Biodiversity and functional variability of the allele pool of horse breed populations

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Abstract. Polysaccharide storage myopathy (PSSM1) is a disease of excessive accumulation of glycogen in muscles, which is encoded by the enzyme glycogen synthase (GYS1). The horses with a mutation at the locus (GYS1) have substandard performance, they often experience muscle pain with intense work, get sweaty and have stiffness in movement, which is generally accompanied by a decrease in performance. The aim of our research was to study the GYS1 mutation (g.18940324 G> A) in glycogen accumulation in horses of different breeds.

For the study, horses (n = 181) of ten factory and local breeds were selected, including Russian Donskaya, Bashkirskaya, Buryatskaya, Vladimirskaya, Vyatskaya, Percheronskaya, Russian heavy draft, Soviet heavy draft, Orlov trotter and Thoroughbred horse breeds. According to the results of testing, the GYS1 mutation was found in Bashkirskaya horse breed (3.2%), Buryatskaya horse breed (10%), Vyatskaya horse breed (9.5%), Soviet heavy draft horse breed (30%), Russian heavy draft horse breed (49.9%) and Percheronskaya horse breed (90%). Donskaya, Thoroughbred and Orlov trotter horse breeds showed no PSSM1 defect. Timely diagnostics and monitoring of horse breeds for the presence of genetic abnormalities that reduce their health and performance allow avoiding the accumulation of genetic burden in populations and contribute to increasing the efficiency of breeding programs.

1. Introduction
At present, molecular technologies serve as a reliable tool for genetic assessment of breeds, study of biodiversity and functional variability of the allelofund of populations. Using DNA analysis, important information about the origin and microevolution of breeds, as well as about the formation of productive and adaptive traits specific to each group of animals can be obtained [1, 2]. The second FAO report on the State of the World’s Livestock Genetic Resources (2015) [2] lists the most significant genetic mutations found in populations of different types of domestic animals, where testing of horses for the GYS1 gene polysaccharide accumulation myopathy (PSSM1) is recommended. PSSM of type 1 is an inherited disorder caused by a mutation in the glycogen synthase gene (GYS1). Polysaccharide storage myopathy (PSSM) is a muscle disorder that causes abnormal accumulation of polysaccharide and glycogen in muscles, resulting in stiffness, pain, unwillingness to
move, weakness, and lying down [3, 4]. Some horses try to dig and roll on the ground immediately after a load. On rare occasions, muscle pain and stiffness can be quite severe, causing the horse to be unable to stand and experience pain even when lying down. The urine of these horses is often brown (rhabdomyolysis). This defect has been diagnosed in representatives of different breeds, including 10% of quarter horses, 60% of Percheron horses and 90% of Belgian draft horses [5]. Polysaccharide accumulation myopathy is associated with the semi-dominant gene GYS1, which encodes the glycogen synthase enzyme (GYS1-R309H), the mutation causes excess glycogen content in muscle tissue [5, 6], leads to disruption of glycogen synthesis and potentially slowed down glycogen metabolism. It is important to take into account that clinical signs of this disease can manifest itself in horses at different ages [7, 8]. The PSSM1 mutation is inherited in an autosomal dominant manner. One copy of this defective gene is enough to show signs of the disease and pass this mutation to offspring.

The horses that have a positive test for the mutation (GYS1-R309H) need a special diet to avoid high sugar foods; physical activity for such animals should also be regulated.

Timely diagnosis and testing of horses for genetic abnormalities will make it possible to avoid the accumulation of genetic burden in populations and breeds and to increase the efficiency of breeding work.

The aim of the research is to study the spread of the GYS1 mutation, which reduces performance as a result of muscle impairment in horses of domestic breeds.

The task of our research included:

- development of a method for testing the GYS1 mutation causing PSSM1 polysaccharide myopathy in horses;
- to determine the incidence of the GYS1 mutation in horses of 10 factory and local breeds.

2. Materials and methods

The material for research was DNA samples isolated from hair follicles of 181 horses of factory and local breeds. The group of factory breeds included Donskaya breed (n = 10), Vladimirskaya breed (n = 30), Russian heavy draft breed (n = 24), Soviet heavy draft breed (n = 10), Persheronskaya breed (n = 10), Orlov trotter breed (n = 20) and Thoroughbred horses (n = 15). Populations of local horses were represented by Bashkirskaya breed (n = 31), Vyatskaya breed (n = 21) and Buryatskaya breed (n = 10). DNA isolation was performed from hair follicles using the ExtraGene DNA Prep 2000 kit (Izogen, Moscow). DNA amplification was carried out according to the method of SNP detection using original primers selected using the Primer3 program [9]. Electrophoresis of amplificates was carried out in 2% agarose gel, followed by staining the fragments with ethidium bromide dye. The results of electrophoresis were recorded visually (Figure 1). Statistical calculations of the frequency of occurrence of alleles and types of PSSM1 were carried out using the MS Excel 10 program.

![Figure 1. Results of horse genotyping for the GYS1 mutation (g.18940324 G> A) in horses of different breeds.](image-url)
3. Results and discussion
When testing 181 horses of different breeds using the SNP marker of the GYS1 gene (g.18940324 G> A), two homozygous individuals for this mutation with genotype AA (1.1%) were found in horses of the Russian heavy draft breed (Table 1). Most of the horses tested, 84% were of the GG genotype and 14.9% were heterozygous with the GA genotype. The GYS1 mutation was found in Bashkirskaya breed (3.2%), Buryatskaya breed (10%), Vyatskaya breed (9.5%), Soviet heavy draft breed (30%), Russian heavy draft breed (49.9%), and the maximum value of the PSSM1 defect was found in horses of the Percheronskaya breed (90%).

Table 1. Distribution of the GYS1 g.18940324 G> A mutation in horses of different breeds

| Breed               | N  | GG | GA | AA | Frequency |
|---------------------|----|----|----|----|-----------|
|                     | N  |    |    |    | G         | A         |
| Bashkirskaya        | 31 | 30 | 1  | 0  | 0.984     | 0.016     |
| Buryatskaya         | 10 | 9  | 1  | 0  | 0.950     | 0.050     |
| Vladimirskaya       | 30 | 30 | 0  | 0  | 1.000     | 0.000     |
| Vyatskaya           | 21 | 19 | 2  | 0  | 0.952     | 0.048     |
| Donskaya            | 10 | 10 | 0  | 0  | 1.000     | 0.000     |
| Orlov trotter       | 20 | 20 | 0  | 0  | 1.000     | 0.000     |
| Percheronskaya      | 10 | 1  | 9  | 0  | 0.550     | 0.450     |
| Russian Heavy Draft | 24 | 11 | 11 | 2  | 0.687     | 0.313     |
| Soviet Heavy Draft  | 10 | 7  | 3  | 0  | 0.850     | 0.150     |
| Thoroughbred        | 15 | 15 | 0  | 0  | 1.000     | 0.000     |

The frequency of occurrence of the g.18940324 G> A nucleotide substitution in horses of ten different breeds varied from 0.016 Bashkirskaya breed to 0.450 Percheronskaya breed (Figure 2). This mutation was most common in hard-draft horses. It is interesting to note that the GYS1 mutation was not detected in the horses of Vladimirskaya, Donskaya, Orlov trotter and thoroughbred horse breeds tested by us.

Figure 2. Distribution of the GYS1 g.18940324 G> A mutation in horses of different breeds.
Noteworthy is the fact that the GYS1 mutation is practically not found in Thoroughbred, Donskaya and Orlov trotter breeds, in which an intensive selection for work capacity is carried out, and animals with signs of polysaccharide myopathy do not fall into the breeding composition. The fixation of this mutation in local horse breeds is most likely due to their long-term improvement with heavy draft breeds. This indicates the need for genotyping of all used stallions-producers for the carriage of defective genes.

4. Conclusions
The results of testing the GYS1 mutation (g.18940324 G> A) in stud horses and local breeds indicate that polysaccharide myopathy PSSM1 is characteristic of heavy draft horses (Russian Heavy Draft, Soviet Heavy Draft, and Percheronskaya). This hereditary defect is also found in horses of local breeds (Bashkirskaya, Buryatskaya and Vyatskaya) as a result of crossing with heavy draft breeds. To prevent the spread of the GYS1 mutation, it is necessary to regularly test stallions of sires in order to further minimize the production of offspring with this defect. When breeding horses of heavy draft breeds, it is advisable to avoid mating of parents who are heterozygous carriers of the GYS1 mutation. Strict rules for keeping, feeding and loading horses must be followed in the presence of PSSM1 polysaccharide myopathy on the farm.

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