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Reference values of hematological and blood biochemical parameters for the Noma horse

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The Noma horse is a Japanese breed from the Noma region of Imabari City, Ehime Prefecture. To obtain reference hematological and biochemical values, we performed examinations in 39 clinically healthy, mature Noma horses managed at the Imabari public ranch. Hematological and biochemical results of Noma horses were close to the normal ranges of horses in the U.S.A. The erythrocyte parameters and hepatobiliary enzyme levels in Noma and Kiso horses were lower than those in Japanese racehorses. Noma horses showed higher erythrocyte parameters and triglyceride concentrations and a lower creatinine concentration compared with those in Kiso horses. These data represent the first report of reference values for Noma horses and may be useful to improve their management.

Key words: *biochemistry, hematology, Noma horse, reference value*

J. Equine Sci.
Vol. 30, No. 3
pp. 69–73, 2019

The Noma horse has the smallest body size of all Japanese native horse breeds (Fig. 1); they are exclusive to the Noma region of Imabari City, Ehime Prefecture [8]. Their physical characteristics include a cylindrical body, oblique buttocks, thin legs, thick and tightened joints, and durable hooves (Fig. 1) [10]. Additionally, they have calm temperaments and endure coarse feed [10]. Noma horses were historically used for transportation in terraced fields and mountainous areas and played an important cultural role in the Ehime region. By the early 1970s, however, their population had fallen to a dozen individuals. Through the efforts of the Noma Horse Preservation Society, the population had increased to 84 by 2006. Recently, however, their numbers have been declining again.

Previous studies on Noma horses were focused on conservation of the breed, characterization of the genetic diversity, and isolation of peripheral blood stem cells [9, 11, 12, 19]. As the struggling population comprises individual animals, careful individualized management is needed to provide proper support. Hematological and biochemical reference values for rare and local horses have been reported for Lipizzan horses in central and eastern Europe [2], Barb horses in Algeria [3], and feral horses in the U.S.A. [15]. The Kiso horse is the only native Japanese breed for which hematological and biochemical data are known [18]. Investigation of the reference values for existing Noma horses will help to improve their population management. Thus, the aim of the present study was to determine reference hematological and blood biochemical values for the Noma horse.

At the time of the study, 49 Noma horses were kept at the Imabari Nomauma Highland public ranch. We excluded 10 horses that were juvenile, under medical treatment, or highly sensitive; thus, the study included 39 healthy horses (17 mares, 11 stallions, and 11 geldings). The mean body condition scores (BCS), measured as described by Henneke *et al*. [7], was 4.8 ± 1.2 (± standard deviation [SD]). The ages of the horses ranged from 3 to 27 years (mean ± SD, 15.4 ± 6.2 years). There were no differences in hematological and blood biochemical data between the three gender groups (mares, stallions, and geldings). All blood samples were taken from the jugular vein on the mornings of two days in September 2018. Blood samples were transferred into EDTA-2K tubes for hematological analyses and into plain tubes for biochemical analyses.

The hematological variables were measured using a cell analyzer (Celltac α, Nihon Kohden, Tokyo, Japan). Leukogram data were assessed by microscopic examinations of

Received: February 20, 2019
Accepted: July 23, 2019
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Fig. 1. Appearance of Noma horse. The Noma horse is smallest body size of all Japanese native horse breeds, and its physical characteristics are a cylindrical body, oblique buttocks, thin legs, thick and tightened joints, and durable hooves.

blood smears and Wright-Giemsa staining (Diff-Quik Stain Kit, Sysmex Corp., Tokyo, Japan). Serum biochemical analyses were performed using an auto analyzer (3100 Auto Analyzer, Hitachi High Technologies Corp., Tokyo, Japan). The normality of the data distribution was assessed by Shapiro-Wilk tests; parameters with non-normal distributions were normalized by Box-Cox transformation [1]. Outliers were detected by Smirnov-Grubbs tests. The data are expressed as means ± 2SD. Since there have been no previous reports on blood tests for Noma horses, the blood profile was compared with the reported reference values for Kiso horses [18] as the only Japanese native breed with reported profiles, Japanese racehorses [4] as a common reference values in Japan, and horses in the U.S.A. [17] as an international standard. The mean values of Noma and Kiso horses [18] were analyzed by Welch's t-tests. Differences were considered statistically significant when the *P*-values were less than 0.05. The data among Noma horses, Japanese racehorses, and horses in the U.S.A. were not compared statistically.

The hematological and biochemical parameters determined in this study are shown in Table 1. The reference ranges (mean ± 2SD) of these parameters in Noma horses were almost within the normal ranges of horses in the U.S.A. The erythrocyte parameters in Noma horses resembled those in Kiso horses, and the parameters of both breeds were lower than those in Japanese racehorses. Higher values in racehorses might reflect the polycythemic state acquired by hard physical training for racing. The low values of erythrocyte parameters in Kiso horses have been considered to be normal; however, it is unknown whether these were characterized by breed-specific or acquired factors [18]. Thus, the lower erythrocyte parameter values did not indicate an anemic state in Noma horses because there were no observable anemic symptoms.

In comparison between the Japanese native breeds, hemoglobin (Hb) and hematocrit (Ht) in Noma horses (12.8 ± 2.6 g/d*l* and 35.5 ± 7.1%, respectively) were slightly higher than those in Kiso horses (11.6 ± 1.5 g/d*l* and 32.9 ± 4.0%, respectively). Urea nitrogen (UN), albumin (Alb), and sodium (Na) levels in Noma horses (15.3 ± 4.5 mg/d*l*, 3.2 ± 0.57 g/d*l*, and 137.7 ± 4.7 mEq/*l*, respectively) were slightly higher than in Kiso horses (12.2 ± 4.8 mg/d*l*, 3.1 ± 0.3 g/d*l*, and 136.7 ± 2.4 mEq/*l*, respectively), although values in both breeds were within the normal ranges for those of horses in the U.S.A. Noma horses are kept in an environment with free access to water. Hence, the higher UN, Alb, and Na levels might not be related to dehydration caused by water loss. The slight differences between Noma and Kiso horses may be attributed to differences in environments, feed, and the physiological features of the two breeds.

Table 1. Hematological and biochemical reference values for the Noma horse

Variable	Noma horse (n=39)		Kiso horse (n=116)	Japanese racehorses	Normal ranges of horses
RBC ($10^4/\mu l$)	712 ± 153	(559–866)	715 ± 105	940 ± 81	620–1,020
Hb (g/d*l*)	12.8 ± 2.6*	(10.2–15.4)	11.6 ± 1.5	16.1 ± 1.4	11.4–17.3
Ht (%)	35.5 ± 7.1*	(28–43)	32.9 ± 4.0	42.2 ± 4.0	31.0–50.0
MCV (f*l*)	49.9 ± 5.1	(45–55)	ND	ND	ND
MCH (pg)	17.9 ± 2.0	(16–20)	ND	ND	ND
MCHC (g/d*l*)	36.0 ± 0.9	(35–37)	ND	ND	ND
WBC ($10^2/\mu l$)	73.3 ± 21.9*	(51.4–95.2)	82.7 ± 21.2	93.6 ± 12.8	49.0–103.0
NEU ($10^2/\mu l$)	45.6 ± 19.0	(26.5–64.6)	ND	ND	22.0–83.0
LYM ($10^2/\mu l$)	22.4 ± 17.5	(4.9–39.9)	ND	ND	17.0–58.0
MON (/μl)	74 ± 123	(2–275)	ND	ND	0–1,000
EOS (/μl)	383 ± 529	(13–1,070)	ND	ND	0–800
BAS (/μl)	0 ± 0	(0–0)	ND	ND	0–300
PLT ($10^4/\mu l$)	15.4 ± 5.6	(10–21)	ND	10.7 ± 2.2	7.2–18.3
AST (U/*l*)	368 ± 165*	(202–533)	277 ± 62	374 ± 198	205–555
LDH (U/*l*)	488 ± 270*	(217–758)	431 ± 161	550 ± 134	ND
ALP (U/*l*)	447 ± 424	(176–948)	630 ± 301	228 ± 31	109–315
GGT (U/*l*)	15.5 ± 9.8	(6–25)	15.0 ± 5.4	23.0 ± 12.0	12–45
CK (U/*l*)	185 ± 95*	(90–279)	305 ± 169	260 ± 186	90–270
UN (mg/d*l*)	15.3 ± 4.5*	(10.8–19.8)	12.2 ± 4.8	12.7 ± 2.9	8.0–27.0
Cre (mg/d*l*)	1.0 ± 0.4*	(0.7–1.4)	1.2 ± 0.3	1.4 ± 0.2	0.6–1.8
TP (g/d*l*)	6.5 ± 1.0*	(5.5–7.5)	6.9 ± 0.6	5.7 ± 0.4	4.6–6.9
Alb (g/d*l*)	3.2 ± 0.6*	(2.6–3.8)	3.1 ± 0.3	3.1 ± 0.3	2.5–4.2
Glu (mg/d*l*)	94.8 ± 21.9*	(73–117)	83.7 ± 13.2	104.0 ± 26.0	72–114
T-Bil (mg/d*l*)	0.9 ± 0.4	(0.6–1.4)	0.7 ± 0.2	2.5 ± 0.7	0.1–1.9
TG (mg/d*l*)	37.2 ± 34.3*	(3–71)	16.4 ± 11.1	24 ± 11	11–52
T-Cho (mg/d*l*)	75.0 ± 25.6	(49–101)	76.6 ± 14.2	83.0 ± 14.0	51–109
Ca (mg/d*l*)	12.1 ± 1.01	(11.1–13.1)	12.0 ± 0.9	11.8 ± 0.5	10.7–13.4
IP (mg/d*l*)	2.5 ± 0.1*	(1.5–3.5)	3.1 ± 0.7	3.7 ± 0.6	1.9–5.4
Mg (mg/d*l*)	2.1 ± 0.3	(1.8–2.5)	2.0 ± 0.3	ND	1.6–2.5
Na (mEq/*l*)	137.7 ± 4.7*	(133.0–142.4)	136.7 ± 2.4	140.0 ± 3.0	132.0–141.0
K (mEq/*l*)	4.6 ± 1.3	(3.3–6.0)	4.6 ± 0.7	3.9 ± 0.5	2.7–4.9
Cl (mEq/*l*)	101.9 ± 3.7	(98.2–105.6)	102.6 ± 2.8	99.0 ± 4.0	94.0–102.0

The data are expressed as means ± 2 standard deviation (2SD) or ranges for Noma horses, and means ± SD for Japanese racehorses. The parentheses are mean−2SD to mean+2SD ranges in Noma horses, and MON, EOS, ALP, T-Bil, Mg, and Cl ranges are calculated based on normalized data by Box-Cox transformation [1]. The data for Japanese racehorses, the normal ranges of horses in U.S.A., and Kiso horses are taken from references [4, 17, 18], respectively. n: number of horses; as a result of outliers, n is 37 for EOS and Cl of Noma horses. *: significant difference from Kiso horses, $P<0.05$. RBC: red blood cells, Hb: hemoglobin, Ht: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean hemoglobin concentration, WBC: white blood cells, NEU: neutrophils, LYM: lymphocytes, MON: monocytes, EOS: eosinophils, BAS: basophils, PLT: pratelets, AST: aspartate aminotransferase, LDH: lactete dehydrogenase, ALP: alkaline phosphatase, GGT: gamma-glutamyl transferase, CK: creatinine kinase, UN: urea nitrogen, Cre: creatinine, TP: total protein, Alb: albumin, Glu: glucose, T-Bil: total bilirubin, TG: triglyceride, T-Cho: total cholesterol, Ca: calcium, IP: inorganic phosphorus, Mg: magnesium, Na: sodium, K: potassium, Cl: chloride, ND: no data.

The white blood cell (WBC) counts in Noma horses (73.3 ± 21.9 × 10^2 cells/μl) were lower than those in Kiso horses and Japanese racehorses. This count was accompanied by a lower lymphocyte count (22.4 ± 17.5 10^2 cells/μl) and higher mean neutrophil:lymphocyte (N:L) ratio (2.5). Minor differences have been reported in WBC counts among various breeds; hot-blooded horses, including Arabian and Thoroughbred breeds, had slightly higher WBC counts than those in cold-blooded horses including draft horse and pony breeds [5]. In adult horses, neutrophil counts were maintained, but lymphocyte counts tended to decrease with age, and the N:L ratio of aged horses tended to be high [5]. A hematological report for Lipizzan horses indicated a significant decrease in WBC counts with age [2]. In Noma horses, blood samples were collected from relatively aged horses (mean age of 15.4 years). Due to their older ages, the WBC and lymphocyte counts may have been lower. Nevertheless, the hematological values of Noma horses were within the

normal ranges of horses in the U.S.A.

The aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities were slightly higher in Noma horses than those in Kiso horses. There were no differences in gamma-glutamyl transpeptidase and alkaline phosphatase (ALP) activities between Noma and Kiso horses. These enzyme activities in Noma horses might have been normal, considering data from other breeds [6, 13, 14, 16]. A previous report also indicated that the hepatobiliary enzyme levels of Kiso horses should be lower than those of racehorses [18]. Horses used for racing and competitions might generally consume more nutritious foods, while Japanese native horses tend to be raised on roughage. Roughage-based feed might be one of the factors related to the lower hepatobiliary enzyme activities in both Noma and Kiso horses. The reason for the slightly higher AST and LDH levels in Noma horses than in Kiso horses is unclear, but they may reflect slight differences in feed content. Conversely, the upper limits of ALP in Noma and Kiso horses were higher than in horses in the U.S.A. These higher activities might not reflect only hepatobiliary function of Noma and Kiso horses, because ALP is commonly known as a liver non-specific enzyme and plasma ALP activities of foals are high due to their skeletal growth [14]. However, the reason for high ALP activity in Noma horses remains unclear. To clarify the cause of higher ALP activity, analyses of ALP isozymes may be useful.

Creatinine kinase (CK) and creatinine (Cre) in Noma horses were within the normal ranges of horses in the U.S.A. but were slightly lower than in Kiso horses and Japanese racehorses. The production and serum concentrations of Cre might depend on absolute muscle mass volume and vital activity. The Noma horses in this study did not undertake any specific exercise. Therefore, the lower CK and Cre levels might reflect the low muscle mass and daily exercise in Noma horses kept in small pastures.

The triglyceride (TG) levels in Noma horses (37.2 ± 34.3 mg/d*l*) were higher than in Kiso horses and Japanese racehorses (16.4 ± 11.1 and 24.0 ± 11 mg/d*l*, respectively). The upper limit of TG in Noma horses was higher than in horses in the U.S.A. Higher TG suggests a negative energy balance. However, most Noma horses had a moderate BCS (mean ± SD; 4.8 ± 1.2) and were not obviously obese or emaciated. In addition, no horses had a negative energy balance, such as lactating mares. For a more specific interpretation, it may be necessary to investigate their detailed body weights and energy requirements.

This study reported reference values of hematological and biochemical parameters for Noma horses. Noma horses were considered to be substantially healthy based on the laboratory test findings: they had no critical clinical symptoms and had moderate BCSs, and their blood profiles were almost within the normal ranges of horses in the U.S.A. The hematological and biochemical characteristics of Noma horses were similar to those of Kiso horses, although their parameters differed slightly. It is difficult to determine whether these differences indicate a specific characteristic of the Noma horse because hematological and blood biochemical properties of horses are affected by various factors including gender, age, season, breed, and management area [2–3, 6, 13–16]. The data for Noma horses in the present study were obtained in summer. Winter, when animals require more energy due to low environmental temperatures, may yield different results. In addition, reference values for young, growing horses should be established separately. It is necessary to further investigate the effects of feed, age, and season. However, the results obtained in this study will be utilized for veterinary practice and appropriate management of Noma horses.

Acknowledgments

We acknowledge the contributions of the members of Imabari City office. We also express gratitude to Mr. Daisuke YAMASHITA of the Japan Equine Affairs Association and Dr. Masaki TAKASU of Gifu University for their valuable comments and suggestions throughout the course of our study.

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