J. Vet. Sci. (2002), 3(4), *255-263*

JOURNAL OF
Veterinary Science

Genetic Polymorphism of the Serum Proteins of Horses in Jeju

Jin-Ah Shin[1], Young-Hoon Yang[2], Hee-Seok Kim[1], Young-Min Yun[1] and Kyoung-Kap Lee[1]*

[1]*Department of Veterinary Medicine, Agriculture & Life Sciences, Cheju National University, Jeju, Korea*
[2]*Department of Animal Biotechnology, Agriculture & Life Sciences, Cheju National University, Jeju, Korea*

Received June 27, 2002 / Accepted November 8, 2002

Abstract

The study was carried out to investigate the genetic polymorphism of the serum proteins of horses in Cheju. They were assigned to three groups; 45 Cheju native horses(CNH), 60 Cheju racing horses(CRH) and 60 Thoroughbreds(TB). We analyzed the phenotypes and gene frequencies of serum proteins which were albumin (Alb), vitamin-D binding protein(GC), esterase (ES), A1B glycoprotein(A1B) and transferrin(TF) loci using horizontal polyacrylamide gel electrophoresis (HPAGE).

All of the loci, except A1B in TB, showed polymorphisms and different allelic and phenotypic frequencies in all three groups. ESS and TFF1 were not observed in CNH. Allelic frequencies of AlbB, ESI, TFD and TFF1 were high in TB. All of the loci, except ES locus in CRH, appeared to be in a state of Hardy-Weinberg equilibrium from *goodness-of-fit* test in all three groups

Heterozygosity estimates at Alb, ES and TF loci were high, but GC and A1B loci were low in all three groups. Average heterozygosities in CNH, CRH and TB were 0.3535, 0.3555 and 0.2726, respectively.

Results showed differences in the frequencies of alleles and phenotypes of several serum protein loci between CNH and CRH, suggested that CRH might be crossed with other breeds of horses in some degree.

Key words : serum protein, polymorphism, phenotype, frequency, heterozygosity, HPAGE, Cheju native horse

Introduction

The Cheju native horses(CNH) are representative of the native horses in Korea, and have a particular hereditariness in process of adaptation to the climate of Cheju. In recent years, it has been assumed that some of CNH have been hybridized with foreign breeds for racing and riding in farms[7]. The CNH had been identified by color, size, shape and hair characteristics[10, 12, 13, 21], but these are relatively difficult to measure[5]. Blood groups and protein polymorphisms can be revealed by laboratory methods which allow precise definition and discriminations of variants[4, 5, 7, 9, 16]. Blood grouping is recognized either by clumping of erythrocytes(agglutination) or by lysis of erythrocytes(hemolysis) in the presence of complement. And several kinds of blood protein are clearly recognized by electrophoresis. Electrophoresis is a technique that uses an electrical current to separate a mixture of molecules embedded in a supporting medium (starch, agarose or acrylamide gel). When applied to blood protein, electrophoresis can reveal genetic differences between animals[4]. The items of blood proteins assay by electrophoresis are usually divided into albumin(Alb), tranferrin(TF), postalbumin(A1B), hemoglobin(Hb), 6-phosphogluconate dehydrogenase(6-PGD) and esterase(ES) loci[3, 5, 6, 7, 8, 11, 23].

The CNH were designated as national monuments, and have been raised specially. Some of them were distributed to farms and have been used as racing horses at the Cheju Racing Track, a branch of Korea Racing Association. Presently, Cheju Institute is very concerned about hybrid of the CNH with foreign breeds artificially for getting excellent records when they are in a race. Therefore the preservation of pure pedigree is very important. There are some reports of morphology[10, 12, 13, 21], genetic phenotypes and frequencies of serum proteins of horses in Cheju[7, 9, 14, 16, 17, 20, 22], but there are few reports of genetic comparison of serum proteins among CNH, CRH and TB.

This study was carried out to find genetic diversity in CNH, CRH and TB by investigating the phenotypes and gene frequencies of Alb, GC, ES, A1B, and TF loci which are authorized internationally among serum proteins, to clarify the distribution and characteristics of serum proteins of CNH and to get a basic data for pedigree establishment and maintenance of purity of the CNH.

Materials and Methods

1) Experimental animals

Three different groups of horses in Cheju used in this study and experimental individuals were gathered at random in each group; 45 Cheju native horses (CNH) which were

*Corresponding author: Kyoung-Kap Lee
Department of Veterinary Medicine, Agriculture & Life Sciences, Cheju National University, Jeju, Korea
Tel : +82-64-754-3368, Fax : +82-64-756-3354
e-mail : leekk@cheju.ac.kr

Table 1. The composition of polyacrylamide gels

Components	A solution	Distilled water	B solution	C solution
12%	44.8 ㎖	15.2 ㎖	30 ㎖	30 ㎖
4%	2 ㎖	8.2 ㎖	2 ㎖ + T10 ㎕	4 ㎖
8%	6 ㎖	9 ㎖	3 ㎖ + T15 ㎕	6 ㎖

precious national monuments in Jeju Institute for Livestock Promotion, 60 Cheju racing horses (CRH) which were racing horses in Jeju Racing Association and 60 Thoroughbreds in (TB) in Jeju equine stud farm and training center.

2) Sampling

Blood samples were collected from 165 horses (CNH: 45, CRH: 60, TB: 60) from jugular vein. The samples were centrifuged at 2,500 rpm for 10 minutes, and then isolated serum and stored in -72℃.

3) Electrophoresis

The polymorphism of serum proteins was analyzed by horizontal polyacrylamide gel electrophoresis(HPAGE)[24]. The gel solutions and electrode buffer contents were as follows;

(1) Gel solution

A solution : Acrylamide 32 g, N'-methylenebisacrylamide 0.8 g/DW 100 ㎖

B solution : 18% Trisaminomethane 50 ㎖, N,N,N',N' - tetramethylethylenedi-amine (TEMED) 300 ㎖, 2-Mercaptoethanol 150 ㎕/DW 100 ㎖, adjust pH 7.9 with 1 M citric acid

C solution : Ammonium persulfate 100 ㎎/DW 50 ㎖

The compositions of solutions for making suitable gels were shown in Table 1.

(2) Electrode buffer : Trisaminomethane 7.87 g, boric acid 1.48 g. pH 9.0

The staining and destaining solutions were as follows;

(1) ES staining : 0.19 M Trisaminomethane 150 ml, 0.05 M Citric acid 200 ml, 1%-Naphthyl acetate (dissolved in Acetone) 8 ml, Fast blue B salt

(2) Protein staining : Coomassie brilliant blue G 1 g, 60 % perchloric acid 60 ml/DW 1000 ml

(3) Destaining : Methanol 200 ml, acetic acid 70 ml/DW 1000 ml

Polyacrylamide gel was cast between glass plates. A step gradient of acrylamide concentration of 12%, 4% and 8% was used in turn. The gel buffer of pH 7.9 was Tris-citrate and the electrode buffer of pH 9.0 was Tris-borate. Samples were run simultaneously on a cooling plate at 5℃. The current was at first set at 500 V, 30 W for 8 minutes, after removing the sample loading papers, and then set at 1200 V, 50 W for 6 hours. The detection of esterase(ES) was stained in ES staining solution and the other proteins were stained in protein solution.

4) Statistical analysis

Statistical methods[18] used in this study were as follows;

(1) Allelic frequency : $2\{ii\} + \{ij\} / 2N = p, q$
($\{ii\}$, the number of ii homozygotes; $\{ij\}$, the number of heterozygotes having an I allele; N, number of individuals)

(2) Expected number : $Ho : p^2 \times N$, $He : 2pq \times N$, $Ho' : q^2 \times N$

(3) Chi-square test : $\chi^2 = \Sigma(O - E)^2 / E$
(O, the observed number; E, the expected number)

(4) Heterozygosity : $H = 1 - \Sigma q_i^2$
(q, the frequency of the I allele of the gene at this locus)

Chi-square tests carried out to check for significant differences between observed and expected numbers for genetic equilibrium of Hardy-Weinberg law.

Results

The image of horizontal polyacrylamide gel electrophoresis at 12% gel to separate horse blood serum protein was presented in Fig. 1. According to mobilities, the protein bands from fast migration to slow migration were albumin(Alb), vitamin-D binding protein(GC), esterase(ES), A1B glycoprotein (A1B) and tranferrin(TF) loci in order.

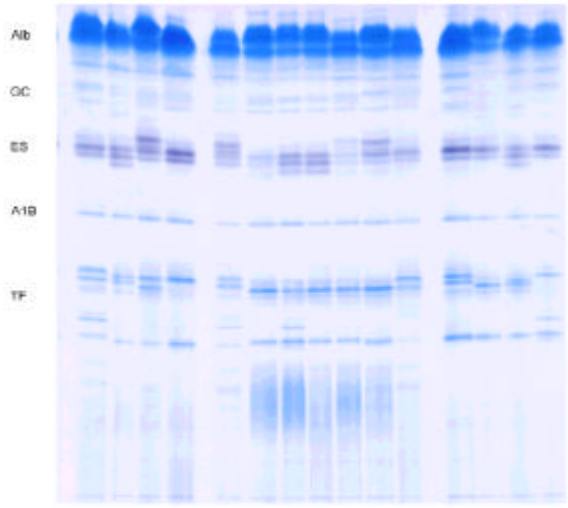

Fig. 1. Serum protein loci separated on the horizontal polyacrylamide gel (HPAGE)
Alb: albumin, GC: vitamin-D binding protein, ES: esterase, A1B: A1B glycoprotein, TF: tranferrin

1) Genetic polymorphism of Albumin(Alb) locus

Albumin is the most fast migrating protein component on gel. This locus was controlled by 2 codominant autosommal allele A and B; phenotypes of albumin were the fast n

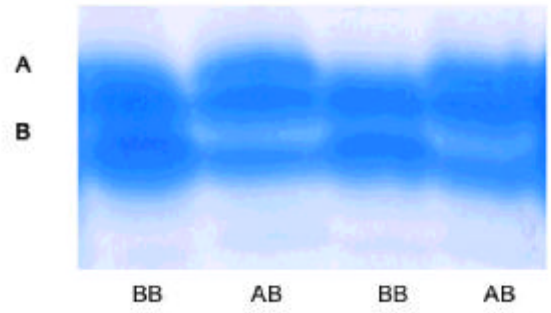

Fig. 2. Phenotypes of Alb locus separated on the HPAGE

The phenotype BB of TB has the highest frequency in all three groups. Over all, the frequency of AlbB was higher than that of AlbA The frequencies of AlbA and AlbB were 0.433 and 0.567 in CNH, 0.450 and 0.550 in CRH, 0.108 and 0.892 in TB, respectively. $\chi 2$ values from Hardy-Weinberg genetic equilibrium test were 0.0742(p>0.05) in CNH, 0.0061(p>0.05) in CRH and 0.1562(p>0.05) in TB.

2) Genetic polymorphism of vitamin-D binding protein

Th ıg
FF, s

Fig. 3. Phenotypes of GC locus separated on the HPAGE

Table 2. Phenotypes and gene frequencies of Alb locus

	Phenotype	No. of heads		Gene frequency	$\chi 2$-test		
		Observed	Expected		$\chi 2$	df	p
CNH	AA	8 (17.8*)	8.450	AlbA= 0.433			
	AB	23 (51.1)	22.10	AlbB= 0.567			
	BB	14 (31.1)	14.450				
	total	45			0.0742	1	0.785
CRH	AA	12 (20)	12.150	AlbA= 0.450			
	AB	30 (50)	29.700	AlbB= 0.550			
	BB	18 (30)	18.150				
	total	60			0.0061	1	0.938
TB	AA	1 (1.7)	0.704	AlbA= 0.108			
	AB	11 (18.3)	11.590	AlbB= 0.892			
	BB	48 (80)	47.70				
	total	60			0.1562	1	0.693

CNH; Cheju native horses, CRH; Cheju racing horses, TB; Thoroughbreds

Table 3. Phenotypes and gene frequencies of GC locus

	Phenotype	No. of heads		Gene frequency	$\chi 2$ -test		
		Observed	Expected		$\chi 2$	df	p
CNH	FF	42 (93.3*)	42.050	GCF = 0.967			
	FS	3 (6.7)	2.900	GCS = 0.033			
	SS	-	0.050				
	total	45			0.0535	1	0.817
CRH	FF	59 (98.3)	59.004	GCF = 0.992			
	FS	1 (1.7)	0.992	GCS = 0.008			
	SS	-	0.004				
	total	60			0.0042	1	0.948
TB	FF	54 (90)	54.150	GCF = 0.950			
	FS	6 (10)	5.700	GCS = 0.050			
	SS	-	0.150				
	total	60			0.1662	1	0.684

The phenotype SS was not observed in all three groups. The frequencies of GCF and GCS were 0.967 and 0.033 in CNH, 0.992 and 0.008 in CRH and 0.950 and 0.050 in TB, respectively. $\chi 2$ values from Hardy-Weinberg equilibrium test were 0.0535 (p>0.05) in CNH, 0.0042 (p>0.05) in CRH and 0.1662 (p>0.05) in TB.

3) Genetic polymorphism of esterase (ES) locus

Three ES variants, F, I and S, showed to be controlled by

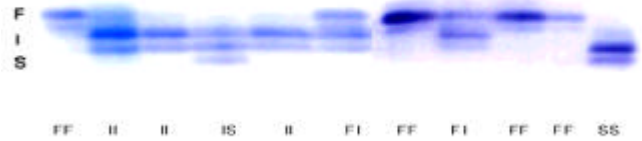

Fig. 4. Phenotypes of ES locus separated on the HPAGE

The frequency of ESI was high in all three groups, and this was the highest in TB. S allele was not observed in CNH. The frequencies of ESF, ESI and ESS, were 0.389, 0.611 and 0 in CNH, 0.308, 0.575 and 0.117 in CRH and 0.108, 0.808 and 0.083 in TB, respectively. $\chi 2$ values from Hardy-Weinberg equilibrium test were 0.5613 (p>0.05) in CNH, 10.3885 (p<0.05) in CRH and 4.5567 (p>0.05) in TB.

4) Genetic polymorphism of A1B glycoprotein(A1B) locus

Generally, three allelic variants F, K and S were detected according to mobilities, but this locus was detected K and S

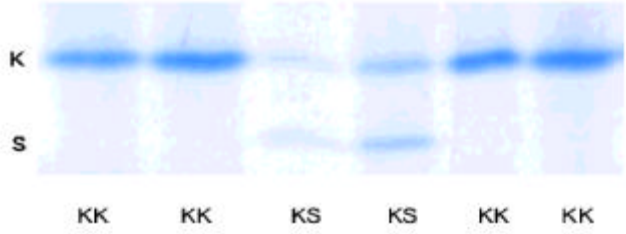

Fig. 5. Phenotypes of A1B locus separated on the HPAGE

In TB only phenotype KK was detected. The frequencies of A1BK and A1BS in CNH, CRH and TB were 0.967 and 0.033, 0.983 and 0.017, 1 and 0, respectively. $\chi 2$ values from Hardy-Weinberg equilibrium test were estimated to be 0.0535 (p>0.05) in CNH, 0.0172(p>0.05) in CRH.

Table 4. Phenotypes and gene frequencies of ES locus

	Phenotype	No. of heads		Gene frequency	$\chi 2$-test		
		Observed	Expected		$\chi 2$	df	p
CNH	FF	8 (17.8*)	6.806				
	II	18 (40)	16.806				
	SS	-	-	ESF= 0.389			
	FI	19 (42.2)	21.389	ESI = 0.611			
	IS	-	-	ESS = 0			
	FS	-	-				
	total	45			0.5613	1	0.454
CRH	FF	11 (18.3)	5.704				
	II	24 (40)	19.838				
	SS	1 (1.7)	0.817	ESF = 0.308			
	FI	12 (20)	21.275	ESI = 0.575			
	IS	9 (15)	8.050	ESS = 0.117			
	FS	3 (5)	4.317				
	total	60			10.3885	3	0.016
TB	FF	2 (3.3)	0.704				
	II	39 (65)	39.204				
	SS	-	0.417	ESF = 0.108			
	FI	9 (15)	10.508	ESI = 0.808			
	IS	10 (16.7)	8.083	ESS = 0.083			
	FS	-	1.083				
	total	60			4.5567	3	0.207

Table 5. Phenotypes and gene frequencies of A1B locus

	Phenotype	No. of heads		Gene frequency	χ2-test		
		Observed	Expected		χ2	df	p
CNH	FF	-	-	A1BF = 0 A1BK = 0.967 A1BS = 0.033			
	KK	42 (93.3*)	42.050				
	SS	-	0.050				
	FK	-	-				
	KS	3 (6.7)	2.900				
	FS	-	-				
	total	45			0.0535	1	0.817
CRH	FF	-	-	A1BF = 0 A1BK = 0.983 A1BS = 0.017			
	KK	58 (96.7)	58.017				
	SS	-	0.017				
	FK	-	-				
	KS	2 (3.3)	1.967				
	SS	-	-				
	total	60			0.0172	1	0.896
TB	FF	-	-	A1BF = 0 A1BK = 1 A1BS = 0			
	KK	60 (100)	60				
	SS	-	-				
	FK	-	-				
	KS	-	-				
	SS	-	-				
	total	60					1

5) Genetic polymorphism of Transferrin(TF) locus

TF locus was detected D, F1, F2, H2, O and R in order of decreasing mobility to the anode (Fig. 6).

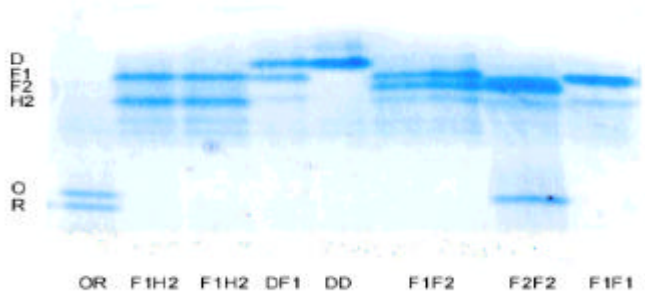

Fig. 6. Phenotypes of TF locus separated on the HPAGE

There were 21 different phenotypes and 6 alleles at TF locus. F1 allele was not observed in CNH, but was observed in CRH. F2 and R alleles were high in CNH, D, F2 and R alleles were high in CRH, D, F1 and F2 alleles were quantitative in TB. χ2 from Hardy-Weinberg equilibrium test were 9.8776(p>0.05) in CNH, 11.5255(p>0.05) in CRH and 12.1406(p>0.05) in TB(Table 6).

6) Average heterozygosity

The heterozygosity reflects the variety of sources from which this breed is being created. Calculated heterozygosity were estimated to be 0.4911, 0.4950 and 0.1932 at Alb locus, 0.0644, 0.0165 and 0.0950 at GC locus, 0.4753, 0.5607 and 0.3279 at ES locus, 0.0646, 0.0328 and 0 at A1B locus 0.6723, 0.6725 and 0.7467 at TF locus in CNH, CRH and TB, respectively. The TF locus showed the highest value at 5 protein loci. Heterozygosity values of TB were low at all loci, especially A1B locus, but value of TF locus was high. Average heterozygosity values ranged from 0.2726(TB) to 0.3555(CRH). TB had the lowest value compared with the other groups. Heterozygosity values of Alb, ES and TF loci were high, but GC and A1B loci were low(Table 7).

Table 6. Phenotypes and gene frequencies of tranferrin(TF) locus.

	Phenotype	No. of heads		Gene frequency	χ^2-test		
		Observed	Expected		χ^2	df	p
CNH	DD	1 (2.2*)	0.356				
	DF1	-	-				
	DF2	5 (11.1)	3.822				
	DH2		0.089				
	DO	1 (2.2)	1.956				
	DR		1.422				
	F1F1	-	-				
	F1F2	-	-				
	F1H2	-	-	TFD = 0.089			
	F1O	-	-	TFF1 = 0			
	F1R	-	-	TFF2 = 0.478			
	F2F2	10 (22.2)	10.272	TFH2 = 0.011			
	F2H2		0.478	TFO = 0.244			
	F2O	10 (22.2)	10.511	TFR = 0.178			
	F2R	8 (17.8)	7.644				
	H2H2	-	0.006				
	H2O	1 (2.2)	0.244				
	H2R		0.178				
	OO	4 (8.9)	2.689				
	OR	2 (4.4)	3.911				
	RR	3 (6.7)	1.422				
	total	45			9.8776	10	0.451
CRH	DD	-	0.817				
	DF1	-	0.583				
	DF2	9 (15)	7.117				
	DH2	2 (3.3)	0.817				
	DO	-	0.817				
	DR	3 (5)	3.033				
	F1F1	-	0.104				
	F1F2	2 (3.3)	2.542				
	F1H2	-	0.292	TFD = 0.117			
	F1O	-	0.292	TFF1 = 0.042			
	F1R	3 (5)	1.083	TFF2 = 0.508			
	F2F2	15 (25)	15.504	TFH2 = 0.058			
	F2H2	3 (5)	3.558	TFO = 0.058			
	F2O	6 (10)	3.558	TFR = 0.217			
	F2R	11 (18.3)	13.217				
	H2H2	-	0.204				
	H2O	-	0.408				
	H2R	2 (3.3)	1.517				
	OO	-	0.204				
	OR	1 (1.7)	1.517				
	RR	3 (5)	2.817				
	total	60			11.5255	15	0.715

	Phenotype	No. of heads		Gene frequency	χ2-test		
		Observed	Expected		χ2	df	p
TB	DD	8 (13.3)	6.338				
	DF1	9 (15)	12.350				
	DF2	8 (13.3)	7.475				
	DH2	2 (3.3)	0.975				
	DO	2 (3.3)	2.925				
	DR	2 (3.3)	2.601				
	F1F1	7 (11.7)	6.017				
	F1F2	7 (11.7)	7.283				
	F1H2	-	0.950	TFD = 0.325			
	F1O	4 (6.7)	2.850	TFF1 = 0.317			
	F1R	4 (6.7)	2.535	TFF2 = 0.192			
	F2F2	3 (5)	2.204	TFH2 = 0.025			
	F2H2	1 (1.7)	0.575	TFO = 0.075			
	F2O	1 (1.7)	1.725	TFR = 0.067			
	F2R	-	1.534				
	H2H2	-	0.038				
	H2O	-	0.225				
	H2R	-	0.200				
	OO	1 (1.7)	0.338				
	OR	-	0.600				
	RR	1 (1.7)	0.267				
	total	60			12.1406	15	0.668

Table 7. Heterozygosity of serum proteins in three groups

Locus	CNH	CRH	TB
Alb	0.4911	0.4950	0.1932
GC	0.0644	0.0165	0.0950
ES	0.4753	0.5607	0.3279
A1B	0.0644	0.0328	0
TF	0.6723	0.6725	0.7466
Average	0.3535	0.3555	0.2726

Discussion

Horizontal polyacrylamide gel electrophoresis was resulted in a separation of proteins, according to mobilities; albumin(Alb), vitamin-D binding protein(GC), esterase(ES), A1B glycoprotein(A1B) and tranferrin(TF) loci were given for CNH, CRH and TB. Mogi *et al* reported that Alb locus is controlled by A and B alleles, and there are genetic differences in frequency between Asia and European's horses [15]. It was reported that GC locus is comprised of F and S alleles[3, 5] and ES locus is comprised of F, G, H, I, S, O and R alleles[5]. Andersson and Cho *et al* reported that A1B locus is controlled by F, K and S alleles and the frequencies were different between breeds[1, 7]. Yokohama *et al* and Schmid Braend reported that TF is identified 14 alleles, C, D1, D2, D, F1, F2, F3, G, H1, H2, J, M, O, R and silent, and phenotypes are different between breeds[19, 22]. In this study, restricted alleles were accomplished by HPAGE.

Studies for CNH have been reported of Alb locus[7, 16, 17], GC locus[14, 7], ES locus[7, 16, 17, 22], A1B locus[7, 14, 17], TF locus[7, 22], almost all of their results appeared to be similar to these results. But at GC locus, results (GCF, 0.411; GCS, 0.589) of Kim *et al* showed differences in frequencies[14], it is probably due to a difference of population examined. And at ES locus, results (ESF, 0.274; ESI, 0.479; ESS, 0) of Cho *et al* showed somewhat different frequencies[7]. It is considered that the differences were due to the electrophoresis method. And S allele of ES locus and F1 allele of TF locus in this study were not observed, this could

be also identified by Yokohama *et al* and Cho *et al*[7, 22].

Cho *et al* reported of CRH at Alb, GC, ES, A1B and TF loci[7]. The phenotypes and frequencies in this study were similar to previous study. But at Alb locus, his results (AlbA, 0.280; AlbB, 0.720) showed differences in frequency. At ES locus, his results (ESF, 0.203; ESI, 0.661; ESS, 0.076) showed slight differences in frequency, it is considered that the differences were due to the electrophoresis method.

Studies for TB have been reported of Alb locus[5, 11, 15], GC locus[5], Es locus[5, 11, 22], A1B locus[5, 11] and TF locus[3, 5, 11, 22], these present results appeared to be similar to previously described results. TB were characterized by a very large preponderance of ESI and TB which had only the phenotype KK showed monomorphism at A1B locus in this study.

Over all, the frequency of AlbB was higher than that of AlbA and especially TB had higher proportions of AlbB than other groups. In this study F allele of GC locus was observed predominantly. Phenotype II was high at ES locus. And phenotype KK was the highest and F allele was not observed at A1B locus. The frequency of TFF1 was about two times higher than that of TFF2 in TB, while F1 allele lacked in CNH and was rare in CRH. In CNH, lacking of F1 allele could be also identified by Yokohama *et al* and Cho *et al*[7, 22]. The frequencies of D and F1 alleles in TB were the highest in all three groups, these results were similar to those of Kaminski *et al* and Yokohama *et al*[11, 22]. The occurrence of ESS and TFF1 in CRH, even though at low frequencies, is one of difference between CRH and CNH, lacking of these variants and the relatively frequencies of ESS and TFF1 in TB were high.

A Chi-square test to determine whether the fit is sufficiently close to expected Hardy-Weinberg proportion revealed that almost of all the polymorphic loci, except ES locus in CRH, showed to be in genetic equilibrium in all three groups. Result of ES in CRH suggested that CRH have been selectively bred as racing horses in farms.

Heterozygosity estimates at Alb, GC, ES, A1B and TF loci were reported previously for CNH and CRH by Cho *et al*[7]. His results appeared to be similar to these results. But these results were different from previous results at GC locus in CNH, and A1B locus in CNH and CRH. TB showed the lowest value all of the loci, except TF locus. It might be from the relationship between individuals within small pedigreed data. Heterozygosity of CNH and CRH showed higher than TB, suggested that these groups are different from TB.

In conclusion, these results of genetic polymorphisms and equilibrium in blood serum proteins loci and the other reports of morphological characteristics[13, 21] indicated that CRH might be a hybrid or mixed population between CNH and TB or other imported breed.

References

1. Andersson, L., Juneja R.K. and Sandberg K. Animal Blood Groups Biochemical Genetics. 14, 45-50, 1983.
2. Bell, K. Blood protein polymorphisms in the donkey [Equus asinus]. Animal Genetics. 1994, 25 suppl 1, 109-113.
3. Bell, K., Pollitt C.C. and Patterson S.D. Subdivision of equine Tf into H1 and H2. Animal Genetics. 1988, 19, 177-183.
4. Bowling, A.T. Horse Genetics. CAB INTERNATIONAL. pp.82-154. 1996.
5. Bowling, A.T. and Clark, R.S. Blood group and protein polymorphism gene frequencies for seven breeds of horses in the United States. Animal Blood Groups and Biochemical Genetics. 16, 93-108, 1985.
6. Bowling, A.T. and Ryder, O.A. Genetic studies of blood markers in Przewalski's horses. The journal of Heredity. 1987, 78, 75-80.
7. Cho, G.J., Kim, B.H., Lee, D.S. and Lee, K.K. Genetic studies of blood markers in Cheju horses II Blood protein types. Korean j Vet Res. 2000, 40(2), 283-290.
8. Cothran, E.G., MacCluer, J.W., Weitkamp, L.R. and Bailey, E. Genetic differentiation associated with gait within American Standardbred horses. Animal Genetic. 1987, 18, 285-296.
9. Han, S.K., Chung, E.Y., Shin, Y.C. and Byun, H.D. Studies on Serum Proteins and Enzyme Polymorphisms for Conservation of Cheju Native Horses. Korean J. Anim. Sci. 1995, 37(1), 52-58.
10. Jung, C.J., Yang, Y.H., Kim, J.K. and Kang, M.S. Studies on the Classification for the Registration of the Cheju Native Horse I. Body measurements by location sex and age. Korean J. Anim. Sci. 1991, 33(6), 418-422.
11. Kaminski, M. and Damian, F. Electrophorestic markers of Andalusian Horses: Comparison of Spanish and lusitanian lineages. Comp. Biochem. Physiol. 1986, 83B(3), 575-588.
12. Kang, M.S., Choung, C.C., Kang, T.S. and Choung, J.J. Coat Color Frequencies in the Cheju Island Pony. Cheju National University. 1988, 3, 129-132
13. Kang, M.S., Choung, C.C., Kang, T.S., Park, H.S., Kim, D.H. and Choung, J.J. Study on the Body Type of Cheju Native Horses. Cheju National University. 1986, 1, 119-125.
14. Kim, S.Y., Kang, M.S. and Jung, C.J. Study of blood protein polymorphism of Cheju native horses. Cheju National University. 1993, 313-317.
15. Mogi, K., Abe, T. and Hosoda, T. Studies on serum protein types in horses. I. Classification and inheritance of transferrin and serum albumin types and efficacy of their typing in determining of doubtful parentage. Jap. J. Zootech. Sci. 1970, 41(8), 400-406.
16. Oh, M.Y., Ko, M.H., Kim, K.O., Kim, S.J., Chung, C.C. and Kim, K.I. Genetic variations of the blood proteins in Cheju Native Horses. Korean J. Genetics. 1992, 14(1), 39-50.
17. Oh, Y.S., Oh, M.Y., Kim, S.J., Kim, K.O., Ko, M.H. and

Yang, Y.H. Polymorphisms of Blood Proteins in Cheju Native Horses and Tsushima Native Horses. Korean J. Zool. 1995, 38, 324-329.
18. Pasteur, N. and Pasteur, G. Practical isozyme genetics: Ellis horwood limited. pp. 165-189, 1988.
19. Schmid, D.O., EK, N. and Braend, M. Further evidence for a silent allele in the transferrin locus of the horse. Animal Genetics. 1990, 21, 423-426.
20. Shin, J.A., Kim, S.H., Kim, Y.H., Chun, C.I. and Lee, K.K. Genetic Polymorphism of Serum Protein in Horses on Cheju. J. Res. Ins. Anim. Sci. 1999, 14, 149-157.
21. Yang, Y.H., Jung, C.J., Lee, H.J. and Kang, T.S. Studies on the Classification for the Registration of the Cheju Native Horse. Korean J. Anim. Sci. 1991, 3(6), 438-443.
22. Yokohama, M., Watanabe, Y. and Kobayashi, E. Classification for Transferrin and Esterase types in Horses. Jpn. J. Zootech. Sci. 1989, 60(2), 115-120.
23. Yokohama, M., Watanabe, Yand. and Mogi, K. A New Equine Transferrin phenotype Detected by Isoelectric Focusing. Jpn J. Zootech, Sci. 1985, 56(2), 116-121.
24. Yokohama, M., Watanabe, Y., Gawahara, H. and Kobayashi, E. Horizontal Polyacrylamide Gel Electrophoresis for Equine Serum Protein Types. ABRI. 1987, 15, 22-27.