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Polymorphism of the BoLA-DRB3 gene in the Ukrainian brown breed

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The article presents the results of a study of polymorphism in the Ukrainian Brown breed (UB) for the BoLA-DRB3 gene. Experimental data were obtained using the PCR-PCRF method. The genotypes of the animals were determined based on the results of typing blood samples from 30 cows and semen from 10 bulls. Twenty-three alleles and 18 genotypes were identified. The proportion of each of the three most common BoLA-DRB3 alleles, *27, *28 and *50, was 10 %. Among other variants, three more stand out with a frequency of more than 5 %: *8, *19 and *36. The most frequently detected genotype was BoLA-DRB3.2*32/*54, which was typed in 9 cows (15.0 %). The total frequency of consolidating alleles was 50 %. Several rare alleles (BoLA-DRB3.2*36, *50, *71 and *98) were detected in the sample, which were rarely found in studies of Major Histocompatibility Complex (MHC) polymorphism in other cattle breeds. A small pool of consolidating alleles and the presence of rare variants with high frequencies indicate the influence of natural selection and regional characteristics of the breed. The observed (0.9) and expected (0.94) heterozygosity indices indicate a high level of genetic variability in the tested animals, which is confirmed by the highest values of the effective number of alleles (16.7) and the efficiency index (0.726). A slight deviation from panmixia detected by Wright's index proved to be unreliable. The high level of biodiversity of UB is confirmed by the Shannon index (2.97). The presence of specific alleles, established indicators of variability and the Shannon index indicate a high level of genetic differentiation and biodiversity of the breed. Autochthonous populations have unique BoLA-DRB3 variants, which serve as a genetic reserve for future selection and development of the dairy industry, especially in the context of climate change or the emergence of new diseases, as well as a source of genetic diversity for the cattle gene pool.

Key words: Brown Carpathian cattle, major histocompatibility complex, polymorphism, BoLA-DRB3 gene, alleles, PCR-RLFP, genetic variability

Introduction

The Carpathian brown cow is a local (indigenous) endangered breed. It was created through selective crossbreeding of local cattle breeds, Ryzhka and Mokana, with brown Alpine breeds (in particular, Swiss breeds). The goal was to obtain animals that were well adapted to the climatic conditions of Ukraine and had increased milk productivity. It was officially registered in 1972. The breed is versatile, producing good quality milk and meat: average milk yield is 4000–5000 kg per year with a fat content of 3.7–4.0 %; meat yield is up to 60 %. The animals are hardy, tolerate both heat and cold well, are adapted to pasture grazing, and are undemanding in terms of feed. They have a calm, obedient temperament. They are most widespread in the central, northern, and western regions of Ukraine.

Aboriginal breeds are usually local, historically formed populations of animals adapted to specific natural and climatic conditions. They preserve a unique gene pool, which is often lost due to intensive selection for productivity in modern industrial herds. Studies show that indigenous Ukrainian cattle breeds have higher genetic variability compared to commercial breeds, which ensures adaptability to stressful conditions such as heat, feed shortages, and disease; genetic stability in small populations; a gene pool reserve for future breeding programmes.

The genetic diversity of cattle breeds is a critically important resource for breeding, adaptability to environmental changes, disease resistance and ensuring the long-term development of the livestock industry. In this context, the BoLA-DRB3 gene, which belongs to MHC and has significant polymorphism of exon 2, is of particular interest. It encodes polymorphic regions of the MHC II molecule that are directly involved in the presentation of antigens to CD4$^+$ T-lymphocytes [9]. The polymorphism of this locus determines the range of peptides that the immune system can present, *i.e.*, it is directly related to resistance or susceptibility to infectious agents. For this reason, BoLA-DRB3 is widely used to assess genetic diversity and as a potential marker in disease resistance selection, *i.e.* its variants can be DNA markers of disease susceptibility.

Aboriginal populations around the world are characterised by high allelic diversity of BoLA-DRB3 alleles, often with region-specific or unique alleles [13]. This indicates a high level of natural selection and adaptive potential in these populations, in contrast to intensively selected dairy breeds such as Holstein, where a reduction in allele diversity for this gene has been observed.

Autochthonous breeds, as carriers of unique BoLA-DRB3 alleles, can serve as a genetic reserve for future selection and development of the dairy industry, especially in the context of climate change or the emergence of new diseases. For their further effective use and support, regular monitoring of DNA markers is necessary, including research into the allelic polymorphism of the BoLA-DRB3 gene.

Analysis of recent research and publications

The work was performed using the PCR-RLFP method [15]. Despite the expansion of advanced modern SBT-typing technologies, the PCR-RLFP scheme is still widely used to detect allelic polymorphism of the BoLA-DRB3 gene. The results of studying BoLA-DRB3.2 allele polymorphism are used to determine three main directions of breeding work [1, 2, 5, 7, 11, 16]:

— identification of associations with cattle diseases and somatic cell count;

— identifying associations with economically useful traits;

— genetic and population analysis of the biodiversity of *Bos taurus* and *Bos indicus*.

In previous studies using the PCR-RLFP method on four Ukrainian breeds (black-and-white dairy, red-and-white dairy, grey and white-headed) with the participation of the authors of the article, 37, 31, 28 and 29 alleles of the BoLA-DRB3 gene were identified, respectively [12]. Over the past two decades, researchers have focused their attention on local and indigenous breeds [6, 8, 9, 10, 14]. To date, the allele spectrum of two local breeds has been established in this direction: Ukrainian grey and Ukrainian white-headed [11, 13]. Research on other domestic breeds, in particular Ukrainian red dairy, as well as river buffalo populations, is continuing.

Purpose

The work is devoted to the study of polymorphism of the BoLA-DRB3 gene of the brown Carpathian breed to replenish the cattle genotype bank based on the results of molecular genetic analysis of MHC. The results deepen the data on the genetic structure and allelic diversity of Ukrainian and world breeds. The formation of more complete and representative genetic databases by supplementing them with new data is an important condition for improving the effectiveness of modern breeding programmes, in particular for the selection of animals with valuable genetic characteristics, optimisation of breeding work and ensuring the sustainable development of animal husbandry.

Materials and Methods

Molecular genetic studies were conducted in the laboratory of genetic of the M. V. Zubets Institute of Animal Breeding and Genetics of the National Academy of Agrarian Sciences of Ukraine. The study used blood and semen samples from 40 Ukrainian Brown (UB) animals, 30 cows and 10 bulls. Blood samples were collected during an expedition in the private sector of the Transcarpathian region. Bull semen samples were collected from the M. V. Zubets Institute of Animal Breeding and Genetics Animal Genetic Resources Bank.

PCR-PCRF studies of BoLA-DRB3 polymorphism were performed based on the following five steps [12, 13]:

1. Isolation of DNA from blood or semen samples.
2. Amplification of specific short DNA fragments of exon 2 of the DRB3 locus, 284 bp in size (281 bp in the presence of a deletion).
3. Restriction treatment of the amplified mixture of DNA fragments using specific restriction endonucleases: *RsaI*, *HaeIII* and *BstY(XhoII)*.
4. Vertical electrophoresis of restriction products in polyacrylamide gel.

5. Deciphering alleles from the obtained electropherograms. PDRF allele numbers were identified based on the extended nomenclature in the format "locus.exon*allele" [3].

For statistical analysis based on direct counting of alleles (N_a) and genotypes (N_G), allele frequency (P_a), genotype frequency (G_a) and observed heterozygosity (H_o) were determined. The expected heterozygosity was determined by the frequencies of all detected alleles using the formula $H_e = 1 - \Sigma P^2_{ai}$. Genetic variability was assessed using Wright's individual fixation index (F_{is}), the effective number of alleles $A_e = 1 / (1 - H_e)$ and the efficiency index A_e/N_a, which shows the proportion of effective alleles among the total number detected in the experimental sample. Biodiversity was assessed based on Shannon's information index $I = -\Sigma (P_{ai} \times InP_{ai})$.

Data processing was performed in the standard *Microsoft Excel 2013* program based on the integrated GenAlEx GenAlEx 6.51 package (http://biology-assets.anu.edu.au/GenAlEx/Download.html). For other calculations, the *PAST 4.03* program (https://www.nhm.uio.no › downloads › past4manual) was used.

Results and Discussion

From the processing of 40 electropherograms, data on the allelic composition of the Ukrainian Brown (Carpathian region) breed were obtained (table 1).

Table 2 shows the typing results and calculated values of genetic differentiation of the UB breed at the DRB3 locus. A total of 23 alleles and 18 genotypes were identified with average frequencies of 4.35 % and 5.56 %, respectively. The most common alleles were BoLA-DRB3.2*27, *28 and *50 with a frequency of P_a = 0.1 and the BoLA-DRB3.2*32/*54 genotype, which was typed in 9 cows. Among other variants, three more stand out with $P_a \geq 5\%$: *8, *19, *36. The total frequency of consolidating alleles was only 50 %, which indicates a fairly high level of biodiversity in the breed. It should also be noted that the BoLA-DRB3.2*36, *50, *71 and *98 alleles are rarely found in scientific literature. The presence of such rare (specific) variants with high frequencies indicates the influence of natural selection and regional characteristics of the breed.

As noted, autochthonous and local breeds have a high level of genetic variability and biodiversity compared to industrial breeds. In different regions of the world (Africa, Asia, Latin America), indigenous breeds demonstrate a rich BoLA-DRB3 allele pool, often with several unique or rare alleles that are absent in industrial breeds [9]. Some alleles of the locus are region-specific and probably arose under the influence of local selection and under the influence of pathogens and climate [4].

The established excess of homozygotes was not statistically significant. Testing for the χ^2 criterion for a single-locus system shows that the deviation from Hardy-Weinberg equilibrium is not reliable ($\chi^2 = 0.0017 < \chi^2_{\min}$). Therefore, statements about possible shifts towards outbreeding should be considered false.

Table 1. Results of typing exon 2 of the BoLA-DRB3 locus in cows and bulls of the Ukrainian Brown breed

Cows (fragment)			Bulls		
Sample No.	General pattern	Genotype	Sample No.	General pattern	Genotype
1	obb	*28	86 Kozel	obb	*28
	tba	*39		uba	*40
2	maa	*32	92 Radist	bbb	*03
	jdb	*54		bba	*02
3	maa	*32	96 Ryck	obb	*28
	jdb	*54		aaa	*01
4	maa	*32	111 Metsor	oba	*37
	jdb	*54		oba	*37
5	oaa	*25	98 Mahovyk	obf	*27
	maa	*32		obe	*98
6	lbb	*20	112 Reid	mba	*22
	nbb	*24		lbb	*20
7	bbb	*03	90 Tsezar	obb	*28
	aaa	*01		oba	*37
9	bba	*02	87 Sjurpryz	faa	*08
	jdb	*54		lba	*36
14к	jbd	*16	91 Kashtan	ibb	*71
	jbd	*16		sbb	*19
15к	jbd	*16	102 Parol'	xba	*50
	rcc	*05		nba	*23

Note. Sample numbers are given in accordance with the numbers of the Genetic Resources Bank of the Institute of Animal Breeding and Genetics.

Table 2. Allele spectrum, genetic variability and biodiversity of Ukrainian brown cattle according to the BoLA-DRB3 gene

Number				Heterozygosity		Wright's F-statistics, F_{is}	effective number of alleles, A_e	efficiency index, A_e/N_a	Shannon's information index, I
genotypes, N_G	alleles, N_a	homozygotes	heterozygotes	observed, H_o	expected, H_e				
18	23	2	18	0.9	0.94	−0.044	16.7	0.726	2.97

The information of the level of genetic variability and biodiversity of UB must be analysed in comparison with other Ukrainian breeds for which the polymorphism of the BoLA-DRB3 gene has been studied (table 3).

The variability of genetic diversity can be conveniently assessed using the number of effective alleles (A_e). This indicator reflects the conditional number of alleles with identical frequencies that would ensure the same level of genetic diversity as actually observed in the sample. In other words, Ae transforms the real, often uneven distribution of allele frequencies into a balanced model in which each allele has an equal probability of appearing. This is a convenient criterion for comparing samples in which the number and structure of allele frequencies may differ significantly. In population genetic studies, with similar heterozygosity values, the number of effective alleles provides a more accurate and unified assessment of the actual level of diversity. The value of A_e = 16.7 established for the brown breed is quite high, which characterises the high level of biodiversity of the studied population.

Table 3. Polymorphism of BoLA-DRB3 gene alleles for autochthonous Ukrainian cattle

Ukrainian breeds	Alleles with a frequency of $P_a \geq 5$ % and their total proportion (%)	$_{N}a$	H_o	He	$_{FI}S$	A_e	A_e/N_a	I
Ukrainian Brown	*8, *19, *27, *28 *36, *50 / 50.0	23	0.9	0.94	−0.044	16.7	0.726	2.97
Grey Ukrainian [12]	*06, *12, *16, *78 / 69.9	28	0.774	0.742	0.041	4.42	0.158	2.21
White-headed Ukrainian [12]	*03, *12, *14, *16, *22, *23, *24 / 65.3	28	0.927	0.959	−0.035	13.7	0.489	2.94

A more accurate assessment of the level of genetic variability is considered to be the efficiency index, which shows the proportion of consolidating alleles among all those detected in the sample. It characterises the overall efficiency of the locus, which does not depend on the number of alleles detected. The results of the study showed that the brown breed has the highest efficiency index value of $A_e/N_a = 0.726$ among domestic populations.

The Wiener-Shannon-Ashby law of requisite variety states that for a biological system to exist sustainably, it must have sufficient internal diversity to compensate for external and internal influences. It can be quantified using the Shannon index, which reflects the entropy of the distribution of elements in the system: maximum values of the index indicate the highest chaos of allele distribution, while lower values indicate its orderliness. The sensitivity of the index to changes in high-frequency allele variants allows for an effective assessment of the biodiversity of the allele-saturated exon 2 of the BoLA-DRB3 gene. The calculated value of the index $I = 2.97$ indicates a high level of biodiversity in the brown breed.

Based on the results obtained, it can be stated that the Ukrainian brown breed is unique in terms of genetic variability for the BoLA-DRB3 gene. Two rare BoLA-DRB3.2*71 and *98 alleles were identified, which had not been previously reported in scientific literature. The presence of specific alleles, heterozygosity indices, effective number and efficiency index indicate a high level of genetic differentiation of the breed, which is confirmed by the high Shannon index.

Indigenous cattle populations harboring unique BoLA-DRB3 variants should be given priority in gene pool conservation programs as vital reservoirs of genetic diversity. These results should be incorporated into dedicated genetic databases and breeding strategies. Moreover, they enhance our understanding of the allelic diversity and distribution of BoLA-DRB3 in Ukrainian cattle and contribute to broader insights into the global polymorphism and variability of the BoLA-DRB3 gene.

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Поліморфізм гена BoLA-DRB3 української бурої породи

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У статті наведено результати дослідження поліморфізму української бурої породи (UB) за геном BoLA-DRB3. Експериментальні дані отримано методом ПЛР-ПДРФ. За результатами типування зразків крові 30 корів і сперми 10 бугаїв встановлено генотипи тварин. Виявлено 23 алеля і 18 генотипів. Частка кожного з трьох найбільш поширених алелів BoLA-DRB3.2*27, *28 і *50 становила 10 %. Серед інших варіантів виділяється ще три з частотою понад 5 %: *8, *19 і *36. Максимально виявлено генотип BoLA-DRB3.2*32/*54, який типовано у 9 корів (15 %). Сумарна кількість частот консолідуючих алелів склала 50 %. У вибірці виявлено декілька малопоширених алелів (BoLA-DRB3.2*36, *50, *71 і *98), які рідко виявляли в дослідженнях поліморфізму МНС інших порід ВРХ. Невеликий пул консолідуючих алелів та наявність малопоширених варіантів з високими частотами свідчать про вплив природного добору та регіональні особливості породи. Показники спостережуваної (0.9) та очікуваної (0.94) гетерозиготності вказують на високий рівень генетичної мінливості протестованих тварин, що підтверджують найбільші значення ефективного числа алелів (16.7) та індексу ефективності (0.726). Незначне відхилення від панміксії виявлене за індексом Райта виявилося недостовірним. Високий рівень біорізноманіття UB підтверджується величиною індексу Шенона (2.97). Наявність специфічних алелів, встановлені показники мінливості та індексу Шенона свідчать про високий рівень генетичної диференціації та біорізноманіття породи. Аборигенні популяції мають унікальні BoLA-DRB3 варіанти, які слугують генетичним резервом для майбутньої селекції та розвитку молочної галузі, особливо в умовах змін клімату чи появи нових захворювань, а також як джерело генетичного різноманіття генофонду ВРХ.

Ключові слова: українська бура худоба, головний комплекс гістосумісності, поліморфізм, ген BoLA-DRB3, алелі, ПРЛ-ПДРФ, генетична мінливість

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