT – 579, 485, and 94 bp; TT – 579 bp.

PstI-polymorphism in exon 11 of gene *SLC11A1* – the individuals were typed by the evaluation of complex genotypes regarding two mutations in the fragment under analysis (SNP5 7400C>G and SNP6 7808A>T) according to Liu K et al (Liu K et al, 2017). There are two SNP in the experimental fragment (SNP5 and SNP6), and SNP5 is presented with variants C and G, SNP6 – A and T. In this case, the complex genotype is a variation of a haplotype. Genotypes GG and TT are presented in the electrophoregram with the fragments of 633 and 303 bp; CC and AA – 227 and 709 bp; CG and AT – 227, 709, 633 and 303 bp; CC and TT – 227, 406 and 303 bp; CG and TT – 227, 406, 709 and 303 bp; CC and AT– 227, 406, 303, 227 and 709 bp.

The electrophoresis of restriction products was conducted with 1.5–3.0 % agarose gel. Ethidium bromide (0,5 mkg/ml) was used to visualize the fragments in the ultraviolet spectrum. The sizes of the restriction fragments were determined using molecular mass markers – 50 bp Ladder DNA Marker (Size Range: 50 to 500 bp) and 100 bp Ladder DNA Marker (Size Range: 100 to 3000 bp) (Simgen).

The results of the study regarding each detected polymorphic locus were used to evaluate the frequencies of genotypes and alleles, the observed and expected distribution of genotypes, the correspondence to the state of genetic equilibrium according to Hardy-Weinberg by the method of χ^2, the parameters of the expected (H_e) and observed (H_o) heterozygosity, the effective number of alleles (n_e), Wright's fixation index (F_{is}). The calculations were done using Popgen32 Version 1.32 software (https://sites.ualberta.ca/~fyeh/popgene_download.html).

Table 1. Primer sequences and restriction endonucleases

SNP	Primer sequence	Amplicon length (bp)	Restriction endonuclease	Reference
TLR1 (1596G>A)	tttagcagcctttccatact; cagatccaggtagatacagag	354	Bc1I	Arslan K et al, 201]
TLR4 (8732G>A; 8834G>C)	gcctaaaccacctctccac; agaagggcttgtagacttct	682	MspI RsaI	Elmaghraby MM et al, 2018
TLR4 (2021C>T)	aacaggtagcccagacagcatttc; gcacgccctcctccaagttc	579	BsiHKAI	Beecher C et al, 2010
SLC11A1 (7400C>G;7808A>T)	tgtgcttcacatctccttccta; agcacattgagcaggtcgtt	936	PstI	Liu K et al, 2017

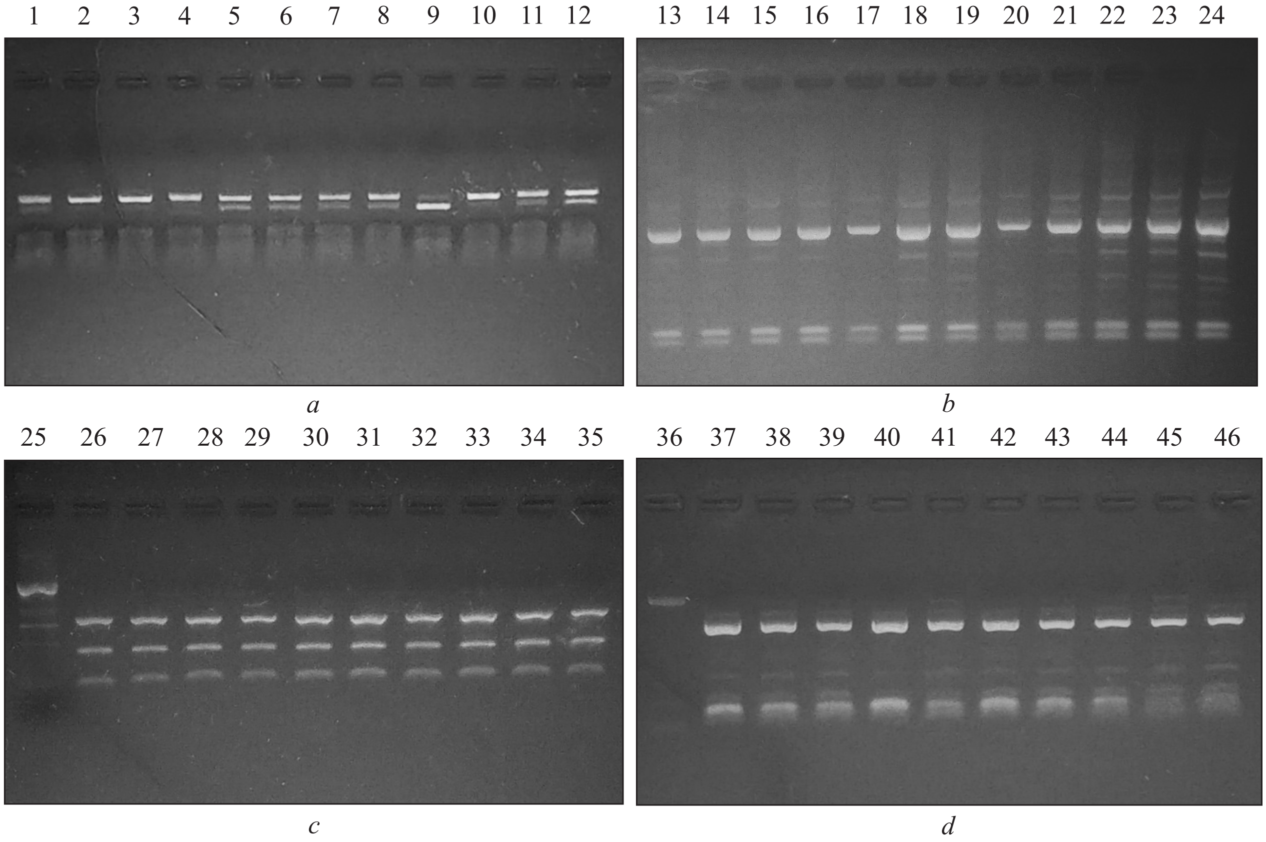

Fig. 1. The electrophoregram of restriction products: *a* – *TLR1* (BclI): 1–4, 10 – genotype GG, 5–8, 11, 12 – AG, 9 – AA; *b* – *TLR4* (MspI): 13–24 – BB; *c* – *TLR4* (RsaI): 25 – amplicon, 26–35 – GG; *d* – *TLR4* (BsiHKAI): 36 – amplicon, 37–46 – CC

RESULTS

Based on the results of the studies, the polymorphism of the *TLR1*, *TLR4* and *SLC11A1* genes was studied in experimental cattle populations. The electrophoregrams of restriction products for *TLR1* and *TLR4* are presented in Fig. 1.

As for BclI-polymorphism of *TLR1,* three possible genotypes (AA, GG, and AG) were found in the studied populations of Ukrainian Black-and-White and Red-and-White dairy breeds, while homozygotes AA were not detected in the population of Ukrainian Grey cattle. All the obtained restriction patterns corresponded to the expected ones completely (which is given in the section "MATERIALS AND METHODS"), which allowed for successful genotyping of all individuals in each experimental population. With direct determination of a genotype, the presence of a monomorphic restriction site for BclI in exon 1 of *TLR1* can be an advantage, since it allows determining the true genotype of an individual with high level of efficiency by the presence of heavy fragments on the electrophoregram, even in the case of incomplete activity of the restriction endonuclease.

Contrary to the findings with *TLR1*, for *TLR4* gene no different alleles were found regarding all potential polymorphic variants (8732G>A, MspI; 8834G>C, RsaI; 2021C>T, BsiHKAI) which demonstrated complete genetic homogeneity of the studied populations. By MspI-polymorphism it was shown that all individuals in all experimental populations had genotype BB; by RsaI – only genotype GG; and BsiHKAI – only genotype CC (Fig. 1). Each obtained restriction pattern corresponded to the expected according to Elmaghraby et al (Elmaghraby MM et al, 2018) and Beecher et al (Beecher C et al, 2010) and no added variations were observed.

The PstI-polymorphism in exon 11 of *SLC11A1* gene demonstrates, that this locus was polymorphic in all experimental populations: individuals with different genotypes for corresponding mutations (7400C>G and 7808A>T) were found in all breeds. The electrophoregram of restriction products for *SLC11A1* is presented in Fig. 2.

In this case, the insufficient restriction endonuclease activity can be observed, that, however, with given the presence of additional monomorphic site for PstI in each allele, practically does not create seriously difficulties for genotyping of individuals. The incomplete activity of PstI can lead to formation an alternate fragment in the restriction pattern which is equal to the amplicon by size. Different variants of genotypes by PstI-polymorphism of *SLC11A1* gene (CC and AA; CG and AT) were shown on the electrophoregram (Fig. 2).

Since *TLR4* showed no variations, only polymorphic variants of *TLR1* and *SLC11A1* genes will be detailed below.

The genetic structure of the experimental populations by *TLR1* gene is presented in Table 2.

All three experimental breeds were characterized by the prevalence of allele G frequency, that reached its maximum in Ukrainian Grey cattle population, in which there were no individuals with the A allele in the homozygous state. By this polymorphism all experimental cattle populations, except Ukrainian Red-and-White dairy breed, showed a deviation from Hardy-Weinberg equilibrium state.

The genetic structure of the experimental cattle populations by *SLC11A1* gene is presented in Table 3.

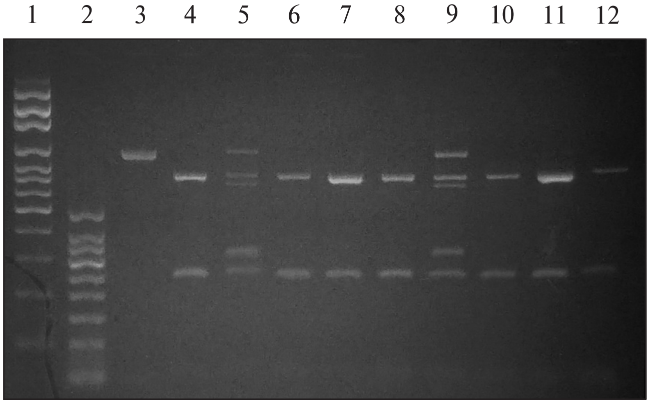

Fig. 2. The electrophoregram of restriction products for exon 11 of *SLC11A1* gene using PstI: 1 – 100 bp Ladder DNA Marker; 2 – 50 bp Ladder DNA Marker; 3 – amplicon; 4, 6–8, 10–12 – genotypes CC and AA; 5, 9 – CG and AT

In this case, the genotyping was conducted simultaneously for two mutations (previously identified with sequencing and described in the article of Liu K. et al.2017). Actually, the complex genotypes (CC-AA, CG-TT and others) belong to the haplotypes, what can lead to the observed ratio of allele frequencies.

In all the experimental populations the complex genotype CC-AA was prevalent. The Ukrainian Grey

Table 2. The genetic structure of experimental populations by *TLR1* gene (1596G>A)

Breed	Genotypes						Allele frequency		HWE (χ^2)
	AA		AG		GG		A	G	
	O	E	O	E	O	E			
Ukrainian Black-and White dairy	12	19.36	64	49.28	24	31.36	0.44	0.56	8.93
Ukrainian Red-and-White dairy	13	16	54	48	33	36	0.40	0.60	1.56
Ukrainian Grey	0	2.52	17	12.35	13	15.12	0.29	0.71	4.57

Note. O – observed and E – expected number of animals with appropriate genotype; HWE – correspondence to Hardy-Weinberg Equilibrium by using χ^2.

Table 3. The genetic structure of experimental populations by *SLC11A1* gene (SNP5 7400C>G and SNP6 7808A>T)

Breed	Genotypes (SNP5-SNP6)				Allele frequency			
	CC-AA	CG-AT	CG-AA	CG-TT	SNP5		SNP6	
					C	G	A	T
Ukrainian Black-and-White dairy	66	32	2	–	0.83	0.17	0.84	0.16
Ukrainian Red-and-White dairy	57	17	26	–	0.79	0.21	0.92	0.08
Ukrainian Grey	21	8	–	1	0.85	0.15	0.83	0.17

cattle appeared to be considerably different from the others, since no animals with genotype CG-AA were detected for it, but genotype CG-TT was found (with the minimal possible frequency). In general, in all cattle populations the prevalence of allele C (SNP5) and allele A (SNP6) frequencies wwere revealed.

To analyze the correspondence of cattle populations to the genetic equilibrium according to Hardy-Weinberg the obtained complex genotypes by *SLC11A1* gene were analyzed separately for SNP5 and SNP6. The results of this analysis are presented in Table 4.

Generally, deviation from the genetic equilibrium was found only in the case of SNP5 (7400C>G) for the Ukrainian Black-and-White and Red-and-White dairy breeds. As for the second mutation (7808A>T), all the experimental populations were in the equilibrium state, which indirectly indicates the absence of expressed active form-generating processes in these groups.

The total genetic-population parameters of the experimental cattle populations by founded polymorphic loci (except for the monomorphic marker for *TLR4*), are presented in Table 5.

In this case, table 4 shows data only on polymorphic loci that describe above.

Our study demonstrates that the experimental cattle populations were considerably different by parameters of genetic variability. A general excess of heterozygous animals was found in all the investigated populations in rather a wide range (from 13 to 39 %) which resulted in deviations from the equilibrium state. The only exception (no excess of heterozygotes) was demonstrated for Ukrainian Grey by *SLC11A1* (SNP6).

In its turn, as for *TLR1* (1596G>A), the maximal level of polymorphism, which value was practically maximal for two-allele systems, was observed for the Ukrainian Black-and-White dairy breed, and the mini-

Table 4. The genotype frequencies by different SNP in *SLC11A1* gene in the experimental cattle populations

Breed	Genotypes						HWE (χ^2)
	O	E	O	E	O	E	
	SNP5 (7400C>G)						
	CC		CG		GC		
Ukrainian Black-and-White dairy	66	68.89	34	28.22	0	2.89	4.13
Ukrainian Red-and-White dairy	57	62.41	43	33.18	0	4.41	7.78
Ukrainian Grey	21	21.67	9	7.65	0	0.68	0.93
Breed	SNP6 (7808A>T)						HWE (χ^2)
	AA		AT		TT		
Ukrainian Black-and-White dairy	68	70.56	32	26.88	0	2.56	3.63
Ukrainian Red-and-White dairy	83	84.64	17	14.72	0	0.64	1.02
Ukrainian Grey	21	20.67	8	8.47	1	0.86	0.06

Note. O – observed and E – expected number of animals with appropriate genotype; HWE – correspondence to Hardy-Weinberg equilibrium by using χ^2.

Table 5. The main genetic-populational parameters of the experimental cattle populations

Breed	TLR1 (BclI-polymorphism)				SLC11A1 (SNP5)				SLC11A1 (SNP6)			
	H_o	H_e	F_{is}	n_e	H_o	H_e	F_{is}	n_e	H_o	H_e	F_{is}	n_e
I	0.64	0.49	–0.31	1.96	0.34	0.28	–0.21	1.39	0.32	0.27	–0.19	1.37
II	0.54	0.48	–0.13	1.92	0.43	0.33	–0.30	1.49	0.17	0.15	–0.13	1.18
III	0.57	0.41	–0.39	1.69	0.30	0.26	–0.15	1.35	0.27	0.28	0.04	1.39

Note. I – Ukrainian Black-and-White dairy breed; II – Ukrainian Red-and-White dairy breed; III – Ukrainian Grey cattle; H_e – expected heterozygosity; H_o – observed heterozygosity; F_{is} – Wright's fixation index; n_e – the effective number of alleles.

mal one – for Ukrainian Grey cattle. The situation was somewhat different for *SLC11A1* – the total level of polymorphism is reduced, which may be caused by linkage, related to the binding of two SNP within one locus.

DISCUSSION

In order for different candidate genes to be used in MAS programs, they must be present as different variants (alleles) relative to a particular site (mutation). Only when polymorphism of candidate genes is detected in experimental animal populations, further studies can be carried out to analyze the associations between various allelic variants and economically valuable traits for the selection of individuals with the desired genotypes. It is for this reason that the study of the genetic-populational parameters of different breeds of livestock is given such close attention by population biology. Thus, it can be noted that the analysis of the genetic variability of populations is an integral part of the overall MAS strategy, in line with which the present work was carried out.

The results of the study demonstrated the genetic structure features of the populations of different cattle breeds of Ukrainian selection by *TLR1*, *TLR4* and *SLC11A1* genes, which allowed for some generalizations and conclusions. For instance, one of the most notable specificities of dairy cattle (Ukrainian Black-and-White and Red-and-White dairy breeds) and dual-purpose breed (Ukrainian Grey cattle) is the monomorphic character of each population by *TLR4* gene. Moreover, it applies to each of the studied mutations (8732G>A, 8834G>C, 2021C>T), which significantly distinguishes Ukrainian breeds from their foreign counterparts. The polymorphic variants of *TLR4* gene are typical for different cattle breeds that indicates a major impact of a breed factor and the direction of the selection work on the genetic structure features of experimental animal populations. For instance, 8732G>A polymorphism was found in a population of Holstein cattle in Iran (Elmaghraby MM et al, 2018). Moreover, according to the results of the study, allele B belongs to the predominant allelic variant, which, in turn, was found in an invariant form in experimental cattle populations in our studies. In this context, the monomorphic character of cattle breeds of Ukrainian selection by this locus potentially reflects the general trends in breeding work in animal husbandry. As for RsaI-polymorphism, our study is consistent with the results obtained by Elmaghraby et al. – the monomorphic character of the locus by this mutation was confirmed. As for 2021C>T polymorphism of *TLR4* gene, different allelic variants were found in Holstein, Jersey, and Montbeliarde cattle as well as in Holstein-Friesian breed in New Zealand (Carvajal AM et al, 2013; Zhou H et al, 2017). However, it should be noted, that the frequency and type of the prevailing allele vary depending on the population and breed of animals. For instance, in most cases, different populations are characterized by high allele C frequency, which may be related to ongoing breeding work in the direction of increasing milk productivity parameters. The association of the C allele with the milk production traits of Holstein cows was confirmed in a study by Chen et al (Chen H et al, 2021). At the same time, in the work of other authors, actually parity values of the C and T allele frequencies for this locus were revealed for Lithuanian Holstein cattle (Mišeikienė R et al, 2020). Such differences in the values of allele frequencies may indicate on influence of the origin of animals (population factor).

All experimental populations of Ukrainian cattle breeds demonstrated a completely different (compared with *TLR4* gene) situation for the *TLR1* gene, namely a high level of polymorphism, the maximum for Black-and-White dairy breed and the minimum for Ukrainian Grey cattle.

The common feature for all studied breeds was the prevalence of the allele G frequency which reached its maximum value in the population of Ukrainian Grey cattle. In its turn, a mutant allele A of *TLR1* gene (1596G>A, V>I) was found in the homozygous and heterozygous state in 76 % individuals of Black-and-White and in 67 % of Red-and-White dairy breed. Individuals with homozygous genotypes by allele A were 12–13 %, and were completely absent in the population of Ukrainian Grey cattle.

Our results differ from those, obtained by other authors while analysing the genetic structure of Holstein cows and local Turkish breeds (Arslan K et al, 2018). An increase of allele G frequency in the cattle populations is also associated with the results of genomic selection for resistance to brucellosis and tuberculosis (Prakash O et al, 2014). A similar situation was also noted for Chinese Holstein cattle (Sun L et al, 2012).

The analysis of *SLC11A1* gene polymorphism considerably differed from the above-mentioned markers due to a more complicated technical procedure (which was described by Liu K et al, 2017), since in this case genotyping is carried out simultaneously for two mutations (7400C>G;7808A>T). Different allele variants were found for both SNPs (SNP5 and SNP6) in each experimental cattle population. In all cases, the prevalence of allele C and A frequencies for SNP5 and SNP6, respectively, were fixed. The frequencies of mutant alleles G and T were within 0.08–0.21 which demonstrated indirectly a relatively high level of possible functional changes in the investigated gene.

The presence of individuals with different genotypes in the experimental cattle populations creates the necessary prerequisites for further studies towards the analysis of the association between the detected polymorphic variants of different genes and animal productive traits. It is also important in the context of issues of studying resistance to diseases, which is especially promising for local/native cattle breeds. In this case, *TLR1* and *SLC11A1* are promising targets for further study due to the presence of different allelic variants of each of these genes in studied breeds of Ukrainian selection. In turn, *TLR4* gene (by 8732G>A, 8834G>C and 2021 C>T polymorphism) cannot be used in breeding programs for populations of Ukrainian Black-and-White dairy breed, Ukrainian Red-and-White dairy breed and Ukrainian Grey cattle, due to its monomorphic state.

CONCLUSIONS

Our studies show that the *TLR4* gene by each investigated mutation (8732G>A, 8834G>C, 2021C>T) is monomorphic, while *TLR1* and *SLC11A1* are polymorphic in the experimental populations of different cattle breeds of Ukrainian selection (Ukrainian Black-and-White dairy breed, Ukrainian Red-and-White dairy breed and Ukrainian Grey cattle). The monomorphic character of *TLR4* gene (8732G>A, 8834G>C, 2021C>T) makes it impossible to further use in breeding programs of studied cattle populations. By BclI-polymorphism in the first exon of *TLR1* gene (1596G>A) allelic variants A and G were found in each studied breed. The populations of Ukrainian Black-and-White dairy breed and Ukrainian Grey cattle demonstrated the deviation from Hardy-Weinberg equilibrium state and a considerable excess (31 and 39 % respectively) of heterozygous individuals was revealed in both breeds. By PstI-polymorphism in exon 11 of *SLC11A1* gene, allelic variants C and G were found for SNP5 (7400C>G), as well as A and T for SNP6 (7808A>T) in all experimental populations. As for SNP5 (7400C>G), a considerable excess of heterozygous individuals (from 15 to 30 %) was determined for all breeds. For SNP6 (7808A>T) there was no excess of heterozygotes (F_{is} = 0,04) in the population of Ukrainian Grey cattle.

Adherence to ethical principles. All procedures performed in the studies involving animal participants were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Strasbourg, 1986.

Conflict of interests. The authors declare the absence of any conflicts of interests.

Financing. This study was not financed by any specific grant from financing institutions in the state, commercial or non-commercial sectors.

Поліморфізм генів *TLR1*, *TLR4* та *SLC11A1* у популяціях корів різних порід української селекції

Р. О. Кулібаба [1], Ю. В. Ляшенко [2], О. Ю. Іващенко [1]

[1] Національний університет біоресурсів
і природокористування України
Вул. Героїв Оборони 15, Київ, Україна, 03041
[2] Інститут тваринництва НААН України
Вул. Тваринників 1А, Харків, Україна, 61026

Мета. Дослідити особливості генетико-популяційної структури української чорно-рябої молочної породи, української червоно-рябої молочної породи та сірої української породи корів за локусами *TLR1*, *TLR4* та *SLC11A1*. **Методи.** Дослідження проведено за використання методів полімеразної ланцюгової реакції та рестрикційного аналізу (PCR-RFLP). **Результати**. За результатами проведених досліджень показано, що у всіх дослідних популяціях корів локус *TLR4* за мутаціями 8732G>A, 8834G>C та 2021C>T відноситься до мономорфних, у той час як локуси *TLR1* (1596G>A) та *SLC11A1* (7400C>G и 7808A>T) – до поліморфних. За локусом *TLR4* за мутацією 8732G>A виявлені особини лише з генотипом BB; за мутацією 8834G>C – із генотипом GG; за 2021C>T – із генотипом CC. За BclI-поліморфізмом у першому екзоні *TLR1* (1596G>A) виявлено алельні варіанти A та G у кожній з досліджених порід. Встановлено відхилення від рівноважного стану за Харді-Вайнбергом популяцій української чорно-рябої молочної та сірої української породи, для обох порід виявлений суттєвий ексцес гетерозиготних особин (31 та 39 % відповідно). За PstI-поліморфізмом у 11 екзоні гену *SLC11A1* виявлено алельні варіанти C та G для SNP5 (7400C>G), а також A і T для SNP6 (7808A>T) у всіх дослідних популяціях корів. Для SNP5 (7400C>G) встановлений суттєвий ексцес гетерозиготних особин (від 15 до 30 %) для всіх дослідних груп. Для SNP6 (7808A>T) показана відсутність ексцесу гетерозигот (F_{is} = 0,04) у популяції сірої української породи. **Висновки.** Досліджено параметри генетичної мінливості популяцій корів української селекції за локусами *TLR1*, *TLR4* та *SLC11A1*. За результатами досліджень доведено неможливість використання локусу *TLR4* за мутаціями 8732G>A, 8834G>C і 2021C>T у програмах маркер-асоційованої селекції досліджених порід корів внаслідок його мономорфності. Аналіз розподілу частот алелів та генотипів за локусами *TLR1* та *SLC11A1* свідчить про можливість проведення подальших досліджень у напряму використання різних алельних варіантів у селекційних програмах на дослідних популяціях корів.

Ключові слова: поліморфізм, рестрикція, мінливість, алель, ген, популяція.

REFERENCES

Arslan K, Akyuz B, Aksel EG, Ozdemir F, Cinar MU. (2018) Polymorphisms of *TLR1*, *TLR4* and *SLC11A1* Genes in Some Cattle Breeds Reared in Turkey. J. Agric. Sci. **24**:547–553. doi: 10.15832/ankutbd.349728

Beecher C, Daly M, Childs S, Berry DP, Magee DA, McCarthy TV, Giblin L. (2010) Polymorphisms in bovine immune genes and their associations with somatic cell count and milk production in dairy cattle. BMC Genet. **11**:99. doi: 10.1186/1471-2156-11-99

Bilgen N, Cinar Kul B, Offord V, Werling D, Ertugrul O. (2016) Determination of Genetic Variations of Toll-Like Receptor (TLR) 2, 4, and 6 with Next-Generation Sequencing in Native Cattle Breeds of Anatolia and Holstein Friesian. Diversity. **8**(4):23. doi: 10.3390/d8040023

Bjelka M, Novák K. (2020) Association of TLR gene variants in a Czech Red Pied cattle population with reproductive traits. Veterin. Immunol. Immunopathol. **220**:109997. doi: 10.1016/j.vetimm.2019.109997

Bordbar F, Jensen J, Du M, Abied A, Guo W, Xu L, Gao H, Zhang L, Li J. (2020) Identification and validation of a novel candidate gene regulating net meat weight in Simmental beef cattle based on imputed next-generation sequencing. Cell Prolif. **53**:e12870. doi: 10.1111/cpr.12870

Canive M, Casais R, Jimenez JA, Blanco-Vazquez C, Amado J, Garrido JM, Alonso-Hearn M. (2020) Correlations between single nucleotide polymorphisms in bovine CD209, SLC11A1, SP110 and TLR2 genes and estimated breeding values for several traits in Spanish Holstein cattle. Heliyon. **6**(6):e04254. doi: 10.1016/j.heliyon.2020.e04254

Carvajal AM, Huircan P, Lepori A. (2013) Single nucleotide polymorphisms in immunity-related genes and their association with mastitis in Chilean dairy cattle. Genet. Mol. Res. **12**(3):2702–2711. doi: 10.4238/2013.july.30.8

Chen H, Liu C, Xiang M, Yu J, Xia Y, Hu X, Wang D, Tao B, Zhang Y, Cheng L. (2021) Contribution of the mutation rs8193069 in TLR4 to mastitis resistance and performance in Holstein cows in southern China. Veterin. Med. Sci. 1–11. https://doi.org/10.1002/vms3.671

Cheng Y, Huang C, Tsai HJ. (2015) Relationship of Bovine SLC11A1 (Formerly NRAMP1) Polymorphisms to the Risk of Bovine Tuberculosis in Holstein Cattle. J. Veterin. Sci. Technol. **6**:247. doi: 10.4172/2157-7579.1000247

Cinar MU, Hizlisoy H, Akyüz B, Arslan K, Aksel EG, Gümüşsoy KS. (2018) Polymorphisms in toll-like receptor (TLR) 1, 4, 9 and SLC11A1 genes and their association with paratuberculosis susceptibility in Holstein and indigenous crossbred cattle in Turkey. J. Genet. doi: 10.1007/s12041-018-1008-7

El-Domany WB, Radwan HA, Ateya AI, Ramadan HH, Marghani BH, Nasr SM. (2019) Genetic Polymorphisms in LTF/Eco RI and TLR4/Alu I loci as candidates for milk and reproductive performance assessment in Holstein cattle. Reproduction in Domestic Animals. **54**:678–686. doi: 10.1111/rda.13408

Elmaghraby MM, El-Nahas AF, Fathala MM. (2018) Association of toll-like receptors 2 and 6 polymorphism with clinical mastitis and production traits in Holstein cattle. Iran. J. Veterin. Res. **19**(3):202–207

El-Zayat SR, Sibaii H, Mannaa FA. (2019) Toll-like receptors activation, signaling, and targeting: an overview. Bull. Nat. Res. Centre. **43**:187. doi: 10.1186/s42269-019-0227-2

Gupta PH, Patel NA, Rank DN, Joshi CG. (2015) Genetic polymorphism of toll-like receptors 4 gene by polymerase chain reaction-restriction fragment length polymorphisms, polymerase chain reaction-single-strand conformational polymorphism to correlate with mastitis cows. Veterinary World. **8**(5):615–620. doi: 10.14202/vetworld.2015.615-620

Holder A, Garty R, Elder C, Mesnard P, Laquerbe C, Bartens M-C, Salavati M, Shabbir MZ, Tzelos T, Connelly T, Villarreal-Ramos B, Werling D. (2020) Analysis of Genetic Variation in the Bovine SLC11A1 Gene, Its Influence on the Expression of NRAMP1 and Potential Association with Resistance to Bovine Tuberculosis. Front. Microbiol. **11**:1420. doi: 10.3389/fmicb.2020.01420

Kadarmideen HN, Ali AA, Thomson PC, Müller B, Zinsstag J. (2011) Polymorphisms of the SLC11A1 gene and resistance to bovine tuberculosis in African Zebu cattle. Animal Genetics. **42**(6):656–658. doi: 10.1111/j.1365-2052.2011.02203.x

Li Z, Zhang H, Wang H, Chen L, Wang L, Liu X, Song A, Ru C. (2014) Polymorphism in the promoter of TLR4 gene by PCR-RFLP and its association with somatic cell score in Chinese Holstein. Arch. Anim. Breed. 57:6. doi: 10.7482/0003-9438-57-006

Liu K, Zhang B, Teng Z, Wang Y, Dong G, Xu C, Qin B, Song C, Chai J, Li Y, Shi X, Shu X, Zhang Y. (2017) Association between SLC11A1 (NRAMP1) polymorphisms and susceptibility to tuberculosis in Chinese Holstein cattle. Tuberculosis. **103**:10–15. doi: 10.1016/j.tube.2016.11.003

Ma Y, Khan MZ, Xiao J, Alugongo GM, Chen X, Chen T, Liu S, He Z, Wang J, Shah MK, Cao Z. (2021) Genetic

Markers Associated with Milk Production Traits in Dairy Cattle. Agriculture. **11**:1018. doi: 10.3390/agriculture 11101018

Mišeikienė R, Ugenskienė R, Pečiulaitienė N, Bižienė R, Švedaitė A. (2020) The influence of TLR4 gene polymorphisms on milk quality and composition of Lithuanian Holstein cows. Mljekarstvo. **70**(2):112–119. doi: 10. 15567/mljekarstvo.2020.020

Novak K, Bjelka M, Samake K, Valcikova T. (2019) Potential of TLR-gene diversity in Czech indigenous cattle for resistance breeding as revealed by hybrid sequencing. Arch. Anim. Breed. **62**(2):477–490. doi: 10.5194/aab-62-477-2019

Okuni JB, Afayoa M, Ojok L. (2021) Survey of Candidate Single-Nucleotide Polymorphisms in SLC11A1, TLR4, NOD2, PGLYRP1, and IFNγ in Ankole Longhorn Cattle in Central Region of Uganda to Determine Their Role in *Mycobacterium avium* Subspecies paratuberculosis Infection Outcome. Front. Vet. Sci. **8**:614518. doi: 10. 3389/fvets.2021.61451

Prakash O, Kumar A, Sonwane A, Rathore R, Singh RV, Chauhan A, Sharma D. (2014) Polymorphism of cytokine and innate immunity genes associated with bovine brucellosis in cattle. Mol. Biol. Rep. **41**(5):2815–2825. doi: 10.1007/s11033-014-3136-3

Razak R, Hussain I, Dar P, Bashir S, Bhat SA, Mir MU. (2015) Association of toll-like receptor 4 gene polymorphism with the severity of mastitis in crossbred dairy cattle. Appl. Biol. Res. **17**:242–249. doi: 10.5958/0974-4517.2015.00035.X

Russell CD, Widdison S, Leigh JA, Coffey TJ. (2012) Identification of single nucleotide polymorphisms in the bovine Toll-like receptor 1 gene and association with health traits in cattle. Veterin. Res. **43**(1):17. doi: 10.1186/1297-9716-43-17

Salisu IB, Olawale AS, Jabbar B, Koloko BL, Abdurrahaman SL, Amin AB, Mahajan T. (2018) Molecular Markers and their Potentials in Animal Breeding and Genetics. Nigerian. J. Anim. Sci. **20**:29–48

Sharma BS, Abo-Ismail MK, Schenkel FS, You Q, Verschoor CP, Pant SD, Karrow NA. (2015) Association ofTLR4 polymorphisms with *Mycobacterium avium* subspecies paratuberculosis infection status in Canadian Holsteins. Anim. Genet. **46**(5):560–565. doi: 10. 1111/age.12333

Sun L, Song Y, Riaz H, Yang H, Hua G, Guo A, Yang L. (2012) Polymorphisms in toll-like receptor 1 and 9 genes and their association with tuberculosis susceptibility in Chinese Holstein cattle. Veterin. Immunol. Immunopathol. **147**(3–4):195–201. doi: 10.1016/j.vetimm.2012. 04.016

Wakchaure R, Ganguly S, Praveen PK, Kumar A, Sharma S, Mahajan T. (2015) Marker Assisted Selection (MAS) in Animal Breeding: A Review. J. Drug. Metab. Toxicol. **6**:e127. doi: 10.4172/2157-7609.1000e127

Zhou H, Cheng L, Gong H, Byun SO, Edwards GR, Hickford JGH. (2017) Variation in the Toll-like Receptor 4 (TLR4) gene affects milk traits in dairy cows. J. Dairy Res. **84**(04):426–429. doi:10.1017/s0022029917000711