UDC 575.17, 619:616.9
IRSTI 34.23.59, 68.41.53 *DOI 10.56339/2305-9397-2022-4-1-53-62*

Beishova I.S., candidate of Agricultural Sciences, Doctor of Biological Sciences, Associate Professor, **main author**, https://orcid.org/0000-0001-5293-2190
NJSC «Zhangir Khan West Kazakhstan Agrarian Technical University», Uralsk, Zhangir Khan street, 51, 090009, Kazakhstan, indira_bei@mail.ru
Nurgaliyev B.E., candidate of Veterinary Sciences, Associate Professor, https://orcid.org/0000-0000-1599-88250
NJSC «Zhangir Khan West Kazakhstan Agrarian Technical University», Uralsk, Zhangir Khan street, 51, 090009, Kazakhstan, nurgaliev.79@mail.ru
Belaya E.V., candidate of Biological Sciences, Associate Professor, https://orcid.org/0000-0003-1786-034
BM «Belarusian State Pedagogical University named after Maxim Tank», Minsk, Sovetskaya street, 18, 220030, Belarus, kolyuchka005@rambler.ru
Ulyanov V.A., Master of Veterinary Sciences, https://orcid.org/0000-0002-7500-1601
NJSC «Zhangir Khan West Kazakhstan Agrarian Technical University», Uralsk, Zhangir Khan street, 51, 090009, Kazakhstan, vadimkst@mail.ru
Ginayatov N.S., PhD, https://orcid.org/0000-0002-9608-002X
NJSC «Zhangir Khan West Kazakhstan Agrarian Technical University», Uralsk, Zhangir Khan street, 51, 090009, Kazakhstan, nginayatov@mail.ru
Zholdasbekova A.Zh., Master of Veterinary Sciences, https://orcid.org/0000-0002-8612-1940
NJSC «Zhangir Khan West Kazakhstan Agrarian Technical University», Uralsk, Zhangir Khan street, 51, 090009, Kazakhstan, aizhan.urazova@mail.ru

ASSOCIATION OF GENETIC POLYMORPHISM OF *LTF, MBL1* AND *TLR -9* WITH RESISTANCE TO CHLAMYDIOSIS IN HOLSTEIN CATTLE

ANNOTATION

Chlamydia is one of the most common and damaging diseases of cattle in the Republic of Kazakhstan. It is caused by intracellular parasites of the genus Chlamidia, different types of which infect individual organs or tissues of the animal. Due to the large number of potential carriers of the infection, work aimed at identifying genetic factors of resistance to chlamydia in livestock and developing test systems that allow identifying resistant and vice versa - susceptible animals in the early stages of ontogenesis. The search for genetic factors primarily focused on genes encoding the main components of innate immunity, which include genes encoding lactoferrin (LTF), the mannose-binding lectin MBL1 and the Toll-like receptor group gene TLR-9. The subjects were 186 cattle of Holstein breed, among whom 93 were cattle with a confirmed diagnosis of chlamydia. The distribution of genotypes for the polymorphic genes studied was analyzed against the theoretically expected distribution according to Hardy-Weinberg law, and a comparative analysis of the distribution of genotypes between groups of sick and healthy animals was carried out. The study identified single markers and paired genotype combinations responsible for an increased risk of morbidity/resistance to chlamydia in cattle.

Keywords: *chlamydia, cattle, Holstein breed, genetic structure, stability, resistance, PCR-RFLP, LTF, MBL1, TLR-9*

Introduction. The leading branch of the agro-industrial complex of the republic, which accounts for about 20% of the volume of food produced in Kazakhstan, has always been and remains the dairy industry, which determines the relevance of research aimed at increasing the efficiency of the industry.

Important role in increasing the productivity of the dairy industry is played not only by modern technologies for breeding and selecting cattle, but also by the use of modern methods of combating infectious diseases, which cause significant damage to the agricultural economy and pose a threat to public health.

Therefore, along with marker-associated breeding activities aimed at increasing the profitability of the industry by increasing the genetic potential of the productivity of farm animals, the use of genetic markers of resistance to bacterial infections can be of great help to veterinary medicine and lead to increased profitability by reducing treatment costs and losses from mortality, abortion and culling of sick animals.

Chlamydia is one of the most common and damaging diseases of cattle in the Republic of Kazakhstan.

Chlamydia in cattle is a complex infectious disease with multiple forms. It is caused by intracellular parasites of the genus Chlamidia, the different species of which affect individual organs or tissues of the animal. Chlamydia is also dangerous for humans. Chlamydia parasites affect all breeds of cattle. Animals become ill at any age and at any time of the year.

Basically, chlamydia lives in the gastrointestinal tract and genital organs, multiply in the lymphoid organs, quickly spread to the liver, kidneys, spleen, and lungs. The development of the disease affects the immune system. Adults are prone to frequent infections, young animals are far behind in development. To infection with chlamydia, damage by viruses, bacteria, mycoplasma is added. Chlamydia has many forms depending on the affected organs and tissues. Mortality depends on the form of the disease. In particular, with the respiratory form it is up to 25%, with the genital form - up to 60% [1, 2, 3].

It is almost impossible to control the infection, since there are extremely many potential carriers. Therefore, works aimed at identifying genetic factors of resistance to chlamydia in livestock and developing test systems that allow identifying resistant and vice versa - susceptible animals in the early stages of ontogenesis.

The search for polymorphisms is primarily focused on genes encoding the main components of innate immunity, which include genes encoding lactoferrin (*LTF*), the MBL1 mannose -binding lectin, and the gene for the Toll -like receptor group *TLR-9.*

Lactoferrin (LTF) is an iron-binding glycoprotein and has a broad spectrum of antimicrobial activity both in vitro and in vivo, and is found in milk and other exocrine secretions such as saliva, tears, bile, urine, semen, vaginal fluids, nasal and bronchial secretions, and in blood plasma [4, 5, 6]. Lactoferrin is a multifunctional protein that stimulates the growth of lymphocytes, plays an important role in the regulation of iron absorption, has antiviral, antibacterial, antifungal, antiparasitic, anti-inflammatory, antioxidant, immunomodulatory and regenerative properties. It is also assumed that lactoferrin has an immunomodulatory effect and may even slow down the growth of tumors [7, 8, 9].

Mannose binding lectin (MBL) is a calcium-dependent collagen protein that is involved in complement activation via the lectin pathway (10). This complement system refers to non-specific resistance factors, while providing immediate protection of the body against infection, including also anti-inflammatory effects. This function is characterized by the secretion of opsonizing components shortly after activation of the complement system, which in turn cover pathogenic microorganisms or immune complexes, while increasing the phagocytosis process (11,12).

Another important function of the compliment system is its participation in the inflammatory processes of the body [13]. The protein mannose-binding lectin is produced in the liver under the influence of inflammatory cytokines. The production of MBL1 occurs as a response to infection, while getting into the blood, it becomes part of the antigen-specific immunity mechanism. This protein is part of many factors defined as acute phase proteins. It plays an important role in innate immunity. MBL1 deficiency in humans is associated with low survival of newborns under the age of one year, which, due to the immaturity of immunity, are very susceptible to infectious diseases.

Studies by a number of authors have shown the relationship between polymorphic variants of the MBL1 gene with resistance to pathogens of various infections, including mastitis.

Genes encoding Toll-like receptors (Toll-like receptors TLRs) form a whole family of innate immunity factors to bacterial and viral infections. For example, because TLR1 and TLR1/2 heterodimers recognize mycobacterial products, both genes are candidates for resistance genes controlling paratuberculosis [14]. The protein product of the TLR4 gene is involved in the respiratory disease of cattle associated with infection with Mannheimia (Pasteurella) haemolytica. A beneficial effect of a single amino acid change in TLR4 on udder disease resistance and lactation tolerance has

been demonstrated in a Canadian population of Holstein cattle [15]. The effect of this polymorphism has been confirmed by an independent assay in Chinese cattle populations (Chinese Simmental, Holstein and Sanhe), which are controlled for the presence of SCS [16]. The role of the TLR4 polymorphism in mastitis resistance is further supported by the association of the detected AluI polymorphism in cattle TLR4 with the incidence of mastitis in Sahiwal cattle [17].

Bovine TLR5 is also a candidate gene for resistance to serious bacterial diseases [18]; however, the necessary experimental support in this direction is lacking.

Thus, the group of polymorphic variants of the family genes is of considerable interest from the point of view of the search for genetic markers of innate resistance to chlamydia in cattle [19].

Conducting research to identify genetic mechanisms for regulating the incidence of chlamydia in cattle will not only increase the efficiency of breeding work with breeding stock and increase the profitability of commercial herds, but also reduce the cost of treatment and culling of sick animals due to breeding measures to increase the innate resistance of animals.

The aim of this study was to investigate the effect of *LTF, MBL1* and *TLR-9* gene polymorphisms on the incidence of chlamydia in Holstein cattle and to identify genotypes that mark increased resistance and increased susceptibility.

Materials and research methods. The study included 93 heads of Holstein cattle diagnosed with chlamydia and a group of healthy animals (control) in the amount of 93 heads. Age, housing and feeding conditions were the same for all animals.

As material for genotyping, hair follicles were selected from animals. DNA extraction was performed with a commercial DNA-Extran-2 kit (Syntol LTD, Russia). This kit provides isolation of DNA of the required quality and concentration for the studies.

Determination of gentypes was carried out by PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism).

The polymerase chain reaction was carried out in a volume of 25 µl. To prepare the PCR mixture, we used the Intifica reagent kit (LLC Alkor Company Bio, Russia) : 10 × Taq buffer - 2.5 µl, MgCl2 solution 25 mM - 2 µl; dNTP mixture - 0.2 µl; Taq DNA polymerase - 0.25 µl; primers (synthesized by Applied Biosystems, USA) was added at a final concentration of 10 pM / µl , DNA material - 1 µl. Amplification was carried out on a three-block thermal cycler ProFlex PCR System (Applied Biosystems, USA).

TLR -9 gene, DNA samples were amplified with primers: F:5'-ATCTTCAACGACCTGACCCA-3' and R:5'-AATCGCCAGACTTCCACCCT-3', for the *MBL1 gene:* F:5'-GTGGTGGCAAATGTTGGCTAAAC-3' and R:5'-TGGCTCCTCCCTTTTCTCCCTT-3', for the *LTF gene:* F:5'-GCCTCATGACAACTCCCACAC-3' and R: 5'-CAGGTTGACACATCGGTTGAC-3'.

Restriction fragments were separated by 2,5% agarose gel electrophoresis; molecular weight marker O'RangeRuller 50 np DNA Ladder (Thermo Scientific, Lithuania).

Animal genotyping by polymorphism *TLR -9- BfaI*.

The *TLR -9* - BfaI polymorphism is a T/C transversion at position + 979 of the *TLR-9 gene*, accompanied by the substitution of the amino acid cysteine for valine at protein position 174, exon 2 [8]. When replacing G → A, a restriction site for BfaI appears (cuts C - TAG). Thus, a rare mutant allele is cut into 2 fragments: 82 and 280 np.

As can be seen from Figure 1, after BfaI restriction and dispersal genotypes are identified: *TLR -9* genotypes BfaI ^{AA} 280 and 82 n.p., *TLR -9* BfaI ^{AG} 362, 280 and 82 n.p. and *TLR -9* BfaI ^{GG} 362 n.p. Fragment 82 n.p. not rendered.

Animal genotyping for the MBL1-HaeIII polymorphism was performed according to the method described by Yuan et al. [20].

Amplification produces a PCR product = 255 np. After endonuclease cleavage with the HaeIII enzyme after electrophoresis in 3% agarose gel, PCR-RFLP fragments are visualized: 255/178/77 n.p. - genotype CC; 178/77 n.p. - TC genotype; 255 - n.p. TT genotype (Figure 2).

Animal genotyping for the LTF - EcoRI polymorphism was carried out according to the method described by Wojdak-Maksymiec et al. [21].

After EcoRI restriction according to the manufacturer's protocol, the following fragments are visualized in a 3% agarose gel: genotype 301 n.p. AA, genotype 201/100 n.p. BB, genotype 301/201/100 n.p. AB. (Figure 3).

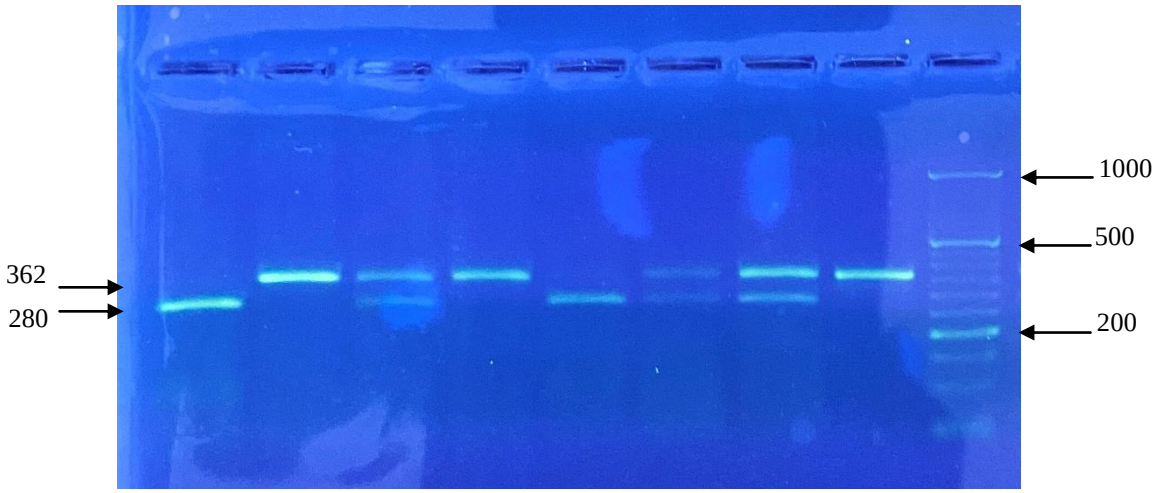

Figure 1 – Restriction fragment length polymorphism visualization BfaI, polymorphic region of the *TLR-9 gene* in 2% agarose gel

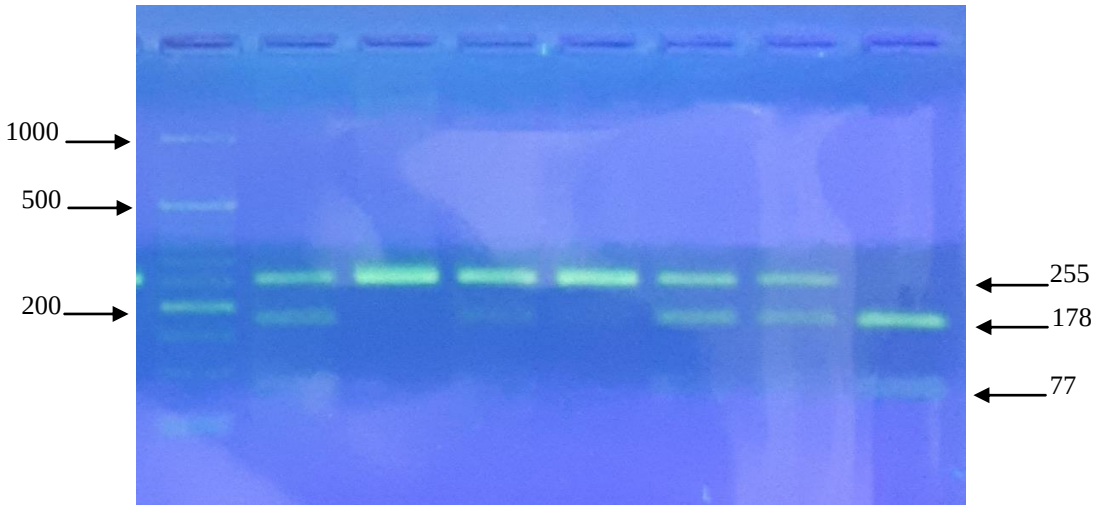

Figure 2 – Electropherogram of the result of PCR-RFLP analysis of the gene, mannose-binding lectin

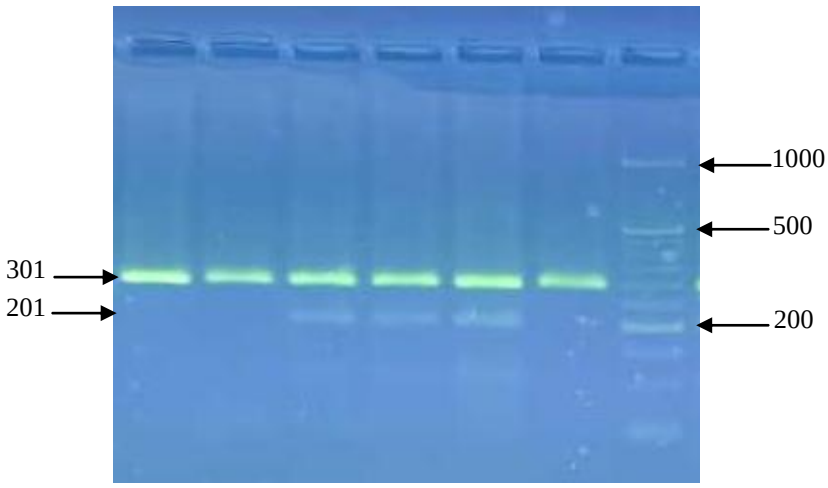

Figure 3 – Electropherogram of the result of PCR-RFLP analysis of the bovine lactoferrin gene with primers LTFf+LTFr and endonuclease digestion with EcoRI

Results and discussion. We have analyzed the compliance of the distribution of genotypes for the studied polymorphic genes with the theoretically expected one, according to the Hardy - Weinberg law. The significance of the observed deviations was assessed using the $\chi^{2.}$

The association of allelic variants of the *TLR-9, MBL1* and *LTF* genes with resistance to chlamydia was assessed by assessing the correspondence of the actual genotype frequencies to those theoretically expected according to the Hardy-Weinberg law, as well as by comparing the distribution of allele frequencies of the studied genes in groups of sick animals and a control group of healthy animals, cows and assessment of the reliability of the observed differences. To do this, the relative frequencies of allelic variants in the group of sick and healthy animals were calculated, and then the calculated significance level P was found by the value of the t-criterion and the number of degrees of freedom from Student's distribution tables. The difference between the samples is significant at P > 0.05.

The results of a comparative analysis of the relative allele frequencies of the studied genes are shown in Table 1.

Table 1 – Distribution of relative allele frequencies of *TLR-9 I, MBL1* and *LTF genes* in groups of sick and healthy cows ($Q \pm S_Q$)

Polymorphism	*TLR- 9BfaI*		*MBL1-HaeIII*		*LTF- EcoRI*	
Allele	*A*	*G*	*T*	*C*	*A*	*B*
Healthy	0,44±0,01	0,57±0,01	0,41±0,01	0,59±0,01	0,78±0,00	0,22±0,00
Chlamydia	0,53±0,01	0,46±0,01	0,40±0.01	0,60±0,01	0,73±0,00	0,26±0,00

The data presented in the table demonstrate the redistribution of allele frequencies according to the *TLR-9-BfaI and* MBL1 *-HaeIII* polymorphisms in the group of healthy and diseased animals. Thus, the ratio of *A* and *G* alleles for *TLR-9 BfaI* polymorphism is 0,44±0,01 to 0,53±0,01 and 0,57±0.00 to 0,46±0,00 in the group of healthy and sick animals respectively. That is, the *G* allele is more common in the group of healthy animals, and the A allele is more common in the group of patients with chlamydia.

According to *MBL1-HaeIII* and *LTF-EcoRI* polymorphisms, the most frequent in the group of healthy animals, it is also the most frequent in the group of cows suffering from chlamydia. Thus, according to the *MBL1-HaeIII* polymorphism in both groups, the T allele is the most frequent. Its ratio with the C allele is 0,41:0,59 and 0,40:0,6 in healthy and diseased animals, respectively.

According to *LTF-EcoRI* polymorphism in both groups, allele A is the most frequent. Its ratio with allele B is 0,78:0,22 and 0,73:0,26 in healthy and diseased animals, respectively.

Table 2 shows the characteristics of samples of sick and healthy animals according to the nature of the distribution of the genotypes of the studied genes. Statistical assessment of the significance of the difference between the sample of sick animals and the control sample of healthy animals according to the nature of the distribution of the genotypes of the *TLR-9, MBL1* and *LTF polymorphic genes* was carried out by finding the calculated significance level P by the value of the t-criterion and the number of degrees of freedom from Student's distribution tables. The difference between the samples is significant at $P \leq 0,05$.

Table 2 – Frequency distribution of genotypes of *TLR-9 I, MBL1* and *LTF* polymorphic genes in the group of healthy Holstein cows.

Polymorphism		*TLR-9 BfaI*			*MBL1-HaeIII*			*LTF-EcoRI*		
Genotype		*AA*	*AG*	*GG*	*TT*	*TC*	*CC*	*AA*	*AB*	*BB*
Healthy (n=93)	n_n	17	47	29	eleven	54	28	51	42	0
	n_o	18	45	29	16	45	33	56	32	5
	χ^1	0,12			3,77			6,23*		
Chlamydia (n= 93)	n_n	23	53	17	13	49	31	43	50	0
	n_o	26	46	20	15	45	33	50	37	7
	χ^2	1,94			0,84			12,57*		
R		0,063			0,916			0,337		

- The value of χ^2 for the significance level of 0,05 is 3,84

The difference between groups is significant at $P \leq \alpha$; $\alpha = 0,05$

*-values of χ^2 were calculated with the Yates correction.

Weinberg law showed a significant redistribution of genotypes in the group of sick and healthy animals according to the *LTF-EcoRI polymorphism*. In the group of healthy animals, a deviation in the distribution is observed for the *LTF-EcoRI polymorphism*.

However, the ratio of homo - and heterozygotes for all three polymorphisms in patients and healthy people is the same: the most common genotype among healthy people is the most common among patients.

Comparative statistical analysis of a group of sick animals with a group of healthy animals in terms of genotype frequencies did not reveal significant differences.

To clarify the data, we carried out a comparative assessment of the relative frequencies of occurrence of genotypes in groups of sick and healthy animals. Numerical data are given in table 3.

Table 3 – Proportion of genotypes of polymorphic genes *TLR-9 I, MBL1* and *LTF* in groups of sick and healthy Holstein cows (n and % of the examined population).

Gene	*TLR-9 - BfaI*			*MBL1-HaeIII*			*LTF-EcoRI*		
Genotype	AA	AG	GG	TT	TC	CC	AA	AB	BB
Healthy	18,3	50,5	31,2	11,9	58,1	30,1	55,9	45,2	0,0
Chlamydia	24,7	57,0	18,3	14,1	52,7	33,3	47,2	53,8	0,0

The data shown in Table 3 quantitatively reflect the nature of the redistribution of genotypes in the group of sick animals compared to the healthy group, and agree with the results of the analysis of the correspondence between the observed frequencies and the expected equilibrium frequencies.

Regarding the *TLR -9 BfaI polymorphism*, it can be noted that in the group of animals with chlamydia there is a significant excess in the proportion of genotypes AG (57,0 % to 50,5 % in patients and healthy people, respectively) and AA (24,7% to 18,3% in sick and healthy, respectively, and in the group of healthy animals there is a significant excess of the proportion of animals with the GG genotype (31,2 % to 18,3 % in healthy and sick, respectively).

In view of the data in Table 2, which show significantly higher observed frequencies of the AG genotype than theoretically expected according to Hardy-Weinberg law, the AG genotype can be considered as an increased risk factor for chlamydia morbidity.

According to Table 3, it can be noted that in the group of animals suffering from chlamydia, the frequency of occurrence of the *TT* genotype is increased (14,1 to 11,9% in the groups of sick and healthy animals, respectively) and the frequency of the TC genotype is significantly reduced (58,1 and 52,7% in healthy and sick people, respectively). According to Table 3, it is in the group of healthy animals that an approximately significant deviation of the observed TC genotypes in relation to the expected equilibrium ones is observed. This allows us to consider the TC genotype as a genetic marker of resistance to chlamydia.

Also, significant differences in the group of healthy and sick animals are observed in the frequency of the *LTF- EcoRI* ^{AA} genotype 55,9 and 47,2%, respectively), as well as in the AB genotype (45,2 and 53,8%, respectively).

Thus, the *LTF- EcoRI* ^{AA} genotype can be considered as a genetic marker of increased resistance to chlamydia, and the *LTF- EcoRI* ^{AB} genotype as a genetic marker of an increased risk of chlamydia in Holstein cattle.

The study of the phenotypic effects of paired combinations of genotypes of polymorphic genes *TLR-9 I, MBL1* and *LTF* included direct calculation of observed frequencies, relative proportions, comparative analysis and statistical assessment of the significance of differences in the frequencies of occurrence of paired combinations of genotypes in the group of sick and healthy animals (Table 4).

Table 4 – The number of heads and the proportion of paired genotypes of polymorphic genes *TLR -9 I, MBL1* and *LTF* in groups of sick and healthy Holstein cows (n and % of the examined population).

No.	Combination of genotypes	Sick	%	Healthy	%	R
1	2	3	4	5	6	7

1	2	3	4	5	6	7
1	TLR9-BfaI ^{GG} - MBL1-HaeIII ^{TC}	6	6,45	16	17,20	0,04
2	TLR9-BfaI ^{GG} - LTF- EcoRI ^{AA}	7	7l53	16	17,20	0,05
3	TLR9-BfaI ^{AG} - MBL1-HaeIII ^{CC}	9	9,68	15	16,13	0,06
4	LTF- EcoRI ^{AA} - MBL1-HaeIII ^{CC}	11	11,83	17	18,28	0,21
5	LTF- EcoRI ^{AA} - MBL1-HaeIII ^{TC}	24	25,81	28	30,11	0,44
6	LTF- EcoRI ^{AB} - MBL1-HaeIII ^{TC}	22	23,66	26	27,96	0,46
7	TLR9-BfaI ^{GG} - LTF- EcoRI ^{AB}	9	9,68	12	12,90	0,52
8	TLR9-BfaI ^{AG} - LTF- EcoRI ^{AA}	25	26,88	27	29,03	0,54
9	TLR9-BfaI ^{AA} - LTF- EcoRI ^{AA}	7	7,53	8	8,60	0,64
10	TLR9-BfaI ^{AA} - MBL1-HaeIII ^{TC}	9	9,68	10	10,75	0,67
11	TLR9-BfaI ^{GG} - MBL1-HaeIII ^{TT}	4	4,30	5	5,38	0,69
12	TLR9-BfaI ^{GG} - MBL1-HaeIII ^{CC}	6	6,45	7	7,53	0,71
13	LTF- EcoRI ^{AA} - MBL1-HaeIII ^{TT}	5	5,38	6	6,45	0,76
14	TLR9-BfaI ^{AA} - MBL1-HaeIII ^{TT}	1	1,08	1	1,08	0,83
15	LTF- EcoRI ^{AB} - MBL1-HaeIII ^{TT}	7	7,53	4	4,30	0,84
16	TLR9-BfaI ^{AG} - LTF- EcoRI ^{AB}	23	24,73	19	20,43	0,85
17	TLR9-BfaI ^{AG} - MBL1-HaeIII ^{TC}	31	33,33	27	29,03	0,86
18	TLR9-BfaI ^{AA} - LTF- EcoRI ^{AB}	14	15,05	9	9,68	0,16
19	TLR9-BfaI ^{AG} - MBL1-HaeIII ^{TT}	9	9,68	4	4,30	0,37
20	TLR9-BfaI ^{AA} - MBL1-HaeIII ^{CC}	12	12,90	6	6,45	0,27
21	LTF- EcoRI ^{AB} - MBL1-HaeIII ^{CC}	20	21,51	11	11,83	0,05

The results of analysis of phenotypic effects of paired combinations of TLR-9 I, MBL1 and LTF polymorphic genotypes in the groups of sick and healthy Holstein cows are a striking confirmation of the assumptions and observations made in the analysis of their individual phenotypic effects.

Thus, a comparative analysis revealed 3 paired genotypes, according to which the group of sick animals differs statistically significantly from the group of healthy animals.

Two paired combinations of TLR9-BfaI^{GG} - MBL1-HaeIII^{TC} and TLR9-BfaI^{GG} -LTF-EcoRI^{AA} are characterized by a significantly higher frequency of occurrence in the group of healthy animals compared to those suffering from chlamydia. It is noteworthy that the structure of these paired combinations includes genotypes that are factors of resistance to chlamydia: TLR9-BfaI^{GG}, MBL1-HaeIII^{TC} and LTF- EcoRI^{AA}.

One paired genotype LTF-EcoRI^{AB} -MBL1-HaeIII^{CC} is characterized by a significantly higher frequency of occurrence in the group of animals suffering from chlamydia compared to the group of healthy ones.

Conclusion. As a result of the conducted study, a sample of Holstein cows diagnosed with chlamydia and a control group of healthy animals were characterized by the nature of compliance of the observed genotype frequencies with the theoretically expected according to Hardy-Weinberg law, by the percentage of genotypes in comparison with the control group of healthy animals, and also by the nature of distribution of relative allele frequencies of the studied genes and their pairwise combinations. Based on the identified features and the results of statistical processing of the data, the following conclusions can be made.

Genotypes TLR-9-BfaI^{AA}, TLR-9-BfaI^{AG}, and LTF- *EcoRI*^{AB} have been identified as genetic markers for an increased risk of chlamydia. Their frequency ratios in the Sick/Healthy groups are 18,3/24,7, 50,5/57,0 and 45,2/53,8%, respectively.

Genetic markers of increased resistance to chlamydia are genotypes *TLR -9-BfaI*^{GG}, *MBL1 - HaeIII* ^{TC}, and *LTF- EcoRI*^{AA}. The ratio of their frequencies is 31,2/18,3, 58,1/52,7 and 55,9/47,2% in the healthy/patient groups, respectively.

Pair combinations of TLR9-BfaI^{GG} -MBL1-HaeIII^{TC} and TLR 9-BfaI^{GG} - LTF - EcoRI^{AA} genotypes are also genetic markers of resistance to chlamydia. Their frequency of occurrence among sick animals is 6,45 and 7,53% respectively and among healthy animals 17,20 and 17,20%, respectively.

Genetic risk factors are the LTF - EcoRI^{AB} -MBL1-HaeII^{CC} pairing. The ratio of its frequency in the sick/healthy group is 21,51/11,83%, respectively.

Information about financing. The work was executed within the framework of the project of grant financing of young scientists of the Ministry of Education and Science of the Republic of Kazakhstan for 2020-2022 "Develop a system for assessing resistance/susceptibility to bacterial infections based on polymorphisms innate immunity genes in Holstein cattle" IRN AP08052983, of state registration 0120RK00042.

REFERENCE

1 Yeleussizova A.T., Ibragimov P.Sh. Analysis of epizootical situation on the chlamidiosis of cattle in the republic of Kazakhstan // 3i: Intellect, Idea, Innovation - интеллект, идея, инновация. - 2017. - № 2-1. - С. 20-26.

2 Madiev D.Zh., Massimov N.A. Monitoring of the epizootic situation on chlamydia of farm animals in the Kostanay region of the Republic of Kazakhstan // Veterinary, zootechnics and biotechnology. - 2020. - № 10. - С. 51-56.

3 Plemyashov K.V., Krutikova A.A. Chlamydia in cattle in breeding farms // Veterinary. - 2018. - № 6. - С. 28-30.

4 Nuijens J.H., van Berkel P.H.C., Schanbacher F. Structure and biological actions of lactoferrin // J. Mammary Gland Biol. Neopl. – 1996 – V. 1. – P. 285-295.

5 Mason P.L., Heremans J.F., Dive C. An iron-binding protein common to many external secretions // Clin. Chim. Acta. – 1996. – V. 14. – P. 735-739.

6 Daly M., Ross P., Giblin L., Buckley F. Polymorphisms within the lactoferrin gene promoter in various cattle breeds // Anim. Biotechnol. - 2006. – V. 17(1). – P. 33-42.

7 Harmsen, M.C., Swart P.J., de Bethune M.P., Pauwels R., De Clercq E., The T.H., Meijer D.K. Antiviral effects of plasma and milk proteins: Lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication in vitro // J. Infect. Dis. – 1995. – V. 172. – P. 380-388.

8 Ellison, R.T., Giehl T.J., LaForce F.M. Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin // Infect. Immun. – 1988. – V. 56. – P. 2774-2781.

9 Crouch, S.P., Slater K.J., Fletcher J. Regulation of cytokine release from mononuclear cells by the iron-binding protein lactoferrin // Blood. – 1992. – V. 80. – P. 235-240.

10 Rupp R., Boichard D. Genetic resistance to mastitis in dairy cattle // D. Vet. Res. – 2003. – V. 34. – P. 671-688.

11 Legrand D., Elass E., Pierce A., Mazurier J. Lactoferin and host defence: on overview of its immune-modulating and anti-inflammarory properties // Biometals. – 2004. – V. 17. – P. 225-229.

12 Hirvonen J., Eklund K., Teppo A.M., Huszenicza G., Kulcsar M., Saloniemi H., Pyorala S. Acute phase response in dairy cows with experimentally induced Escherichia coli mastitis // Acta Veterinaria Scandinavia. – 1999. – V. 40. – P. 35-46.

13 Seyfert, H.M., Henke M., Interthal H., Klussmann U., Koczan D., Natour S., Pusch W., Senft B., Steinhoff U.M., Tuckoricz A., Hobom G. Defining candidate genes for mastitis resistance in cattle: the role of lactoferrin and lysozyme // Journal of Animal Breeding and Genetics (Zeitschrift fur Tierzuchtung und Zuchtungsbiologie). – 1996. – V. 113. – P. 269-276.

14 Bhide M.R., Mucha R., Mikula Jr.I., Kiˇsova L., ˇSkrabana R., Novak M., Mikula Sr.I. Novel mutations in TLR genes cause hyporesponsiveness to Mycobacterium avium subsp. paratuberculosis infection // BMC Genet. - 2009. – V.10 (21).

15 Sharma B.S., Leyva I., Schenkel F., Karrow N.A. Association of Toll- Like receptor 4 polymorphisms with somatic cell score and lactation persistency in Holstein bulls // Dairy Sci. - 2006. – V. 89 – P. 3626–3630

16 Wang X.P., Xu S.Z., Gao X., Li J.Y., Ren H.Y., Luoren Z.M. Cloning and SNP screening of the TLR4 gene and the association between its polymorphism and somatic cell score in dairy cattle // S. Afr. J. Anim. Sci. - 2008. – V. 38. – P. 101–109.

17 Wakchaure R.S., Gupta I.D., Verma A., Kumar D., Sonawane G.S. Association of genetic variants of partial exon 3 region of TLR4 gene with mastitis in Sahiwal cattle // Indian J. Anim. Res. - 2012. – V. 46. – P. 208-209

18 Hayashi F., Smith K.D., Ozinsky A., Hawn T.R., Yi E.C., David R., Goodlett D.R., Eng J.K., Akira S., Underhill D.M., Aderem A. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5 // Nature - 2001. – V. 410. - P. 1099–1103

19 Cargill E.J., Womack J.E. Detection of polymorphisms in bovine toll-like receptors 3, 7, 8, and 9 // Genomics - 2007. – V. 89. – P. 745–755.

20 Zhengrong Y., Jiao L., Junya L., Xue G., Shangzhong X. SNPs identification and its correlation analysis with milk somatic cell score in bovine MBL1 gene // Mol. Biol. Rep. – 2013 – V. 40. – P. 7–12.

21 Wojdak-Maksymiec K., Kmiec M., Ziemak J. Associations between bovine lactoferrin gene polymorphism and somatic cell count in milk // Veterinarni Medicina. – 2006. – V. 51. – P. 14-20.

ТҮЙІН

Хламидиоз Қазақстан Республикасындағы ірі қара малдың ең кең тараған және зиянды ауруларының бірі болып табылады. Оны Chlamidia тұқымдасының жасушаішілік паразиттері тудырады, олардың әртүрлі түрлері жануардың жеке мүшелеріне немесе тіндеріне әсер етеді. Инфекцияның әлеуетті тасымалдаушыларының көптігіне байланысты малдың хламидиозға төзімділігінің генетикалық факторларын анықтауға және онтогенездің бастапқы кезеңдерінде төзімді және керісінше сезімтал жануарларды анықтауға мүмкіндік беретін тест-жүйелерін әзірлеуге бағытталған жұмыстар маңызды болуда. Генетикалық факторларды іздеу, ең алдымен, туа біткен иммунитеттің негізгі компоненттерін кодтайтын гендерге бағытталған, олар лактоферринді (LTF), манноза байланыстыратын лектинді MBL1 және TLR-9 Toll-тәрізді рецепторлар тобының генін кодтайды. Зерттеу объектілері 186 бас голштин ірі қара малы болды, оның ішінде 93-нің хламидиоз диагнозы расталған. Зерттелетін полиморфты гендер бойынша генотиптердің таралуының теориялық күтілетінге сәйкестігі талданды, Харди-Вайнберг заңы бойынша ауру және сау жануарлар топтары арасында генотиптердің таралуына салыстырмалы талдау жүргізілді. Зерттеу нәтижесінде ірі қара малда хламидиоз ауруының/резистенттігінің жоғарылау қаупін тудыратын бір маркерлер мен генотиптердің жұптық комбинациялары анықталды.

РЕЗЮМЕ

Одним из наиболее распространенных и наносящих значительный урон заболеваний крупного рогатого скота в Республике Казахстан является хламидиоз. Его вызывают внутриклеточные паразиты рода Chlamidia, разные виды которых поражают отдельные органы или ткани животного. Вследствии большого количество потенциальных носителей инфекции, важное значение приобретают работы, направленные на выявление генетических факторов резистентности к хламидиозу у скота и разработка тест–систем, позволяющих идентировать резистентых и наоборот – восприимчвых животных на ранних стадиях онтогенеза. Поиск генетических факторов в первую очередь сосредоточен на генах, кодирующих основные компоненты врожденного иммунитета, к которым относятся гены, кодирующие лактоферрин

(*LTF*), маннозосвязывающий лектин *MBL1* и ген группы Toll-подобных рецепторов *TLR-9*. Объектами исследования стали 186 голов крупного рогатого скота голштинской породы, среди которых 93 были с подтвержденным диагнозом хламидиоз. Было проанализировано соответствие распределения генотипов для исследуемых полиморфных генов теоретически ожидаемому, согласно закону Харди-Вайнберга, проведен сравнительный анализ распределения генотипов между группами больных и здоровых животных. В результате проведенного исследования выявлены одиночные маркеры и парные сочетания генотипов, обуславливающие повышенный риск заболеваемости /резистентности к хламидиозу КРС.

UDC 578.74
IRSTI 34.03.47 34.25.37 ***DOI 10.56339/2305-9397-2022-4-1-62-69***

Sarsenkulova N.A., senior laboratory assistant, **main author,** https://orcid.org/0000-0001-9930-0297
Research Institute of Biological Safety Problems MH RK Guardeyskiy uts, 080409, Kazakhstan, nuraiym.sarsenkulova@mail.ru
Tuyskanova M.S., junior researcher**,** https://orcid.org/0000-0001-6565-082X
Research Institute of Biological Safety Problems MH RK Guardeyskiy uts, 080409, Kazakhstan, monica_94@list.ru
Tabys Sh.T., senior laboratory assistant, https://orcid.org/0000-0002-4909-6598
Research Institute of Biological Safety Problems MH RK Guardeyskiy uts, 080409, Kazakhstan, shalkar.tabys@bk.ru
Hussen J., Associate Professor, https://orcid.org/0000-0001-8942-005X
King Faisal University, Al-Ahsa, Saudi Arabia, jhussen@kfu.edu.sa
Zhugunissov K.D., PhD, head of laboratory, https://orcid.org/0000-0003-4238-5116 Research Institute of Biological Safety Problems MH RK Guardeyskiy uts, 080409, Kazakhstan, kuandyk_83@mail.ru

DEVELOPMENT OF OPTIMAL PARAMETERS OF INACTIVATION OF CAMELPOX VIRUS

ANNOTATION

The article presents the results of the development of optimal parameters for virus inactivation for the development of an inactivated pox vaccine. The study used inactivating agents (formaldehyde, beta-propiolactone, and dimerethylenemine), which are widely used in vaccine production to inactivate pox virus activity. During the study, beta-propiolactone and formaldehyde were selected as the most effective inactivators for inactivating the pox virus. The final concentration of beta-propiolactone 0.05% and formaldehyde 0.2% at 22°C completely inactivation the activity of the virus and retained its antigenic properties. Binaryethyleneimine acted 0.2% slower than the two previous agents and had a prolonged effect on the virus, reducing the antigenic activity of the virus. For virus inactivation based on the two selected inactivating agents, a 0.05% solution of beta-propiolactone inactivated the virus at 22°C in 5 hours, and a 0.2% formaldehyde solution at the same temperature completely inactivated the virus in 8 hours. Inactivated samples based on effective inactivation parameters were able to induce an immune response against camelpox in goats. Samples of inactivated virus produced antiviral neutralizing antibodies on day 7 (0.5 log2), which ranged maximum on day 28 (3.0 log2). The results of the study will allow in the future to widely use the inactivated pox vaccine in production.

Key words: *camelpox virus, inactivation, camelpox vaccine, beta-propiolactone, formaldehyde, dimerethyleneimine*

Introduction. According to the World Organization for Animal Health (WAHO or, as it was previously called, Office Internationale des Epizootics), camelpox (CMLP) is one of the rare, but very