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Abstract. The article highlighted the problem of genetic defect of Aberdeen Angus cattle. Previous investigation showed the presence in Russian population of Aberdeen Angus cattle of animals carrying in their genotypes the mutant alleles associated with Arthrogriposis multiplex, Developmental Duplication and Osteopetrosis. In some populations, the frequencies of the animals-genetic defects carriers were sufficiently high. The aim of the study was to evaluate the risk of spread of the mutant alleles causing the genetic disorders by their transfer from parents to offspring under absence of control over the genetic defects in the farm, breeding Aberdeen Angus cattle. The analysis has shown increasing of the frequency of animals-Arthrogriposis multiplex carriers about on 10.3% in the population of heifers comparing with population of their mothers and the raising of the mutant allele frequency in six times in F2 generation.

1. Introduction

Resolution of the government of the Russian Federation "On approval of the Federal scientific and technical program for 2017-2025» was published on 25th of August 2017. The main objective of this program is to ensure stable growth of agricultural production, in particular, through the creation of modern diagnostic tools and the introduction of domestic competitive technologies for the examination of genetic material [1].

Currently in Russia to improve the profitability of beef cattle is widely used the breeding material of animals of Aberdeen Angus breed. On the one hand, the animals of this breed have excellent acclimatization abilities and high genetic potential [2], but on the other one – they have been found a number of genetic defects, the manifestation of which instead of the expected benefits can cause serious economic damage [3].

The investigation of Aberdeen Angus cattle genetic defects was started in Russia in 2017 [4]. We were studying genetic defects of Arthrogriposis multiplex, Developmental Duplication and Osteopetrosis. Brief description of them has been given below.

Arthrogriposis multiplex (AM) – lethal genetic defect with 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 genes – 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) [5].

Osteopetrosis (OS) – lethal genetic defect. The defining characteristics of Osteopetrosis (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) [6].

Developmental duplication (DD) – nonlethal genetic defect which 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 [7]. The disease caused by single nucleotide polymorphism g.34618072T>C in the second gene contained NHL repeats (NHLRC2) (OMIA 002103-9913) [8].

The result of the study was a development in 2018 of the test systems based on DNA analysis for diagnostics of genetic defects of Arthrogriposis multiplex and Developmental Duplication [9, 10] and in 2019 the test system for diagnostics of Osteopetrosis has been developed [unpublished data].

The results of the large-scale screening of Aberdeen Angus cattle breeding on the Russian Federation territory revealed enough high frequencies of occurrence of animals carrying the mutant alleles causing the Arthrogriposis multiplex and Developmental duplication [9, 10]. In addition, we have revealed the animals-carriers of Osteopetrosis [unpublished data].

The aim of the study was to evaluate the risk of spread of the mutant alleles causing the genetic disorders by their transfer from parents to offspring under absence of control over the genetic defects in the farm, breeding Aberdeen Angus cattle.

2. Material and methods

The investigation has been conducted in 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 purebred Aberdeen Angus cattle (n=214) belonged to the farm of Central Federal district of Russia which name, status and location are not indicated on ethical reasons. Two populations of the animals were studied in the investigation: I - cows (n=107) and II - heifers (n=107).

All of the animals were genotyped on the genetic defects of arthrogriposis multiplex, developmental duplication and Osteopetrosis by test-systems developed in L.K. Ernst Federal Center of Animal Husbandry. The test systems for diagnostics of arthrogriposis multiplex and developmental duplication based on allele specific polymerase chain reaction (AS-PCR) and polymerase chain reaction with the further restriction length polymorphism analysis (PCR-RFLP) methods were developed in 2018 [9, 10]. The AS-PCR test system for diagnostics of Osteopetrosis has been developed in 2019 [unpublished data].

For the evaluation of the spread of the animals-carriers of Arthrogryposis multiplex and the accumulation of the AM-mutant allele in progenies, we counted as the percent of animal-AM-carriers in populations II and I as the frequencies of the mutant allele associated with AM in both populations in according to Hardy-Weinberg law [11]. For the statistical evaluation of the obtained data we used student`s t-test (t) showing the validity of difference in percent of AMC-animals frequencies and $\chi 2$ (chi squared) illustrating the likelihood of the observing of the determined allele frequencies by chance. All calculations have been conducted by on line calculator [12].

The difference was considered as significant ($p<0.05$) when $t \geq 1.972$ at the significance level $\alpha = 0.05$. The allele frequencies considered not random ($P \leq 0.05$) when the $\chi 2 \geq 3.84$.

3. Results

The results of the PCR analysis of genetic defects were shown on the figure 1.

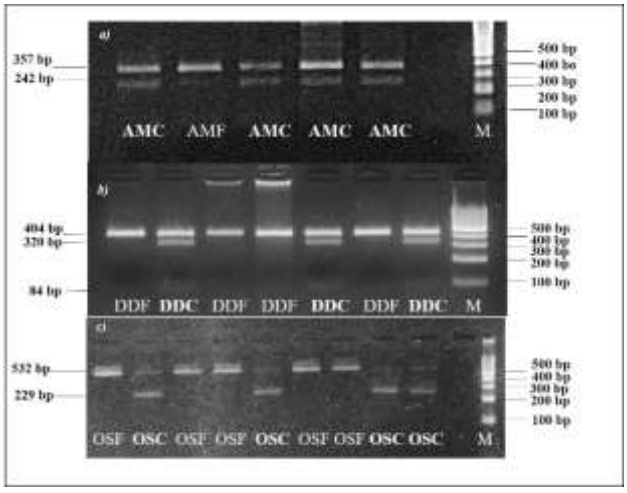

Figure 1. Results of gel electrophoresis. a) – results of AS-PCR for diagnostics of Arthrogriposis multiples; b) – results of PCR-RFLP for diagnostics of Developmental Duplication and c) – results of AS-PCR for diagnostics of Osteopetrosis. AMC – arthrogriposis multiplex carrier, AMF - arthrogriposis multiplex free; DDC – Developmental duplication carrier, DDF – Developmental duplication free; OSF – osteopetrosis free, OSC – osteopetrosis carrier; M – marker of molecular weight (500 bp).

Developed test systems allow the amplification as normal as mutant allele and after gel electrophoresis we can visualize in animals-carriers of the mutations two DNA fragments of different sizes (dependent from the designs of the test systems).

The analysis of two investigated populations by the developed test system has shown a presence of the animals-carriers of the mutations associated with AM, DD and OS in both populations (table 1).

In the population of cows (number I) the frequencies of AMC- and DDC-animals were on 0.93% and OSC-animals – 1.87%. In population of the heifers (number II) frequencies of AMC-, OSC- and DDC-animals were 11.2, 0.93 and 0.93%, respectively.

Table 1. Frequencies of AMC-, OSC- and DDC animals in Russian Aberdeen Angus populations.

Population N	Frequency of the genetic defect (%)		
	AM	OS	DD
I	0.93	1.87	0.93
II	11.2	0.93	0.93

In comparing of the genetic defects frequencies between two investigated populations, we haven`t observed much differences for OS and DD. However, it is noteworthy that if the frequency of AMC-animals in the cow population was 0.93%, then this figure in heifers was 11.2%, i.e. it has increased by almost 10.3% that was statistically significant ($t=2.85$; $p=0.004863$ or $p<0.05$).

For the evaluation the accumulation of the mutant allele associated with AM, in the offspring, we calculated it`s frequencies in both study populations, considering cows as the F1 generation, and heifers as the F2 generation and observed a six fold increasing in the frequency of the mutant allele in the F2 generation ($P>0.05$) (figure 2).

The values of the $\chi 2$ criterion were relatively low and amounted to 0.01 for generation F1 and 0.38 for generation F2, which is evidence of any selection influence absence to the AM genetic defect in populations.

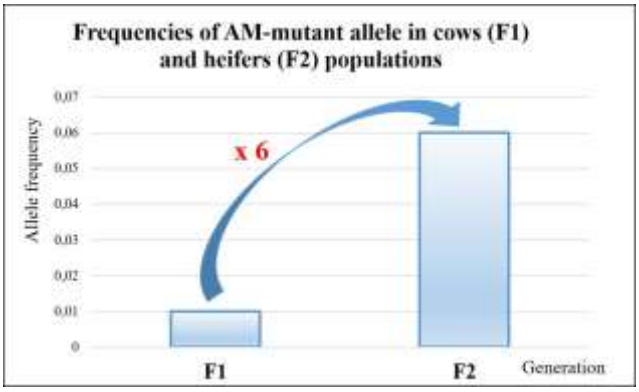

Figure 2. The demonstration of the accumulation of the mutant allele associated with Arthrogryposis multiplex, in the offspring population.

The practical value of the results is that we have obtained data on animals-carriers of the studied genetic defects on this farm. Using of the information about genotypes of animals on genes associated with congenital disease will be the best means of preventing hereditary abnormalities, as it will allow the use of animals-carriers of genetic defects in the selection programs without their culling and losses of genetic potential of the herd.

4. Discussion

Today the biggest information resource of cattle of Aberdeen Angus breed is American Angus Association, which has a huge database on productivity and genetic defects, in particular "Policy regarding specific genetic conditions and factors", clearly prescribing rules for the registration and use of breeding material in which mutant alleles of certain genetic diseases have been detected. In accordance with the Policy, ten genetic defects including AM, OS and DD, are mandatory for the control. Data on the carriers are recording in the pedigrees and breeding certificates of animals. Homozygous animals considering sick due the mutation are not allowed to the registration, and the previously issued certificates cancel [13].

At the beginning of our work on the problem, we analyzed American Angus Association database on animals free and carriers of genetic defect and concluded that none of the defects has been eliminated. We have analyzed several genetic defects and noted that the maximum frequencies of the animals-genetic defects carriers was in the first year of genotyping of some defect. As the annual DNA control frequency of occurrence of the animal-defect carriers in most cases gradually decreased [14] that allow to summary about the benefits of DNA testing. We continued to track the dynamics of the animal-carriers of genetic anomalies in American Angus Association data and found that the frequency of AMC- and DDC-animals were in 2018 5.0 and 23.3%, respectively [14].

In 2018 we conducted large-scale screening on genetic defects of Arthrogriposis multiplex (n=1205) and Developmental Duplication (n=1342) of several Aberdeen Angus cattle populations located in Russia. The frequency of AM-genetic defect were not high – from six investigated populations the AMC-animals were found in three in frequencies 0.94-1.06% [9]. However, the frequency of Developmental duplication were higher. DDC-animals were found in all of ten investigated populations with frequencies 0.9-12.8% [10]. In 2019, we have conducted the analysis of 2585 heads of Aberdeen Angus cattle of 38 Russian populations. If the frequency of AMC-animals almost haven`t different from previous our data, then the frequencies of DDC-animals were very high (20.6-38.2%) in the some populations [15]. The findings is additional indication of the need for genetic defect control measures.

In present time thanks to rapid development of DNA technologies, the most the most advanced foreign laboratories (GeneSeek Co., Zoetis Co., Neogen Co., USA) investigate gene mutations widely using of quantitative polymerase chain reaction method (RT-PCR or qPCR) allowed the quantitative determination of DNA copies in the sample. The main advantage of the method is speed of the result obtaining by reducing the analysis time due to the absence of the need for detection of gel electrophoresis. Due this fact, the method is more suitable for high-flow genotyping.

In the future, we plan to upgrade the developed test systems by applying of RT-PCR method for the increasing of laboratory productivity. We also consider a possibility of digital PCR method using allowed to optimize the analysis for simultaneously detection several mutation in one tube.

But, it is necessary to understand that none of the most advanced test systems based on RT-PCR or dPCR cannot been created without the initial development of test systems based on classical PCR.

The test systems developed in L.K. Ernst Federal Science Center for Animal Husbandry are the initial stage of work on creation of means of prevention and control over genetic defects. Taking into account the fact that there is no control over genetic defects in Russia, our work in this direction is a great breakthrough in solving this problem.

5. Conclusions

Carried out investigation shows the high risk of spread of genetic anomalies by transfer of mutant alleles causing the congenital defects from parents to offspring that point on necessity of control for genetic defects in Russia.

As known, the best method in the fight against congenital diseases is their prevention by avoiding of the mating of animals-genetic defect carriers among each other. This approach allows using of animals carrying of the mutant alleles without their culling and losses of genetic potential of population [16].

Today, DNA analysis is the only method to obtain accurate information about the animal genotypes, including in relation of genetic defects, and its conducting is impossible without development of high sensitive and specific test systems.

The developed in L.K. Ernst Federal Science Center for Animal Husbandry test systems are cheap and highly sensitive tools for the genotyping of the animals on genetic defects Arthrogriposis multiplex, Osteopetrosis and Developmental Duplication. Today in Russian circumstances the development fully consensus with “Federal scientific and technical program for 2017-2025” is the most optimal decision for solve the problem of genetic defects in cattle of Aberdeen Angus breed.

It is possible that someone will offer to use test systems already developed abroad, however, foreign test systems in most cases are not available or their use is regulated by the legislation of the origin country and requires payment of the relevant patent fees.

We convince, that for the competitiveness of Russia in the world market, it is necessary to develop and actively implement our own science developments, in particular molecular genetic technologies allowed to control of cattle breeding material independently of any assistance from abroad. Only in this case our country will be able to ensure stable profitability of the beef cattle industry in the future.

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