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Marking of Genetic Resistance to Chlamydia, Brucellosis and Mastitis in Holstein Cows by Using Polymorphic Variants of LTF, MBL1 and TLR9 Genes

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Abstract: The purpose of the research is to study the association of polymorphic variants of the Toll-Like Receptor 9 (TLR9), Mannose-Binding Lectin 1 (MBL1), and Lactoferrin (LTF) genes with the incidence of chlamydia, brucellosis and mastitis in Holstein cows. Samples of Holstein cows with established diagnoses of chlamydia, brucellosis, and mastitis were characterized by the nature of the correspondence of the observed frequencies of genotypes theoretically expected by the Hardy Weinberg law, by the percentage of genotypes in comparison with the control group of healthy animals, as well as by the nature of the distribution of relative frequencies of alleles of the studied genes. It was found that the genotypes TLR9-BfaI^{AA} and MBL1-HaeIII^{CC} were the genetic markers of increased resistance to chlamydia. The genotypes TLR9-BfaI ^{AG} and MBL1-HaeIII^{TT} were the genetic markers of an increased risk of chlamydia. The genotypes MBL1-HaeIII^{TT} and LTF-EcoRI^{AA} were the genetic markers of an increased risk of brucellosis. The genotypes MBL1-HaeIII^{TC} and LTF-EcoRI^{AB} were the genetic markers of increased brucellosis resistance. The LTF-EcoRI^{AA} genotype was a genetic marker of an increased risk of mastitis and the LTF-EcoRI^{AB} genotype marked increased resistance to mastitis in Holstein cattle.

Keywords: Cattle, Holstein Breed, Lactoferrin, Mannose-Binding Lectin, Toll-Like Receptors

Introduction

An important role in increasing the productivity of the dairy industry is played not only by modern technologies of cattle breeding and selection but also by the use of modern methods of infectious disease control (Zhanabayev *et al*., 2022).

The most infectious bacterial diseases of cattle in Kazakhstan are brucellosis, chlamydia, and mastitis. As noted by Jakipov *et al.* (2021). Brucellosis is a zoonotic disease affecting human and animal health. The disease causes great economic damage to livestock, expressed by large losses of livestock from abortions, infertility, and reduced productivity in animals (Jakipov *et al.*, 2021).

Chlamydia is one of the most common diseases in cattle worldwide. The disease can manifest itself both sub clinically and in an acute sporadic form, expressed as endometritis, conjunctivitis, and other infections. With a subclinical manifestation of the disease in infected calves under the age of 15 weeks, there is a decrease in growth rate and body weight by up to 48%. In dairy cattle, chlamydial infection can cause inflammation of the mammary gland, characterized by an increase in the number of somatic cells in the milk (Anstey *et al.*, 2019).

Mastitis also causes inflammation of the mammary gland, as a result of which the physical, chemical, and microbiological composition of milk changes and pathological transformations in the tissues of the mammary gland occurs. Mastitis has a negative impact on the development of animal husbandry due to the presence of bacteria and their toxins in milk and also causes the resistance of animals to pathogens to reduce.

One of the main causes of mastitis is the microbial factor, which accounts for about 85% of all cases of morbidity. In Kazakhstan, the incidence of mastitis varies from 20-40%. Losses associated with mastitis in cattle include reduced productivity, high treatment costs, and high mortality (Zhylkaidar *et al.*, 2021).

It is also worth noting that the elimination of the studied diseases requires a lot of effort, time, and money. Apart from the losses from the culling of confirmed sick animals, the annual costs of screening studies of livestock are estimated at billions of Kazakh tenge, which undoubtedly affects the cost of production and, consequently, its competitiveness in the international market.

Therefore, we believe that along with marker-associated breeding activities aimed at increasing the profitability of the industry by increasing the genetic potential of the farm animal productivity, the use of genetic markers of resistance to bacterial infections as a significant help for veterinary medicine leads to increased profitability by reducing treatment costs and losses from mortality, abortions and culling of sick animals. This significantly contributes to the development of Kazakh breeding and veterinary medicine.

The search for genetic markers of resistance to infection is primarily focused on the genes encoding the major components of innate immunity, especially the family receptor recognition of the types of infections, which among others called Toll-Like Receptors (TLRs), as well as genes of Lactoferrin (LTF) and Mannose Binding Lectin (MBL1).

TLR1-10 genes are involved in the recognition of pathogen associated molecular patterns of viral, bacterial, and fungal pathogens and complement each other (Ghaffari *et al*., 2021).

LTF participates in the mechanism of food immunity since infection factors have limited availability of iron, as well as other growth agents, such as phosphorus and zinc. This mineral contributed to the intracellular destruction of bacteria, carried out by inducing the formation of hydroxyl radicals, which are catalyzed by iron (Musaev *et al*., 2021).

The MBL protein was 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 mechanism of antigen-specific immunity. This protein is part of many factors defined as acute phase proteins. It plays an important role in innate immunity. MBL1 deficiency is associated with low survival of newborns under one year of age, who, due to immaturity of immunity, are very sensitive to infectious diseases (Yuan *et al.*, 2013).

The purpose of the research is to study the association of polymorphic variants of the TLR9, MBL1, and LTF genes with the incidence of chlamydia, brucellosis, and mastitis in Holstein cows. The use of the identified associations allows for identifying animals with the desired genotype at an early stage of development and forming a herd with resistant qualities to the above diseases or culling animals with genotypes predisposed to chlamydia, brucellosis, and mastitis. The study consists of five sections, namely the introduction, materials and methods, results, discussion, and conclusions.

Materials and Methods

The protocol of the study was discussed and approved at the meeting of the local ethical committee of the Jangir Khan West Kazakhstan Agrarian and technical university, protocol No. 5 dated April 09, 2020.

The experiment was conducted in the period from 2019-2021. Animals with established diagnoses of brucellosis, chlamydia, and mastitis were selected by farms in the Kostanay region of the Republic of Kazakhstan. Samples of biomaterial from healthy animals were selected from the same farms to form a control group.

The experiment involved 34 Holstein cows born in 2017-2018 (1-3 lactations) diagnosed with chlamydia, 117 cows diagnosed with brucellosis, and 67 cows diagnosed with mastitis. The control group consisted of 93 healthy cows.

Samples of hair follicles served as the material for the study. DNA isolation from the studied animal samples was carried out using a commercial reagent kit DNK-ekstran-2 (manufactured by "syntol" LLC, Russian federation).

Detection of the genotype by the selected polymorphisms was carried out by the method of Polymerase Chain Reaction product Restriction Fragment Length Polymorphism (PCR RFLP).

The primer sequences and PCR conditions for the analysis of each polymorphism are given in Table 1.

Table 1: Individual characteristics of PCR conditions for the studied polymorphic loci of somatotropin cascade genes

Polymorphism	Annealing t (C°)	Primers
TLR9-BfaI	56	F: 5′-ATCTTCAACGACCTGACCCA-3′
		R: 5′-AATCGCCAGACTTCCACCCT-3′
MBL1-HaeIII	61	F: 5′-GTGGTGGCAAATGTTGGCTAAAC-3′
		R: 5′-TGGCTCTCCCTTTTCTCCCTT-3′
LTF-EcoRI	59	F: 5′-GCCTCATGACAACTCCCACAC-3′
		R: 5′-CAGGTTGACACATCGGTTGAC-3′

Genotyping of Animals

The TLR9-BfaI polymorphism is a T/C transversion at position +979 of the TLR9 gene, accompanied by the substitution of the amino acid cysteine for valine at the position of protein 174, exon 2 (Elmaghraby *et al.*, 2018). At the time of the G → A replacement, a site for BfaI emerges (which cuts the C-TAG).

Genotyping of animals by MBL1-HaeIII polymorphism was carried out according to the method described by Yuan *et al.* (2013).

Genotyping of animals by LTF-EcoRI polymorphism was carried out according to the method described by Wojdak-Maksymiec *et al.* (2006).

Data Analysis

During the experiment, we analyzed the correspondence of the genotype distribution for the studied polymorphic genes of the somatotropin cascade to the theoretically expected one, according to the Hardy-Weinberg law. The significance of the observed deviations was assessed using the criterion χ^2.

The association of allelic variants of the TLR9, MBL1, and LTF genes with resistance to bacterial infections was assessed by evaluating the correspondence of the actual frequencies of genotypes theoretically expected by the Hardy-Weinberg law, as well as by comparing the frequency distribution of alleles of the studied genes in groups of sick animals and a control group of healthy cows and assessing the reliability of the observed differences. The relative frequencies of allelic variants in each group were calculated and the calculated significance level P was calculated 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 $p>0.05$.

Statistical data processing was carried out using the standard Statistica 6.0 software package (stat soft, Inc. 1994-2001).

Results

Figure 1 shows the electrophoregram of the results of the PCR-RFLP analysis of the TLR9 gene; after BfaI restriction and dispersal, the following genotypes were identified: TLR9-BfaI^{AA} with 280/82 bp, TLR9-BfaI^{AG} with 362/280/82 bp and TLR9-BfaI^{GG} with 362 bp. Thus, a rare mutant allele is cut into 2 fragments, 82 and 280 base pairs (bp).

Figure 2 shows the results of animal genotyping for the MBL1-HaeIII polymorphism. During amplification, a PCR product = 255 pp is formed. After endonuclease cleavage by the HaeIII enzyme, after electrophoresis, PCR RFLP fragments are visualized in 3% agarose gel: 255/178/77 bp for the TC genotype; 178/77 bp for the CC genotype; and 255 bp for the TT genotype.

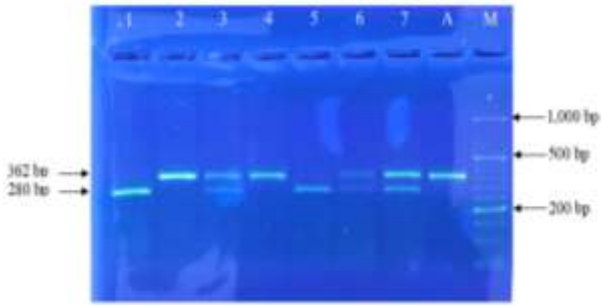

Fig. 1: Electrophoregram of the result of PCR RFLP analysis of the TLR9 gene; M is the DNA marker with 1,000-50 bp; A is amplification; 3, 6, 7 are the AG genotype (362/280/82 bp); 2, 4 are the GG genotype (362 bp); 1, 5 are the AA genotype (280/82 bp)

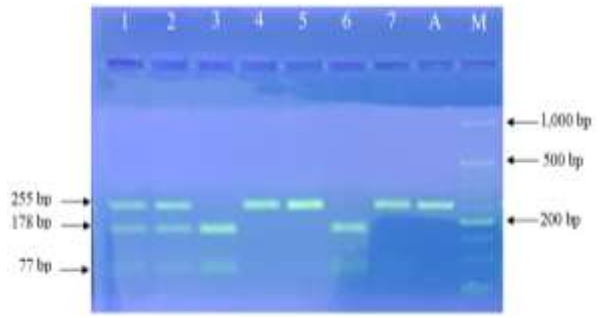

Fig. 2: Electrophoregram of the result of PCR RFLP analysis of the MBL gene (MBL1); M is the DNA marker with 1,000-50 bp; A is amplification; 1, 2 are genotype TC (255/178/77 bp); 3, 6 are genotype CC (178/77 bp); and 4, 5, 7 are genotype TT (255 bp)

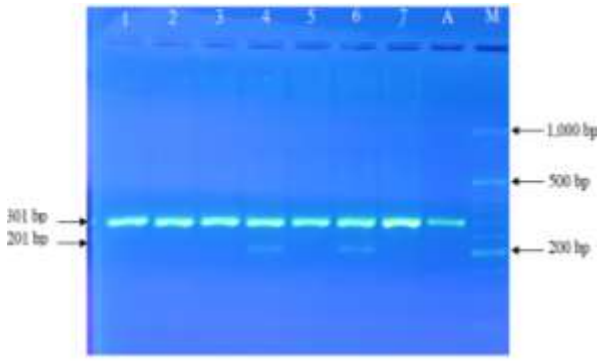

Fig. 3: Electrophoregram of the result of PCR RFLP analysis of the LTF gene (LTF); M is the DNA marker with 1,000-50 bp; A is amplification; 1, 2, 3, 5, 7 are genotype AA (301 bp); 4, 6 are genotype AB (301/201/100 bp)

Genotyping of animals according to LTF-EcoRI polymorphism is shown in Fig. 3. After EcoRI restriction, according to the protocol of the manufacturer, the following fragments were visualized in a 3% agarose gel: Genotype 301 bp-AA, genotype 201/100 bp-BB and genotype 301/201/100 bp-AB.

Table 2 shows the characteristics of samples of sick and healthy animals by the nature of the distribution of genotypes of the studied genes.

The analysis of data in the control group of healthy cows according to Table 2 demonstrates the correspondence of the observed genotype frequencies for TLR9-BfaI and LTF-EcoRI polymorphisms to the ones theoretically expected by the Hardy Weinberg law. According to LTF-EcoRI polymorphism, a significant deviation from the norm towards an increase in the number of LTF-EcoRI^{AB} heterozygotes is observed in the group of healthy animals. This suggests that a heterozygous genotype may be associated with resistance to bacterial infections.

In the group of animals with chlamydia, the deviation of the observed genotype frequencies from those theoretically expected according to the Hardy-Weinberg law is demonstrated in the form of an excess of the number of TLR9-BfaI^{AG} heterozygotes by TLR9-BfaI polymorphism (23 observed compared to 17 theoretically calculated). This observation suggests that the TLR9-BfaI^{AG} genotype is associated with a decrease in resistance to chlamydia in Holstein cows.

In the population of Holstein cows with brucellosis, a statistically significant redistribution of genotype frequencies is observed for the polymorphic TLR9 gene. In particular, the number of observed TLR9-BfaI^{AG} heterozygotes in this group equals 68 and significantly exceeds the one theoretically expected.

In the group of animals with mastitis, statistically significant deviations of the observed frequencies of genotypes compared to the ones theoretically expected by the Hardy-Weinberg law were not found.

To assess the nature of the influence of the genotype on the incidence of bacterial infections, we performed a comparative assessment of the frequency of occurrence of genotypes in groups of sick and healthy animals. The numbers are shown in Table 3.

The data in Table 3 numerically reflect the nature of the redistribution of genotypes in groups of sick animals compared with the group of healthy ones.

It has been shown that according to the TLR9 gene, the proportion of heterozygous TLR9-BfaI^{AG} animals is 17% higher compared to the healthy group (Fig. 4).

According to the data given in Table 3, it can be noted that in the groups of animals diagnosed with chlamydia and brucellosis according to the polymorphic gene MBL1, there is an increase in the frequency of occurrence of MBL1-HaeIII^{TT} genotypes and a decrease in the frequency of occurrence of MBL1-HaeIII^{CC} genotypes compared with the group of healthy animals (Fig. 5).

Table 2: Distribution of genotype frequencies of polymorphic genes TLR9, MBL1, and LTF in a group of healthy and sick animals

		Healthy			Chlamydia			Brucellosis			Mastitis		
Polymorphism	Genotype	n_n	n_o	χ^2	n_n	n_o	χ^2	n_n	n_o	χ^2	n_n	n_o	χ^2
TLR9-BfaI	AA	17	18	0.12	2	6	4.39	26	31	3.91	11	11	0.02
	AG	47	45		23	17		68	58		33	32	
	GG	29	29		9	12		23	28		23	32	
MBL1-HaeIII	TT	11	16	3.77	6	6	0.12	25	23	0.55	12	14	0.90
	TC	54	45		10	9		39	42		39	35	
	CC	28	33		3	3		21	19		20	22	
LTF-EcoRI	AA	51	56	6.23*	16	18	3.07*	60	62	2.53*	48	50	2.88*
	AB	41	32		17	13		25	21		24	20	
	BB	0	5		0	2		0	2		0	2	

Notes: The value of χ^2 for a significance level of 0.05 is 3.84 * the values of χ^2 were calculated with the Yates correction
n_n-observed genotype frequency of polymorphic genes; n_o-expected genotype frequency of polymorphic genes

Table 3: The proportion of genotypes of polymorphic genes TLR9, MBL1, and LTF in groups of sick and healthy Holstein cows (% of the surveyed livestock)

Gene	Genotype	Chlamydia	Brucellosis	Mastitis	Healthy
TLR9-BfaI	TLR9-BfaI^{AA}	5.88	22.22	16.42	18.28
	TLR9-BfaI^{AG}	67.65	58.12	49.25	50.54
	TLR9-BfaI^{GG}	26.47	19.66	34.33	31.18
MBL1-HaeIII	MBL1-HaeIII^{TT}	31.58	29.41	16.90	11.83
	MBL1-HaeIII^{TC}	52.63	45.88	54.93	58.06
	MBL1-HaeIII^{CC}	15.79	24.71	28.17	30.11
LTF-EcoRI	LTF-EcoRI^{AA}	48.48	70.59	66.67	55.43
	LTF-EcoRI^{AB}	51.52	29.41	33.33	44.57
	LTF-EcoRI^{BB}	0.00	0.00	0.00	0.00

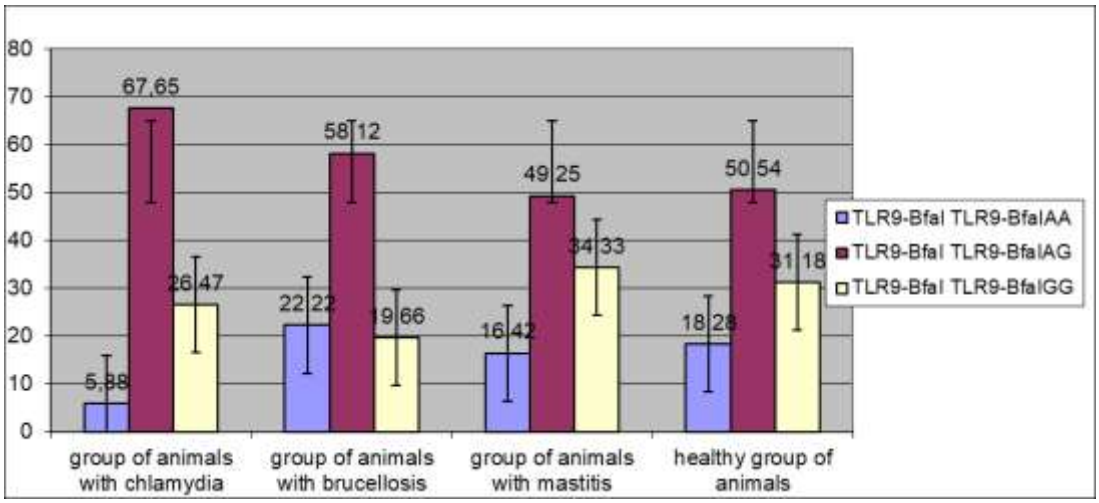

Fig. 4: The degree of redistribution of genotype frequencies compared to healthy animals by the TLR9 gene

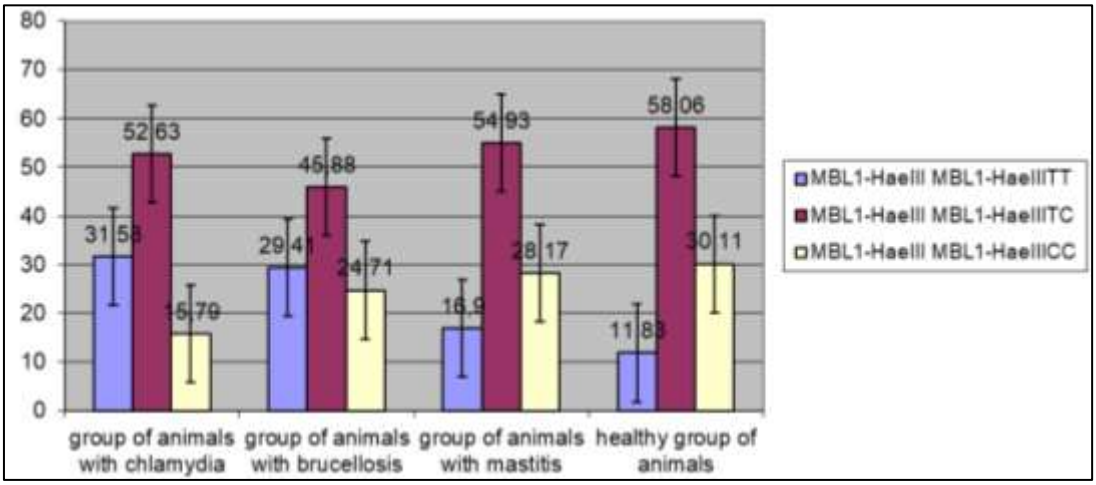

Fig. 5: The degree of redistribution of genotype frequencies compared to healthy animals by the MBL1 gene

We noted that in the group of animals with brucellosis, there was an increase in the proportion of homozygous animals MBL1-HaeIII^{TT} (29.41% sick and 11.83% healthy animals) and in the group of healthy animals the frequency of heterozygous genotypes, MBL1-HaeIII^{TC} was significantly increased (45.88% in sick and 58.06% in healthy animals, respectively). Thus, the MBL1-HaeIII^{TT} genotype can be considered a genetic marker of an increased risk of brucellosis, and the MBL1-HaeIII^{TC} genotype is a genetic marker of brucellosis resistance in Holstein cattle.

Regarding the EcoRI polymorphism of the LTF gene, it should be noted that differences from the group of healthy animals in the proportion of polymorphic genotypes are observed in groups of animals with brucellosis and mastitis (Fig. 6).

In the group of animals with brucellosis, there is an increase in the proportion of homozygous animals with the LTF-EcoRI^{AA} genotype (70.59% sick and 55.43% healthy animals) and in the group of healthy animals, the frequency of heterozygous LTF-EcoRI^{AB} genotypes is significantly increased (29.41% in sick and 44.57% in healthy animals). Thus, the LTF-EcoRI^{AA} genotype can be considered as a genetic marker of an increased risk of brucellosis, and the LTF-EcoRI^{AB} genotype as a genetic marker of brucellosis resistance in Holstein cattle.

In particular, in groups of sick animals, in comparison with the group of healthy animals, there is a significant increase in the number of LTF-EcoRI^{AA} homozygotes, which may indicate a negative phenotypic effect of this genotype on the resistance of animals to brucellosis and mastitis.

The characteristic of the distribution of relative frequencies of alleles of polymorphic genes TLR9, MBL1, and LTF is shown in Table 4.

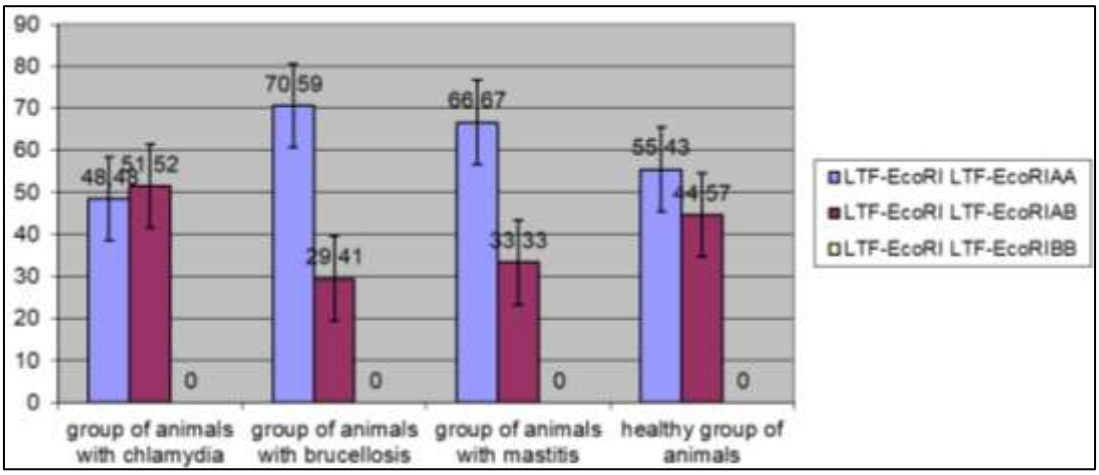

Fig. 6: The degree of redistribution of genotype frequencies compared to healthy animals by the LTF gene

Table 4: Distribution of relative frequencies of alleles of the studied TLR9, MBL1, and LTF genes in groups of sick and healthy Holstein cows ($Q \pm S_Q$)

		Relative frequencies of alleles			
Polymorphism	Allele	Chlamydia	Brucellosis	Mastitis	Healthy
TLR9-BfaI	TLR9-BfaI^A	0.39±0.01	0.51±0.00	0.41±0.01	0.44±0.01
	TLR9-BfaI^G	0.61±0.01	0.49±0.00	0.59±0.01	0.57±0.01
MBL1-HaeIII	MBL1-HaeIII^T	0.58±0.03	0.52±0.01	0.44±0.01	0.41±0.01
	MBL1-HaeIII^C	0.42±0.03	0.48±0.01	0.56±0.01	0.59±0.01
LTF-EcoRI	LTF-EcoRI^A	0.74±0.01	0.85±0.00	0.83±0.01	0.78±0.00
	LTF-EcoRI^B	0.26±0.01	0.15±0.00	0.17±0.01	0.22±0.00

Table 5: Values of the calculated significance level P for assessing the statistical reliability of the difference between samples of sick animals from the control group by the nature of the distribution of alleles of polymorphic genes TLR9, MBL1, and LTF

	Calculated significance level P		
Polymorphism	Chlamydia	Brucellosis	Mastitis
TLR9-BfaI	0.05	0.12	0.65
MBL1-HaeIII	0.04	0.031	0.52
LTF-EcoRI	0.56	0.05	0.02

Note: The difference between the groups is significant at $p \leq \alpha$; $\alpha = 0.05$

For the MBL1 gene, according to Table 4, the frequency ratio of the MBL1-HaeIII^T and MBL1-HaeIII^C alleles in the group of animals with brucellosis was 0.52±0.01 and 0.48±0.01, respectively, while in the group of healthy animals, it equaled 0.41±0.01 and 0.59±0.01, respectively.

According to our data, it can be noted that the frequency ratio of LTF-EcoRI^A and LTF-*E* coRI^B alleles in the group of animals with brucellosis is 0.85±0.00 and 0.15±0.00, while in the group of healthy animals, it equals 0.78±0.00 and 0.22±0.00, respectively. Regarding the incidence of mastitis, according to the data given in Table 5, it can be noted that statistically significant differences between the group of sick and healthy animals are observed in the polymorphism of the LTF gene.

For the LTF gene, the frequency ratio of LTF-EcoRI^A and LTF-EcoRI^B alleles in the group of animals with mastitis is 0.83±0.01 and 0.17±0.01, while in the group of healthy animals, it equals 0.78±0.00 and 0.22±0.00, respectively.

Statistical evaluation of the reliability of the difference between samples of sick animals from a control sample of healthy animals by the nature of the distribution of genotypes of polymorphic genes TLR9, MBL1, and LTF 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 the student's distribution tables (Table 5). The difference between the samples is significant at $p \leq 0.05$.

From the data given in Table 5, it follows that in the group of animals with chlamydia, there are significant differences in the distribution of relative frequencies of allelic variants of the TLR9 and MBL1 genes compared to those in the group of healthy animals.

Discussion

Our data are only partially consistent with the results of other researchers since representatives of different breeds have different genomic patterns of breed-specific SNP. Such polymorphisms, being scattered over different genes, form a genomic background that affects the manifestation of the phenotypic effects of individual polymorphisms. This makes it necessary to conduct studies not only for individual breeds but also for individual populations of animals of the same breed bred in different territories. Any information obtained brings us closer to understanding the genetic mechanisms of control of traits such as innate resistance to viral and bacterial infections in cattle. In our research, we studied the association of polymorphic variants of LR9, MBL1, and LTF genes with the diseases of cattle of the Holstein breed in Kazakhstan.

For the TLR9 gene, according to our experimental data, the frequency ratio of TLR9-BfaI^{A} and TLR9-BfaI^{G} alleles in the group of animals with chlamydia amounted to 0.39±0.01 and 0.61±0.01, while in the group of healthy animals, it was 0.44±0.01 and 0.57±0.01, respectively. In the group of animals with chlamydia, an increase in the proportion of heterozygous TLR9-BfaI^{AG} animals was observed (67.65% sick and 50.54% healthy animals) and in the group of healthy animals, the frequency of TLR9-BfaI^{AA} genotypes was significantly increased (5.88% in sick animals and 18.28% in healthy ones). Thus, the TLR9-BfaI^{AG} genotype can be considered a genetic marker of an increased risk of chlamydia, and the TLR9-BfaI^{AA} genotype is a genetic marker of resistance to chlamydia in Holstein cattle. There was no association with diseases such as brucellosis and mastitis for the TLR9 polymorphic gene.

According to the authors, in particular, Cinar *et al.* (2018), in the study of 53 heads of Holstein cows' heterozygous animals with the TLR9-BfaI^{AG} genotype were not found. According to Sun *et al.* (2012), no significant association was found in the study of the association of the polymorphism gene in the TLR9 gene with the sensitivity or resistance to tuberculosis of Chinese cattle of the Holstein breed. The relationship of the TLR9 gene with sensitivity or resistance to tuberculosis has also been studied by Prakash *et al.* (2014). The authors studied 300 heads of local cattle from Sri Mataji Goshala, Barsana. The results of the research revealed the absence of statistically significant differences in the frequency of TLR9-BfaI^{A} and TLR9-BfaI^{G} alleles in groups of animals with serological signs of brucellosis and the control group.

Comparing the above results with our data, we can conclude that in the countries where the analysis of associations of the TLR9 gene genotypes with the diseases presented in the studies was carried out, no associations were found. However, we found an association of genotypes with chlamydia. Studies on the search for the association of the TLR9 gene with chlamydia were not noted in other scientific papers analyzed by us. Based on this, we can conclude that it is necessary to conduct additional studies in other populations of Holstein cattle on the association of the TLR9 gene with chlamydia.

Regarding the polymorphism of the MBL1 gene, according to our results, it can be noted that the frequency ratio of the MBL1-HaeIII^{T} and MBL1-HaeIII^{C} alleles in the group of animals with chlamydia was 0.58±0.03 and 0.42±0.03, while in the group of healthy animals, it amounted to 0.41±0.01 and 0.59±0.01, respectively. In the group of animals with chlamydia, there was an increase in the proportion of heterozygous animals MBL1-HaeIII^{TT} (31.58% sick and 11.83% healthy animals), and in the group of healthy animals, the frequency of MBL1-HaeIII^{CC} genotypes was significantly increased (15.79% in sick and 30.11% in healthy cows). Thus, the genotype MBL1-HaeIII^{TT} can be considered as a genetic marker of an increased risk of chlamydia, and the genotype MBL1-HaeIII^{CC} as a genetic marker of resistance to chlamydia in Holstein cattle.

The association of polymorphism of the MBL1 gene with brucellosis or mastitis was not established in our study. However, according to Yuan *et al.* (2013), the MBL1 gene turned out to be one of the promising indirect markers for improving mastitis resistance in cattle. According to Kumar *et al.* (2018), the association of the MBL1 gene with mastitis resistance in cattle has been described. According to Dhundwal *et al.* (2019), when studying the relationship between the polymorphism of the MBL1 gene in the Murra buffalo with mastitis at the molecular level, no significant association between the Single-Nucleotide Polymorphism (SNP) candidate with resistance to mastitis was found, which may be a characteristic of a particular breed. It was found that all animals were monomorphic, that is, of the CC genotype (Dhundwal *et al*., 2019). This may be due to the location of the cattle population and the acquisition of resistance to mastitis during the evolution of the development of a particular population.

According to our data, in the group of animals with brucellosis, there were significant differences in the distribution of relative frequencies of allelic variants of the LTF gene compared to those in the group of healthy animals.

Regarding the polymorphism of the LTF gene, a study of the frequencies of allelic variants of the gene in Holstein cows was conducted by Safina *et al.* (2019). The authors have shown that the LTF gene is polymorphic for the studied population. During the work, the following allelic variants and genotypes were identified: A-0.84 and B-0.16; AA-67.5% (401 animals), AB-32.5% (193 animals), and BB-0.0% (0 animals). A chi-squared check between the observed and expected genotype distribution indicates a genetic equilibrium in the population under study. For all groups of cattle, the predominance of allele A over allele B has been established.

According to our results, in the group of animals with mastitis, there is an increase in the proportion of homozygous LTF-EcoRI^{AA} animals (66.67% sick and 55.43% healthy animals), and in the group of healthy animals, the frequency of heterozygous LTF-EcoRI^{AB} genotypes is significantly increased (33.33% in sick and 44.57% in healthy animals). Thus, the LTF-EcoRI^{AA} genotype can be considered a genetic marker of an increased risk of mastitis, and the LTF-EcoRI^{AB} genotype is a genetic marker of resistance to mastitis in Holstein cattle.

The association of the LTF gene with mastitis resistance was studied by Wojdak-Maksymiec *et al.* (2013). The authors showed that the effects of LTF varied depending on age (parity). Alleles that were associated with high immunity to mastitis at lower parity turned out to be less favorable at higher parity. The authors concluded that the observed phenomena might be associated with inflammation aging, that is, increased susceptibility to infection due to the deregulation of the immune system, which progresses with age. Such a pattern of interactions makes it impossible to use the genes in question in animal breeding using markers aimed at reducing hereditary predisposition to mastitis. This is since the immune mechanisms of resistance to infections turned out to be too complex. This may be due, as in the case of the previous gene, to the location of the cattle population and the acquisition of resistance to mastitis during the evolution of the development of a particular population.

Welderufael *et al.* (2017; 2018) as a result of the study involving a genome-wide search for associations with mastitis in Danish Holstein cows showed the relationship of polymorphic variants of genes BTA13, TNF-α, E3 ubiquitin-protein ligase MARCH 3, STAB2 (stabilin-2 precursor), gene SLX4IP (SLX4 interacting protein), ATG16L2 (autophagy-related 16-like 2), gene PDGFD (platelet-derived growth factor D), PTX3 (pentraxin 3). The association with mastitis in cattle can be found in combination with various genes and not only with those presented in the current study (Kurz *et al*., 2019). Further research on this issue is required.

Thus, it can be noted that the sign of resistance to brucellosis, chlamydia, and mastitis is under the control of various genes belonging to gene networks that regulate various biological processes.

Conclusion

According to our results, associations of the genotypes of the studied genes with resistance and an increased risk of chlamydia, brucellosis, and mastitis were established. Namely, the association of the TLR9-BfaI^{AA} and MBL1-HaeIII^{CC} genotypes with increased resistance to chlamydia has been established. The genotypes TLR9-BfaI^{AG} and MBL1-HaeIII^{TT} are the genetic markers of an increased risk of chlamydia. The genotypes MBL1-HaeIII^{TT} and LTF-EcoRI^{AA} are the genetic markers of an increased risk of brucellosis. The genotypes MBL1-HaeIII^{TC} and LTF-EcoRI^{AB} are the genetic markers of increased brucellosis resistance. The LTF-EcoRI^{AA} genotype is a genetic marker of an increased risk of mastitis and the LTF-EcoRI^{AB} genotype marks increased resistance to mastitis in Holstein cattle.

The data obtained makes it possible to understand and compare the work of the studied genes with other studies, as well as to evaluate their use in practice. The use of the identified associations allows for identifying animals with the desired genotype at an early stage of development and forming a herd with resistant qualities to the above diseases or culling animals with genotypes predisposed to chlamydia, brucellosis, and mastitis. This, in turn, allows farmer units to reduce the financial costs of keeping animals with unwanted genotypes. Since the results in this study are formulated based on a relatively small sample, further studies on a large scale are required.

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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