Allelic polymorphisms of the BoLA-DRB3 gene and resistance to brucellosis in Kazakh cattle breeds

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Genetic resistance/
susceptibility,
PCR-RFLP.

Summary
The Authors investigated polymorphism of the bovine BoLA-DRB3 gene in connection with resistance or susceptibility to brucellosis of two Kazakh meat breeds, Auliekol and Kazakh Whiteheaded breeds, using PCR-RFLP. In Auliekol cattle (n = 158), 22 alleles were detected in the brucellosis group, and 24 alleles were shown in the healthy group. *BoLA-DRB3* alleles *3, *4, *19, *21 were more common in healthy animals, while *Brucella*-positive cattle were more frequently carriers of alleles *7, *10, *18. In Kazakh Whiteheaded cattle (n = 146), 21 alleles were detected in infected and 23 alleles in healthy cattle. Alleles *3, *8, *21 significantly predominate in healthy cattle, while alleles *7, *11, *16 are typical for animals with brucellosis. This study identified *BoLA-DRB3* alleles associated with genetic resistance (*3 and *21) and susceptibility (*7) to brucellosis; remarkably, resistance alleles are shared by two important meat breeds of Kazakhstan.

Introduction

One of the most promising areas of animal breeding is marker-assisted genetic selection used, in particular, to control common infectious diseases. The existence of several forms of genes that occur with a certain frequency reflects the level of genetic polymorphism in the animal populations (Sulimova *et al.* 1992, Nam *et al.* 2014).

The Bola genetic system is characterized by a high polymorphism of DNA sequences, and it is responsible for the formation of defense reactions of cattle (Lewin 1989, Lewin 1999). For the BoLA-DRB3 gene, which is associated with resistance/susceptibility of cattle to various infectious diseases, more than 100 alleles were detected. Fifty four of them can be discriminated by the PCR-RFLP method (polymerase chain reaction - restriction fragment length polymorphism) (Van Eijk *et al.* 1992, Xu *et al.* 1993, Nassiry *et al.* 2005).

The application of PCR-RFLP to study allelic polymorphism of the BoLA-DRB3 gene has made it possible to test different breeds of cattle in Russia, Belarus, and Kazakhstan for the presence / absence of genetic resistance or predisposition of animals to bovine leukemia virus (Nam *et al.* 2014, Nam *et al.* 2015, Smaznova 2014, Latypova *et al.* 2017).

The problem of combating brucellosis is one of the priorities for the world veterinary medicine and healthcare, including Russia and Kazakhstan (Corbel 1997, Gordienko 2014). Brucellosis is eradicated in most regions of Russia, but in recent decades, this disease has become increasingly common in livestock farms in the Southern, North Caucasian, Siberian, and Far Eastern federal districts (Gordienko *et al.* 2017, Gulyukin *et al.* 2013, Gulyukin *et al.* 2016). In Kazakhstan, brucellosis is still spread in all regions and more than 1,500 newly diagnosed brucellosis cases among people are registered annually in the country (Sultanov 2012, Ivanov *et al.* 2013).

This study was focused on cattle belonging to two beef breeds of the Republic of Kazakhstan, i.e., Auliekol and Kazakh Whiteheaded breeds. Auliekol cattle are well-adapted to severe conditions of the North Kazakhstan but no data regarding population consistency are available. Kazakh Whiteheaded was developed between 1930 and 1950 by crossing Hereford cattle with local Kazakh and Kalmyk stock, and total population in Kazakhstan was estimated at 1,334,000 in 1990.

We aimed to identify alleles in the BoLA-DRB3 gene associated with genetic resistance and susceptibility

to brucellosis by genotyping healthy and infected cattle; statistical analysis was employed to determine allelic polymorphisms associated with each group of animals.

Materials and methods

A total of 304 whole blood samples from livestock farms in five regions of the Republic of Kazakhstan were used for this study: West Kazakhstan (n = 62), Karaganda (n = 56), Kostanay (n = 62), East Kazakhstan (n = 62), and Almaty (n = 62) regions. Samples were equally distributed between Auliekol and Kazakh Whiteheaded breeds. Control and infected animals were categorized based on the results of routine serological and bacteriological analyses carried out during screening for *Brucella* infection. Specifically, Rose-Bengal test, agglutination reaction, complement fixation reaction and inoculation directly on both Farrell medium and modified Thayer-Martin medium were carried out. The final sample panel included: 157 control cattle (Auliekol, n = 84 animals; Kazakh Whiteheaded, n = 73 animals) and 147 *Brucella*-infected cattle (Auliekol, n = 74 animals; Kazakh Whiteheaded, n = 73 animals).

DNA was isolated using the DIAtom™ DNA Prep kit by Biokom (Smaznova 2015) and checked by horizontal agarose electrophoresis with ethidium bromide for DNA visualization. Genetic analysis of allelic polymorphism of the BoLA-DRB3 gene in case and control groups was performed by PCR-RFLP using the method described by Sulimova and colleagues (Sulimova *et al.* 1995). In brief, PCR amplification of a 284 base pair (bp) fragment of the gene was performed, followed by restriction using *RsaI*, *BstIY* and / or *HaeIII* endonucleases; finally, restriction fragments were visualized by vertical electrophoresis in acrylamide gel at a current strength of 50-100 mA, an electric field of 100-150 V for 1.5-2 hours.

Allelic polymorphism of the BoLA-DRB3 gene (i.e., differences in nucleotide sequences of different alleles of a gene) is expressed as different position of restriction sites within the analysed gene fragment. To determine alleles, a previous table of restriction fragments of the BoLA-DRB3.2 gene was used (Sulimova *et al.* 1995).

Statistical analysis of results and determination of significant differences in the frequency of *BoLA-DRB3* alleles between the studied groups was carried out according to Student's criteria as previously described (Dospekhov 1985, Usmanov and Proshina 2013). The frequency of occurrence of alleles and confidence intervals were determined at 5% and 1% significance levels. Allele frequency errors were calculated using the formula:

$$Si = \sqrt{pi * (1 - pi) / N}$$

where

Si is the frequency error of the *i* allele;

Pi the frequency of occurrence of the *i* allele;

N is the sample size.

Confidence intervals were calculated using the standard method.

In the tables, data are presented as:

$$Pi \pm t * Si$$

where

Pi is the frequency of occurrence expressed in percentage;

Si is the error of frequency of *i* allele in percentage;

Student's *t* is the coefficient for significance levels of 0.05 or 0.01.

Results

Agarose gel visualization of extracted genomic DNA showed a single band of high molecular weight indicating good quality and integrity of nucleic acid. PCR amplification provided an amplicon of the expected size of 284 bp (Figure 1). As a first step, parallel restriction by *RsaI* and *HaeIII* endonucleases was performed. If the obtained restriction spectra did not allow determining the allele, then amplicon restriction was additionally performed using the *BstX2I* enzyme. Examples of restriction spectra by RsaI and HaeIII are shown in Figure 2.

In Auliekol cattle breed, 24 alleles were revealed in the control group, and 22 alleles were detected in the brucellosis-affected cows. Kazakh Whiteheaded animals showed 23 alleles in the control group, while 21 alleles were identified in brucellosis cases. Table I shows comparative *BoLA-DRB3* allele frequencies in cases and controls in the two cattle breeds under study. Table II shows the results of statistical analysis performed on most common alleles to identify significant differences in allele frequencies between groups. Thus, the results presented in Table II allow to determine the presence of alleles of the BoLA-DBD3 gene mainly common in healthy

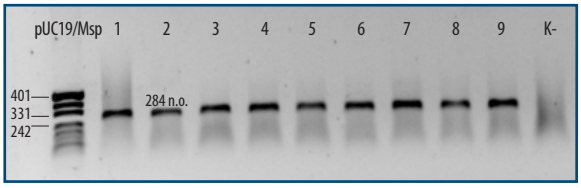

Figure 1. *Electropherogram of PCR products of the* BoLA DRB3 *gene in 1% agarose gel.* pUC19/Msp = Molecular weight marker; K = No amplification control; 1-9 =Test samples.

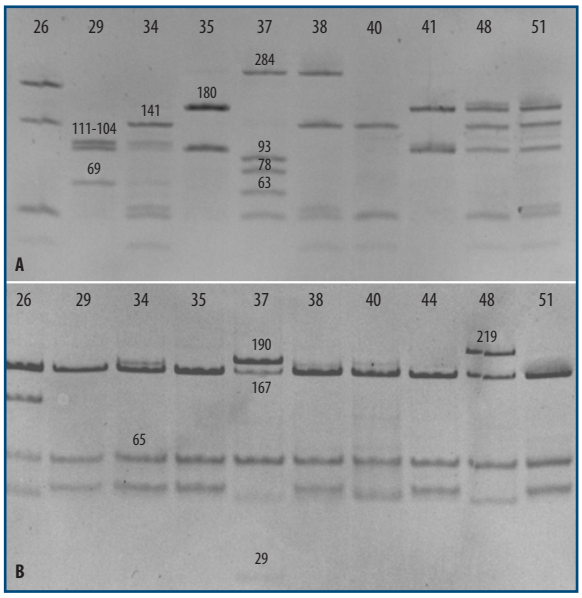

Figure 2. *Electropherograms of restriction products of the* BoLA-DRB3 *gene by Rsa I (***A***) and HaeIII (***B***) endonucleases for Kazakh Whiteheaded cows.*

cows (R, resistance alleles) and *Brucella* carriers (S, susceptibility alleles).

Figures 3 and 4 show the distribution in the different animal groups/breeds of *BoLA-DRB3* alleles associated with resistance or susceptibility, respectively. As shown in Figure 3, allele *3 is present with high frequency in healthy cattle of both breeds. This allele is instead absent in *Brucella*-positive cows of the Kazakh Whiteheaded breed, and with a significantly lower frequency occurs in the Auliekol breed. Allele *21 is found with a high frequency in healthy cows, significantly exceeding its level of occurrence in cases.

These results allow us to draw a statistically confirmed conclusion about the influence of alleles *3 and *21 on the formation of brucellosis resistance in the Auliekol and Kazakh Whiteheaded breeds. Alleles *4 and *19 can play a positive role in the development of a protective reaction in the Aueliekol breed, and allele *8 in the Kazakh Whiteheaded breed. Figure 5 shows that the allele *7 occurs with high frequency in *Brucella*-positive cows of both breeds, significantly exceeding the level of its occurrence in groups of healthy animals. Alleles *10, *11, *16 and *18 occur more frequently in cases but are not shared by both breeds.

Discussion

This study aimed to investigate the genetic resistance/susceptibility of cattle to brucellosis by analyzing the BoLA-DRB3 gene using PCR-RFLP method. Previously, the study of the bovine

Table I. *Frequency of the* BoLA-DBD3 *alleles in* Brucella*-positive and control cattle of Auliekol and Kazakh White-headed breeds.* Allele numbering according to Sulimova and colleagues (Sulimova *et al.* 1995).

Allele no.	Auliekol (%)		Kazakh white-headed (%)	
	Control	*Brucella*-positive	Control	*Brucella*-positive
1	-	-	0	1.4
3	**8.9**	2.7	**8.2**	0
4	5.9	1.3	3.5	3.5
7	**5.9**	**16.2**	**8.9**	**20.5**
8	4.1	0	6.2	0
9	-	-	0	2.7
10	2.4	**13.5**	4.2	0
11	1.2	2.0	0.7	9.6
12	5.3	6.1	2.1	2.7
13	-	-	0	2.7
14	0	4.0	-	-
15	0	2.7	0	4.2
16	**7.7**	4.7	4.8	**15.1**
17	0.6	2.0	4.8	0
18	3.0	**11.5**	4.8	**6.8**
19	6.6	1.3	2.8	0
20	**5.3**	1.3	5.6	2.7
21	**6.6**	1.3	**6.8**	0
22	4.2	0	2.7	2.7
23	2.4	0	4.2	3.5
24	4.2	4.0	2.7	2.1
27	4.2	0	4.2	2.7
28	3.6	1.3	0	2.7
29	4.1	3.3	4.2	3.4
31	0.6	2.7	-	-
32	3.6	0	6.2	2.7
33	1.2	0	4.2	0
34	0	3.4	-	-
36	**6.6**	**10.8**	**6.8**	2.7
42	1.8	2.7	0.7	0
45	0	1.2	0.7	0
50	-	-	0	2.7
52	-	-	0	2.7

histocompatibility complex showed the presence of a high genetic diversity of the BoLA-DRB 3 gene, which is an important indicator of the adaptability of populations and is responsible for resistance or susceptibility to infectious diseases (Lewin 1989, Van Eijk *et al.* 1992). To date, an association of BoLA-DRB3 alleles and several infectious diseases has been proved, such as hemoblastosis, mastitis (Suprovych *et al.* 1997, Zhang *et al.* 2007), necrobiosis (Suprovych *et al.* 2016), dermatophilosis (Maillard *et al.* 1996, Maillard *et al.* 2003). Moreover, a number of markers related to the resistance of cattle to parasites have been reliably established

Table II. *Most common alleles of the* BoLA-DRB3 *gene identified in control and* Brucella*-positive cattle of Auliekol and Kazakh White-Headed breeds.*

Allele no.	Auliekol (Aul)		Kazakh white-headed (KWH)	
	Control	*Brucella*-positive	Control	*Brucella*-positive
3	8.9 ± 2.2*	2.7 ± 1.3	8.2 ± 2.2**	0
4	5.9 ± 1.8*	1.3 ± 0.93	0	0
8	0	0	6.2 ± 1.9*	0
19	6.6 ± 1.9*	1.3 ± 0.93	0	0
21	6.6 ± 1.9*	1.3 ± 0.93	6.8 ± 2.1**	0
7	5.9 ± 1.8	16.2 ± 3.02**	8.9 ± 2.3	20.5 ± 3.3**
10	2.4 ± 1.2	13.5 ± 2.8**	0	0
11	0	0	0.7 ± 0.6	9.6 ± 2.4**
16	0	0	4.8 ± 1.8	15.1 ± 3.0**
18	3.6 ± 1.4	11.5 ± 2.6**	6.8 ± 2.1	2.7 ± 1.3

*The difference is significant between groups at level 0.90;
**The difference is significant between groups at level 0.95.

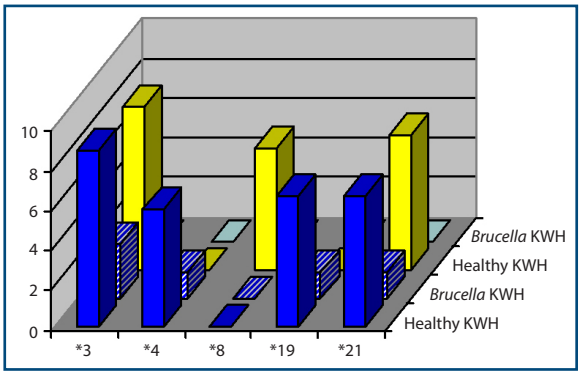

Figure 3. *Frequency comparison of* BoLA-DRB3 *allele for brucellosis resistance in Auliekol (Aul) and Kazakh Whiteheaded (KWH) breeds.*

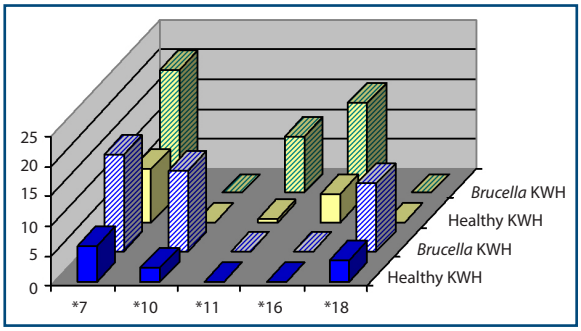

Figure 4. *Frequency comparison of* BoLA-DRB3 *allele for brucellosis susceptibility in Auliekol (Aul) and Kazakh Whiteheaded (KWH) breeds.*

(Zhang *et al.* 2007, Nassiry *et al.* 2005), in particular to *Theileria parva* (Ballingall *et al.* 2004).

Scientists have most actively studied alleles of the BoLA-DRB3 gene associated with resistance to bovine leukemia, a dangerous viral disease (Sulimova *et al.* 1995, Udina 2003, Satsuk 2009, Nam *et al.* 2014, Nam *et al.* 2015, Smaznova 2015, Latypova *et al.* 2017). It has been shown that there are alleles that can determine resistance or susceptibility of cattle to leukemia. Animals with alleles *22, *24, *16, *8 are susceptible to leukemia, they occur more often than others in the groups of hematological patients, while alleles *11, *23, *28 determine resistance to leukemia. Importantly, these alleles are dominant, and animals that carry alleles in heterozygosis do not get leukemia.

In this study, the comparison of BoLA-DRB3 allelic profiles in healthy and brucellosis-affected cattle of two Kazakh meat breeds was carried out. In Auliekol breed, 24 alleles were detected in the control population; among them, the most common was allele *3 (8.9%), while alleles *4, *7, *12, *16, *19, *20, *21, *36 occurred with a frequency > 5%. In the group of brucellosis-affected animals, 22 alleles were identified, among which the most common were alleles *7 (16.2%), *10 (13.5%), *18 (11.5%), *36 (10.8%), and *12 (6.1%). Comparison between the groups has shown that, in healthy animals of the Auliekol breed, the frequency of alleles *3, *4, *19, and *21 is significantly higher (3.0-5.0 times) than in the group of brucellosis cases. On the contrary, a higher frequency of alleles *7, *10, *18 and *36 is observed in brucellosis cases; these alleles are found several times less frequently (1.6-5.6 times) in the control group. These findings may indicate that BoLA-DRB3 alleles can be associated with resistance and susceptibility of animals to brucellosis, respectively. In Kazakh Whiteheaded breed, 23 alleles were identified in the healthy cattle group, among them the most common was allele *7 (8.9%), while alleles *3, *8, *32, and *36 were found with a frequency > 5%. In the group of brucellosis cases, 21 alleles were identified and the most common were alleles *7 (20.5%), *16 (15.1%), *11 (9.6%), and *18 (6.8%). Interestingly, three alleles of the BoLA-DRB3 gene (*3, *8, *21) are characteristic only for healthy individuals, and three alleles (*7, *11, *16) were found mainly in brucellosis cases.

The results of this study, carried out on two important meat breeds of Kazakhstan, show that BoLA-DRB3 alleles *3 and *21 are associated with resistance to brucellosis in both the Auliekol and Kazakh Whiteheaded breeds. At the same time, alleles *4 and *19 seem to play a positive role in the development of a protective reaction in Auliekol breed, while allele *8 in Kazakh white-headed breed.

Allele *7 is associated with susceptibility since it occurs with higher frequency in sick cows of both breeds. Alleles *10, *18 in Auliekol breed, and alleles *11, *16 in Kazakh Whiteheaded breeds may also be associated with susceptibility.

In conclusion, the Authors first identified alleles

of the BoLA-DRB3 gene that are associated with resistance and susceptibility to brucellosis. Allelic polymorphisms of the BoLA-DRB3 gene might be exploited to implement veterinary approaches for the management of infected herds, as well as for the selection of brucellosis-resistant animals. Further studies, by expanding the range of cattle breeds, would be advisable before to carry our selective breeding to increase the genetic resistance to brucellosis of the general bovine population in Kazakhstan.

References

Ballingall K.T., Luyai A., Rowlands G.J., Sales J., Musoke A.J., Morzaria S.P. & McKeever D.J. 2004. Bovine leukocyte antigen major histocompatibility complex class II DRB3*2703 and DRB3*1501 alleles are associated with variation in levels of protection against *Theileria parva* challenge following immunization with the sporozoite p67 antigen. *Infect Immun*, **72** (5), 2738-2741.

Corbel M.J. 1997. Brucellosis: an overview. *Emerg Infect Dis*, **3**, 213-221.

Gordienko L.N. 2014. The intensity of the spread of brucellosis in cattle meat breed under range content. *Vet Sci*, **11**, 17-20.

Gordienko L.N., Kulikova E.V., Gaidutskaya G.M., Elantseva N.B., Volkova T.I. & Guskova T.V. 2017. Assessment of the immune status of imported cattle treated for brucellosis. *Veterinary*, **2**, 19-22.

Gulyukin M.I., Albertyan M.P., Iskandarov M.I., Fedorov A.I & Kolomytsev S.A. 2013. Brucellosis of farm animals in the Russian Federation. *Veterinary*, **6**, 23-28.

Gulyukin M.I., Albertyan M.P., Iskandarov M.I., Fedorov A.I. & Iskandarova S.S. 2016. Effectiveness of measures taken against bovine brucellosis in the Russian Federation. *Veterinary*, **12**, 24-28.

Dospekhov B.A. 1985. Field experiment methodology (with the basics of statistical processing of research results). *M Agropromizdat*, 352.

Ivanov A.V., Salmakov K.M. & Fomin A.M. 2013. State and prospects of specific prevention and elimination of brucellosis of cattle. *Veterinary*, **7**, 10-13.

Kerimov C.H. & Iskandarov M.I. 1999. Prevention and improvement of camels and cattle from brucellosis. Development of animal husbandry scientific support, Ashgabat.

Latypova Z., Sarbakanova Sh., Sultanov A., Nam I., Zayakin V., Achmedov R., Smaznova I., Egiazaryan A., Sheiko I. & Budevich A. 2017. DNA-polymorphism of the BoLA-DRB3 gene in crossbreeding of Holstein and Black Pied cattle from the Bryansk region of Russia, Belarus and Kazakhstan. *Current Science*, **5**, 2173-2186.

Lewin H.A. 1989. Disease resistance and immune response genes in cattle: strategies for their detection and evidence of their existence. *J Dairy Sci*, **72** (5), 1334-1348.

Lewin H.A., Russel G.C. & Glass E.J. 1999. Comparative organization and function of the major histicompatibility complex of domesticated cattle. *Immunol Rev*, **167**, 145-158.

Maillard J.C., Martinez D. & Bensaid A. 1996. An amino acid sequence coded by the exon 2 of the BoLA DRB3 gene associated with a BoLA class I specificity constitutes a likely genetic marker of resistance to dermatophilosis in Brahman Zebu cattle of Martinique (FWI). *Ann New York Acad Sci*, **791**, 185-197.

Jean-Charles M., Berthier D., Chantal I., Thevenon S., Sidibй I., Stachurski F., Belemsaga, Hanta Razafindranьbi D. & Elsen J.M. 2003. Selection assisted by a BoLA-DR/DQ haplotype against susceptibility to bovine dermatophilosis. *Genet Sel Evol*, **35** (1), S193-S200.

Nam I.Y., Zayakin V.V., Smaznova I.A., Egiazaryan A.V., Sulimova G.E., Sheiko I.P. & Budevich A.I. 2014. High genetic susceptibility to leukemia in breeding black pied and holstein cattle. *Middle-East Journal of Scientific Research*, **20** (10), 1297-1301.

Nam I.Y., Zayakin V.V., Smaznova I.A., Kozlov A.L., Achmedov R.B. & Kobozeva M.S. 2015. The genetic polymorphism of BoLA-DRB3 gene and the resistance to virus leukemia in different herds of cattle at Bryansk region. *Res J Pharmac Biol Chem Sci*, **6** (1), 1903-1907.

Nam I.a., Zayakin V.V. & Drozdov E.V. 2014. Allelic polymorphism of the somatotropin gene among Holstein-friesian breed in the livesock farming in the Bryansk region. *World Appl Sci J*, **30** (7), 802- 805.

Nassiry M.R., Eftekhar Shahroodi F., Mosafer J., Mohammadi A., Manshad E., Ghazanfari S., Mohammad Abadi M.R. & Sulimova G.E. 2005. Analysis and frequency of bovine lymphocyte antigen (BoLA-DRB3) alleles in Iranian holstein cattle. *Genetics*, **41** (6), 817-822.

Satsuk V.F. 2009. Using the Bola-DRB3 marker in the selection and breeding work with cattle. *Cand* Dissertation in Biol Sci, Stavropol, 118 pp.

Smaznova I.A. 2015. Characteristics of allelic polymorphism of the BoLA-DRB3 gene, which affects resistance to leukemia, and genes of milk productivity in producing bulls of the Bryansk region. PhD Thesis, Saint Petersburg, 126 pp.

Sulimova G.E., Sokolova S.S., Semikozova O.P., Nguet L.M. & Berberov E.M. 1992. Analysis of DNA polymorphism of cluster genes in cattle: casein genes and major histocompatibility complex (MHC) genes. *Genetika*, **26** (5), 18-26.

Sulimova G.E., Udina I.G., Shaïkhaev G.O. & Zakharov I.A.

1995. DNA polymorphism of the BoLA-DRB3 gene in cattle in connection with resistance and susceptibility to leukemia. *Genetika*, **31** (9), 1294-1299.

Suprovych T., Suprovych N., Karchevska T., Chornyy I. & Chepurna V. 2016. Allelic polymorphism gene BOLA-DRB3.2 in connection with the types of excretory ducts udder and mastitis cows Ukrainian black-pied dairy breed. *Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj*, **3** (71), 117-123. https://nvlvet.com.ua/index.php/journal/article/view/943.

Sultanov A.A. 2012. Veterinary issues of development of meat cattle breeding in Kazakhstan. *Agrarian sector*, **1**, 11.

Usmanov R.R. & Proshina E.T. 2013. Features of statistical processing of field experience: Study guide. M .: FSBEI of AE FCEBC, 96.

Van Eijk M.J., Stewart-Haynes J.A. & Lewin H.A. 1992. Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. *Anim Genet*, **23** (6), 483-496.

Xu A., Van Eijk M.J., Park C. & Lewin H.A. 1993. Polymorphism in BoLA-DRB3 exon 2 correlates with resistance to persistent lymphocytosis caused by bovine leukemia virus. *J Immunol*, **151** (12), 6977-6985.