Monitoring of the allele fund of erythrocyte antigens and DNA polymorphism of genes - markers of beef quality

S D Tyulebaev, M D Kadysheva and L G Surundaeva

"Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences“ FSSI FRC BST RAS, 29, January 9 St., Orenburg, 460000, Russia

E-mail: s-tyulebaev@mail.ru

Abstract. The article presents the results of the analysis of animals of the Bredy meat type by the allele fund of erythrocyte antigens of blood groups and DNA - polymorphism of the GDF5 and TG5 genes, which are valuable for beef cattle. The studies were carried out on the basis of blood analysis of cows from three micropopulations belonging to well-known breeding enterprises in Russia. Alleles of blood groups were identified for all systems, and the frequency of their occurrence was shown. The work focuses on the ratio of homozygous and heterozygous genotypes, shows the degree of their displacement depending on the micropopulation, determines the indices of genetic similarity (I) and genetic distance (Dn) between micropopulations. The revealed levels of polymorphism of animals of the Bredy meat type for genes GDF5 and TG5 showed an insignificant frequency of occurrence of the desired genotypes, this indicator for the first gene was 8.7, and for the second - 4.3%. Comparison of the frequency of occurrence of the most common alleles of the EAB-system of blood groups of the herd, with the frequency of occurrence of genotypes for genes GDF5 and TG5 in a random sample of cows of the Bredy meat type, did not show any dependence of the polymorphic state of genes on the specific content of alleles.

1. Introduction
For the temperate latitudes of Russia, characterized by a sharply continental climate for the development of beef cattle breeding, it is advisable to use the qualities of breeds adapted for these zones. To improve the quantitative and qualitative characteristics of the productivity of local livestock in these conditions, from a business point of view, it is enough to use the genetics of highly productive beef cattle of imported selection in local populations, so the Bredy meat type was created (RF Patent No. 3098 dated 04/25/2006), which received a positive review from farmers of the South Urals, in particular the Chelyabinsk region [1-3]. Confirmation of the origin of animals on the basis of immunogenetic tests is still an important element of breeding work with animals, but it is being replaced by new genetic methods designed not only to confirm the origin, but also to predict productivity, to identify and knowingly, before the birth of offspring, to design future quality and quantitative indicators of animal productivity [4-8]. Consideration of these questions in a complex will help to obtain new knowledge of the evolutionary processes within the created type, determination of its genetic and immunogenetic identity and genetic distance between individual herds, will allow to correctly regulate breeding activities for improving this new breeding form.
2. Materials and methods

The object of the study was animals of the Bredy meat type of two populations: LLC “State farm Bredy” (n = 147), LLC “Borovoe” (n = 60) and LLC “Splav” (n = 60). Blood samples were extracted as biosubstrates, while efforts were made to cause minimal harm to animals, in accordance with the provisions of Russian Regulations, 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and “The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996)”. In all cases, the animals were kept according to the technology adopted in beef cattle breeding.

Blood for analysis was taken from the root of the tail into 2 vacuum tubes with the anticoagulant EDTA. At LLC “State Farm Bredy” 46 5-year-old cows took blood into an additional test tube. The labeled tubes corresponded to the number of the animal. The samples were delivered: part to the Regional Information and Breeding Center of the Orenburg Region (RISC) at the Federal Research Center of Biological Systems and Agrotechnologies of the Russian Academy of Sciences (FSSI FRC BST RAS), the other to the Collective Use Center (CUC), genetic laboratory of the FSSI FRC BST Testing Center RAS (certificate of accreditation RA.RU.21PF59 dated 10/12/2015; www.tskp-bst.rf; http://ckp-rf.ru/ckp/77384). In the RISC, the analysis was carried out on the basis of the Methodological Recommendations “Use of immunogenetic markers in the breeding process in cattle breeding” (Orenburg, 1995). The blood group of each animal was determined by a hemolytic test using immune sera specific to each individual antigen in the presence of complement (rabbit blood serum). Reagents for serological tests were ordered from OOO “Novomoskovskoye” for breeding, which contained complements to identify 55 antigenic factors in 11 systems (loci) of blood groups. Based on the results of the immunogenetic test, the coefficient of genetic similarity of populations (total) was calculated using the Mayal-Lindstrem algorithm:

\[ r = \frac{\sum x y}{\sqrt{\sum x^2 \sum y^2}} \]  

where \( x \) and \( y \) are the frequencies of the same alleles in animals of two compared populations. Genetic distances between populations (\( D_n \)) were determined by the formula:

\[ D_n = -\ln r_{total} \]

The blood that entered the genetic laboratory of the FSSI FRC BST RAS Testing Center was immediately subjected to a DNA extraction procedure. Canned blood samples were used as a DNA source. Isolation of DNA from blood was carried out using a set of reagents "DNA-Extran-1" ("Syntol", Russia). An ANK-32 programmable amplifier (“Syntol, Russia”) was used for genotyping. To carry out a polymerase chain reaction (PCR) to replicate the genome region of the GDF5 and TG5 gene components, primers from the DNA – OMIM databases were used, which were synthesized in the Russian company "SYNTOL" (table 1). Statistical calculations were carried out based on the use of the Microsoft Office software package with the use of “Excel” and “Statistica 10” software (Stat Soft Inc.) USA.

| Primer | The sequence of nucleotides |
|--------|-----------------------------|
| GDF5   | 5’ - TGTCGTGAGTGGACAGAACAG - 3’ |
|        | 5’ - GAGTGAGTTAATCCCAGATACCA - 3’ |
| TG5    | F: 5’-GTGAAAATCTTGTGGAGGCTGTA-3’ |
|        | R: 5’-GGGGATGACTACGAGTATGACTG-3’ |

3. Research results

When studying the allele pool of the Bredy meat type of simmentals, the blood groups of animals of three micropopulations of this cattle, located in different breeding farms, were studied: LLC “State Farm
Bredy” - group I, LLC “Borovoe” - group II, LLC SPK “Splav” – group III. The analysis of the level of homo and heterozygosity of herds showed the following results (table 2). In simple systems, which include F-V, L, Z, the concentration of one of the alleles is often much higher than the other and ranges from 6.1% (group I) in the L system to 63.3% (group II) in the Z system of blood groups.

Table 2. Frequency of occurrence of alleles in simple blood group systems.

| System blood | Allele | Group I | | Group II | | Group III |
|--------------|--------|---------|---------|---------|---------|---------|
|              |        | frequency of occurrence | ratio of genotypes, % | frequency of occurrence | ratio of genotypes, % | frequency of occurrence | ratio of genotypes, % |
| F-V          | F      | 0.846   | 39.800  | 46.600  | 1.00    | 100.00  | -        | 0.2841  | 2.317   | 25.808  |
|              | f      | 0.136   | 13.600  | -        | -       | -       | -        | 0.719   | 71.875  | -       |
|              | V      | 0.136   | 0.50    | 13.108  | 0.276   | 2.063   | 24.603   | -       | -       | -       |
|              | v      | 0.846   | 86.39   | -        | 0.733   | 73.333  | -        | -       | -       | -       |
| L            | L      | 0.061   | 0.097   | 6.030   | 0.333   | 3.367   | 29.966   | 0.264   | 0.0248  | 3.10    |
|              | l      | 0.939   | 93.88   | -        | 0.677   | 66.667  | -        | 0.9688  | 96.880  | -       |
| Z            | Z      | 0.537   | 10.21   | 43.500  | 0.633   | 15.560  | 47.772   | 0.0625  | 0.101   | 6.149   |
|              | z      | 0.463   | 46.290  | -        | 0.367   | 36.667  | -        | 0.937   | 93.750  | -       |

So, the genotypes were also distributed according to the indicated systems. Homozygotes for F, v, l, z alleles were found with the highest frequency of occurrence, except for the F allele in animals belonging to LLC SPK “Splav”. For the less common L allele, the frequency of distribution ranged from 2.48% (LLC SPK “Splav”) to 33.67% (LLC “Borovoe”). The distribution of heterozygotes also varied significantly from 0 to 46.6 - 48.27 (F allele) to 2.19 - 47.77% (Z allele). In the M system, the data for which are not reflected in the table, one allele m and the absence of the M allele are identified, which means 100% of homozygotes for the m allele.

Thus, according to the traits, the variability of which is due to two alternative alleles in the analyzed herds, homozygous forms predominated, while the ratio between homo- and heterozygotes varies and is 2.6: 1 for animals belonging to LLC “State Farm Bredy”, LLC “Borovoe” - 2.91: 1 and LLC SPK “Splav” - 7.56: 1.

In multi-allelic systems of blood groups A, C, S, the following situation has developed (table 3). In system A, for allele A, the lowest concentration was typical for animals from LLC SPK “Splav”, 9.4%, and the highest, 63.3%, for heifers from LLC “Borovoe”. The latter, had the same concentration that was characteristic for the A2 allele, while in animals of the third group it was 21.9%. As for the animals from LLC “State Farm Bredy”, the frequency of the A1 allele was 57.1%, and the A2 allele, respectively, 42.8%. For the A1 allele, homozygotes on average for micropopulations turned out to be 9.24%, heterozygotes - 34.04%, for the A2 allele, respectively, homozygotes - 7.62% and heterozygotes - 35.06%. In the C - system, a relatively high frequency of occurrence was noted for the C2 allele - 57.75 on average for micropopulations.

Table 3. Frequencies of alleles in A, C and S systems of blood groups in micropopulations of individual farms.

| System blood | Allele | Group I | | Group II | | Group III |
|--------------|--------|---------|---------|---------|---------|---------|
|              |        | frequency of occurrence | ratio of genotypes, % | frequency of occurrence | ratio of genotypes, % | frequency of occurrence | ratio of genotypes, % |
| A            | A1     | 0.571   | 11.926  | 45.216  | 0.633   | 15.561  | 47.773   | 0.094   | 0.231   | 9.144   |
|              | a1     | 0.429   | 42.9    | -        | 0.367   | 36.7    | -        | 0.906   | 90.6    | -       |
The fluctuations ranged from 12.5 (LLC SPK “Splav”) to 63.3% (LLC “Borovoe”). In the S - system, the S₁ and H alleles were distributed with a frequency of 3.1%. In this system, there was an insignificant shift of genotypes towards heterozygotes and their ratio, respectively, was 0.013: 1.59% and 0.056: 2.922%.

In the largest and most informative system B, 33 alleles were analyzed with significant variations in their frequencies (table 4). This system is much more complete than others reflecting hereditary differences both between individual genotypes, and in general between the analyzed populations. As you can see, in general, the following alleles were most widespread for the Bredy meat type: O₄ - 37.4%, Y₂ - 13.2%, E₃ - 27.5%, and J₂ - 11.0%.

The ratio of homo- and heterozygotes in individual herds was biased towards homozygotes. So, this indicator in animals belonging to LLC “Borovoe” was the following ratio - 3.26: 1. In the micropopulation of animals of LLC “State Farm Bredy”, this indicator was higher - 6.08: 1, And in animals from LLC SPK "Splav", respectively - 3.23: 1.

**Table 4.** Frequencies of alleles in the B - blood group system in micropopulations of individual farms.

| System blood | Allele | Group | I |  | II |  | III |  |
|--------------|--------|-------|---|---|---|---|---|---|
|              |        |       | frequency | ratio of occurrence | genotypes, % | frequency | ratio of occurrence | genotypes, % | frequency | ratio of occurrence | genotypes, % |
| B₂           | 0.204  | 1.163 | 19,24      | 1.163 | 0.333 | 3.367 | 29.96 |
| b₂           | 0.796  | 79.6  | -          | -     | 0.667 | 66.7  | -    |
| G₂           | 0.156  | 0.665 | 14.98      | 0.665 | 0.267 | 2.064 | 24.60 |
| g₂           | 0.844  | 84.4  | -          | -     | 0.733 | 73.3  | -    |
| IL           | 0.15   | 0.606 | 14.36      | 0.606 | 0.433 | 6.112 | 37.22 |
| il           | 0.85   | 85    | -          | -     | 0.567 | 56.7  | -    |
| O₂           | 0.279  | 2.275 | 25.61      | 2.275 | 0.267 | 2.064 | 24.60 |
| o₂           | 0.721  | 72.1  | -          | -     | 0.733 | 73.3  | -    |
| O₄           | 1      | 100   | -          | -     | 0.969 | 96.9  | -    |
The distribution of frequencies in different loci of the studied populations showed that the genetic similarity between the populations of cattle of the Bredy meat type varies significantly. In view of the fact that these herds were created on the basis of the purchase of livestock and, first of all, the semen of bulls - producers and from LLC "Experimental", naturally their immunogenetic identity. However, their further development and selection and breeding work in herds was carried out more autonomously. In addition, the farms periodically carried out a cross-exchange of bulls-producers, and also, sometimes improved herds using the semen of the same outstanding bulls-producers of meat simmentals of Canadian selection (table 5).

Table 5. Indices of genetic similarity (I) and genetic distance (Dn) between micropopulations of the cattle of Bredy meat type.

| Micropopulation | LLC“State Farm Bredy” | LLC “Borovoe” | LLC “Splav” |
|-----------------|-----------------------|--------------|-------------|
| Dn              |                       |              |             |
| LLC“State Farm Bredy” | -               | 0.278        | 0.643       |
| LLC “Borovoe”   | 0.757                 | -            | 0.536       |
| LLC “Splav”     | 0.442                 | 0.624        | -           |

As a result, the following picture emerged, the highest genetic similarity index was found between the populations of meat simmentals belonging to LLC“State Farm Bredy”and LLC “Borovoe” (I_2 = 0.757, D_n = 0.278). This circumstance is due to the fact that these farms having a long history of
communication and exchange of genetic information, are the country’s leading breeding plants for this type of animals and coordinate selection and genetic activities. LLC SPK “Splav” began its activity with the purchase of heifers in the amount of more than 100 heads from LLC “State Farm Bredy”, at the same time it often used semen and bulls from other sources, which was probably reflected in more modest indicators.

Between the micropopulations of animals from LLC "State Farm Bredy" and LLC JV "Splav", the indices of genetic similarity and differences were as follows \( I_r = 0.643, D_n = 0.442 \), and the same indicators between LLC "Borovoe" and LLC JV "Splav" had the following values \( I_r = 0.536, D_n = 0.624 \).

Meanwhile, the gene pool of animals is interesting for some marker DNA associated with some indicators related to the productivity and quality of meat. For example, the GDF5 gene is related to the exterior and constitution, the formation of active tissues, and the proportions of animal bodies, and TG5 may be responsible for the nature of adipose tissue deposition, including in animal muscles (table 6).

**Table 6.** The level of polymorphism of some genes in animals of the Bredy meat type (LLC "State Farm Bredy").

| DNA marker | Genotype | CC\(^a\) | CG | GG |
|-----------|-----------|----------|----|----|
|           | frequency of occurrence of the genotype | n | % | n | % | n | % |
| GDF5      | allele frequency | C\(^a\) |      | G |
|           |                  | 0.196   |    | 0.804 |
| TG5       | genotype        | TT\(^a\) | TA | AA |
|           | frequency of occurrence of the genotype | 2 | 4.3 | 24 | 52.2 | 20 | 43.5 |
|           | allele frequency | T\(^a\) |      | A |
|           |                  | 0.305   |    | 0.695 |

\(^a\) - desired genotype and allele.

In combination, these genes in animals can characterize their meat forms and the marbling of beef, which is important for animals of the meat production direction. Analyzing the data obtained, it should be said that of all the studied bulls, 4 heads (8.7 %) had the desired genotype of the studied gene, while 10 heads (21.7%) had a heterozygous form. However, the largest number of bulls – 32 heads, which is 69.6 % of all animals, had the homozygous undesirable genotype GG. The desired polymorphism for the TG5 gene is expressed only in 4.3 % of animals, with 52.2% of heterozygous forms, the rest of the livestock is represented by homozygous AA. Analysis of alleles showed a slight shift in the balance towards heterozygotes for the TG5 gene.

Also, the work presents materials for comparing the frequency of occurrence of the most common alleles of the EAB system of blood groups of the herd, with the frequency of occurrence of genotypes for the GDF5 and TG5 genes in a random sample of Bredy meat type cows (table 7). The analysis showed that in the blood of carriers of the desired genotype of the GDF5 gene, alleles of the EAB system of groups are significantly represented: B\(_2\), E\(_3\)', Q'. However, in general, no significant correlative relationships of DNA markers with alleles of blood groups were found.
Table 7. Frequency of distribution of the most common alleles of the EAB-system of blood groups in the environment of carriers of genotypes for the GDF5 and TG5 gene, %.

| Alleles of blood groups | Allele frequency in the herd | SNP - marker | GDF5 | TG5 |
|-------------------------|-------------------------------|--------------|------|-----|
|                         |                               |              | CC   | CG  | GG  | TT  | TA  | AA  |
| B2                     | 0.204                         | 2.17         | 4.35 | 13.04 | 2.17 | 10.87 | 8.70 |
| O2                     | 0.279                         | -            | 6.52 | 19.56 | -   | 17.39 | 10.86 |
| Y2                     | 0.252                         | -            | 4.34 | 17.39 | 2.17 | 13.04 | 10.86 |
| E1’                    | 0.354                         | 2.17         | 6.52 | 23.91 | -   | 19.56 | 15.22 |
| K’                     | 0.272                         | -            | -    | 17.39 | -   | 15.22 | 13.04 |
| Q’                     | 0.218                         | 2.17         | 4.35 | 10.87 | -   | 8.70  | 8.70 |

4. Conclusion

Thus, we have presented a detailed immunogenetic analysis of the new Bredy beef type of cattle, as well as the analysis of polymorphic genotypes of two important genes for beef cattle, GDF5 and TG5. The frequency of occurrence of genotypes of the studied genes, their alleles, as well as alleles of blood group systems are shown. The search for establishing any dependence of the polymorphic state of genes on the specific content of alleles in the B-system of blood groups did not give positive results.

Acknowledgments

The studies were carried out in accordance with the research plan for 2021-2023. FSSI FRC BST RAS (No. 0526-2021-0001).

References

[1] Kayumov F G, Kadysheva M D and Tyulebaev S D 2011 Selection and genetic parameters of the productivity of young animals in the creation of meat type simmentals Izvestia of the Orenburg State Agrarian University 31(3) 151-3
[2] Novikova N V, Kanatpaev S M, Tyulebaev S D and Kononenko S I 2010 Using combination variability in increasing the meat productivity of heifers Proceedings of the Kuban State Agrarian University 25 131-4
[3] Kayumov F G, Kanatpaev S M, Tyulebaev S D and Kadysheva M D 2008 The first breeding farm for breeding "Bredy meat" type of simmentals Herald of beef cattle breeding 1(61) 117-9
[4] Tait R G, Shackelford S D, Wheeler T L et al. 2014 CAPN1, CAST, and DGAT1 genetic effects on preweaning performance, carcass quality traits, and residual variance of tenderness in a beef cattle population selected for haplotype and allele equalization J. Anim. Sci 92 5382-93
[5] Tyulebaev S D, Kadysheva M D, Litovchenko V G, Kosilov V I and Gabidulin V M 2019 The use of single-nucleotide polymorphism in creating a crossline of meat simmentals IOP Conference Series: Earth and Environmental Sci 341(1) 012188
[6] Xia X, Yao Y, Li C, Zhang F, Qu K, Chen, H, Lei C et al. 2019 Genetic diversity of Chinese cattle revealed by Y-SNP and Y-STR markers Animal genetics 50(1) 64-9
[7] Tyulebaev S D, Kadysheva M D, Kosilov V I and Gabidulin V M 2021 The state of polymorphism of genes affecting the meat quality in micropopulations of meat simmentals IOP Conf. Series: Earth and Environmental Sci 624(1) 012045
[8] Boichard D, Boussaha M, Capitan A et al. 2018 Experience from large scale use of the EuroGenomics custom SNP chip in cattle World Congress of Genetics Applied to Livestock Production 11 675