Kafkas Univ Vet Fak Derg
15 (6): 913-918, 2009
DOI:10.9775/kvfd.2009.244

RESEARCH ARTICLE

Blood Protein Polymorphism of Horse Types Being Bred by Public at Villages in Three Different Regions of Turkey

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Makale Kodu (Article Code): KVFD-2009-244

Summary

Different breeds and types of horses are raised in different regions of Turkey. The numbers and types of native horses have declined steadily over time because of increased agricultural mechanization. The blood samples of 85 native Turkish horses of different types from different geographical regions (Kars-Erzurum 30, Şanlıurfa 20 and Adapazarı 35) were analysed for blood protein polymorphism. Starch gel electrophoresis, polyacrylamide gel electrophoresis and polyacrylamide isoelectric focusing were used to identify genotypic variants of albumin (ALB), transferrin (TF), hemoglobin alpha (HBA), vitamin D-binding protein (GC), serum carboxylesterase (ES), A1B-glycoprotein (A1B), 6-phosphogluconate dehyrogenase (6-PGD) and phosphoglucomutase (PGM) loci. The direct counting method was used to calculate the frequency of genes for blood protein systems. Homozygosity degrees and chi-square were calculated with the frequency of genes for blood protein systems. According to the results of chi-square testing on eight systems of three different regions, 6-PGD system was significant P<0.001 in the eastern population (Kars-Erzurum). Among the local horses in the south-east region (Şanlıurfa) 6-PGD system (P<0.001) and HBA system (P<0.05) were found to be significant. On the other hand, in the western region (Adapazarı), chi-square analyses of 6-PGD and HBA systems were found to be significant at P<0.05. In this study, it was found that the three different local horse types in the three regions descended from the same origin.

Keywords: *Electrophoresis, Horse, Polymorphism, Sera protein*

Türkiye'nin Üç Farklı Bölgesinde Halk Elinde Yetiştirilen Yerli At Tiplerinde Kan Protein Polimorfizmi

Özet

Türkiye'de halk elinde yetiştirilen çeşitli at tipleri mevcuttur. Tarımda mekanizasyonun artması sonucunda Türkiye'de yetiştirilen yerli tip atların sayıları her geçen gün azalmaktadır. Çalışmada 35 baş Adapazarı, 30 baş Kars-Erzurum ve 20 baş Şanlıurfa menşeli toplam 85 baş yerli at kullanılmıştır. Çalışmada nişasta jel elektroforezi, poliakrilamid jel elektroforezi ve poliakrilamid izoelektrik odaklama yapılarak, albümin (ALB), transferin (TF), hemoglobin alfa (HBA), vitamin D-bağlayıcı protein (GC), serum karboksilesteraz (ES), A1B-glikoprotein (A1B), 6-fosfoglukonat dehidrojenaz (6-PGD) ve fosfoglukomutaz (PGM) olmak üzere sekiz sistem çalışılmıştır. İncelenen kan protein sistemlerinin gen frekansları direk hesaplama yöntemi ile hesaplanmıştır. Hesaplanan gen frekansları kullanılarak, bu sistemlere ait homozigotluk dereceleri ve ki-kare testi yapılmıştır. İncelenen üç farklı bölgedeki yerli atlarda çalışılan sekiz sistemin ki-kare test sonuçlarına göre 6-PGD sistemi, Kars-Erzurum ve Şanlıurfa bölgelerine ait yerli atlarda P<0.001, Adapazarı bölgesine ait atlarda ise P<0.05 güven düzeyinde önemli bulunmuştur. HBA sisteminin ise Şanlıurfa ve Adapazarı bölgesi yerli atlarında P<0.05 güven düzeyinde istatistikî olarak önemli olduğu görülmüştür. Bu çalışmada, Türkiye'nin üç faklı bölgesinden getirilen yerli at tiplerinin aynı orijinden kök aldıkları belirlenmiştir.

Anahtar sözcükler: *At, Elektroforez, Polimorfizm, Serum proteini*

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INTRODUCTION

In the past, the Anatolian peninsula was an important bridge for armies as well as tradesmen moving between east and west. All these movements resulted in a mixture of animal populations, including horses. As a consequence, Persian, Arabian and Thracian horses as well as Russian, Mongolian and Caucasian horse breeds have contributed to the formation of Anatolian horse breeds [1].

The domestic horse breeds in Turkey are classified as Anatolian, Uzunyayla, Çukurova, Pony breeds and there are also some local types like Malakan Atı, Canik Atı, Hınıs Kolu Kısası and others [1,2]. After the establishment of the Turkish Republic, the Nonius, Ardene and Halflinger breeds were brought to Turkey to improve the Anatolian horse population. Thoroughbred and Arabian horses have also been introduced for the improvement of native horses, creating half-breed populations [1]. In view of the fact that there are many breeds currently at risk in the world, there is a need to access the genetic uniqueness of each breed for the purpose of determining which breeds should be prioritized in conservation programs [3]. According to the Turkish Institute of Statistics, the horse population decreased from 1132000 in 1960 to 188640 in 2007 [4].

Blood groups and blood protein loci have been traditionally used in order to evaluate intra and interbreed genetic diversity in horse breeds [5,6]. There are 25 blood plasma and red cell protein systems in horse. Some systems have been examined in only small a number of breeds (e.g. Arabian horses and Thoroughbred horses) and in individuals, while others, especially those routinely used in parentage testing, have been studied in a wide variety of breeds [5]. These standardized systems make it possible to compare results between the studies.

Protein-polymorphism studies in horses were started with hemoglobin-polymorphism study in 1955 [5,7]. The International Society for Animal Genetics (ISAG) has accepted three alleles for albumin (ALB) [8-12], 15 alleles for transferrin (TF) [13-16], seven alleles for serum carboxyl-esterase (ES) [12,15,17,18], four alleles for hemoglobin alpha (HBA) [8,19,20], three alleles for A1B-glycoprotein (A1B) [20], two alleles for vitamin D-binding protein (GC) [5-10,21,22], three alleles for phosphoglucomutase (PGM) [8,15,18], and three alleles for 6-phosphogluconat dehydrogenase (6-PGD) [9,12,18,19].

The present study aims to assess the genetic relationships among local horse populations in three different regions of Turkey based on allelic diversity of 8 of the ISAG blood and sera protein systems; namely ALB, TF, ES, HBA, A1B, GC, PGM and 6-PGD.

MATERIAL and METHODS

Blood samples of the horses analysed in the present study were collected from 35 local horses (22 male and 13 female) in the Adapazarı region, 30 local horses (15 male and 15 female) in the Kars-Erzurum region and 20 local horses (12 male and 8 female) in the Şanlıurfa region, which represent the western, the eastern and the southeastern regions of Turkey respectively.

Blood samples were stored in tubes containing sodium citrate and taken to the laboratory, where blood plasma and erythrocytes were separated to analyse serum protein systems and HBA polymorphism, respectively. In the context of the present study ALB, 6-PGD and PGM systems were analysed by starch-gel electrophoresis, HBA polymorphism was analysed by isoelectric focusing, and TF, A1B, ES and GC systems were analysed by polyacrilyamide-gel electrophoresis. Isoelectric focusing, starch and polyacrilyamide-gel electrophoresis techniques were performed as proposed by Kopar [8]. Isoelectric focusing, starch and polyacrilyamide-gel electrophoresis, images are presented in *Figures 1, 2* and *3*.

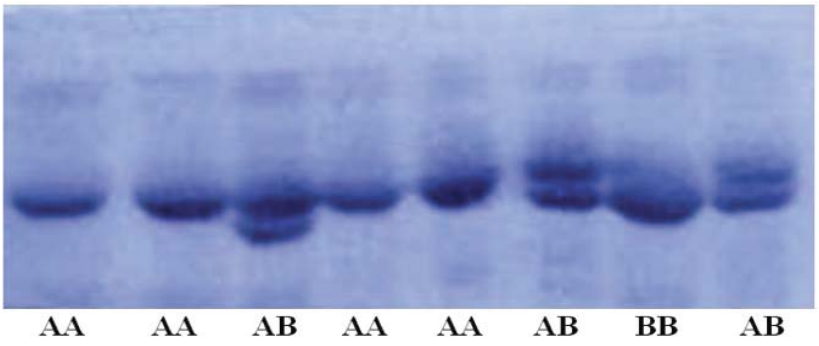

Fig 1. ALB genotypes in starch gel electrophoresis

Şekil 1. Nişasta jel elektroforezindeki ALB genotipleri

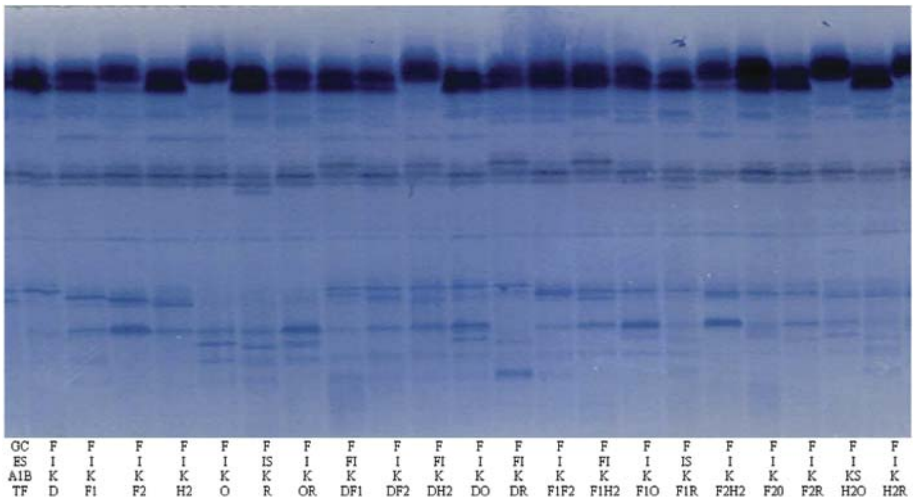

Fig 2. GC, ES, A1B and TF genotypes in polyacrylamide gel electrophoresis

Şekil 2. Poliakrilamid jel elektroforezinde GC, ES, A1B ve TF genotipleri

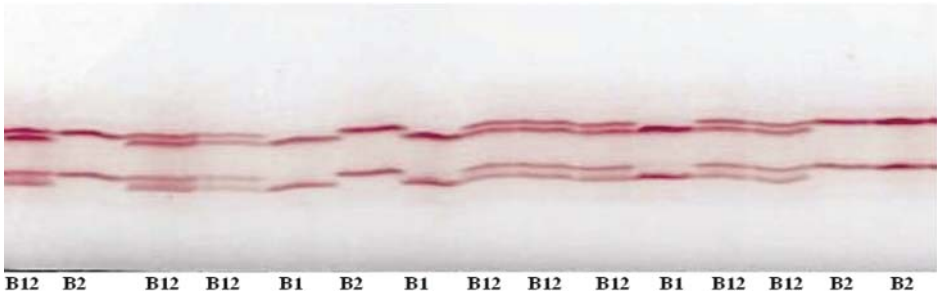

Fig 3. HBA genotypes in isoelectric focusing

Şekil 3. İzoelektrik fokuslamada HBAgenotipleri

The allele frequencies, as well as observed and expected heterozygosities were estimated using the Genetix 4.00 computer program [22]. The same software was used to asses the genetic differentiation between the populations based on Wright's F-statistics (1965) and its significance was tested by permuting the data 1000 times [22]. The Factorial Correspondence Analysis (FCA) in the Genetix 4.00 allowed us to evaluate the genetic data of each individual in populations and put them in a two-dimensional plane. Thus, relationships among the individuals in and out of the populations were interpreted [22]. Then, the chi-square test was applied to assess Hardy-Weinberg equilibrium 6 for each protein system. Afterwards, the Populations 1.0 computer program [23] was used to estimate pairwise genetic distances among the populations based on Nei's standard genetic distance [24].

The following formulas were used in the estimations; for the exclusion probability of each system (P): $P=\Sigma p(1-p)^2 - \Sigma(p_i p_j)^2 [4-3 (p_i+p_j)]$ [8,25], for exclusion of whole systems: $P=1-(1-p1)(1-p2)\ldots\ldots(1-pn)$ [8,25]. In these formulas, p_i is the i^{th} allele frequency in the defined systems.

RESULTS

Statistical analysis results of alleles detected by electrophoresis analyses of 85 local horses from three different regions were given in *Table 1*. The results showed the S allele in the ES system was found in two horses in the eastern region (Kars-Erzurum). In the other two populations, only I and F alleles of the ES system were observed. In the A1B system, only the K allele was observed in local horses from Kars-Erzurum and Adapazarı. However, both K and S alleles were observed in the south-eastern region (Şanlıurfa).

According to Wright's F statistics, the value F_{IS} was only found to be significant in the Kars-Erzurum *(Table 2)*.

The F_{ST} value, which was used to test the genetic differentiation-divergence and was calculated by mutual comparison, was found to be 0, so no significant differentiation was observed among the populations. Similarly, the FCA analysis of the individuals' genotypes from all three regions did not reveal any genetically distinct grouping based on geographic origin *(Figure 4)*. Except for few samples (within

Table 1. *Gene frequencies, exclusion percentages and chi-square test results of blood protein systems belonging to local horse types from three different regions*

Tablo 1. *Üç farklı bölgeden örneklenen yerli at tiplerinde kan protein sistemlerine ait gen frekansları, dışlama yüzdeleri ve ki-kare test sonuçları*

Systems	Alleles	POPULATIONS											
		Kars-Erzurum				Şanlıurfa				Adapazarı			
		Gen Freq.	SE	Σx^2	P	Gen Freq.	SE	Σx^2	P	Gen Freq.	SE	Σx^2	P
HBA	BI BII	0.533 0.467	0.064	1.157	0.187	0.525 0.475	0.079	**4.576 ***	0.187	0.543 0.457	0.059	**4.719 ***	0.187
ES	I F S	0.784 0.183 0.033	0.053 0.049 0.023	3.540	0.167	0.800 0.200	0.063	3.420	0.134	0.757 0.243	0.051	3.556	0.15
ALB	A B	0.500 0.500	0.065	0.209	0.188	0.475 0.525	0.079	0.305	0.187	0.514 0.486	0.06	0.196	0.188
A1B	K S	1.000 0.000	0.000	0.000	0.000	0.975 0.025	0.025	0.000	0.024	1.000 0.000	0.000	0.000	0.000
6-PGD	F S	0.933 0.067	0.023	**39.37 *****	0.031	0.925 0.075	0.042	**12.324 *****	0.065	0.929 0.071	0.024	**5.690 ***	0.039
PGM	F S	0.983 0.017	0.000	0.000	0.000	0.900 0.100	0.047	0.181	0.082	0.914 0.086	0.036	0.719	0.082
GC	F S	0.950 0.050	0.028	0.055	0.045	0.95 0.05	0.035	0.027	0.045	0.943 0.057	0.028	3.279	0.051
TF	F_1 F_2 H_2 D O R	0.117 0.416 0.067 0.250 0.133 0.017	0.042 0.064 0.032 0.056 0.044 0.016	8.709	0.544	0.100 0.375 0.075 0.275 0.100 0.075	0.047 0.077 0.042 0.071 0.047 0.042	16.70	0.540	0.014 0.457 0.071 0.358 0.071 0.029	0.014 0.457 0.071 0.358 0.071 0.029	13.777	0.395
Total exclusion percentage					**0.768**				**0.779**				**0.716**

** Significant at P<0.05; *** Significant at P<0.001; Σ x^2= Total Chi-Square*

Table 2. *The F_{ST} valves among the local horse types in the present study*

Tablo 2. *İncelenen yerli at tipleri arasında hesaplanan F_{ST} değerleri*

Populations	F_{IS}
Adapazarı	0.008
Kars-Erzurum	0.130*
Şanlıurfa	0.038

** Significant at P<0.05*

squares), all the samples grouped together (within the circle). The samples in the squares show some differentiation from the rest regardless of their geographic origin.

The greatest exclusion percentage (P) was computed for the TF system in all populations. According to the results of the chi-square test of eight systems of three different regions, the 6-PGD system was significant

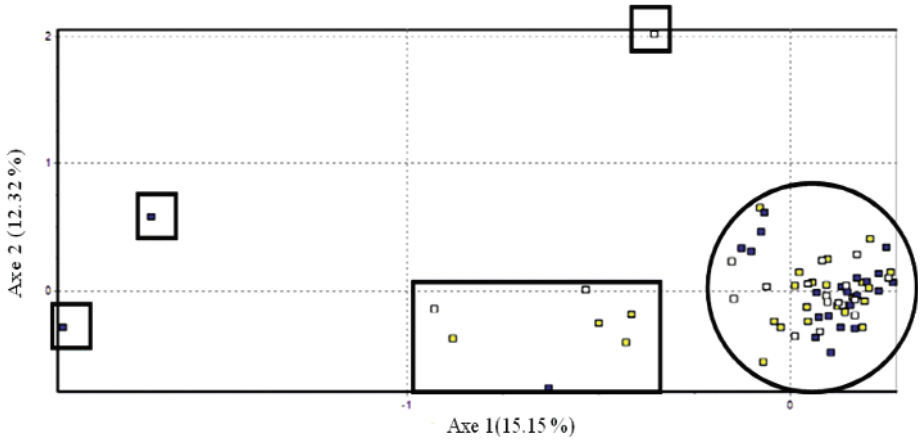

Fig 4. FCA results for local horses from Adapazarı (yellow boxes), Kars-Erzurum (blue boxes), Şanlıurfa (white boxes)

Şekil 4. Adapazarı (sarı kutular), Kars-Erzurum (mavi kutular), Şanlıurfa (beyaz kutular) bölgelerinden getirilen yerli at tipleri için hazırlanan FCA analiz sonuçları

(P<0.001) in the eastern population (Kars-Erzurum). Among the horses in the south-east region (Şanlıurfa) the 6-PGD system (P<0.001) and HBA system (P<0.05) were also found to be significant. Among the horses found in the western region (Adapazarı), the 6-PGD system and HBA systems were found to be significant at P<0.05. Furthermore, chi-square analyses of other systems were not found to be statistically significant.

DISCUSSION

In ALB system, the I allele was not found in this study. Otherwise, in this study the frequency of A and B alleles were found to be same. In TF systems, G, M and I alleles were not found in Andalusian horse, Thoroughbred and Arabian breeds [26]. Similarly, these three alleles were not found in this study. In Arabian horses the frequency of the I allele in the ES system was reported to be higher than all of the other alleles [11,16,18]. In this study, the same result was found that I allele frequency was the highest. In Thoroughbred, Arabian and Andalusian horses, all three alleles of the A1B system were identified but the frequency of the K allele was found to be higher than the other alleles [8,17,18]. In this study, only K and S alleles were identified and the frequency of the K allele was higher than that of the S allele. In the GC system, F and S alleles were identified in Thoroughbred, Arabian and Andalusian horse breeds but the frequency of the F allele was found to be higher than that of the S allele [17,18]. In this study, both of these alleles were identified and the frequency of the F allele was also found to be higher than that of the S allele. In 6-PGD polymorphism studies on the Arabian breed, both F and S alleles were detected and the frequency of the S allele was found to be lower than that of the F allele [8,17,19]. Likewise, the frequency of the F allele was found to be higher than the S allele in this study.

There is a general understanding that the horse population in Turkey has been influenced by the genetic make-up of the Arabian breed. The findings in this study may strengthen the argument that Turkish domestic horses have been influenced by the Arabian breed, but the influences of cold-blooded Russian and Caucasian horses must also be considered.

There is no sufficient information on native Turkish horses in the literature. Only a few detailed studies on the morphological characteristics of native breeds [1] were carried out in the first half of the XX. century. These studies and the records of the Ministry of Agriculture, including the current breeding practises of owners, points to the significant effect that the Arabian horse has had on native breeds [1]. On the other hand, the effect of cold-blooded Russian and Caucasian horses on the horses of the Kars-Erzurum region should not be overlooked [1]. Still, the serum protein polymorphism results provided no differentiation between these three breeds in the present study. Hill et al.[27] found a close genetic relationship between Anatolian and Çukurova breeds in a study which included 19 families of British horses, 5 breeds from the Far East, 4 breeds from the Middle East (including Anatolia and Çukurova breeds) and 4 European breeds of horses based on mtDNA variation. However, it should be noted that the F_{IS} estimation for the Kars-Erzurum population was found to be significant. Kars and Erzurum were adjacent and as they were both affected by the same cold-blooded breeds, they were expected to be genetically similar. But this significant F_{IS} value might be explained by the "Wahlund effect" [28], indicating that there are actually two different populations.

Due to the agricultural policies implemented after the establishment of the Republic in 1923, the mechanization of agriculture sharply increased, causing a sharp decline in the number and size of the horse populations. According to the State Institute of Statistics, there has been a 74% decline in the horse population over the last four decades [4]. Among the native Turkish horses that were counted, there are Arabian and Thoroughbred horses as well. This means the decline in the number of native Turkish horses may be even worse. As a consequence, this sharp decrease in the population has caused a decrease in the number of "pure" breed populations. As there is no controlled breeding system and no studbooks are kept for native breeds, the breeders relentlessly hybridize native breeds with each other and with foreign breeds, mostly with Arabian and Thoroughbred horses. These in turn has led to the high genetic similarities between the populations analyzed in the present study.

There is global concern regarding the characterization and conservation of native animal genetic resources. The decrease in numbers may result in the decrease of genetic variation, which may help the species to adapt in changing environments. The loss of variation might cause the extinction of the species. The necessary conservation strategies must be implemented to preserve existing genetic resources, which requires that the present variation first be characterized.

The accumulation of data may help future studies develop an effective conservation and management strategies. Studies based on more markers and markers of different types (like microsatellites, mtDNA haplotypes, etc) are urgently needed so that the necessary precautions can be taken before more livestock breeds are lost.

The decline in the horse population in Turkey may also bring the extinction of genetic resources. The necessary conservation strategies must be implemented to preserve the existing genetic resources.

REFERENCES

1. Batu S: Türk Atları ve At Yetiştirme Bilgisi. s. 79-95, Ankara Yüksek Ziraat Enstitüsü, Ders Kitabı Sayı: 3, Ankara, 1938.

2. Arpacık R: At Yetiştiriciliği. s. 29-31, Şahin Matbaası, Ankara, 1994.

3. Ibeagha-Awemu EM, Jäger S, Erhardt G: Polymorphisms in blood proteins of *Bos indicus* and Bos taurus cattle breeds of Cameroon and Nigeria, and description of new albumin variants. *Biochem Genet,* 42 (5-6): 181-97, 2004.

4. Anonim: http://www.tuik.gov.tr/VeriBilgi.do. *Erişim tarihi:* 01. 04. 2009

5. Sandberg K, Cothran EG: Blood groups and biochemical polymorphisms. **In,** Bowling AT, Ruvinsky A (Eds): The Genetics of the Horse. pp. 91-97, CABI Publishing, Oxon, 2000.

6. Kelly L, Postiglioni A, de Andrés DF, Vega-Plá JL, Gagliardi R, Biagetti R, Franco J: Genetic characterisation of the Uruguayan Creole horse and analysis of relationships among horse breeds. *Res Vet Sci,* 72 (1): 69-73, 2002.

7. Özbeyaz C, Ertugrul O, Alpan O, Doğrul F, Sönmez C: Tek tırnaklılarda bazı polimorfik sistemlerin kalıtımı. *Ankara Üniv Vet Fak Derg,* 40 (1): 132-141, 1993.

8. Kopar A: Türkiye'de yetiştirilen İngiliz atlarında kan grubu ve kan protein gen frekanslarının tahmin edilmesi. Ankara Üniversitesi Sağlık Bilimleri Enstitüsü, Biyometri Anabilim Dalı, 2000.

9. Sandberg K: Guidelines for the interpretation of blood typing tests in horses. Dept. of Anim. Breed. and Genet. Swedish Univ. of Agri. Sci. Uppsala, Sweden, 1994.

10. Weitkamp LR, MacCluer JW, Guttormsen SA, King RH: Standardbred stallion gene transmission for twelve protein systems: Evidence for selection in trotters. *Anim Genet,* 19, 317-330, 1988.

11. Cristofalo C, Cozzi MC, Valiati P: Blood marker gene frequencies for five Italian horse breeds. Red cell group serology. 23rd Conference of ISAG, 1989.

12. Bowling AT: Population genetics of Great Basin feral horses. *Anim Genet,* 25 (Suppl 1): 67-74, 1994.

13. Colthran EG, Henney PJ, King JA: Inheritance of equine Tf F3 allele. *Anim Genet,* 22, 187-190, 1991.

14. Schmid DO, Ek N, Braend M: Further evidence for a silent allele in the transferrin locus of the horse. *Anim Genet,* 21, 423-426, 1990.

15. Lubas G, Gugliucci B, Mengozzi G, De Berardinis T: Genetic markers in the blood of four Italian horse breeds. *Anim Blood Grps Biochem Genet,* 15, 133-135, 1984.

16. McGuire TR, Weitkamp LR: Equine marker genes. Polymorphism for transferrin alleles, TfF1 TfF2, in Thoroughbreds. *Anim Blood Grps Biochem Genet,* 11, 113-117, 1980.

17. Uzun M, Sulu N: Türkiye'deki safkan at ırklarında bazı enzim sistemlerinin elektroforetik incelenmesi. *Kafkas Üniv Vet Fak Derg,* 6 (1-2): 89-96, 2000.

18. Rodríguez-Gallardo PP, Aguilar Sánchez P, Vega Plá JL, de Andrés Cara DF: Blood group and protein polymorphism gene frequencies for the Andalusian horse breed. A comparison with four American horse breeds. *Arch Zootec,* 41, 433-442, 1992.

19. Bowling AT, Clark RS: Blood group and protein polymorphism gene frequencies for seven breeds of horses in the United States. *Anim Blood Grps Biochem Genet,* 16, 93-108, 1985.

20. Andersson L, Juneja RK, Sandberg K: Genetic linkage between the loci for phosphohexose isomerase (PHI) and a serum protein (Xk) in horses. *Anim Blood Grps Biochem Genet,* 14, 45-50, 1983.

21. Uzun M, Çelebi F, Çarhan A, Kopar A: Albumin and vitamin D binding protein systems polymorphisms of Arabian Horses in Turkey. *Kafkas Üniv Vet Fak Derg,* 6 (1-2): 29-33, 2000.

22. Belkhir K, Borsa P, Chikhi L, Goudet J, Bonhomme F: (1996-2000) GENETIX 4.00 WindowsTM software for sample genetics. Laboratoire Génome, Populations, Intéractions. University of Montpellier, France. Université Montpellier II. http://www.univ-montp2.fr/~genetix/genetix/genetix.htm. *Accessed:* 25.03.2006.

23. Langella O: Populations software, v. 1.2.28. CNRS UPR9034, France, copyright (C) 1999. http://www.pge.cnrs-gif.fr/bioninfo/samples/index. *Accessed:* 12.05.2002.

24. Nei M: Genetic distance between populations. *Am Natur,* 106, 283-292, 1972.

25. Jamieson A: The genetics of transferrins in cattle. *Heredity,* 20, 419-441, 1965.

26. Kaminski M: Genetic structure of populations of horses based on distribution of hemotypes. *Biochem Sys Ecol*, 10, 377-385, 1982.

27. Hill EW, Bradley DG, Al-Baroy A, Ertugrul O, Splan RK, Zakharov I, Cunningham EP: History and integrity of thoroughbred dam lines revealed in equine mtDNA variation. *Anim Genet,* 33, 287-294, 2002.

28. Wahlund S: Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungslehre aus Betrachtet. *Hereditas,* 11, 65-106, 1928.