Assessment of the population structure of horses of the Priobskaya breed based on modern technologies

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Abstract. Priobskaya horse belongs to the group of northern forest breed and is located in Khanty-Mansi Autonomous Region along Ob River and its tributaries. Among other local horse breeds of Siberia this breed stands out for its endurance and resistance to mosquitoes and other insects. The aim of our work was to study the features of the population structure of Priobskaya horses by 17 distinctive markers: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1 and CA425. The results of biotechnological DNA typing of 25 Priobskaya horses by STR loci show that gene pool of the studied population is represented by 121 alleles, typical of local horse breeds. Horses of this breed had a number of rare alleles, including unique variant HMS1R, which was not found in horses of European origin (Van de Goor et al., 2010). The breed had fairly high rates of genetic diversity, the mean number of alleles per loci (MNA) reached 7.12, the effective number of alleles (Aₑ) – 4.16. The analysis demonstrated good compliance between the observed (Hₒ=0.741) and the expected (Hₑ=0.731) heterozygosity level and the absence of population inbreeding (Fᵢₛ=−0.017). Analysis of the phylogenetic relations between local horse breeds showed that Priobskaya breed has the highest coefficient of genetic similarity with Tuvinskaya (0.845) and Narymskaya (0.844) horses. Cluster analysis confirmed genetic uniqueness of Priobskaya.

1. Introduction
The Priobskaya horse belongs to the group of horses of Northern forest breeds. The breeding area of these horses is limited mainly to the Khanty-Mansiysk national district, while the main livestock is concentrated in the territory around Ob and Irtysh rivers. Taiga, located east and west of these rivers, gives place to moist water meadows, intersected by numerous channels.

Since the XVII century these horses were used as the main transport in summer and winter works in forests and in agricultural works. Today in the conditions of the Far North preference is given to the use of snowmobiles and other modern equipment; there is no need to breed horses as a labor force. Now these horses are kept outdoor in herds all year round and are bred mainly for meat [1, 2].

The severe climate of the North, great frosts, deep snows and almost complete absence of concentrated feed create extremely difficult conditions for the survival of the horse and require it to have outstanding endurance and resistance to natural adversity. The Priobskaya horse is characterized by the flat hooves form, which helps to move on viscous soil.
The Priobskaya horse has good fertility and longevity. In the favorable years the fertility percentage of mares was 80–90 %, with a fairly primitive organization of reproduction. Horses keep their ability to work up to 18–20 years [1].

According to the latest FAO recommendations [3] the number of typical Priobskaya horses corresponds to the critical status. To save this local breed of horses it is necessary to establish breeding records and genetic monitoring of population.

At the present stage of study of the genetic diversity of horse populations microsatellite DNA loci are mainly used, which are an effective tool for assessing the biological uniqueness of various breeds and for studying their microevolution [4–11]. The use of DNA markers is particularly important in the genetic monitoring programs for cultural and native populations, as well as rare and endangered species. Microsatellite markers are more likely than other methods to detect small differences between populations due to their high levels of allelic variation, being able to discriminate in both mean number of alleles and overall heterozygosity [3, 6, 11].

In our country DNA microsatellites have found wide practical application, both in controlling the origin of horses and in assessing the diversity of cultural and native horse populations [4, 6, 12–16], but still genetic studies of Priobskaya horse have been conducted. The studied horse populations differed in their genetic structure and degree of differentiation. Nei’s genetic distances were in interval 0.075–0.690 [12]. The analysis showed that all Russian native horse breeds from Eastern Europe and Asia form overall cluster with Orlov Trotter and Russian Heavy Draft breeds.

The purpose of our research was to study the genetic features of the Priobskaya horse breed and to assess the genetic similarity with other local native breeds.

2. Materials and Methods

The hair follicles were taken from horses of Priobskaya native breed (n=25), bred in private farm, located in the Belogorye village near Khanty-Mansiysk. The results of DNA testing of other local horses of Siberia were taken, such as Narymskaya (n=20), Pechorskaya (n=31), Tuvinskaya (n=20) and Yakutskaya (n=20) [12–15] for comparison. All horses were tested using 17 DNA loci: VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1, CA425, recommended by International Society for Animal Genetics (ISAG). The study was performed in the Laboratory of Genetics of the All Russian Research Institute for Horse Breeding.

DNA was extracted from samples using "Extragen™ DNA Prep 200" kits (Laboratory “Isogen”, Moscow). Extracted DNA probes were amplified using a 17-Plex set for genotyping horses Equine-STR ("Gordis", Moscow), optimized for use on automated laboratory equipment.

Amplification was performed on Termocycler 2730 (Applied Biosystems). PCR program included 30 cycles at the following method: the first 4 cycles: 58 °C (30 sec.), 59 °C (120 sec.), 72 °C (75 sec); the next 6 cycles: 94 °C (30 sec), 59 °C (120 sec), 72 °C (75 sec); the next 20 cycles: 90 °C (30 sec), 59 °C (120 sec), 72 °C (75 sec). The extension lasted 5 minutes at 68 °C, and then the samples were cooled to 4 °C.

Separation of amplification products was performed by capillary electrophoresis on an automatic genetic analyzer AB 3130 (Applied Biosystems), using GeneMapper™ program v. 4.0 program. The results of amplification DNA fragments were interpreted using a control DNA profile with a known genotype and data from international comparative tests (Horse Comparison Tests) conducted by ISAG.

Genetic analysis was performed according to basic parameters: the number of alleles in total (N_a) means the number of alleles in at each locus (MNA), the number of effective alleles (A_e), observed (H_o) and expected (H_e) heterozygosity. Genetic distances within populations were calculated according to Nei [17]. Inbreeding coefficient Fis and genetic differentiations were determined by methods of F-statistics [18] using MS Excel 2010, Statistica 12 (www. StatSoftStatistica) and FSTAT 1.2. (www2.unil.ch/popgen/fstat.htm).
3. Results
In Priobskaya horses (n=25), tested by 17 STR loci 121 alleles were found in total. Polymorphism of tested STR loci was high and ranged from 4 (HTG7) to 11 (ASB17). The allele’s variants of LEX3 loci, located in X-chromosome, were represented by 8 alleles, indicating the participation of many female lines in formation of the breed. In the genetic structure of Priobskaya horse breed a unique allele HMS7S (0.060) was detected, missing in standardized equine DNA allele’s nomenclature of 17 panel STR loci [19]. Rare allele ASB23V (0.040) was found only in horses of this breed and was absent in other local horse breeds. Allele ASB17D (0.020) was detected in Bashkirskaya, Tuvinskaya and Mongolian horses [12]. A characteristic feature of horses of Priobskaya breed was the relatively high frequency of alleles VHL20M (0.380), HTG4M (0.500), HTG6O (0.760), HMS2H (0.360) and HMS1J (0.500).

| Table 1. Allele’s spectrum of microsatellite loci in Priobskaya horses |
|-----------------------------|-----------------|-----------------|
| Loci           | Typical alleles | Rare alleles    |
|                | p>0.05          | p<0.05          |
| VHL20          | I,L,M,N,O,P,Q,R | H               |
| HTG4           | L,M,O,P         |                 |
| AHT4           | H,J,K,L,N,O     | I,R             |
| HMS7           | J,L,N,O,S       | K               |
| HTG6           | J,O             | G,I,M,P,R       |
| AHT5           | J,K,L,M,N,O     | I               |
| HMS6           | L,M,N,O,P,Q     |                 |
| ASB23          | J,K,L,S,U       | G,R,V           |
| ASB2           | K,M,N,O,Q       | I,R             |
| HTG10          | I,K,L,M,O,Q,R,S | N,P             |
| HTG7           | K,M,N,O         |                 |
| HMS3           | M,O,P,Q,R       | I,N,S           |
| HMS2           | H,I,K,L         | R               |
| ASB17          | F,H,K,M,N,O,P,R | D,I,J           |
| LEX3           | F,H,K,L,M,O     | P,Q             |
| HMS1           | I,J,M,Q         | K,N             |
| CA425          | M,N,O           | G,I,K,L         |

| Table 2. Assessment of STR loci polymorphism in horses of Priobskaya and other local breeds |
|-----------------|-----------------|-----------------|
| Breed           | N   | MNA       | A_e  | H_o  | H_e  | F_is | Na  |
| Priobskaya      | 25  | 7.118     | 4.157| 0.741| 0.731| -0.017| 121 |
| Narymskaya      | 20  | 6.765     | 4.611| 0.740| 0.758| 0.034 | 115 |
| Pechorskaya     | 31  | 7.118     | 4.173| 0.732| 0.705| -0.038| 121 |
| Tuvinskaya      | 55  | 6.650     | 4.200| 0.748| 0.742| -0.008| 113 |
| Yakytskaya      | 42  | 7.000     | 4.271| 0.734| 0.732| -0.032| 119 |

Note: N – number of horses; Na – number of alleles; A_e – effective number of alleles; H_e – expected heterozygosity; H_o – observed heterozygosity; F_is – population inbreeding level; MNA – average amount alleles per locus.

Comparative analysis of five local horse breeds according to base genetic characteristics (Table 2) showed that the Priobskaya population is leading in the parameter of allele’s number (Na=121), but has a more lower level of polymorphism (A_e = 4.157). The level of observed heterozygosity Ho exceeded expected heterozygosity H_o that ensured a negative F_is value (~0.017) and indicated the absence of intra-bred inbreeding.

Observed heterozygosity (H_o) values among studied horse breeds ranged from 0.732 (Pechorskaya) to 0.748 (Tuvinskaya). Four from five local horse breeds were in Hardy-Weinberg equilibrium (HWE)
and had negative $F_{is}$ values (Fig. 1). The reason of positive $F_{is}$ values for Narymskaya horses may be due to a small number of tested animals.

**Figure 1.** The dendrogram of genetic distances between of Priobskaya horse and other local horse breeds by 17 STR loci

The coefficients of genetic similarity between the studied local horse breeds varied in interval 0.770–0.871. Horses of Priobskaya breed had the highest coefficients of genetic similarity with Tuvinskaya (0.849) and Narymskaya (0.844) breeds. It should be noted that Priobskaya and Narymskaya horses are the closest geographical neighbors with an area in West Siberian lowland. The lowest level of genetic similarity was found between Narymskaya and Yakytskaya horse (0.770), territorially far removed each from other.

The genetic differences between the studied populations of local horse by 17 STR loci confirm the values of genetic distances, which varied in the range of 0.094–0.135. The dendrogram presents the result of cluster analysis conducted on the basis of the matrix of genetic distances, which demonstrated genetic uniqueness of Priobskaya horse and other local breeds. The results of our study confirm the previously obtained data [6, 12] of the originality of allele’s pool of Russian local horse populations and their significant differences with cultural breeds.

**4. Conclusion**

Horses of Priobskaya breed demonstrated a high level of variability studied by STR loci, 121 alleles were calculated in local horse breeds. Two unique alleles HMS7S (0.060) and ASB23V (0.040) were detected in genetic structure of the breed, which were absent in other Russian local horse breeds. Comparative analysis of five local horse breeds according to base genetic characteristics showed that Priobskaya population had high enough parameters of genetic variability according to allele’s number $N_a$ (121), the effective number of alleles $A_e$ (4.157) and observed heterozygosity $H_o$ (0.741). The level of observed heterozygosity $H_o$ exceeded expected heterozygosity $H_e$, that ensured a negative $F_{is}$ value (−0.017) and indicated the absence of intrabred inbreeding.

Genetic analysis of phylogenetic relationships of Priobskaya horse with other local breed demonstrated that this population had the highest coefficients of genetic similarity with Tuvinskaya (0.849) and Narymskaya (0.844) breeds. The lowest level of genetic similarity was found between Narymskaya and Yakytskaya horse (0.770), territorially far removed from each other. The results of our study confirm the previously obtained data of the originality of allele’s pool of Russian local horse populations and their significant differences from European cultural breeds and indicate the promising use of STR’s markers in breeding and conservation programs.
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