Control over the inherited diseases of Ayrshire and Aberdeen Angus cattle breeds on the Russian Federation territory

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Abstract. In the paper have been described the inherited diseases AH1 and FMO3 charactering for Ayrshire cattle breed and AM, OS and DD, charactering for Aberdeen Angus one, particularly, the clinical signs and causing gene mutations. The aim of the study was a genotyping of Ayrshire and Aberdeen Angus breeds cattle of Russian populations by earlier developed DNA tests and the evaluation of the spread of animal carriers. The analysis of two populations of Ayrshire cows (n=268) and three populations of Aberdeen Angus (n=772) sire bulls has shown the presence of animals carriers of the genetic defect of AH1 and FMO3 among the Russian Ayrshire populations in 6.33-15.79% and 7.69-46.15% frequencies, respectively. In addition, in Subpopulation 5b three FMO-affected animals were found (3.03%). The animals carriers of AM and DD genetic defects have been found in Russian Aberdeen Angus populations in the frequencies of 0.63-2.73 and 1.13-9.49% dependently from the populations, respectively. In addition, we have observed the increasing of the frequencies of AMC- and DDC-animals in the subpopulations of descendants of populations 1 and 2. The increasing of AMC-animals in Subpopulation 2b comparing with 2a was statistically significant.

1. Introduction
As it is known, cattle breeding is one of the most important branches of animal husbandry, which, depending on natural and economic conditions has a dairy or meat direction of productivity. Since the use of cattle in the national economy, there have been great changes in increasing its milk and meat productivity, and now the outstanding animals of dairy breeds are able to give more than 15 000 kg of milk per year, and the gains of the best animals-representatives of meat cattle reach more than 1500 g per day [1]. Undoubtedly, a great role in the growth of these indicators is played by a huge breeding work aimed at using the genetic potential of the best domestic and foreign breeds of cattle.

One of the best in the dairy direction is Ayrshire breed of cattle, which is characterized by high productivity, resistance to infectious diseases, good feed conversion and milk with high quality indicators. In some cases, the animals of this breed are more comfortable in the content compared to Holstein breed, due to the fact, that they are less demanding to the conditions of maintenance and feed [2].

To increase the profitability of beef cattle breeding, the use of Aberdeen Angus breed is considered promising, the animals of which are very hardy, easily acclimatized to difficult climatic conditions, are characterized by high meat productivity, in particular, from they could receive meat of excellent quality [3].
However, one of the problems observed in the breeding of cattle of both the above breeds is the tendency to reduce the fertility of cows and output of calves due to the manifestation of congenital hereditary anomalies. The cause of hereditary diseases are various gene mutations, often called genetic defects, which result in the termination or premature synthesis of immature proteins, which leads to the loss of function of certain organs and tissues (LOF-mutations). Depending on the severity of respective diseases, the genetic defects have been classified on class I (lethal) and class II (non-lethal) [4].

2. Inherited diseases of Ayrshire and Aberdeen Angus cattle

In the frame of our work have been studied the genetic defect of AH1 and FMO3, characterizing for Ayrshire cattle and ones of AM, OS and DD, appearing in Aberdeen Angus cattle. Their common feature is that they have a simple recessive type of inheritance, meaning that the disease manifests only in animals homozygous for the recessive mutant allele.

Below is a brief description of the mutations and clinical manifestations of the above defects.

2.1. Genetic diseases of Ayrshire breed cattle

Based on the clinical traits, genetic abnormalities associated with fertility haplotypes AH1 and AH2 are lethal genetic defects, and trimethylaminuria is nonlethal.

AH1 (Ayrshire haplotype 1) – the main clinical manifestation is delayed growth and mortality syndrome (PIRM), as well as the manifestation of facial ptosis (due to the abnormally large eyelid, sick animals look constantly asleep). Venhoranta et al. (2014) first described the disease [5]. The cause is polymorphism rs475678587, consisting in a point mutation G>A in the third base of codon 692 ubiquitin protein ligase E3B gene (UBE3B) (BTA 17) (OMIA 001934-9913).

Trimethylaminuria (FMO3) is monogeneous disease manifesting in fish-off flavor of milk from the affected cows. The reason of the disease is the mutation (R238X) in the flavin-containing monooxydase 3 gene (FMO3) (OMIA001360-9913). Result of the mutation is impaired oxidation of the trimethylamine (TMA) and the elevated levels of the TMA in milk of affected cows [6]. It was observed that the milk of the cows homozygous on recessive X allele have fish odor independently of diet and the milk of the heterozygous cows can appear fish-off flavor in under some environmental factors as the presence of TMA precursors of FMO inhibitors in feed [6].

2.2. Genetic diseases of Aberdeen Angus breed cattle

Described genetic defects of arthrogryposis multiplex and osteopetrosis are lethal and development duplication is nonlethal.

AM – Arthrogriposis multiplex - clinical signs of abnormal curve of the back (kyphosis or scoliosis), muscle hypoplasia and less common moderate hydrocephalus. Sick calves are stillborn or die soon after birth. The reason of the defect is large deletion of 23347 bp encompasses three gene – completely gene of ubiquitin like modifier (ISG15), 5 regulatory region of hair and cleavage enhancer gene (HES4) and two first exons of agrin gene (AGRN) (OMIA 002135-9913) [7].

OS – Osteopetrosis - The defining characteristics of the condition (also known as "marble bone disease") are the defective activity of osteoclasts, large multinucleated cells that resorb bone, and the resulting accumulation of primary spongiosa in marrow cavities. Affected calves are typically stillborn prematurely (250-275 days of gestation). They often have a small body size, flat skull, impacted molars, shortened lower jaw, protruding tongue; the leg bones are easily broken. The reason of the disease is deletion of 2784 bp in SLC4A2 gene (Solute carrier family 4 (anion exchanger), member 2) (OMIA 000755-9913) [8].

DD – Developmental duplication - appearance of calves with additional parts of body (most often limbs). Other phenotypes of the disease are birth of conjoined twins, the manifestation of the vices of the middle and front brain (e.g., no split-brain hemisphere), craniofacial dysmorphogenesis, microphthalmia, diprosopus, embryogenic teratomas, dermoid cyst [9]. The disease caused by single
nucleotide polymorphism g.34618072T>C in the second gene contained NHL repeats (NHLRC2) (OMIA 002103-9913) [10].

Earlier in L.K. Ernst Federal Center for Animal Husbandry have been developed the test systems based on DNA analysis for the identification of animal carriers of the genetic defects of AH1, FMO3, AM, OS and DD among the animals of Ayrshire and Aberdeen Angus breeds [11-12].

The aim of our work was the investigation of Ayrshire an Aberdeen Angus cattle of Russian populations on studied genetic defects and determining frequencies of the animals carriers on the Russian Federation territory.

3. Material and methods

3.1. Material

The investigation has been conducted in 2008-2019 at the Laboratory of molecular selection basis of L.K. Ernst Federal Science Center for Animal Husbandry. Material of the study were DNA samples of Ayrshire cows (n=268) and Aberdeen Angus sire bulls (n=772) belonging to the different farms of Russia and obtaining by DNA extraction from the different biomaterial (skin, blood, milk and other) by means DNA extraction sets DNA-Extran-1 and DNA-Extran-2 (Syntol Co., Russia) in according to the manufactory recommendations. The description of the material presented in the Table 1.

| Breed          | Population No. | n  | Year of birth |
|----------------|----------------|----|---------------|
| Aberdeen Angus | 1a             | 55 | 2015          |
|                | 1b             | 73 | 2019          |
|                | 2a             | 152| 2013          |
|                | 2b             | 176| 2017          |
|                | 3              | 316| 2018          |
| Ayrshire       | 4a             | 19 | 2008          |
|                | 4b             | 13 | 2012          |
|                | 4c             | 23 | 2014          |
|                | 4d             | 22 | 2017          |
|                | 5a             | 78 | 2014          |
|                | 5b             | 99 | 2015          |
|                | 5c             | 13 | 2016          |

As can be seen from the table 1, the Aberdeen Angus populations No. 1 and 2 included the animals born in 2013-2017 (Subpopulations No. 1a, 1b, 2a and 2b) and the Ayrshire populations No. 4 and 5 included the animals born in 2008-2017 (Subpopulations No. 4a-4d and 5a-5c).

3.2. Genotyping

The genotyping of the material has been conducted by means earlier developed test systems based on the using of AS-PCR and PCR-RFLP methods [11-12].

3.3. Statistical evaluation

For the statistical evaluation of the obtained data we used student’s t-test (t) showing the validity of difference in percent of AMC- and DDC-animals frequencies between the subpopulations of populations No. 1 and 2 and χ2 (chi squared) illustrating the likelihood of the observing of the determined allele frequencies by chance. All calculations have been conducted by on line calculator [13, 14]. The difference was considered as significant (p<0.05) when t ≥1.972 at the significance level α = 0.05. The allele frequencies considered not random (P≤0.05) when the χ2 ≥3.84.
4. Results

The analysis of Aberdeen Angus cattle has revealed the animals carriers of Arthrogriposis multiplex (AMC) and Development duplication (DDC) in Populations No. 1b, 2b and 3 (table 2) with the frequencies of 0.63-2.73 and 1.13-9.47%, respectively.

Table 2. The frequencies of AMC-, OSC- and DDC-animals in Russian Aberdeen Angus cattle populations.

| Population No. | Frequency of the genetic defect carriers, n (%) |
|----------------|-----------------------------------------------|
|                | AM       | OS       | DD       |
| 1a             | 0.00     | 0.00     | 0.00     |
| 1b             | 2 (2.73) | 0.00     | 2 (2.73) |
| 2a             | 0.00     | 0.00     | 0.00     |
| 2b             | 3 (1.70) | 0.00     | 2 (1.13) |
| 3              | 2 (0.63) | 0.00     | 30 (9.49)|

Also noteworthy is the appearance in Subpopulations 1b and 2b of AMC- and DDC animals, which in previous generations (Subpopulations 1a and 2a) were not observed (figure 1).

![Figure 1](image_url)  
**Figure 1.** The frequencies of AMC- and DDC-animals in subpopulations of populations 1 and 2.

This fact has been demonstrated on the figure 1. The frequency of AMC-animals in Subpopulation 1b has increased on 2.73% and in Subpopulation 2b – on 1.7% compared with the Subpopulations 1a and 2a. The frequency of DDC-animals in Subpopulations 1b and 2b has increased on 2.73 and 1.13%, respectively. Moreover, if in the difference in frequency of DDC- and AMC-animals between Subpopulations 1a and 1b and we have observed a tendency of increasing of carriers percent ($t=1.0-1.50$ at $p=0.1361-0.1380$) but the increasing of AMC-animals in Subpopulation 2b comparing with 2a was statistically significant ($t=2.0$ at $p=0.046$). The meaning of $\chi^2$ criterion was in the range of 0.0-0.0137 for the each subpopulation, confirming the absence of the selection in relation to AM and DD genetic defects.

Among the animals of Ayrshire breed, the analysis has revealed the carriers as AH haplotype as FMO (table 3), in this case, the frequencies of the recessive alleles A of $UBE3B$ gene and X of $FMO3$ gene were 3.17-15.91 and 3.85-23.08% dependent from the year of birth, respectively.
Table 3. The frequencies of the carriers of AH1 and FMO genetic defects in Russian Ayrshire cattle populations.

| Population No. | Frequency of the genetic defect carriers, n (%) | AH1C | RX | XX |
|----------------|-----------------------------------------------|------|----|----|
| 4a             |                                                | 3 (15.79) | 3 (15.79) | 0 |
| 4b             |                                                | 2 (15.38) | 1 (7.69) | 0 |
| 4c             |                                                | 6 (26.09) | - | - |
| 4d             |                                                | 7 (31.82) | - | - |
| 5a             |                                                | 5 (6.33) | 11 (13.92) | 0 |
| 5b             |                                                | 14 (14.14) | 25 (25.25) | 3 (3.03) |
| 5c             |                                                | 1 (7.69) | 6 (46.15) | 0 |

It is worth noting that in population 4 (Subpopulations 4a-4d) for 10 years, an increase in the number of heterozygous carriers of the haplotype of fertility AH1 (AH1C) was noted in 2 times, while in it there was a decrease in animal carriers of the allele X of FMO3 gene. However, in the population 5 of Ayrshire animals (Subpopulations 5a - 5c) on the FMO3 gene, the opposite trend was observed. In addition, in Subpopulation 5b were found the animals homozygous on X allele of FMO3 gene, causing the appearance of trimethylaminuria (figure 2).

Figure 2. The frequencies of AH1C- and FMO3 RX/XX -animals in the Populations 4 and 5.

Thus, the frequency of AH1C-animals in the Population 4 increased from 15.38 to 31.82% for 5 years and the frequency of the animals with RX genotype of FMO3 gene also increased in three times for period of 2014-2016. This trends were not statistically significant (t<1.0 at p=0.05).

5. Discussion
The results of the investigation has shown the presence of the animals carriers of the considered genetic defects as in Ayrshire as in Aberdeen Angus cattle breeding on the Russian territory. Also in one of the Ayrshire population, we have found the affected of trimethylaminuria animals.

In the Ayrshire cattle populations the increasing of the frequencies of AH1C- and R238X –animals probably bound with bull sires using in the artificial insemination system.

As had been expected we have not found any animal carrier of Osteopetrosis genetic defect. In 2019 we have carried out the large screening of several populations of Aberdeen Angus, Hereford and Simmental breeds resulting it was found only 6 OSC-animals among the three populations of Aberdeen Angus bulls (n=220) which was 1.9-3.3% dependently from the population.
In addition, we must note that despite the frequency of DDC-animals in the current investigation was 2.73-9.49%, earlier carried out investigation has shown that the frequency of this disease carriers among the Russian populations is sufficiently high and in the same herds exceed 20% [12].

The observed tendencies of increasing over time of heterozygotes on genes associated with the appearance of the genetic defects as in Ayrshire as in Aberdeen Angus cattle also consistent with the previous studies [15].

6. Conclusions

The presence in the cattle herds independently of breed and productivity direction of the animals-carriers of the mutant alleles associated with the genetic diseases is the worrying sign pointing to the possibility of birth of the calves with the clinical patterns of the inherited anomalies and further economic damage. In this study, we have already found FMO-affected animals and in this regard, our task is to prevent the further spread of the disease. We have also found the animals, carrying the mutant alleles, associated with AH1, FMO, AM, OS and DD genetic defects, and observed the dynamics of their frequencies raising over time. Due these facts, it’s necessary to control of the breeding material of Ayrshire and Aberdeen Angus cattle on the relevant genetic defects.

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Aberdeen Angus Cattle *Genetics and Breeding of Animals* 1 16-21

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