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POLYMORPHISM OF THE GENE BOLA-DRB3 UKRAINIAN BREEDS OF CATTLE

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KEYWORDS

polymorphism, alleles, BoLA-DRB3.2 gene, DNA marker, cattle, Ukrainian cattle breeds

ABSTRACT

Polymorphism of alleles of exon 2 of the BoLA-DRB3 gene of three Ukrainian cattle breeds was investigated. the cows of the Ukrainian black-pied dairy breed detected 32 alleles out of 54 possible, which were typed using the PCR-RFLP method and 74 genotypes.

In the red-pied dairy breed population, the allele spectrum consists of 22 alleles and 35 genotypes are identified. 28 alleles of BoLA-DRB3.2 were established for two studs of aboriginal Ukrainian gray breed, of which five non-nominated PCR-RFLP (* jab, * jba, * jbb, * nad and * nda). the received results are needed in further studies to establish associations between the alleles of the BoLA-DRB3.2 gene, diseases and utility-useful signs.

1. INTRODUCTION

The population of the Earth is growing rapidly, which aggravates the problems associated with supplying mankind with food. Now almost 800 million people in the world are inadequately fed. the UN predicts that in 2050 the world's population will grow to 9.1 billion people. to provide such a large number of people with food, the agricultural products production should increase to 70% [1].

Animal husbandry is one of the most dynamic branches of agriculture. Over the past decades, it has developed rapidly. it is expected that by the middle of this century, the demand for livestock products will continue to grow rapidly due to population growth, increased prosperity and urbanization. in the food balance, livestock products are constantly growing. at the global level, livestock provides 15% of food the energy value and 25% of food protein [34].

Modern tasks of accelerating the livestock production development to increase production require the new approaches development to the animal genetic resources

management. Great hopes are placed on modern achievements in the field of genetics and biotechnology. the animal husbandry development at the present stage is impossible without the introduction of new biotechnological methods for assessing the signs of the farm animals' productivity, based directly on the analysis of hereditary information. Advances in molecular biology, molecular genetics and genetic engineering have led to the widespread use of molecular genetic methods in various fields of science and practice, including livestock breeding [4,41]. Therefore, the development and introduction in animal husbandry of DNA diagnostics based on genetic markers is an urgent task of breeding and selection of farm animals.

A genetic marker is any biological carrier of information, which makes it possible to distinguish one individual (or cell, virus) from another on the polymorphic system basis. Since the transfer and realization of hereditary information is a fundamental basis of biological diversity, genetic markers can be classified according to the level of the genetic structure (DNA, RNA, cytogenetic and protein markers) [12, 27]. a genetic marker is a broad term for any apparent phenotype or genetic basis for assessing the observed phenotypic variability.

The bigger part economically valuable breeding features have a polygenic character, which is controlled by a group of genes, which leads to a significant variability in the phenotypic manifestation of the desired features under the environmental factors influence. at the same time, there are genes or a group of genes whose alleles in postnatal ontogenesis cause a clear manifestation of the desired performance characteristics regardless of the environmental conditions in which the animal is located. DNA sections containing genes or linked to genes that are responsible for a certain quantitative trait are called quantitative traits loci (QTL).

Molecular markers (DNA markers) are genetic markers considered at the DNA level. DNA markers are the third generation of genetic markers. They were preceded by classical genetic and protein markers.

With the introduction of DNA markers, the greatest scope has been acquired, among others, by building molecular maps of individual chromosomes and genomes, mapping the genes and loci of quantitative traits on them. Mass distribution of works on gene mapping, as well as loci of quantitative traits began with the appearance of cheaper and more convenient PCR markers. it was with them that the widespread introduction of DNA markers into the selection process began [11].

With the development of molecular biotechnology, molecular markers are rapidly progressing and becoming increasingly informative. Various types of molecular markers are used to evaluate polymorphisms of DNA. They make it possible to solve the problem of genome saturation with markers and mark almost any part of the DNA. with their help, you can analyze any tissues and organs, regardless of the stage of body development.

In modern breeding, based on DNA markers, there are two main directions: MAS (Marker Assisted Selection) and genomic selection.

The MAS method involves the use of DNA markers closely linked to the target gene, with the possible use of phenotypic analysis. Markers closely linked to the target gene are the reliable tool for predicting the phenotype. Selection of the desired allele of the target gene is carried out because of the closely related allele of the neighboring marker locus [11].

Genomic selection allows using genomes of DNA markers that are evenly distributed across the genome to select genotypes in the absence of data on genes that affect a particular feature.

MAS is a combined product of traditional genetics and molecular biology, allowing the selection of genes coding for utility signs. the gene variants identification allows, in addition to the traditional animals' selection, to select directly at the DNA level.

Marker selection in livestock has a number of advantages over traditional methods, namely:

- does not depend on the variability caused by the external environment;
- the possibility of assessing and selecting animals at an early age in industry;
- lack of dependence on the sex of the animal;
- reduction of costs and breeding timing.
- facilitates identification and rapid introduction of the desired alleles from resource populations in the recipient population to increase productivity and resistance to diseases of improving animal breeds [11].

The improvement of complex quantitative traits of economic importance in dairy cattle breeding depends on the identification of genes controlling these features, as well as polymorphic DNA variants in these genes that directly affect their phenotypic manifestation. Currently, 11 543 QTL are known, representing 481 signs of cattle. the efforts of researchers are aimed at finding candidate genes localized near QTL for economically important signs or genes that affect the physiological effects of these traits, as well as the nucleotide substitutions in them that contribute to phenotypic differences. When the association between DNA polymorphism and an economically important feature

is reliably established, such polymorphism can be included in MAS selection programs. the use of such a marker can significantly improve the quality of selection [39].

Most species of genes are quite conservative in nature. However, there are several genetic groups in which polymorphism is evolutionarily entrenched. the most polymorphic of these are genes responsible for blood groups and genes of the main histocompatibility complex.

The Major Histocompatibility Complex (MHC) is the main part of the immune system of almost all vertebrates. This is one of the most important genetic systems of resistance to infectious diseases in vertebrates. Therefore, the definition of structure, function and its diversity is important for understanding the immune response. There are three groups of histocompatibility antigens, classes I, II and III.

The MHC genes of cattle (BoLA) are located on the 23 chromosome (Fig. 1) and encode lymphocytic antigens (class i and II molecules)

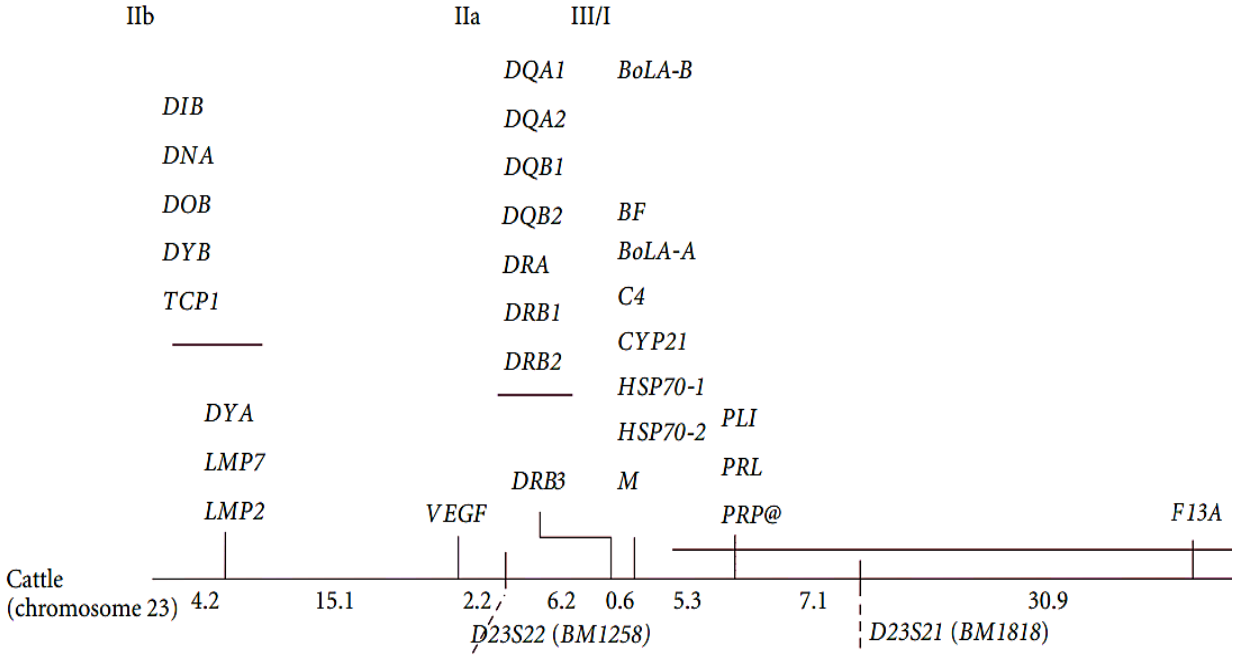

Fig.1. Genetic links map of the main histocompatibility complex in cattle

Class i molecules consist of an alpha chain with a molecular mass of about 45 kDa, which is associated non-covalently with the β2-microglobulin chain, which is about 12 kDa. Class i molecules are expressed in all nucleophilic cells, and their main function is to present peptides to CD8+T-lymphocytes that kill virus-infected and neoplastic cells.

Class II molecules are formed non-covalently by the association of α- and β-chains encoded by individual genes in the MHC. Both strands of class II molecules (33 kDa

and 28 kDa, respectively) are encoded by genes within the MHC. Class II molecules are expressed in antigen-presenting cells, such as dendritic cells and macrophages. Class II moleculeson these cells represent peptides derived from extracellular pathogens, to CD4+T cells, which simultaneously stimulate the activation of macrophages and B-cells to create anti-inflammatory and antibodies, respectively.

Class III molecules include products that differ somewhat from the MHC molecule, but are also associated with the immune process, for example, components of the complement system, steroidal 21-hydroxylase enzymes and tumor necrosis factors [3].

The genomic organization of MHC ruminants differs from the level of mice and humans, since the ruminant class II region is split into two subregions that are separated by at least 15 sM (from DYA in the class IIb region to DRB3 in region class IIa). Class IIa subregion includes two clusters of DR and DQ genes. the organization of class II cattle genes arose through chromosomal inversions in the ancestors of mammals.

Class IIb region includes the genes DMA, DMB, LMP2, LMP7 and TAP, which are involved in the processing and transport of antigens and other type II genes such as DNA, DOB, DIB, DYA and DYB, whose function is unknown.

Thus, BoLA exists as a system, but in the form of separate clusters; some locus are closely related, while others are relatively remote. There are at least three DRB locus in cattle, but only one DRB3 gene is functional. the study of polymorphism of the locus is important, since this region represents the antigen and the variability in this region can be related to the immune response to various pathogens. Also, the analysis of polymorphism DRB3 is useful for studying the evolutionary history of MHC in the ruminant species.

The alleles of the second exon of the BoLA-DRB3 gene as DNA markers are used in many studies of cattle. They became the most widespread in connection with the search for "allele-disease" associations. the alleles were found to be closely related to leukemia [10, 25, 35], mastitis [20, 23, 32], necrobacteriosis [31, 30], and the content of somatic cells in milk [2, 5, 24, 26]. Relationships between the BoLA-DRB3 gene and the less common cattle diseases are studied: FMD [8], tick-borne diseases [6, 16], theileriosis [14], tuberculosis [10], etc. Actively conducted research on the influence of the gene BoLA-DRB3.2 on the economically useful signs of cattle [9, 13, 21, 22, 23, 25, 26, 27, 40] (Table 1).

Tab. 1. the association of the alleles of BoLA-DRB3.2 with utility characteristics and resistance (susceptibility) to diseases [5, 9, 13,15,16,21, 22, 23, 26, 27, 31]

Alleles	linking	
	positive	negative
03	Resistance to placental abruption, reduced number of somatic cells (SCC - somatic cell count) and high protein content in milk, resistance to necrobacteriosis	-
07	High-quality milk, easy calving, resistance to mastitis	Low resistance to viral and bacterial infections
08	High milk production, easy calving	Low resistance to viral and bacterial infections, increased quantity of SCC in milk, susceptibility to mastitis
09	High protein content in milk	-
11	High resistance to viral and bacterial infections, the high level of fat in milk, resistance to mastitis, reduced the number of SCC	Low milk productivity
13	Resistant to mastitis	-
16	High milk production and protein content in milk, resistance to cystitis	Low resistance to viral and bacterial infections (in particular in mastitis and necrobacteriosis)
18	Resistant to mastitis and tick-borne diseases	-
21	Growth, strong constitution	Heavy calving, large fetus
22	High milk productivity, resistance to cystitis, necrobacteriosis and mastitis	Low resistance to viral and bacterial infections, increased amount of SCC in milk, low protein content in milk
23	High resistance to viral and bacterial infections	Low milk productivity, increased quantity of SCC, the tendency to mastitis and necrobacteriosis
24	High milk productivity	Low resistance to viral and bacterial infections, susceptibility to mastitis
26	High protein content in milk, easy calving	Susceptibility to mastitis
27	High levels of fat in milk, easy calving, resistance to mastitis and tick-borne diseases	-
28	High resistance to viral and bacterial infections	Low milk production

The following statistics testify to the importance of the BoLA-DRB3 gene. the study of polymorphism by this gene using the PCR-RFLP method in more than 30 samples of cattle. More than 10 samples were analyzed by sequencing. the most thoroughly studied Holstein and Holsteinized breeds. All studied rocks (and rock samples) differ in both spectrum and frequency of the alleles of the BoLA-DRB3 gene. the number of RFLP-alleles varies in a wide range from 9 in Argentine Holstein [17] to 32 in Sistani [19] and Ukrainian black-pied [31], SBT alleles from 14 in Japanese jersey to 33 in Japanese black [18] breeds. Some of the alleles were present in most of the studied species (SBT alleles BoLA-DRB3 *0101, *0201, *0301, *0501, *0502, *0503, *0504, *0601, *0701, *0801, *0901, *0902, *1001, *1101, *1103, *1201, *1301, *1302, *14011 and the RFLP-alleles of BoLA-DRB3 *08, *15, *23), others were found in separate rocks. the spectrum of BoLA-DRB3 alleles

in European breeds (Bos Taurus) of cattle and in zebra-like cattle (Bos indicus) differ significantly. All this points to the high information content of the polymorphism of the BoLA-DRB3 gene as a genetic marker.

2. MATERIALS AND METHODS

The maximum accuracy of typing alleles was achieved using three independent approaches:

- restriction analysis of PCR-RFLP amplification products [29];
- allele-specific PCR with primers ER-17 and VD-19 [18];
- allele-specific PCR with primers HLO-07 and HLO-24d [28].

RFLP analysis reduces to DNA digestion with restriction enzymes, followed by electrophoretic distribution of the resulting mixture and determination of the length of the resulting fragments after blot-hybridization with a specific labeled probe. Endonucleases have strictly specific cleavage sites. Therefore, the genetic differences between the nucleotide sequences of DNA between individuals (polymorphism at the DNA level) lead to a different distribution of restriction sites along the corresponding DNA molecules and the production of restriction products in which the length of homologous fragments will be different. Thus, DNA polymorphism is tested as RFLP. the advantage of this type of markers is the high reproducibility of the results, as well as the codominant type of inheritance. RFLP-loci can have multiple alleles, which significantly increases their informatively.

PCR-RFLP. This method refers to enzymatic methods for analyzing SNPs and is similar to the RFLP method, but it is based on the use of PCR. Restriction (one or more restriction enzymes) in this case are subjected to amplification products, rather than DNA genomes. Due to its simplicity and reliability, the method has become widely used and is still used to analyze allelic polymorphism of genes in the objects' variety [23, 29].

DNA isolation was carried out using "DIAtomTMDNA Prep200" kits from Isogen Laboratory Ltd. in accordance with the manufacturer's requirements. DNA isolated from fresh biological material (yield is 5-10 mg with 200 ml from whole blood) high molecular weight (40-50 tis.pn) and pure (OD260 / 280 nm = 1.6-2.0) substance. Isolation of DNA was carried out in an isolated room under the laminaria using a separate set of pipettes to avoid contamination of the samples.

The DNA concentration was determined visually. to this end, on 1% agarose gel was applied to the phage λ in an amount of 25, 50, 100 ng and aliquots from a solution with an unknown concentration.

Electrophoresis was performed in 1xTrisborate (TBE) buffer (89mM Tris-OH, 89mMH3BO3, 2mMEDTA) with ethidium bromide (10-3 mg/ml) added to the gel to stain DNA at a constant voltage of 120V. to avoid contamination, electrophoresis was performed in the separate room.

The DNA concentrations of the test samples were determined by comparing the fluorescence intensity of the aliquots from solutions with unknown concentration and control phage λ phage DNA.

For further analysis by nested PCR, the exon 2 region of the BoLA-DRB3 gene, 284 bp in size, was amplified. (281 bp for deletion alleles).

For the first round of the reaction primers were used: HLO-30 (5'-3 ': TCCTCTCTCTGCAGCACATTTCC) and HLO-31 (5'-3': ATTCGCGCTCACCTC GCCGCT). 5 ml DNA was used as a template, regardless of its concentration. For the second round, PCR primers were used: HLO-30 and HLO-32 (5'-3 ': TCGCCGCTGCACAGTGAAACTCTC).

For the restriction analysis of the exon 2 fragment of the BoLA-DRB3 gene, restriction end nucleases RsaI, HaeIII, XhoII of Promega, USA, New England BioLabs, RsaI, HaeIII, XhoII of Promega, USA, New England BioLabs, USA and SibEnzim, Russia. the restriction fragments were separated by electrophoresis in a 4% agarose gel (Fig. 2).

Based on the restriction patterns, 54 allelic variants of the BoLA-DRB3 gene were developed [36, 37].

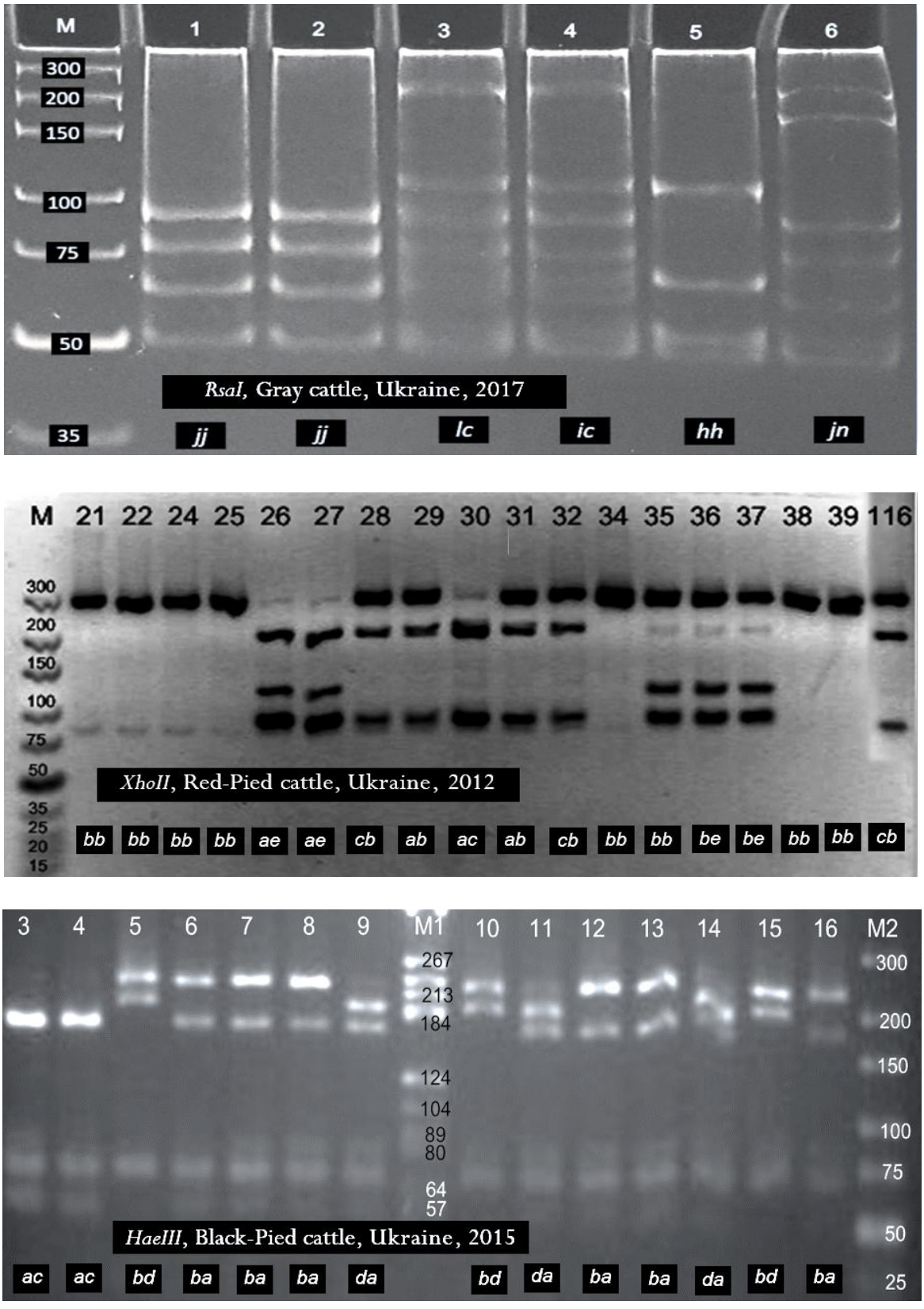

Fig.2. Foregrams of amplification products of the BoLA-DRB3 of exon 2 gene obtained on the DNA of Ukrainian breeds cows using various end nucleases (below are the variants of DNA patterns).

For today, there are two variants of allele names for the BoLA-DRB3 gene. Alleles determined by sequencing are called four-digit sequence numbers. the alleles described

by the PCR-RFLP method are numbered in order from first to fifty-fourth. If alleles that are not included in the nomenclature of PCR-RFLP are detected as a result of the fore gram processing, then the record of the allele without the established nomenclature is carried out in the form of three consecutive letters that correspond to certain restriction patterns RsaI, HaeIII and XhoII. the paper uses the RFLP nomenclature.

Allele-specific PCR (AC-PCR). in cases where it was impossible to determine the genotype of the animal using restriction analysis, the allele-specific PCR method was used (Fig. 3).

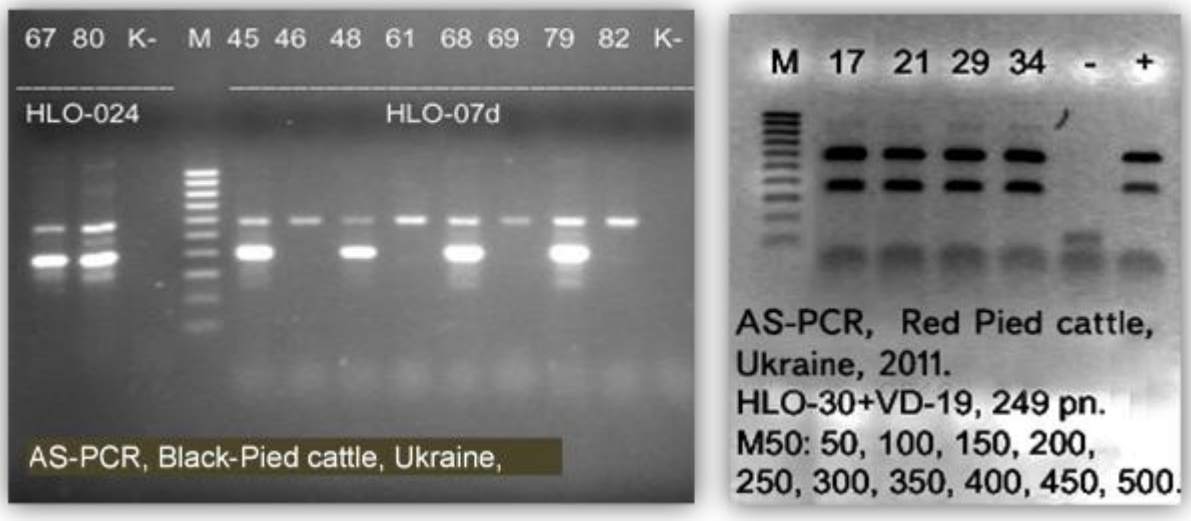

Fig.3. Variants of AS-PCR with different primers

Allelic variants differ due to the fact that the 3'-terminal nucleotide of one of the primers hybridizes directly to the variable nucleotide (SNP position), which causes the presence or absence of PCR. the specificity of the reaction can be increased by introducing an additional, not paired, nucleotide in the second or third position from the 3 'end of the same primer or using a competitive PCR in the same tube.

Allele-specific PCR was carried out using ready-made sets of "GenPakR PCR Core" from the company "Isogen Laboratory". the PCR products of the first round of nested PCR were used as a template.

As a result of typing, cow genotypes were obtained and the exon 2 polymorphism of the BoLA-DRB3 gene was analyzed.

The allele frequencies were counted taking into account the number of homozygotes and heterozygotes found from the corresponding allele of formula

$$P(A) = \frac{2N_1 + N_2}{2n} \quad (1)$$

where N1 and N2 are, respectively, the number of homozygotes and heterozygotes for the allele under investigation;

n - is the sample size.

The frequency of genotypes is determined by the formula

$$P(G) = N/n \quad (2)$$

where N - is the number of corresponding genotypes in the sample.

The statistical error in the frequencies of alleles of the gene is determined by the formula:

$$S_p = \sqrt{pq/n} \quad (3)$$

where p is the frequency of the allele, and q = 1 - p;

n - is the sample size.

3. RESULTS AND DISCUSSION

At the present time polymorphism by gene BOLA-DRB3 is studied in three Ukrainian breeds: black-pied dairy, red-pied dairy and gray. Ukrainian black-pied and red-pied dairy cattle refer to industrial breeds. Ukrainian black-pied dairy livestock is spread throughout the country. She has the largest number of livestock among other breeds of cattle. the total number of the breed is 2500000, including 1800000 cows. Ukrainian red-pied dairy breed is common in 14 regions of Ukraine. the total number of broodstock is about 1500000 head, of which about 500 thousand cows. the Ukrainian gray breed is a native aboriginal breed. at present, the population is 850 animals, including 13 bulls and 364 cows.

Ukrainian black-and-white dairy breed. the allelic spectrum of Ukrainian black-pied dairy cattle was studied in 2009-2015 on five samples in various farms of the Khmelnitsky region. the study was conducted in two stages: 1st - 2009-2011; 2nd - 2014-2015 years. During the first stage, 162 were tested, and the second stage - 114 heads.

The data for the total sample are presented in Table. 2. it was found that 28 alleles (mean frequency 3.57%) of 54 PCR-RFLP and allele-specific PCR for the BoLA-DRB3.2 gene, encoding antigens of class II of the main complex, were determined in cows of Ukrainian black-pied milk breed histocompatibility of cattle.

With a frequency of more than 5% ("weighty" alleles), there were 7 alleles in the general group. Most often in the sample presented is the allele BoLA-DRB3.2*22, which is found in 31 cows, that is, its carriers are 19.1% of animals (including homozygotes). a higher frequency was recorded only for the allele *24, found in 34 cows (21% of the animals tested). Quite often it appeared among the total number of identified alleles - 38 cases (11.7%).

The border in P (A) ≥ 5% exceeded, also the alleles of BoLA-DRB3.2: *28-25 (7.7%), *08-24 (7.4%), *03-19 (5.9%), *10 and *13 - 17 cases (5.3%). the overall frequency of finding 7 "weighty" alleles was 59.6%. Fewer variants of 2 (0.6%) were found for alleles *16, *25, *31, *41 and *42.

The overall frequency of the detection of rare alleles (P (A) <5%) was 40.4%. There are no allele populations in the allele fund *05, *06, *09, *14, *17, *19, *27, *29, *30, *33-* 35, * 38-* 40, * 43-* 47, * 49 * 54.

Tab. 2. the distribution of the frequencies of the alleles of BoLA-DRB 3.2 in the population of Ukrainian black-pied dairy breed cows (n = 162)

Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)	Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)
***01**	5	0,015	0,685	***21**	6	0,019	0,749
***02**	8	0,025	0,862	***22**	39	0,12	1,808
***03**	19	0,059	1,305	***23**	6	0,019	0,749
***04**	7	0,022	0,808	***24**	38	0,117	1,788
***07**	16	0,049	1,204	***25**	2	0,006	0,435
***08**	24	0,074	1,455	***26**	14	0,043	1,13
***10**	17	0,053	1,239	***28**	25	0,077	1,482
***11**	5	0,015	0,685	***31**	2	0,006	0,435
***12**	12	0,037	1,049	***32**	10	0,031	0,961
***13**	17	0,053	1,239	***36**	10	0,031	0,961
***15**	6	0,019	0,749	***37**	11	0,034	1,006
***16**	2	0,006	0,435	***41**	2	0,006	0,435
***18**	8	0,025	0,862	***42**	2	0,006	0,435
***20**	3	0,009	0,532	***48**	8	0,024	0,862

The modern herd of Ukrainian black-pied dairy cattle is quite diverse in its genealogical structure. Most researchers define it as an open population. in the breed there are several species genotypes - Dutch, Estonian, Lithuanian, black-pied Moscow and other selections, and now there is a large-scale golshtinization of cattle [38]. Therefore, the presence of 28 alleles of the gene, which we determined, in the cows of this breed is consistent with its genealogy.

The study of the next stage confirms this conclusion. Repeated study of the allelic spectrum showed the following results (Table 3). the total number of detected alleles

increased to 32 (mean frequency 3.13%). in the allele fund of the population, new alleles appeared: BoLA-DRB3.2*06, *14, *19, and *51.

With a frequency greater than 5% in the general population, there were also 7 alleles, which together occupy 56.1% of the allele fund. the most common is the allele BoLA-DRB3.2*24, which carriers are 18% of animals. Alleles *22 (7.9%) and *28 (7.5%) were also often detected. the threshold in 5% exceeded the alleles BoLA-DRB3.2*08 and *09 (6.1% each), *03 and *16 (5.3% each). Alleles *06, *25, *31 and *41 were detected in less than one case at a frequency of 0.4%.

Tab. 3. the distribution of the frequencies of the alleles of BoLA-DRB 3.2 in the population of Ukrainian black-pied dairy breed cows (n = 114)

Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)	Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)
***01**	2	0,009	0,618	***20**	2	0,009	0,618
***02**	4	0,018	0,869	***21**	3	0,013	0,755
***03**	12	0,053	1,479	***22**	18	0,079	1,786
***04**	4	0,018	0,869	***23**	10	0,044	1,356
***06**	1	0,004	0,438	***24**	41	0,180	2,543
***07**	10	0,044	1,356	***25**	1	0,004	0,438
***08**	14	0,061	1,59	***26**	5	0,022	0,97
***10**	14	0,061	1,59	***28**	17	0,075	1,74
***11**	2	0,009	0,618	***31**	1	0,004	0,438
***12**	6	0,026	1,06	***32**	5	0,022	0,97
***13**	8	0,035	1,219	***36**	7	0,031	1,142
***14**	2	0,009	0,618	***37**	8	0,035	1,219
***15**	4	0,018	0,869	***41**	1	0,004	0,438
***16**	12	0,053	1,479	***42**	2	0,009	0,618
***18**	4	0,018	0,869	***48**	4	0,018	0,869
***19**	2	0,009	0,618	***51**	2	0,009	0,618

Comparison of the two-stage study results shows changes in the allelic spectrum of the black and pied breed (Fig. 4)

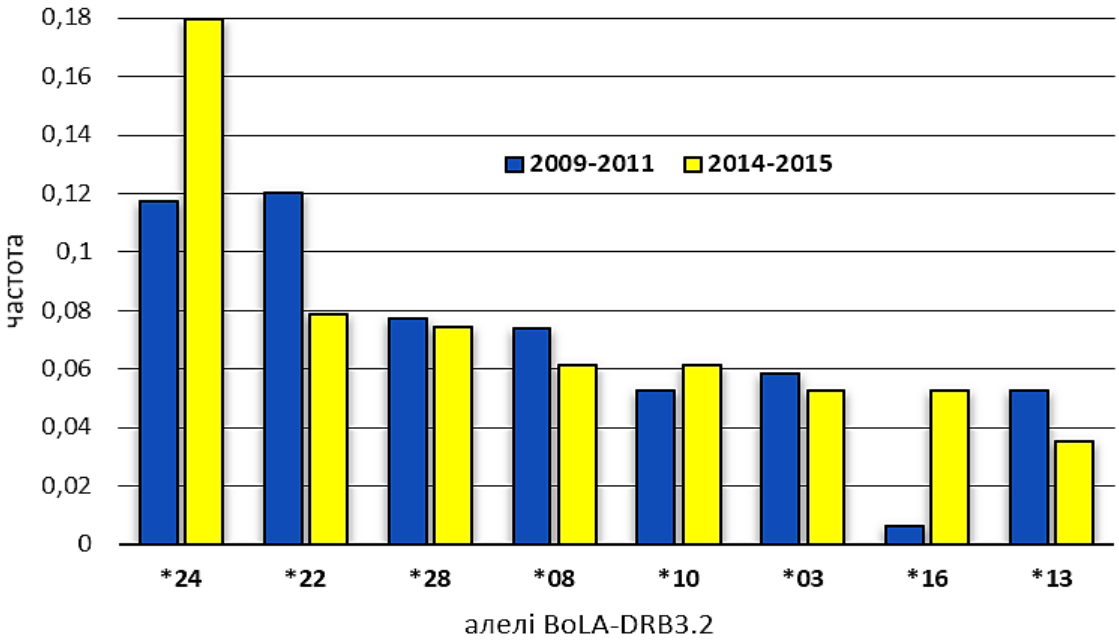

Fig.4. Changing the frequencies of "weighty" alleles of BoLA-DRB 3.2 of Ukrainian black-pied dairy breed at different stages of the study

There is a slight difference in the detection of alleles *03, *08, *10 and *28, the change in frequencies of which does not exceed 1.3%, while other "weighty" alleles are characterized by significant transformations. the most common of them show one of the highest increments *24 (+ 6.25%) and the maximum elimination *22 (-4.14%). the maximum frequency increase was established for allele *16 (4.64%), which due to this became "weighty". the share of allele *13 decreased by 1.74%, which translated it into the category of rare.

It should be noted that the most common alleles identified in our study are more often found in the Holstein breed of the USA and Canada. They display 8 alleles *03, *07, *08, *11, *16, *22, *23, *24 with a frequency of 4.15 to 16.69% [5, 26].

As the research result, 74 genotypes were identified in cows of Ukrainian black-pied dairy breed (Table 4).

genotype	number of genotype / %)	genotype	number of genotype / %)	genotype	number of genotype / %)	genotype	number of genotype / %)
***01/*03**	2 / 1,23	***07/*36**	2 / 1,23	***12/*18**	2 / 1,23	***22/*36**	2 / 1,23
***01/*22**	2 / 1,23	***08/*08**	2 / 1,23	***12/*28**	4 / 2,47	***22/*37**	2 / 1,23
***01/*48**	1 / 0,62	***08/*10**	2 / 1,23	***12/*42**	1 / 0,62	***23/*26**	2 / 1,23
***02/*13**	1 / 0,62	***08/*12**	4 / 2,47	***13/*21**	2 / 1,23	***24/*24**	4 / 2,47
***02/*22**	1 / 0,62	***08/*13**	3 / 1,85	***13/*23**	4 / 2,47	***24/*25**	1 / 0,62
***02/*28**	6 / 3,70	***08/*20**	2 / 1,23	***13/*24**	1 / 0,62	***24/*26**	2 / 1,23
***03/*11**	3 / 1,85	***08/*21**	2 / 1,23	***13/*26**	3 / 1,85	***24/*28**	2 / 1,23
***03/*13**	2 / 1,23	***08/*22**	3 / 1,85	***15/*24**	3 / 1,85	***24/*31**	1 / 0,62
***03/*24**	3 / 1,85	***08/*24**	2 / 1,23	***15/*37**	3 / 1,85	***24/*36**	2 / 1,23
***03/*32**	6 / 3,70	***08/*25**	1 / 0,62	***16/*36**	2 / 1,23	***24/*42**	1 / 0,62
***03/*48**	3 / 1,85	***08/*26**	1 / 0,62	***18/*24**	2 / 1,23	***26/*26**	1 / 0,62
***04/*07**	3 / 1,85	***10/*10**	5 / 3,09	***18/*37**	2 / 1,23	***26/*48**	1 / 0,62
***04/*24**	4 / 2,47	***10/*13**	1 / 0,62	***20/*48**	1 / 0,62	***28/*28**	1 / 0,62
***07/*07**	1 / 0,62	***10/*24**	1 / 0,62	***21/*22**	2 / 1,23	***32/*37**	2 / 1,23
***07/*18**	2 / 1,23	***10/*28**	3 / 1,85	***22/*22**	8 / 4,94	***32/*41**	2 / 1,23
***07/*22**	1 / 0,62	***11/*12**	1 / 0,62	***22/*24**	3 / 1,85	***36/*37**	2 / 1,23
***07/*24**	2 / 1,23	***11/*26**	1 / 0,62	***22/*26**	1 / 0,62	***48/*48**	1 / 0,62
***07/*26**	1 / 0,62	***12/*18**	2 / 1,23	***22/*28**	5 / 3,09		
***07/*28**	3 / 1,85	***11/*26**	1 / 0,62	***22/*31**	1 / 0,62		

Tab. 4. Distribution of genotypes in Ukrainian black-pied dairy breeds cows

The distribution of genotypes has the uniform character, since there are no allelic pairs that are sharply distinguished by the frequency of detection. There are no genotypes with the frequency of more than 5%. the most common cases are allelic pairs *22/*22 - 8 cases (4.94%), *02/*28 and *03/*32 for 6 cases (3.7%) and *10/*10 and *22/*28 for 5 cases (3.09%).

Ukrainian red-pied dairy breed. the allelic spectrum of Ukrainian red-pied dairy cattle was studied in 2010-2012 on 2 samples in farms of Khmelnitsky and Chernivtsi regions with a total number of 117 animals.

It was found that 22 alleles are found in the investigated populations (the average frequency is 4.55%) (Table 5). with a frequency of more than 5%, there are 7 alleles. the most "informative" in the presented breed is the allele BoLA-DRB3.2*07, which occurs 36 times (15.4%). it was found in 30 cows, it affects every fourth animal of the research sample. Ranked by the highest frequency of detection, the series for the remaining 6 alleles is as follows: *22 to 31 cases (13.1%), *11 to 22 cases (9.4%), *24 to 20 cases (8.7%), *01 - 18 cases (7.7%), *03 and *16 - for 12 cases (5.1%). Totally "weighty" alleles make up 64.5% of the allele fund of the population. Rarely three times (1.6%) identified seven alleles *04, *09, *12, *20, *32, *35 and *43.

Tab. 5. the frequencies of the alleles of the BoLA-DRB 3.2 gene in Ukrainian red-pocked dairy breed cows (n = 117)

Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)	Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)
***01**	18	0,077	1,74	***20**	3	0,013	0,74
***03**	12	0,051	1,44	***22**	31	0,131	2,19
***04**	3	0,013	0,74	***24**	20	0,087	1,87
***07**	36	0,154	2,36	***25**	6	0,026	1,03
***08**	11	0,047	1,38	***27**	6	0,026	1,03
***09**	3	0,013	0,74	***28**	8	0,034	1,26
***10**	11	0,047	1,38	***32**	4	0,017	0,74
***11**	22	0,094	1,91	***35**	3	0,013	0,74
***12**	3	0,013	0,74	***42**	11	0,047	1,38
***15**	4	0,017	0,85	***43**	3	0,013	0,74
***16**	12	0,051	1,44	***45**	4	0,017	0,85

35 genotypes were identified in the rocks (Table 6). the frequency of finding more than 5% was found for 4 genotypes: *01/*07 and *16/*24 - 9 cases (7.69%), *07/*07 and *22/*24 - 6 cases (5, 13%).

Tab.6. Distribution of genotypes in cows of Ukrainian red-pied dairy breeds

genotype	number of genotype / %)	genotype	number of genotype / %)	genotype	number of genotype / %)	genotype	number of genotype / %)
***01/*03**	3 / 2,56	***07/*22**	5 / 4,27	***10/*28**	2 / 1,71	***16/*24**	9 / 7,69
***01/*07**	9 / 7,69	***07/*27**	4 / 3,42	***10/*32**	3 / 2,56	***22/*24**	6 / 5,13
***01/*11**	3 / 2,56	***07/*42**	4 / 3,42	***11/*11**	3 / 2,56	***22/*27**	3 / 2,56
***01/*42**	4 / 3,42	***08/*08**	3 / 1,71	***11/*15**	2 / 1,71	***22/*28**	3 / 2,56
***03/*07**	4 / 3,42	***08/*22**	1 / 0,85	***11/*22**	3 / 2,56	***22/*42**	5 / 4,27
***03/*24**	2 / 1,71	***08/*28**	1 / 0,85	***11/*24**	3 / 2,56	***22/*43**	3 / 2,56
***03/*28**	3 / 2,56	***09/*11**	3 / 2,56	***11/*45**	2 / 1,71	***25/*35**	3 / 2,56
***04/*08**	3 / 2,56	***10/*12**	3 / 2,56	***15/*15**	1 / 0,85	***45/*45**	1 / 0,85
***07/*07**	6 / 5,13	***10/*25**	3 / 2,56	***16/*20**	3 / 2,56		

Ukrainian gray breed. Gray cattle is an aboriginal native breed, which in many ways has preserved the features of its wild descendant - the European tour. in the past, it is widespread in a large part of the country. the main distribution area is the steppe zone. the breed became the basis for the creation of native breeds: Simmental, Red Steppe and Lebedynska. in recent years, the number of gray Ukrainian breeds has been reduced to a minimum. This breed is considered by breeders as a genetic material source for creating populations with valuable economic and useful attributes.

Allelic fund of Ukrainian gray cattle was studied in 2016-2017 on two samples (Table 7). Blood samples from 48 cows were examined in the experimental farm

"Polyvanivka" (Dnipropetrovsk region), and 28 alleles (average frequency of finding 3.57%) were found in the research farm "Markeevo" of the Institute of Animal Breeding in the Steppe Regions of Askania-Nova - 40 cows.

Tab. 7. the frequencies of the alleles of the BoLA-DRB 3.2 gene in Ukrainian gray breed cows (n = 88)

Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)	Allele BoLA-DRB 3.2	allele amount	Frequency, *P(A)*	Statistical error, S_p (%)
*01	1	0,006	0,57	*30	2	0,011	0,8
*04	2	0,011	0,8	*32	1	0,006	0,57
*06	14	0,080	2,04	*34	1	0,006	0,57
*07	1	0,006	0,57	*36	3	0,017	0,98
*12	22	0,125	2,49	*39	1	0,006	0,57
*13	2	0,011	0,8	*43	1	0,006	0,57
*14	5	0,028	1,25	*44	1	0,006	0,57
*15	8	0,045	1,57	*53	2	0,011	0,8
*16	76	0,432	3,73	*54	1	0,006	0,57
*17	1	0,006	0,57	*jab	1	0,006	0,57
*20	3	0,017	0,98	*jba	9	0,051	1,66
*22	2	0,011	0,8	*jbb	3	0,017	0,98
*23	4	0,023	1,12	*nad	1	0,006	0,57
*24	7	0,040	1,47	*nda	1	0,006	0,57

With a frequency of more than 5%, there are 4 alleles with a total frequency of 68.8%. the main part of the rock allele fund is the BoLA-DRB3.2 * 16 allele (43.2%), which is consistent with the genealogy of populations developing under monohybrid crosses conditions. it was found in 57 cows, which it occurs in the genotype of 64.8% of the animals in the experimental sample. the border in P (A) ≥ 5% exceeded, as well the alleles of BoLA-DRB3.2*12 - 25 (12.5%), *06 - 24 (7.95%) and * jba (5.11%). in the genotype of gray cows, there are 5 alleles with a total frequency of 8.5% that do not belong to the nomenclature of PCR-RFLP: *jab, *jba, *jbb, *nad and *nda.

In the gray cattle identified 40 genotypes (Table 8). it was found that the frequency of 4 genotypes exceeds the threshold in 5% *16/*16 - 17 cases (19.3%), *12/*16 - 11 cases (12.5%), *06/*16 - 7 cases 7.95%) and *16/*24 - 5 cases (5.68%), which is more than 45% in total. the prevalence of the allele BoLA-DRB3.2*16 leads to the fact that all prevailing genotypes have it in its composition. Obviously, this allele can serve as a marker for the gray breed.

Tab. 8. Distribution of genotypes in cows of Ukrainian gray breed

Genotype	Number of Genotypes / %)	Genotypes	Number of Genotypes / %)	Genotype	Number of Genotypes / %)	Genotype	Number of Genotypes / %)
***01/*16**	1 / 1,14	***12/*14**	1 / 1,14	***16/*23**	2 / 2,27	***jba/*14**	1 / 1,14
***04/*36**	2 / 2,27	***12/*16**	11 / 12,5	***16/*24**	5 / 5,68	***jba/*16**	3 / 3,41
***06/*06**	1 / 1,14	***12/*24**	1 / 1,14	***16/*30**	1 / 1,14	***jba/*20**	1 / 1,14
***06/*12**	3 / 3,41	***12/*54**	1 / 1,14	***16/*36**	1 / 1,14	***jba/*24**	1 / 1,14
***06/*15**	1 / 1,14	***13/*43**	1 / 1,14	***16/*44**	1 / 1,14	***jba/*34**	1 / 1,14
***06/*16**	7 / 7,95	***14/*16**	2 / 2,27	***16/*53**	2 / 2,27	***jba/*39**	1 / 1,14
***06/*23**	1 / 1,14	***15/*16**	3 / 3,41	***17/*32**	1 / 1,14	***jba/*jbb**	1 / 1,14
***07/*16**	1 / 1,14	***15/*22**	2 / 2,27	***20/*31**	1 / 1,14	***jbb/*jbb**	1 / 1,14
***12/*12**	2 / 2,27	***16/*16**	17 / 19,32	***23/*36**	1 / 1,14	***nad/*14**	1 / 1,14
***12/*13**	1 / 1,14	***16/*20**	1 / 1,14	***jab/*16**	1 / 1,14	***nda/*30**	1 / 1,14

4. CONCLUSIONS

The allele spectrum of cattle behind this gene has been studied for most of the world's breeds. in this paper, we present data on the polymorphism of the BoLA-DRB3.2 gene from three Ukrainian breeds: black-and-white dairy, red-pied dairy and gray. the data bank of the Bos Taurus allele spectrum is supplemented with new information that may be useful in further studies of the relationships between the alleles of the BoLA-DRB3.2 gene, diseases and utility useful signs.

High information content of the gene alleles BoLA-DRB3.2 is fully used in breeding programs, it requires continued study of the allele fund of Ukrainian cattle breeds. This should occur both due to the geography expansion of the rocks that had been already studied, and the other Ukrainian breeds involvement, including indigenous ones, in research programs.

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