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## Genetic Polymorphism of Blood Proteins in Iranian Kurd and Turkoman Horse Populations

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**Abstract:** Blood protein polymorphism of two Iranian indigenous horse populations, Kurd (KD) and Turkoman (TM) were electrophoretically examined. Gene frequencies were analyzed at Albumin (*Alb*),  $\alpha$ -1  $\beta$  glycoprotein (*A1B*), Pre-albumin (*Pr*), Alkaline phosphatase (*Alp*), Catalase (*Cat*), Esterase (*Es*), Cell-esterase (*CEs*), Hemoglobin (*Hb*), Transferrin (*Tf*) and Pre-transferrin (*Ptf*) loci. Among these 10 loci, 4 blood protein loci (*Alp*, *CEs*, *Hb* and *Ptf*) were monomorphic. In KD population, the frequency of *Alb*<sup>A</sup> (0.6057) was higher than *Alb*<sup>B</sup> (0.3943), while the frequencies of these two alleles in TM were almost the same. In TM population; the *Pr*<sup>3</sup> and *Pr*<sup>1</sup> alleles showed the highest (0.5469) and the lowest (0.0156) frequencies, respectively. In *Es* system frequency difference of I and F alleles was relatively high in both populations. *Tf*<sup>B2</sup> locus was highly polymorphic. Five alleles were observed for this locus., *Tf*<sup>B2</sup> showed higher frequency in both populations and *Tf*<sup>C</sup> and *Tf*<sup>E</sup> were in the lowest frequencies.

**Key words:** Protein polymorphism, electrophoresis, Turkoman, Kurd, Iranian horses

### INTRODUCTION

Electrophoresis applied to blood proteins, can reveal genetic differences between animals<sup>[1]</sup>

Serum samples and RBC lysates are complex mixtures of proteins sorted out by electrophoresis and then visualized by protein-specific dyes or histochemical stains<sup>[1]</sup>

Blood plasma and erythrocyte proteins polymorphism among native horses has been amply demonstrated by several investigators for various purposes<sup>[2-11]</sup>. Therefore it has become possible, with genes controlling blood proteins as markers, to elucidate difference within and between populations. Sandberg and Cothran<sup>[12]</sup> documented 26 biochemical polymorphism in horse.

In Iran several breeds or local populations are existing and some of them are unique, e.g. Turkoman and Kurd that have not been studied genetically. In addition to, such kind of pure native horse are dramatically decreasing in number. It is very important for practical use in future to survey the genetical characteristics of the native horses and to evaluate it from a viewpoint of genetic resources.

In the previous study it has reported that some blood proteins of Caspian miniature and Arab were highly polymorphic<sup>[2]</sup>. This study was conducted to clarify the gene constitutions of two above mentioned famous native populations namely, Kurd and Turkoman populations.

### MATERIALS AND METHODS

**Horse and blood collection:** The blood samples were collected from two populations of horse namely, Kurd (KD) and Turkoman (TM) from the following areas: Kermanshah, 51 horses and Horse Racing Court, 3 horses, for KD., Turkoman Sahra, 50, Raz and Jargalan, 36 and Horse Racing Court, 10 horses for TM. The blood samples taken in the tube containing anticoagulant were fractionated immediately into plasma and erythrocytes by centrifugation at 1200 rpm for 5 min.. The erythrocyte fraction was washed two or three times with saline solution and both fractions were stored separately in the freezer at -20°C until examinations.

Biochemical genetic variation in blood proteins was examined for 10 genetic loci controlling 9 kinds of plasma and erythrocytes (Table 1). Following the protocols described by Nozawa *et al.*<sup>[10]</sup> and Patterson *et al.*<sup>[13]</sup>.

The allele frequencies were calculated by the simple gene counting method.

### RESULTS

**Protein polymorphism:** The electrophoretic patters of 10 loci examined in the present experiment (Table 1) are shown in Fig. 1-8. No polymorphism was observed for loci *Alp*, *CEs*, *Hb* and *Ptf* (Fig. 1-4). The other 6 loci were polymorphic, both within and between populations (Fig. 5-8).

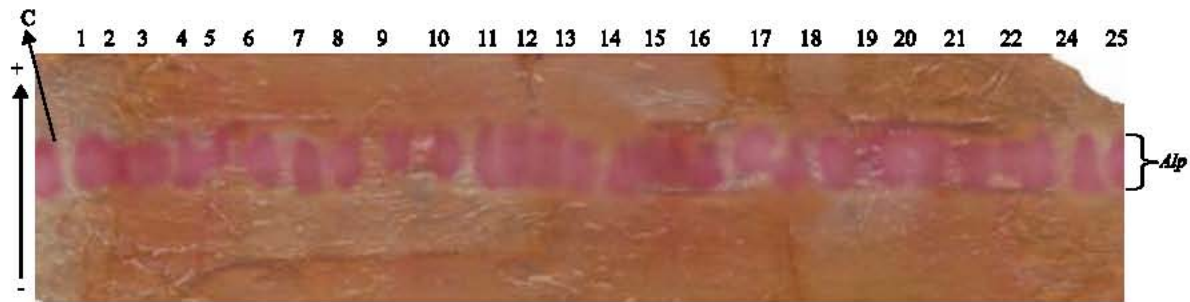


Fig. 1: Electrophoretic patterns of *Alp* (only *Alp*<sup>A</sup> allele) in two populations (C: Control., KD, 1-12., TM, 13-25)

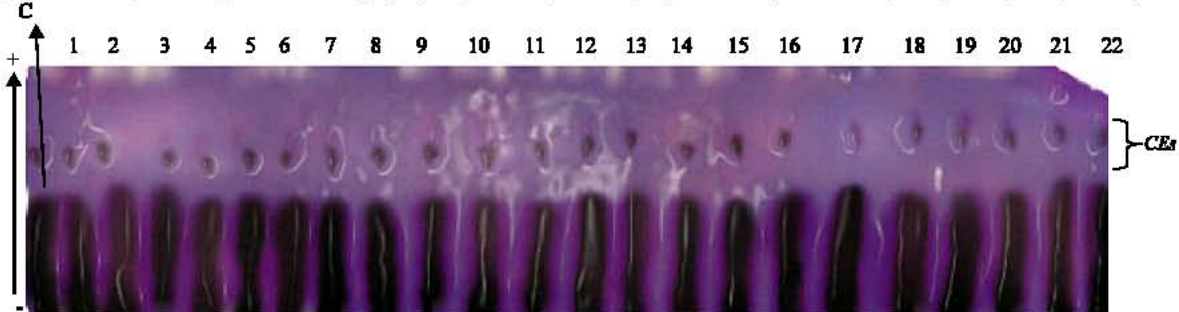


Fig. 2: Electrophoretic patterns of *CEs* (only *CEs*<sup>1</sup> allele) in two populations (C: Control., KD, 11, TM, 12-22)

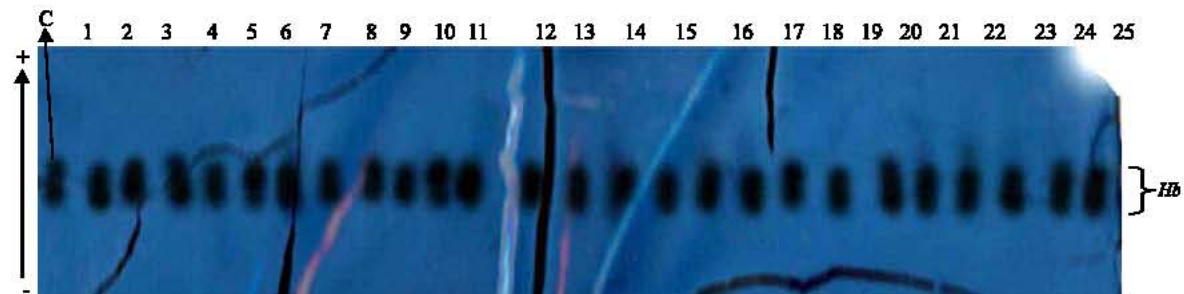


Fig. 3: Electrophoretic patterns of *Hb* (only *Hb*<sup>0</sup> allele) in two populations (C: Control., KD, 1-12., TM, 13-25)

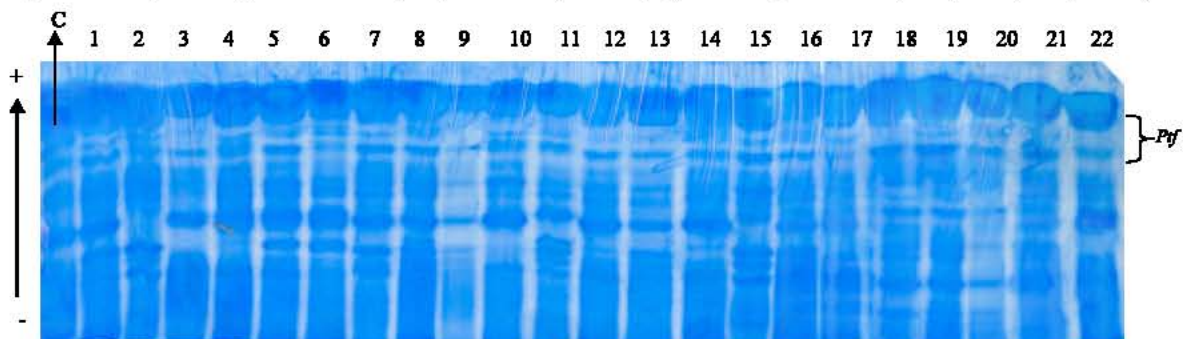


Fig. 4: Electrophoretic patterns of *Ptf* (only *Ptf*<sup>F</sup> allele) in two populations (C: Control., KD, 1-11., TM, 12-22)

At *Alp*, *CEs*, *Hb* and *Ptf* loci, both populations were fixed in A, 1, O and F alleles, respectively (Table 2). At *Alb* locus, the two frequent alleles, *Alb*<sup>A</sup> and *Alb*<sup>B</sup> were observed in both populations. KD breed showed higher allele frequency of A allele than B, while there was no difference in TM horses. In *AlB* system only *AlB*<sup>K</sup> and

*AlB*<sup>S</sup> were observed. *AlB*<sup>S</sup> had lowest frequency in both populations.

Two codominant alleles *Cat*<sup>F</sup> and *Cat*<sup>S</sup> therefore three FF, SS and FS phenotypes were found in KD and TM horses. Frequency of *Cat*<sup>F</sup> was higher than *Cat*<sup>S</sup>.

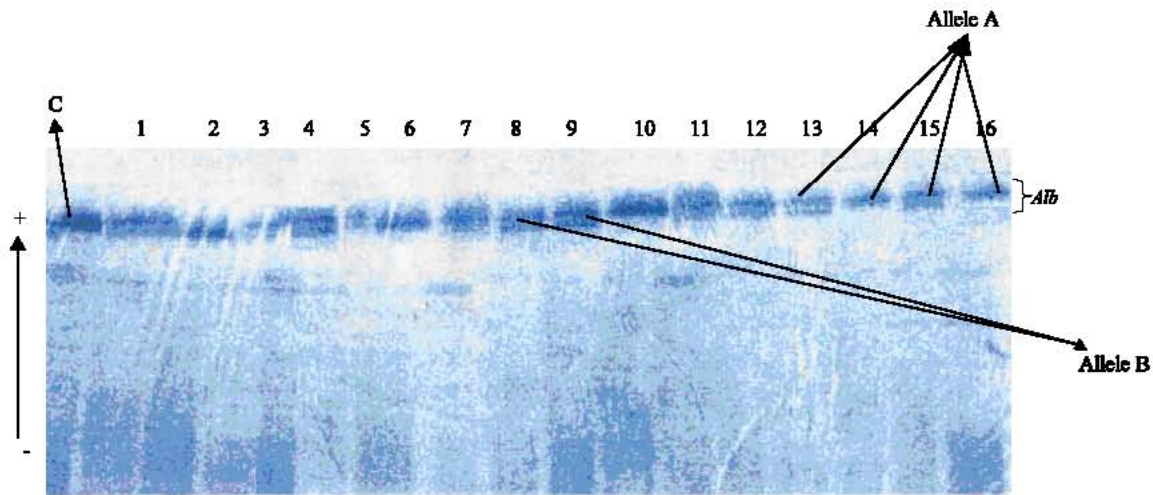


Fig. 5: Electrophoretic patterns of *Alb* in two populations (C: Control., KD, 1-8., TM, 9-16)

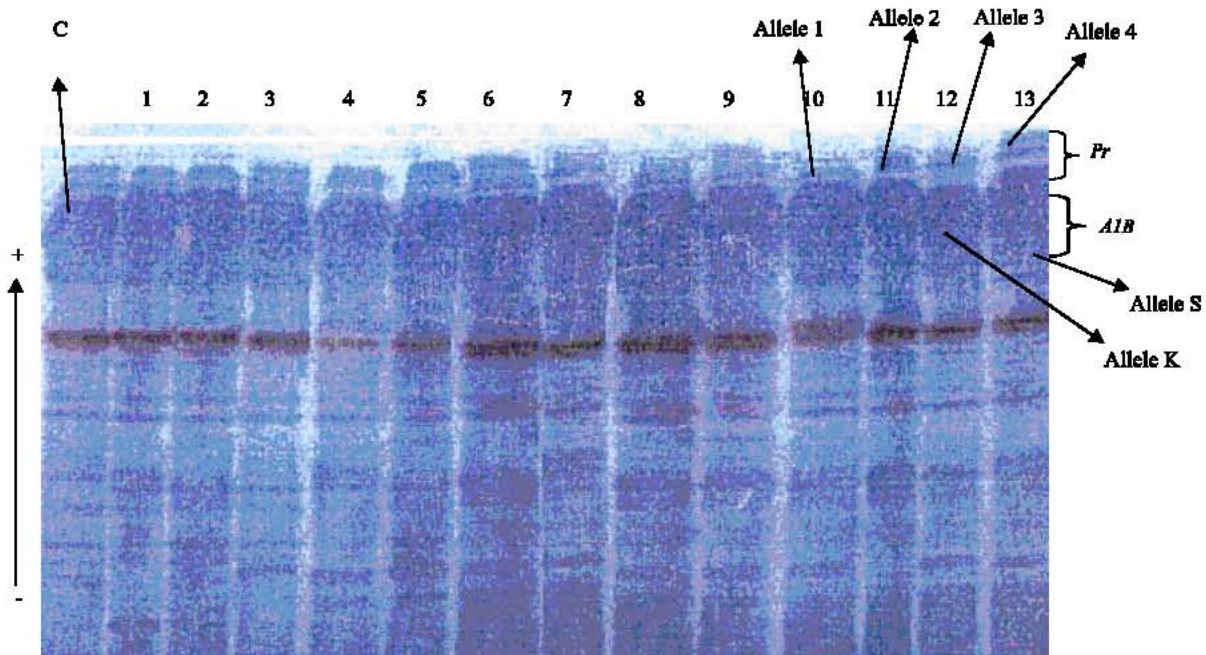


Fig. 6: Electrophoretic patterns of *Pr* and *AIB* in two populations (C: Control., KD, 1-7., TM, 8-13)

Table 1: Blood protein loci electrophoretically examined

| Protein                          | Locus      | References No. |
|----------------------------------|------------|----------------|
| Albumin                          | <i>Alb</i> | [10]           |
| $\alpha$ -1 $\beta$ glycoprotein | <i>AIB</i> | [13]           |
| Pre-albumin                      | <i>Pr</i>  | [10]           |
| Alkaline phosphatase             | <i>Alp</i> | [10]           |
| Catalase                         | <i>Cat</i> | [10]           |
| Esterase                         | <i>Es</i>  | [10]           |
| Cell-esterase                    | <i>Ces</i> | [10]           |
| Hemoglobin                       | <i>Hb</i>  | [10]           |
| Transferrin                      | <i>Tf</i>  | [10]           |
| Pre-transferrin                  | <i>Ptf</i> | [10]           |

$Es^I$  showed higher frequency in both populations however,  $Es^S$  was found at lowest frequency in TM population.

Plasma transferrin and pre-albumin in comparison with the other examined loci were highly polymorphic in both populations. The  $Tf^E$  and  $Pr^1$  showed the lowest frequencies in TM breed. On the contrary  $Tf^{B2}$  and  $Pr^3$  alleles showed the highest frequencies in both populations.



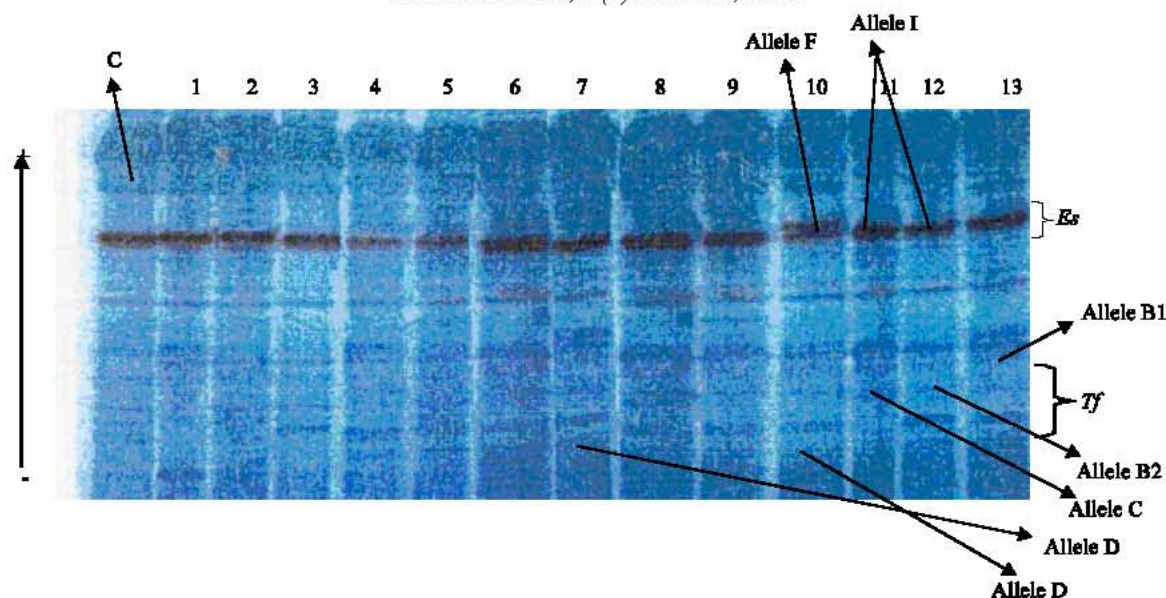


Fig. 7: Electrophoretic patterns of *Es* and *Tf* in two populations (C: Control., KD, 1-6., TM, 7-13)

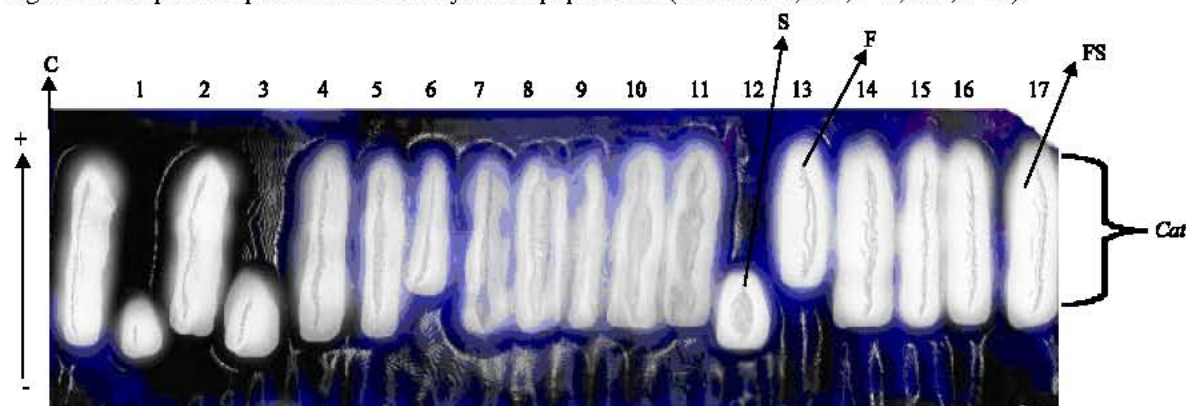


Fig. 8: Electrophoretic patterns of *Cat* in two populations (C: Control., KD, 1-9., TM, 10-17)

Table 2: Gene frequencies at the electrophoretic polymorphic loci of the Iranian KD and TM horse populations

| Allele                 | Breed  |        |
|------------------------|--------|--------|
|                        | KD     | TM     |
| <i>Alb<sup>A</sup></i> | 0.6057 | 0.4921 |
| <i>Alb<sup>B</sup></i> | 0.3943 | 0.5079 |
| <i>Alb<sup>K</sup></i> | 0.5714 | 0.5506 |
| <i>Alb<sup>S</sup></i> | 0.4286 | 0.4494 |
| <i>Pp<sup>1</sup></i>  | -      | 0.0156 |
| <i>Pp<sup>2</sup></i>  | 0.0863 | 0.3125 |
| <i>Pp<sup>3</sup></i>  | 0.8103 | 0.5469 |
| <i>Pp<sup>4</sup></i>  | 0.1034 | 0.1250 |
| <i>Cat<sup>F</sup></i> | 0.5280 | 0.5680 |
| <i>Cat<sup>S</sup></i> | 0.4720 | 0.4320 |
| <i>Es<sup>F</sup></i>  | 0.1981 | 0.2000 |
| <i>Es<sup>I</sup></i>  | 0.6604 | 0.7684 |
| <i>Es<sup>S</sup></i>  | 0.1415 | 0.0316 |
| <i>Tf<sup>B1</sup></i> | 0.1932 | 0.2500 |
| <i>Tf<sup>B2</sup></i> | 0.6478 | 0.4949 |
| <i>Tf<sup>C</sup></i>  | 0.0227 | -      |
| <i>Tf<sup>D</sup></i>  | 0.0568 | 0.1581 |
| <i>Tf<sup>E</sup></i>  | 0.0795 | 0.0970 |

## DISCUSSION

*Alb<sup>A</sup>*, *CEs<sup>1</sup>*, *Hb<sup>O</sup>* and *Ptf<sup>F</sup>* alleles were fixed in many Asian native breeds<sup>[10]</sup>. The similar results were found for two examined populations, indicating the presence of similarity with Asian native populations from the viewpoint of these alleles.

The serum *Alb* locus was one of the first polymorphisms described in the horse. Initially, two codominant alleles, *Alb<sup>A</sup>* and *Alb<sup>B</sup>*, were observed. A third allele, very rare in the few breeds has been found<sup>[4]</sup>. The population of KD showed higher allele frequency of *Alb<sup>A</sup>* allele than *Alb<sup>B</sup>* one, while it was vice versa in TM population. The same results was reported for Asian and European horse populations<sup>[10]</sup>.

*Alb* functions as a metalloproteinase inhibitor<sup>[11]</sup>. Three *Alb<sup>F</sup>*, *Alb<sup>K</sup>* and *Alb<sup>S</sup>* alleles were found in wide variety of horse breeds. Additionally, up to four

other rare alleles have been described<sup>[5, 11, 13]</sup>. In this study only two  $A1B^K$  and  $A1B^L$  alleles were appeared in both populations that their frequencies were similar to previous reports.

Initial description of variation at the horse red cell catalase locus was given by Kelly *et al.*<sup>[8]</sup> Two codominant alleles were found in Quarter Horses, Thoroughbred and Shetlands. Bowling *et al.*<sup>[14]</sup> examined Cat variation in 27 domestic horse breeds and seven feral horse populations. Again, two alleles were observed, with the  $Cat^S$  allele the most common in all populations. Nozawa *et al.*<sup>[10]</sup> examined Cat variation in 8 populations of Japanese horses, 18 populations of Asian native horses and 8 breeds of European horses. They found two  $Cat^F$  and  $Cat^S$  alleles too, however  $Cat^S$  allele was not the most common in all populations<sup>[10]</sup>. All Indonesian native populations and some Japanese native horses had higher  $Cat^F$  allele than  $Cat^S$  allele<sup>[10]</sup>. In the KD and TM populations the frequency of  $Cat^F$  was higher than  $Cat^S$ , which is in accordance with results reported by Nozawa *et al.*<sup>[10]</sup>

$Pr^1$ ,  $Es^S$  and  $Ty^E$  are rare alleles in horse breeds<sup>[5, 10, 11, 17]</sup>. The results in this article provide further support for this view. Plasma transferrin and pre-albumin were highly polymorphic loci (with many alleles) in most examined horse<sup>[5, 9, 11]</sup>. The results presented here confirmed these findings and the suggestion is that these systems could be chosen for parentage testing too.

From the presented data it can be concluded that most of the studied loci were polymorphic in both populations. Therefore, if the other polymorphic loci are added the genetic variation comparison between populations will be possible.

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#### REFERENCES

1. Bowling, A.T., 2000. Parentage testing of horses. *Horse Gene.*, pp: 82-96.
2. Afraz, F., S. Esmailkhanian, F. Aminy and E. Rastgary, 2002. Investigation on genetic blood markers in different horse populations of Iran: Polymorphism of albumin, prealbumin, postalbumin, esterase and transferrin proteins of Arab and Caspian miniature, horses. *Pajouhesh and Sazandegi (in Farsi)*, 54: 40-44.
3. Ban, B., A. Gurman and C. Jozsa, 2002. Thoroughbred and Tratter blood group and DNA allele frequency in Hungary. 28th ISAG Conference Germany, pp: 83.
4. Cothran, E.G., 2002. Genetic diversity in feral horse and burro populations. 28th ISAG Conference Germany, pp: 92.
5. Cothran, E.G. and E. van Dyk, 1998. Genetic analysis of three South African horse breeds. *J. S. Afr. Vet. Ass.*, 69: 120-125.
6. Cholewinski, G., B. Gralak, E.G. Cothran and E. Iwanczyk, 2002. Genetic diversity of two rare horse breeds from Poland. 28th ISAG Conference Germany, pp: 90.
7. Ertugrul, O., B. Akyuz and A. Kopar, 2002. Blood protein polymorphism in some native horse types in Turkey. 28th ISAG Conference Germany, pp: 94.
8. Kelly, E.P., C. Stormont and Y. Suzuki, 1971. Catalase polymorphism in the red cells of horses. *Anim. Blood Groups Biochem. Genet.*, 2: 135-143.
9. Niinni, T., A. Stratil, D. Cizowa-Scroffelow and K. Sandberg, 197. A partially deficient and a typical equine transferrin variant, TF N. *Anim. Genet.*, 28: 233-234.
10. Nozawa, K., T. Shotake, S. Ito and Y. Kawamodo, 1998. Phylogenetic relationship among Japanese native and alien horses estimated by protein polymorphisms. *J. Equine Sci.*, 9: 53-69.
11. Ouragh, L., J.E. Meriau and J.P. Braun, 1994. Genetic blood markers in Arabian, Barb and Arab-Barb horse in Morocco. *Anim. Genet.*, 25: 45-47.
12. Sandberg, K. and E.G. Cothran, 2000. Blood groups and biochemical polymorphisms. In: *The Genetics of the Horse*, Bowling, A.T. and A. Ruvinsky (Eds.) pp: 85-108.
13. Patterson, S.D., K. Bell and D.C. Shaw, 1991. Donkey and horse  $A1B$ -glycoprotein: Partial characterization and new alleles. *Comp. Biochem. Physiol.*, 98: 523-528.
14. Bowling, A.T. and R.S. Clark, 1988. Excess of heterozygotes at albumin locus in American Standard bred horse. *Anim. Genet.*, 19: 331-341.
15. Catanese, J.J. and L.F. Kress, 1992. Isolation from opossum serum of a Metalloproteinase inhibitor homologous to human  $A1B$ -glycoprotein. *Biochemistry*, 31: 410-418.
16. Bowling, A.T., L. Gordon, M.C.T. Penedo, E. Wictum and J. Beebout, 1990. A single gel for determining genetic variants of equine erythrocyte Carbonic Anhydrase (CA) and Catalase (Cat). *Anim. Genet.*, 21: 191-197.
17. Blokhuis, H.J. and C. Buis, 1979. Genetic relationship between breeds of horses and ponies in the Netherlands. *Anim. Blood Groups Biochem. Genet.*, 10: 21-38.