Hemoglobin Type Analysis of Ryukyu and Tokara Native Horses

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Hemoglobin phenotypes in horses were first classified by BRAEND¹⁾ into three groups, in which the fast component/slow component ratios were approximately 60/40, 80/20 and 100/0, respectively. KILMARTIN and CLEGG⁵⁾ identified amino acid variation in both tyrosine and phenylalanine at position 24 and glutamine and lysine at position 60 of the equine α globins which were encoded by two closely linked α genes. CLEGG³⁾ suggested three haplotypes; BI, BII and A. Later much rarer haplotypes AII, N and C were also reported²⁻⁴⁾. Recently YOKOHAMA and MOGI⁷⁾ and YOKOHAMA *et al.*⁸⁾ reported hemoglobin types in Japanese native horses and noted three haplotypes, BI, BII and A. This paper describes the quantitative analysis of the hemoglobin types of native horses in the Ryukyu Islands (Miyako horses and Yonaguni horses) and of the Iriki group of Tokara horses.

Materials and Methods

Blood samples were collected from 9 Miyako horses, 8 Yonaguni horses and 19 Tokara horses at the Iriki Livestock Farm of the Kagoshima University. Hemolysates were prepared in the standard manner, and KCN and (*p*-Amidinophenyl) Methanesulfonyl Fluoride HCl were added to the hemoglobin solution. Isoelectric focusing (IEF) was carried out on a 5% polyacrylamide gel containing glycerol (13%) and pH 6-8 Pharmalyte (5%) and pH 3-10 Pharmalyte (1.25%) solutions. 0.02 M NaOH and 0.01 M H₃PO₄ were used as a catholyte and an anolyte, respectively. A typical electrophoresis was performed for 3 hours (initially 200 V, 25 mA, 5 W, and finally 720 V, 7 mA, 5 W). After IEF, a gel was fixed in 10% trichloroacetic acid. The density of the hemoglobin bands was measured using a Biomed soft laser

琉球在来馬およびトカラ在来馬のヘモグロビン型分析: 江口幸典・中嶋安嗣・大城 稔・菅又昌雄・武居 洋・柳田宏一*・橋口 勉** (琉球大学医学部 沖縄県西原町, 鹿児島大学 鹿児島市 890)

Table 1. Mean peak height ratio and distribution of the hemoglobin types identified in the Ryukyu and Tokara native horses following isoelectric focusing

Hemoglobin type	Mean peak height ratio				Frequency distribution		
	Band position				Miyako horses	Yonaguni horses	Tokara horses
	1	2	3	4			
BI/BI		60.2		39.8	3	0	0
BI/BII	30.8	34.2	17.0	18.0	3	3	0
BII/BII	59.0		41.0		2	4	6
A/A		100.0			0	0	9
A/BII	35.9	47.1	17.0		0	1	4
AII/BI	47.4	33.7		18.9	1	0	0
Total					9	8	19

Band position is numbered from the most acidic band

densitometer (model SLR-TRER).

Results and Discussion

Table 1 presents the mean value for the peak height ratio of hemoglobin components in various hemoglobin types and distribution of the hemoglobin types in the three groups of horses. The haplotype frequencies calculated from the present data are BI 0.56, BII 0.39 and AII 0.06 in Miyako horses and BI 0.19, BII 0.75 and A 0.06 in Yonaguni horses. YOKOHAMA *et al.*⁸⁾ reported the frequencies of haplotypes in 13 Yonaguni horses as BI 0.462, BII 0.500 and A 0.038. The numbers of samples examined by both YOKOHAMA *et al.*⁸⁾ and us represent a small portion of the Yonaguni horse population. Hemoglobin type analysis on a substantial number of Yonaguni native horses is therefore needed. A rare hemoglobin type, AII/BI, was found in the Miyako horses. The peak height ratio of hemoglobin components in this type was similar to that of AII/BI type reported by PATTERSON and BELL⁶⁾. However, the AII haplotype was not detected by YOKOHAMA *et al.*⁸⁾ who examined 250 Japanese native horses from seven different breeds. We found that the haplotype frequencies in 19 Tokara horses were A 0.58 and BII 0.42. Similar results were reported by YOKOHAMA *et al.*⁸⁾ on 15 Tokara native horses. All the

Tokara horses we analysed in this study have been raised at the Iriki Livestock Farm of Kagoshima University. Two other clusters of Tokara horses are known, one in Nakanoshima and another in Mt. Kaimon Natural Park of Kagoshima prefecture. It will be of interest to examine the hemoglobin type distribution among these horses.

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