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Electrophoretic Study of Haemoglobin Phenotypes in Caspian Miniature Horses

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Abstract. In order to study the haemoglobin types of the Caspian miniature horse, blood samples were collected from 35 such horses, and haemoglobin electrophoresis was carried out on lysates. Two types of haemoglobin, A and B, were fractionated, and the levels of these types were determined for sex and for various age groups. No significant differences were seen between the sexes for the measured parameters. Total hemoglobin and absolute level of hemoglobin A, however, showed significant differences between age groups ($p<0.05$). Correlations between measured parameters were also determined.

Keywords: Caspian miniature horse; Electrophoresis; Haemoglobin type

Introduction

Perhaps the most exciting equine discovery of the 20th century was that the Caspian is not a pony, but an ancient breed of miniature horse, previously believed to have been extinct for over a thousand years. This tiny horse was probably one direct ancestor of the oriental breeds, and subsequently of all light horse breeds . These animals are extremely rare and were pulled back from the brink of extinction in 1965 by Louise Firouze, an American living in Iran (Hendricks 1995).

The Caspian miniature horse is a native of the area around the Elborz Mountains and Caspian Sea in Iran. The current theory is that the Caspian is the ancient miniature horse of the Mesopotamia which, after being used by the Mesopotamians in the third millenium BC until the seventh century AD, was believed to have become extinct (Edwards 1985).

Establishing continuity for a breed for more than 3000 years will be difficult, but failure to take an exhaustive look into the past of this unique little horse may result in the loss of the vestige of the wild horse of the Middle East. The greatest importance of the Caspian lies in the fact that if continuity can be proven, the breed is likely the ancestor of all modern breeds of hot-blooded horses (Edwards 1992; Hendricks 1995; Draper 1996).

In human medicine haemoglobin electrophoresis is the main method for the detection of haemoglobin variants, and may also be of importance in the differential diagnosis of various haemoglobinopathies (Chanarin 1989). Both qualitative and quantitative abnormalities have been reported in humans but not in animals (Jain 1993). Electrophoretic markers (such as haemoglobin) are used extensively in horses because red blood cell antigens fail to provide the same degree of discrimination among individuals in this species (McClure and Parish 1996). Thus, the present study was performed for the detection of haemoglobin phenotypes of Caspian miniature horses and their levels for sex and for various age groups. To our knowledge this study is the first report of Caspian haemoglobin phenotypes.

Materials and Methods

A total of 35 Caspian miniature horses (15 female and 20 male) from a private stud belonging to Miss Louise Firooze were considered for this study. These horses were devided into three groups based on age: 1–3 years, 4–6 years and 7 or more years.

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Sampling was conducted from the jugular vein using disposable syringes and blood was added to EDTA-containing tubes and transferred to the laboratory on ice as soon as possible. The amounts of total haemoglobin (tHb) and haematocrit (PCV) were determined by cyanmethaemoglobin and microhaematocrit methods, respectively, after which lysates were prepared by adding distilled water and chloroform to the samples, followed by shaking and centrifugation. Electrophoresis was performed on cellulose acetate strips (350 V, 45 min) and after staining with Ponceau S, destaining, clearing and drying, the levels of haemoglobin types were determined by densitometery.

The mean $\pm$ standard deviation of tHb, PCV and types of haemoglobin were determined for sex and age groups. Independent t-test and ANOVA with multiple range Duncan test were used for statistical analysis; $p<0.05$ was considered to be significant. Correlation between measured parameters was detected by Pearson's method.

Results

After electrophoresis on cellulose acetate, two types of haemoglobin were fractionated: fast, with high levels, and slow, with lower levels. These were named A and B, respectively. The rate of migration of the haemoglobin types was identical for the Caspian miniature and thoroughbred horse, but migration was higher than haemoglobin of other domestic animals (cattle, sheep and dog). Regarding the above results, the haemoglobin phenotype of the Caspian is AB, the mean ratio of A: B being 1.75 $\pm$ 0.61.

No significant differences were seen for tHb, PCV, percentage of HbA and HbB, absolute levels of HbA and HbB and A: B ratio between the sexes. Total haemoglobin and the absolute level of haemoglobin A showed significant differences between age groups, with horses aged 7 years or more having higher tHb ($p<0.05$), and horses in age group 1–3 years having a lower amount of HbA ($p<0.05$). The results are shown in Table 1. Correlation between the measured parameters is presented in Table 2.

Discussion

Information about the haematology and serum biochemistry of Caspian miniature horses is limited, especially for haemoglobin phenotypes.

In this study, the levels of PCV and tHb for Caspian horese were 32.6% and 9.22 g/dl, respectively, the levels of tHb being lower than previously reported (Rezakhani 1978; Atyabi et al. 1999). Total haemoglobin and HbA levels showed significant differences between age groups ($p<0.05$), with advancing age resulting in increased tHb and HbA. The correlation coefficient also confirmed these results. This increase may be related to the effects of sex hormones. Atyabi et al.

Table 1. Mean $\pm$ standard deviation of measured parameter with statistical analysis between various groups of Caspian miniature horse

Group	*n*	PCV(%)	tHb(g/dl)	HbA(%)	HbA(g/dl)	HbB(%)	HbB(g/dl)	A: B
Male	15	31.64 ± 5.1	9.29 ± 2.12	64.42 ± 7.62	5.96 ± 1.35	35.58 ± 7.62	3.33 ± 1.15	1.98 ± 0.86
Female	20	33.29 ± 4.1	9.18 ± 1.38	60.83 ± 2.84	5.60 ± 0.87	39.18 ± 2.84	3.60 ± 0.61	1.57 ± 0.20
p value	–	NS	NS	NS	NS	NS	NS	NS
1–3 years	15	30.92 ± 5.13^{a}	8.49 ± 1.65^{a}	60.47 ± 4.97^{a}	5.16 ± 1.12^{a}	39.53 ± 4.97^{a}	3.35 ± 0.77^{a}	1.59 ± 0.49^{a}
4–6 years	16	34.14 ± 3.76^{a}	9.55 ± 2.35^{ab}	64.19 ± 6.25^{a}	6.10 ± 0.74^{b}	35.81 ± 6.25^{a}	3.46 ± 0.92^{a}	1.91 ± 0.74^{a}
⩾7 years	4	32.50 ± 2.12^{a}	10.68 ± 2.32^{b}	62.18 ± 3.50^{a}	6.62 ± 1.29^{b}	37.83 ± 3.50^{a}	4.10 ± 1.11^{a}	1.66 ± 0.27^{a}
p value	–	NS	S	NS	S	NS	NS	NS
Total	35	32.64 ± 4.50	9.22 ± 1.71	62.37 ± 5.63	5.75 ± 1.10	37.63 ± 5.63	3.48 ± 0.88	1.75 ± 0.61

[a,b,] Means in the same column with different superscripts are significantly different ($p<0.05$).

Table 2. Correlation coefficients between measured parameters in caspian miniature horse

	Age	A: B	HbB(%)	HbB(g/dl)	HbA(%)	HbA(g/dl)	tHb(g/dl)	PCV(%)
PCV	0.21	0.09	−0.13	0.50*	0.13	0.77*	0.75*	–
tHb	0.43*	−0.13	0.12	0.83*	−0.12	0.90*	–	0.75*
HbA(%)	0.21	0.98*	−1.00*	−0.64*	–	0.33	−0.12	0.13
HbA(g/dl)	0.49*	0.30	−0.33	0.50*	0.33	–	0.90*	0.77*
HbB(%)	−0.21	−0.98*	–	0.64*	−1.00*	−0.33	0.12	−0.13
HbB(g/dl)	0.21	−0.64*	0.64*	–	−0.64*	0.50*	0.83*	0.50*
A: B	0.14	–	−0.98*	−0.64*	0.98*	0.30	−0.13	0.10
Age	–	0.14	−0.21	0.21	0.21	0.49*	0.43*	0.27

* Significant correlation ($p<0.05$).

(1999) also reported this relationship. Electrophoresis of haemoglobin revealed two types of haemoglobin for Caspians: HbA, at higher levels and with faster migration, and HbB with lower amounts and slow migration. Thus, the haemoglobin phenotype of Caspians is AB. Jain (1993) reported that the majority of horses have two types of haemoglobin, but that certain breeds may have only one component. Ezcurra and Mitat (1973) studied haemoglobin of quarter horses by starch gel electrophoresis and reported two types of haemoglobin: A1 and A2, and that A1 was predominant. Nishimura et al. (1975) reported three haemoglobin phenotypes for south-east Asian ponies, and Santiago-Lagona et al. (1974) also found two types of haemoglobin in Arab horses, with higher levels of the faster type. Our results are consistent with the above reports for various breeds of horses.

Horses generally have two types of haemoglobin in the ratio of 70:30 (2.5:1) (Jain, 1986, 1993). The ratio for Caspian horses was 1.75:1, and therefore lower than the ratio reported for other breeds. To our knowledge, this is the first report for haemoglobin phenotypes and levels of haemoglobin types in Caspian miniature horse, but further studies will be required.

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