Genetic characterization of Akhal-Teke horse subpopulations using 17 microsatellite loci

A V Ustyantseva¹, L A Khrabrova¹, N V Abramova¹, T N Ryabova¹

¹ All-Russian Research Institute for Horse breeding, Divovo, Ryazan Region, 391105, Russia

E-mail: ustavanna@yandex.ru

Abstract. Akhal-Teke is the ancient cultural horse breed of oriental origin. The study of genetic features different subpopulations of Akhal-Teke breed was carried out on 17 loci of DNA microsatellites (VHL20, HTG4, AHT4, HMS7, HTG6, AHT5, HMS6, ASB23, ASB2, HTG10, HTG7, HMS3, HMS2, ASB17, LEX3, HMS1 and CA425) to assess their genetic differentiation. The results of DNA typing of 5457 Akhal-Teke horses on 17 microsatellite loci show that gene pool of world population is represented by 121 alleles, among which 98 are found in all countries. Comparative analysis of genotypes of horses representing different subpopulations indicates that they differ in the number of alleles (100-117), allele frequencies, the level of polymorphism Ae (3.40-3.91) and the degree of observed heterozygosity Ho (0.661-0.724). The genetic distances between the subpopulations varied in range from 0.007 (Russia – CIS) to 0.051 (Czechia – Turkmenistan). Correspondence to HWE was maintained in all countries, which confirms the negative value Fis. Genetic options of the Akhal-Teke by index Fst varied in a range 0.001-0.078 at the mean value Fst=0.022. Cluster analysis demonstrated more close relationship between Russian, CIS and European subpopulations.

Key words: Akhal-Teke, differentiation, genetic diversity, horse, microsatellite DNA, population analysis.

1. Introduction

Akhal-Teke is one of the oldest, most distinctive and unusual horse in the world. It is bred around the oases of the Turkmenistan Desert, north of Iran. Horses were bred and raced there 3,000 years ago. There is nothing in the world quite like this mystery horse. This extraordinary feat has never been equaled. Today the Akhal-Teke is a racehorse, a long-distance performer and a horse for equestrian sports, including the dressage and jumping disciplines [1], [2].

Increased interest in this breed began in the early twentieth century. The first society of Akhal-Teke horse lovers appeared in Germany. Currently the horses of this breed are popular in many countries of Europe, Asia and America. The selection of Akhal-Teke horses is supervised by International Association of the Akhal-Teke Horse Breeding engaged at the All-Russian Research Institute for Horse Breeding. Only in the Russian Federation Akhal-Teke horses are bred at 3 studs, 12 breeding farms and by more than 60 private owners. More than 200 stallions and 650 mares are used for breeding purposes.

Since the mid-70s of the last century, the Laboratory of genetics of the institute of horse breeding has been monitoring the origin of Akhal-Teke horses using polymorphic blood systems [3]. For the last ten years, all horses of this breed have been tested on DNA microsatellites, which allow them to conduct research on the genetics of the Akhal-Teke breed [3], [5], [6]. The loci of microsatellite DNA in its properties are universal genetic markers and can be used to solve many research problems, including...
parentage verification, phylogenetic relationships and origin of the breeds [7], [8], [9], [10], [11], [12]. According to microsatellite analysis, the Akhal-Teke form a common cluster with Caspian Pony and Arabian horse, which have oriental origin [13].

The aim of this study was to conduct a comparative analysis of the genetic diversity and relationships of different subpopulations of Akhal-Teke breed horse using microsatellite markers and the data on other domestic horse breeds.

2. Materials and methods

For genetic and population analysis there were used the data of DNA typing of 5457 Akhal-Teke horses, issued in the form of DNA-certificate by the genetic laboratories of different countries, including Italy, Germany, France, Czech Republic, Russia, USA and Turkmenistan. All tested horses were registered in the database of International Association of the Akhal-Teke Horse Breeding. For comparison were taken the results of DNA testing of 420 Arab horses in the laboratory of the All-Russian Research Institute for Horse Breeding [5].

DNA were prepared from hair loops or blood samples of Akhal-Teke horses by standard procedure using “Extra Gene ТМ DNA Prep 200” and “Diatom ТМ DNA Prep 200” kits (Laboratory Isogene, Russia) in the Laboratory of Genetics of the All-Russian Research Institute for Horse Breeding, certified by ISAG based on the results of Horse Comparison Test (HCT).

PCR products of 17 panel microsatellite markers (AHT4, AHT5, ASB2, ASB17, ASB23, CA425, HMS1, HMS2, HMS3, HMS6, HMS7, HTG4, HTG6, HTG7, HTG10, LEX3 and VHL20) were amplified with the fluorescently labeled primers (StockMarks®, Applied Biosystems) and then analyzed on the automated DNA sequencer ABI 3130. The size of STR marker was determined using Gene Mapper version 4.0 (Applied Biosystems). Detailed information on microsatellite markers used to parentage control of horses is presented in the article Van de Goor and van Haeringen W.A. [14].

Genetic diversity within the line was evaluated by the basic parameters including total number of allele variants (Na), effective number of allele (Ae), number of allele per loci (MNA), observed (H0) and expected heterozygosity (He) and Hardy-Weinberg equilibrium (HWE). The coefficients of genetic similarity and genetic distances between the lines were determined by the Nei’s methods [15] using software Statistica 12 ver.10 (www.StatSoftStatistica). Intrabreed inbreeding and genetic differentiations among lines were estimated by methods of F-Statistics [16] using MS Excel 2010 and Statistica 12.

3. Results and discussion

The study of genetic diversity of alleles of STR loci in horses of Akhal-Teke breed is of particular interest, as these breed had a huge impact on the world horse breeding. Analysis of polymorphism of 17 panel microsatellite loci in the horses show that the oldest Akhal-Teke horse breed has the wide range of alleles, total 121, that is significantly more than found in Arabian horses (Table 1). The numbers of alleles at loci were high and ranged from 4 (HMS1, HTG7) to 9 (ASB2, ASB17, HMS2, VHL20 and LEX3). In the locus LEX3 there were determined 9 of the 14 known alleles that indicates a fairly wide parent the maternal foundation of this breed. Private allele HMS2 N has not been detected in horses of other studied breeds [14], [17].

Genetic structure of Akhal-Teke breed is characterized by a high frequency of alleles AHT4 H (0.538), AHT5 N (0.380), ASB17 N (0.278), ASB23 J (0.445), HMS1 M (0.593), HMS2 K (0.258), HMS3 P (0.356), HMS6 O (0.463), HTG4 M (0.763), HTG6 G (0.550), HTG10 O (0.500) and VHL20 R (0.241). The number of alleles on average amounted to 7.12 per locus, Ae level ranged from 0.72 (HMS7) to 6.97 (ASB2). The observed heterozygosity (H0) was 0.694 and varied from 0.405 (HTG42) to 0.865 (ASB2).
Table 1. The range of alleles of 17 microsatellite loci in Akhal-Teke and Arabian breeds

| Loci | Number of alleles | Akhal-Teke n=5457 | Arabian n=420 |
|------|------------------|-------------------|---------------|
|      | Typical alleles p > 0.05 | Rare alleles q < 0.05 | Typical alleles p > 0.05 | Rare alleles q < 0.05 |
| AHT4 | 11 | H, J, O | I, K, M, N, P | H, J, K, M, O | I, P |
| AHT5 | 11 | J, K, N, O | M, Q | J, K, M, N | O |
| ASB2 | 16 | I, K, M, N, O, P, Q, R | B | B, J, K, N, O, Q | C, I, L, M, R |
| ASB17 | 15-19 | G, H, N, Q, R | K, M, O, S | N, O, R | G, K, M, Q |
| ASB23 | 9-14 | I, J, K, L, U | R, S | I, J, K, L, S | U |
| CA425 | 11 | J, M, N, O | I, K, L | J, N, O | I, L, M |
| HMS1 | 8 | I, J, M | K | I, J, L, M |
| HMS2 | 12 | H, I, K, L, M, P, R | J, N | H, L, M, P | I, K, R |
| HMS3 | 13 | I, M, N, O, P, Q | R, S | I, M, N, O, P | Q |
| HMS6 | 8 | L, M, O, P | K | L, P | K, M, O |
| HMS7 | 11 | J, K, L, M, N, O | - | J, K, L, M, N | O |
| HTG4 | 12 | K, M, P, Q | L, N, O | K, M, L, N | P |
| HTG6 | 13 | G, J, O | I, M, P | G, J, O | - |
| HTG7 | 8 | K, N, O | M | K, N, O | - |
| HTG10 | 14 | K, L, O, R | I, M, P, Q | I, K, L, O, R | M, N, S |
| LEX3 | 14 | F, H, L, M, N, O, P | I, K | F, H, I, M, P | K, L, N, O |
| VHL20 | 12 | J, M, N, Q, R | I, L, O, P | I, L, M, N, R | P |

Note: a Private allele HMS2 N has not been detected in horses of other breeds.

Comparison Akhal-Teke horses from different regions demonstrate that each subpopulation has allele pool typical for this breed. Genetic characteristics of the horse’s subpopulations varied within a narrow range (Table 2). A wide spectrum of alleles, including rare alleles (q < 0.05), was found in Russian (Na=117) and CIS horses (Na=119). American population of Akhal-Teke horses is characterized by the lowest level of genetic variation (Na=108). All of the regional populations of Akhal-Teke horses have a typical genetic structure and it is available rather large variability of resource for the successful development of this breed.

Comparative analysis of representatives of different subpopulations of Akhal-Teke horse on the base of genetic and population parameters shows (Table 2) that they differ in number of alleles (Na), level of polymorphism (Ae) and degree of observed heterozygosity (H0). In European countries the number of alleles varied in the range of 100-117, polymorphism level (Ae) - 3.401-3.780. Akhal-Teke horse of Turkmenistan had the highest level Ae (3.907).

Among the Akhal-Teke breed in Russia, horses in the Krasnodar region were characterized by the highest rates of genetic diversity (the level of polymorphism Ae = 3.808, the degree of observed heterozygosity Ho = 0.699). A relatively wide allele’s spectrum of microsatellite loci was registered in the Akhal-Teke of Dagestan and Moscow Regions. The small subpopulation Akhal-Teke horses of Kalmykia had the lowest parameters of genetic diversity (Ae = 3.365, Ho = 0.667), but it stands out the highest differentiation index (FST = 0.050).
Table 2. Polymorphism indices of microsatellite loci in subpopulations of Akhal-Teke horses

| Country, region | N | Na | MNA | $A_e$ | $H_o$ | $H_e$ | $F_{is}$ | $F_{st}$ |
|----------------|---|----|-----|------|------|------|--------|--------|
| Russia         | 2994 | 117 | 6.882 | 3.780 | 0.693 | 0.690 | -0.005 | 0.002  |
| Dagestan       | 832  | 108 | 6.353 | 3.793 | 0.696 | 0.690 | -0.006 | 0.003  |
| Kalmykia       | 146  | 98  | 5.765 | 3.365 | 0.667 | 0.657 | -0.018 | 0.050  |
| Krasnodar region | 333  | 111 | 6.529 | 3.808 | 0.699 | 0.691 | -0.013 | 0.004  |
| Moscow region  | 395  | 109 | 6.412 | 3.747 | 0.704 | 0.692 | -0.017 | 0.003  |
| Rostov region  | 273  | 106 | 6.235 | 3.517 | 0.687 | 0.671 | -0.023 | 0.035  |
| Stavropol region | 553  | 107 | 6.294 | 3.527 | 0.687 | 0.669 | -0.028 | 0.032  |
| CIS $^a$       | 1001 | 119 | 7.000 | 3.797 | 0.697 | 0.691 | -0.008 | 0.000  |
| Kazakhstan     | 645  | 117 | 6.882 | 3.782 | 0.696 | 0.689 | -0.010 | 0.004  |
| Uzbekistan     | 261  | 108 | 6.353 | 3.777 | 0.708 | 0.696 | -0.016 | -0.015 |
| Europe         | 1137 | 115 | 6.765 | 3.688 | 0.688 | 0.686 | -0.004 | 0.010  |
| Czechia        | 159  | 103 | 6.059 | 3.401 | 0.661 | 0.649 | -0.017 | 0.078  |
| France         | 297  | 101 | 5.941 | 3.450 | 0.703 | 0.660 | -0.074 | 0.054  |
| Germany        | 112  | 100 | 5.882 | 3.505 | 0.714 | 0.674 | -0.057 | 0.026  |
| Slovakia       | 133  | 102 | 6.000 | 3.576 | 0.685 | 0.680 | -0.005 | 0.017  |
| America        | 203  | 108 | 6.353 | 3.724 | 0.718 | 0.691 | -0.040 | 0.001  |
| Turkmenistan   | 109  | 104 | 6.118 | 3.907 | 0.724 | 0.709 | -0.019 | 0.033  |
| Total          | 5457 | 121 | 7.059 | 3.799 | 0.694 | 0.692 | -0.003 | 0.000  |

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; $F_{st}$ – index differentiation: MNA – average amount alleles per locus.

$^a$ CIS - The Commonwealth of Independent States (Azerbaijan, Armenia, Kazakhstan, etc.).

The observed heterozygosity ($H_o$) values among studied subpopulations ranged from 0.661 (Czechia) to 0.724 (Turkmenistan). All subpopulations were in Hardy-Weinberg equilibrium (HWE) and had negative $F_{is}$ values, indicating an excess of heterozygous genotypes (Figure 1). High polymorphism of microsatellite, blood group and biochemical markers in Akhal-Teke horses proves the ancient origin of the breed [6].

![Figure 1. Observed ($H_o$) and expected ($H_e$) heterozygosity in subpopulation of Akhal-Teke horses from different regions.](image-url)
Analysis of genetic differentiation of Akhal-Teke subpopulations based on F-statistics showed that index $F_{st}$ varied in the range of 0.001-0.078 and averaged 0.022. Index $F_{st}$ indicate the level of genetic differentiation among population and always has positive values that range from 0 to 1. The small Czechia subpopulations had the highest index of fixation ($F_{st} = 0.078$). Relatively low values of differentiation index ($F_{st} = 0.001$) and the highest level of consolidation was determined in the horses of America, geographically the most remote from the center of breeding Akhal-Teke horses.

The coefficients of genetic similarity demonstrate more close relationship between Russian and CIS populations of Akhal-Teke horses (0.993) and a more significant differentiation between Turkmenistan and American horses (0.966). Maximum index of differentiation is observed in the Akhal-Teke of America. The genetic Nei’s distances between the subpopulation varied in range from range from 0.007 (Russia – CIS) to 0.051 (Czechia – Turkmenistan).

The revealed genetic differences between the subpopulation of Akhal-Teke horses by STR-loci are graphically illustrated the dendrogram of linkage distances (Figure 2). In the center of the dendrogram it is clearly visible a cluster, including Russian, CIS and European subpopulations.

![Dendrogram](image)

**Figure 2.** The dendrogram of genetic distances between Akhal-Teke horses from different regions.

Comparative evaluation of the Akhal-Teke horses of different subpopulations on a number of parameters, including the number of alleles, genotypes, polymorphism level and degree of heterozygosity, as well as the dendrogram of genetic distances show that they have some differences, but in general are rather consolidated on the genetic structure.

**4. Conclusion**

Akhal-Teke horse breed has the high level of genetic diversity and retains its characteristics when breeding in different countries. The results of DNA typing of 5457 Akhal-Teke horses on 17 microsatellite loci show that gene pool of world population is represented by 121 alleles, among which 98 are found in all countries. Comparative analysis of genotypes of horses representing different subpopulations indicates that they differ in the number of alleles (100-117), allele frequencies, the level of polymorphism $Ae$ (3.40-3.91) and the degree of observed heterozygosity $Ho$ (0.661-0.724), but in general are rather consolidated on the genetic structure. It is important to be concerned about the genetic diversity of horse breeds on the basis of effective management, especially in respect to small subpopulations.
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