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GENETIC DISEASES IN THE BEEF CATTLE POPULATION OF KAZAKHSTAN

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Annotation

54 animals of different beef breeds of domestic and foreign breeding from a variety of business entities of Akmola and North Kazakhstan oblasts of Kazakhstanwereselected for the purpose of the research.These breedsare: 18 Angus, 20Hereford, 5 Angler (англер) and 21 Kazakh white.54 biological samples (hairs) were collected respectively. Genotyping was carried out in cooperation withthe laboratory "Labogena"in France (with an international accreditation ICAR). The results of the genotyping revealed that a bull of the Kazakh white is a carrier of recessive alleles of two diseases: hypotrichosis with SNP in the HELPH1 gene and glycogenosis 5 with SNP in the PYGM gene.

Keywords: dna, genotyping, hypotrichosis, glycogenosisv, cattle

Introduction

A timely diagnosis and eradication of the source causing the genetically determined diseasesare one of the main issuesin the development of healthy and highly productive breeding livestock animals. It is required to achieve the goals set by the President in Address to the people of Kazakhstan on January 17, 2014 "Kazakhstan 2050: The common goal, common interests, common future" («…Kazakhstan should become one of the major regional exporters of meat, dairy and other products») [1].

At the present stage of development of animal husbandry, issues of particular interest are congenital abnormalities, which are directly connected with the intensification of livestock production, on the one hand, and with the increase of anthropogenic burden on the environment, on the other. Therefore, in order to develop healthy and highly productive breeding livestock animals, veterinarians and specialists in purebred breeding have to deal with an issue of atimely diagnosis and an eradication of the source causing the genetically caused diseases. An effective fight against hereditary disease is based on knowledge of the molecular structure of the genes, and theproper determination of heterozygous carrier and mutant organisms.

In a number of countries there are genetic monitoring services that control the genetic health of livestock

populations. For decades, genetic and selection studies arebeing carried out to improve the genetic resistance of animals to a number of diseases and to identify carrier animals of harmful genes or chromosomal abnormalities, howeversimilar studies have not yet been conducted in our country.

Since the beginning of the industrialization of the livestock sector, the number of individual populations and the general livestock population has beensignificantly increased. Therefore, the objective conditions for the accumulation of recessive mutations and for their transition in a homozygous state have been formed. Particularly, the widespread introduction of artificial insemination and embryo transplantation facilitated these processes. The introduction of artificial insemination has led to the fact that the number of offspring produced by the same seed bull has increased from tens to hundreds of thousands. Even if 90% of the produced offspring is not used for a reproduction, the rest of the population is sufficient for stable preservation of some mutations.

There are dozens (more than 60) genetic abnormalities and mutations of farm animals, the occurrence of which is associated with a recessive or dominant gene mutations [2,3]. These mutations occur in specific populations at different rates, depending on the mutation frequency, breeding systems, etc.

The genetic diversity of the population is shrinking because of the intensive use of the limited number of producers in the economic entities. Also, cross breeding on farms will inevitably lead to spontaneous inbreeding in commercial farms, thereby increasing the frequency of deformities and abnormalities in populations.

Monitoring and detection of the genetic abnormalities, mutations and pathologies are an integral part of animal breeding. One of the important aspects in controlling genetic abnormalities after the disease has been found is a control of its manifestations by phenotypic characteristics.Some genetic diseases do not manifest themselves immediately after the formation of mutations duringa crossing of parents butmany years later. By this time, genetic diseases might spread throughout the population. For this reason, it is important to identify genetic changes of the animal as early as possible.

Symptoms and occurrence of genetic mutations, pathologies and diseases are also characteristic for the near and far abroad imported livestock, which is still actively imported into Kazakhstan. Most of the foreign cattle has a genetic certificate that contains information about the most common genetic diseases.

To prevent a distribution of harmful genes, it is necessary to test genotypes of producer animals and to exclude mutation carriers from the further use.

Genetic DNA diagnostics of the cattle at an early age is requiredforan identification ofthe inherited diseasecarriers in order to avoid the economic losses.

HEPHL1 - hypotrichosis, a hereditary anomaly, characterized by the absence of hair in the newborn animal. Hypotrichosis is transmitted by an autosomal recessive inheritance, when the mutant allele (gene) is localized in the sex chromosome (autosome) of both parents [4].

13 types of hypotrichosis are described in cattle breeds Angus, Brangus, Holstein-Friesian, Hereford, Jersey, Simmental, etc.Hereditythe diseaseisan autosomal recessive or sex-linked[5].

Marron and Beeversuggest that a mutation in the hephaestin-like 1 (HEPHL1) geneon BTA29 is causative [6].This disease can vary in severity and may be accompanied by other symptoms such as lack of teeth.

Congenital defects of the hair are harmful for all segments of the livestock industry, as the affected animals are more vulnerable to environmental exposure, skin infections, insect pests, sunburn, cold stress, and have a lower economic value, regardless of where they are grown.

GlycogenosisV is the most common disorder of carbohydrate metabolism in skeletal muscle, and one of the most frequent genetic myopathies (frequency ~ 1:100000).The characteristic symptoms of GSD-V are zero tolerance to exercises, myalgia (muscle pain), muscle stiffness and contractures, fatigue and giperkemiya and myoglobinuria (dark burgundy color of urine due to the presence of myoglobin, a protein found in the heart and muscles) [7].

GSD-V is caused by mutations in the *PYGM gene*, which encodes an enzyme myophosphorylase (a musclular form of glycogenphosphorylase). PYGM is located on the chromosome 11 at the 11q13 position.

The spread of infectious and genetic diseases significantly affects the animals breeding [8].

Materials and methods

The studyis carried out within the grant project of MES of the Republic of Kazakhstan No.1645/GF4(2015-2017) under agreementNo.76 from 12.02.2015"Study of heritability of the genetic diseases of foreign and domestic breedingcattle"».

54 animals of different beef breeds of domestic and foreign breeding from a variety of business entities of Akmola and North Kazakhstan oblasts of Kazakhstan were selected for the purpose of the research. These breeds are: 18 Angus, 20Hereford, 5 Angler (англер) and 21 Kazakh white.

54 biological samples (hairs) were collected forgenotyping.

DNAextractionwasperformedatt heResearchinstituteof agricultural biotechnology, JSC “S.Seifullin Kazakh Agro Technical University”. A commercial kit “Qiagen” was used fortheextractionofgenomicDNAfromh airsamples.

AlsoamodifiedmethodofDNAisolation (by Glowatzki) wasused: 50 µl lysis

buffer (10 mMTrispH 8.3, 50 mMKCL, 0.5% Tween) and 10-20 µl proteinase K were added, the mixtures were vortexed and centrifuged at 13 000 rpm. Thenthesampleswereincubatedat 60^0C in water bath overnight and at 95^0Cfor 45 minutes. Intotal 54 DNAsampleswereextracted.

Genotyping ofthe DNA samples for SNP identification was performed by using DNA chips and an Illuminasequenceratthe laboratory «Labogena» inFrance.Theprocessbeganfrom 16-hoursamplificationofDNA. The amplified product was then fragmented during the controlled enzymatic cleavage process that does not require gel electrophoresis.After alcohol precipitation and resuspensionof DNA, the DNA chip was prepared for hybridization;the samples were applied to it and incubated overnight. During the night hybridization, DNA samples were hybridized with specific 50-mer oligonucleotides, covalently bound to the beads. After hybridization, the allele specificity was confirmed by further enzymatic elongation.The products were consistently colored by a fluorescent label. The intensity of the fluorescence emission was measured by iScan system, after thatobtained data was analyzed using the Illuminasoftware.

An interpretation of the results of genotyping was carried out in collaboration with specialists of the “Labogena”.

Results

Genotyping was performed for the following autosomal recessive diseases of beef cattle breeds: dwarfism with 3 SNPs in the GH1, RNF11 and PRKG2 genes, hypotrichosis with a SNP in the HEPHL1 gene, glycogen storage disease (glycogenosis)with a SNP in the PYGM gene, subfertility of male individuals with a SNP in the TMEM95 gene and maple syrup urine diseasewith 2 SNPs in the BCKDHAgene. Table 1 shows the results of genotyping of 54 samples of beef cattle breeds for autosomal recessive diseases in both alleles.

Table 1 – Genotyping results of 54 samples of Kazakhstanbeef cattlepopulation

KZ animal number	**ID number of the sample (France)**	**SNP**															
		BCKDHA_1		BCKDHA_2		GH1		HEPHL1		PRKG2		PYGM		RNF11		TMEM95	
		A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18

KZC158257183	COLA22334	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109168661	COLA22342	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC110000051	COLA22358	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183039288	COLA22366	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157356781	COLA22374	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157356878	COLA22382	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158380586	COLA22390	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109007561	COLA22398	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183043819	COLA22327	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158256624	COLA22335	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109168663	COLA22343	G	G	G	G	-	-	**A**	**T**	-	-	**A**	**G**	A	A	-	-
KZC158380572	COLA22351	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109168428	COLA22359	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183039338	COLA22367	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157356789	COLA22375	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP57356792	COLA22383	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158257960	COLA22391	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183043820	COLA22328	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C

Continuation of Table 1

KZC158257109	COLA22336	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158462014	COLA22344	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183043749	COLA22360	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157	COLA	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C

356697	22376																
KZP157 356793	COLA 22384	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 380744	COLA 22392	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 380610	COLA 22400	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183 043907	COLA 22329	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 258283	COLA 22337	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 462013	COLA 22345	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC102 968168	COLA 22353	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 358148	COLA 22377	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 356751	COLA 22385	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 257742	COLA 22393	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC183 043611	COLA 22401	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183 043822	COLA 22330	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109 168664	COLA 22338	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT100 402412	COLA 22346	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC102 967966	COLA 22354	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 356798	COLA 22378	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 356833	COLA 22386	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 380430	COLA 22394	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC183 089211	COLA 22402	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183 043839	COLA 22331	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109 168673	COLA 22339	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109	COLA	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C

168439	22355																
KZS157 356845	COLA 22371	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 356788	COLA 22379	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP573 56865	COLA 22387	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 256630	COLA 22395	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC183 043605	COLA 22403	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZT183 043824	COLA 22332	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC109 168667	COLA 22340	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZC158 462017	COLA 22356	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 356848	COLA 22372	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C
KZP157 356701	COLA 22380	G	G	G	G	G	G	T	T	G	G	G	G	A	A	C	C

*Note:A_1 – allele_1, A_2 – allele_2

Accordingtothegenotypingresults, abull of the Kazakh white breed is a carrier of recessive alleles of 2 diseases: hypotrichosis with a SNP in the HEPHL1 gene andglycogenosis 5with a SNP in the PYGM gene(Table 2).

Table2 – The number of identified carriers of recessive genes in animal meat breeds

A breed	The number of animals	BCKDHA_1	BCKDHA_2	GH1	HEPHL1	PRKG2	PYGM	RNF11	TMEM95
Angus	18	0	0	0	0	0	0	0	0
Hereford	20	0	0	0	0	0	0	0	0
Angler	5	0	0	0	0	0	0	0	0
KW	21	0	0	0	1	0	1	0	0

*Note: KW – Kazakh white

Since these diseases have not previously been observed and studiedin the population of Kazakh Whiteheaded breed in our country, it is likely that they wereinherited from the Hereford breed, which is the ancestor of the Kazakh Whiteheaded. In our opinion, the caused mutations might be transmitted during the wide-scale “Herefordization” of the Kazakh Whiteheaded in 2000s.

A reliable method of preventing an occurrence of inherited hairless disease among young animals is an avoidingof mating of cows with bulls-producers that carry the mutant hairless gene. In order to achieve a goal of breeding and to prevent the spread of these diseases, it is necessary to carefully analyze the pedigree of the parents, as well as to carry out a careful selection of parents in the herd, breed, or in the cattle population.

Conclusion

Based on the study results, it can be concluded that there are mutation carriers in the beef cattle population.Theywereidentifieddespite the small sample sizeof studiedanimals. Excludingthemfromthe useseems to be the most sensible decision.Otherwiseaspreadofthesemutationstothepopulationwilleventuallybe increased.

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Түйін

Зерттеуге 54 биологиялық сынама (құйрық шашы) алынды. Генотиптеу Лабожена, ICAR халықаралық аккредитациясы бар, зертханасымен бірлесіп жасалынды. Генотиптеу нәтижесі бойынша қазақтың ақ бас тұқымының бір бұқасы HEPHL1 геніндегі SNP бар гипотрихоз және PYGM геніндегі 5 SNP бар гликогеноз ауруларының рецессивті аллельдерінің тасымалдаушысы екені анықталды.

Summary

54 biological samples (hairs) were collected. Genotyping was carried out in cooperation with the laboratory “Labogena”in France (with an international accreditation ICAR). The results of the genotyping revealed that a bull of the Kazakh white is a carrier of recessive alleles of two diseases: hypotrichosis with SNP in the HELPH1 gene and glycogenosis 5 with SNP in the PYGM gene.

Резюме

Были отобраны биологические образцы (волосы), в количестве 54 образца. Генотипированиепроводились совместно с зарубежной лабораторией Лабожена (Франция), с международной аккредитацией ICAR. По результатам генотипирования было выявлено, что бык казахской белоголовой породы является носителем рецессивных аллелей 2-х заболеваний: гипотрихоз с SNP на гене HEPHL1, гликогеноз 5 с SNP на гене PYGM.

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