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Relationships between the age and blood test results or body sizes in Noma horses

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The objective of this study is to analyze the relationships between the age and blood test results or body sizes in Noma horses by using the results of periodical health examination. Out of 45 hematological or physical items examined, statistically significant, but loose correlations were observed in 14 items. Red blood cell count, activities of aspartate aminotransferase, alkaline phosphatase, and creatinine kinase, concentrations of calcium and inorganic phosphorus decreased with aging. Conversely, mean corpuscular volume, mean corpuscular hemoglobin, lipase activity, γ-globulin and chloride concentrations, body height, chest circumference and cannon bone circumference increased with aging. The changes in a few items seemed unique to Noma horse. However, most age-related changes found in this study might be considered as a common trend in horse breeds rather than distinctive characteristic in Noma horse.

Key words: *aging, blood test, body size, Noma horse*

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The Noma horse is one of the Japanese native horses, reared and bred exclusively in the Noma region of Imabari city, Ehime Prefecture, Japan [8]. Nowadays, they are bred only at the preservation ranch except several kept at zoo. Recently, the number of Noma horses has been declining to approximately 50 animals [9], and it is endangered despite the efforts to increase population by the preservation society. Figure 1 shows the age distributions of Noma horses at the time of this study. The populations of each constitution were very small; from 0 to 3 horses in all ages. Their age range was 0–28 years old with an average of 13.5 ± 7.7 years old. Out of these, 54% of the group member were 14-years or older (mare: 56.5%, stallion: 35.3%, gelding: 80.0%).

It is important to understand the standard range of hematological and physical examination in diagnosing the animal health condition. There are several reports about the relation between age and hematological or physical examinations in Equidae [1–6, 10–13, 16], but general tendency is not provided about some items among each report. In this study we attempted to characterize the age-related changes of hematological and physical examination results in Noma horses, which the age structure of the population deviates to the old side.

The study included 50 clinically healthy horses (23 mares, 17 stallions, and 10 geldings) kept at the Nomauma Highland public ranch in the Imabari city. Their ages were from 173 to 10,424 days (0.5 to 28-years-old). Peripheral blood samples were collected from the jugular vein of 49 horses, and body sizes were determined in all 50 horses in October 2019 as periodical health examination. The hematological items were measured using a cell analyzer (Celltac α, Nihon Kohden, Tokyo, Japan) in the equine setting. Leukogram data were assessed by microscopic examinations of blood smears and Wright-Giemsa staining (Diff-Quik Stain Kit, Sysmex Corp., Tokyo, Japan). Serum biochemical analysis were performed using an auto analyzer (3100 Auto Analyzer, Hitachi High Technologies Corp., Tokyo, Japan). Correlation coefficients between the age in days and blood test results or body sizes were calculated by Spearman's rank correlation coefficient (BellCurve in Excel, Social Survey Research Information Co., Ltd., Tokyo, Japan). Correlations and differences were consid-

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ered statistically significant when the *P*-values were less than 0.05. In several reports about the relationship between aging and blood profiles in horse [1–6, 10–13, 16], horses investigated were categorized in some age groups, and blood test results were compared among those groups by the significant difference examination. In small population such as Noma horse, however, there is difficulty to carry out comparative statistical analysis because of a few numbers of horses in each age group. So, in this study, correlation between age and the results of hematological and physical examination were analyzed.

The experimental protocols of this study were approved by the Animal Care and Use Committee of Okayama University of Science (approval number, 2019-13).

The mean values of hematological and serum biochemical test results and body sizes, and correlation coefficients with ages in days are shown in Table 1. Among 45 items inspected in the present study, there were statistically significant correlations with aging in 14 items. The items detected significantly positive correlation with aging were

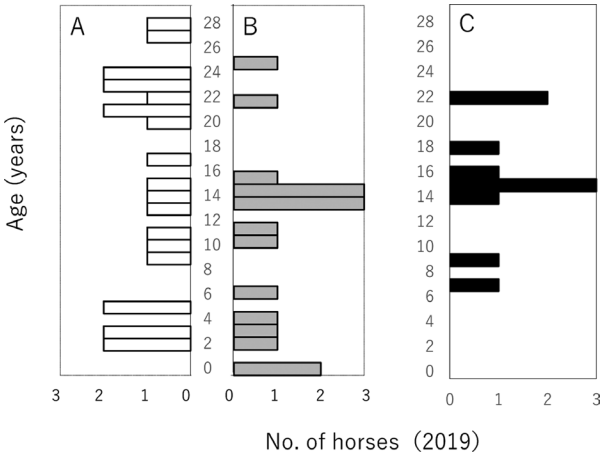

Fig. 1. Age compositions of each gender used in the present study. Columns indicate the number of horses. A: mares, B: stallions, C: geldings.

Table 1. Means, standard deviations, and correlation coefficients of blood test results and body sizes in Noma horse

Variable	n	Mean	SD	r	*P*	Variable	n	Mean	SD	r	*P*
RBC ($10^4/\mu l$)	49	773.4	106.4	−0.581	<0.001	GLU (mg/d*l*)	49	111.6	17.9	−0.280	0.051
Hb (g/d*l*)	49	13.2	1.3	−0.206	0.156	T−BIL (mg/d*l*)	49	0.8	0.2	0.210	0.148
Ht (%)	49	37.8	3.7	−0.250	0.083	TG (mg/d*l*)	49	43.1	21.5	−0.231	0.110
MCV (f*l*)	49	49.3	4.0	0.727	<0.001	T−CHO (mg/d*l*)	49	83.4	16.0	0.040	0.784
MCH (pg)	49	17.2	1.4	0.673	<0.001	TP (g/d*l*)	49	7.3	0.7	0.104	0.475
MCHC (g/d*l*)	49	34.8	0.4	0.100	0.494	ALB (g/d*l*)	49	3.7	0.4	−0.123	0.400
WBC ($10^2/\mu l$)	49	70.7	13.4	−0.271	0.059	GLOB (g/d*l*)	49	3.7	0.6	0.161	0.268
Neu ($10^2/\mu l$)	49	41.6	9.8	−0.116	0.428	A/G	49	1.0	0.2	−0.171	0.239
Eos (/μl)	49	346.1	233.2	−0.252	0.081	α (g/d*l*)	49	1.1	0.3	−0.044	0.766
Baso (/μl)	49	6.4	20.3	−0.226	0.119	β (g/d*l*)	49	1.3	0.2	0.121	0.407
Mono (/μl)	49	69.8	79.8	−0.225	0.120	γ (g/d*l*)	49	1.4	0.3	0.314	0.028
Lym ($10^2/\mu l$)	49	24.8	9.1	−0.220	0.128	Ca (mg/d*l*)	49	12.8	0.7	−0.317	0.026
PLT ($10^4/\mu l$)	49	13.4	3.4	−0.130	0.374	IP (mg/d*l*)	49	3.3	0.9	−0.545	<0.001
LIPA (U/*l*)	49	17.2	4.8	0.318	0.026	Mg (mg/d*l*)	49	2.2	0.2	0.123	0.400
AST (U/*l*)	49	399.6	106.7	−0.294	0.040	Fe (μg/d*l*)	49	168.3	39.3	0.158	0.278
LD (U/*l*)	49	536.2	188.0	−0.231	0.110	TBA (μmol/*l*)	49	6.0	2.6	0.117	0.425
ALP (U/*l*)	49	501.6	262.9	−0.397	0.005	Na (mEq/*l*)	49	140.8	2.8	−0.099	0.498
γ-GT (U/*l*)	49	18.2	6.4	−0.013	0.930	K (mEq/*l*)	49	3.8	0.9	−0.089	0.545
CK (U/*l*)	49	218.5	56.2	−0.394	0.005	Cl (mEq/*l*)	49	101.1	2.3	0.373	0.008
AMY (U/*l*)	49	10.2	3.5	0.149	0.308	BCS	50	4.7	1.3	−0.172	0.232
BUN (mg/d*l*)	49	17.2	4.3	0.122	0.402	BH (cm)	50	108.6	6.4	0.291	0.040
CRE (mg/d*l*)	49	1.0	0.2	0.237	0.101	CC (cm)	50	127.1	9.5	0.293	0.039
						Cannon (cm)	50	13.6	0.9	0.417	0.003

The explanations of each abbribiation are as follows; number of horses tested (n), correlation coefficient (r), *P*-value (*P*), red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), neutrophil (Neu), eosinophil (Eos), basophil (Baso), monocyte (Mono), lymphocyte (Lym), platelet (PLT), lipase (LIPA), aspartate aminotransferase (AST), lactate dehydrogenase (LD), alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), creatinine kinase (CK), amylase (AMY), blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total bilirubin (T-BIL), triglyceride (TG), total cholesterol (TCHO), total protein (TP), albumin (ALB), globulin (GLOB), albumin/globulin ratio (A/G), α-, β-, and γ-globulins (α, β, and γ), calcium (Ca), inorganic phosphorus (IP), magnesium (Mg), iron (Fe), total bile acid (TBA), sodium (Na), potassium (K), chloride (Cl), nine-point scale body condition score (BCS), body height (BH), and chest circumferences (CC) and cannon bone circumferences (Cannon).

mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), lipase activity (LIPA), concentration of γ-globulin (γ) and chloride (Cl), body height (BH), chest circumferences (CC) and cannon bone circumferences (Cannon). Adversely, items having significantly negative correlations were red blood cell count (RBC), activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine kinase (CK), concentrations of calcium (Ca) and inorganic phosphorus (IP).

The changes of the hematological items in aged horse, i.e., lower RBC and higher MCV and MCH, showed a logarithm approximation and major change by 6-year-old (data not shown). Therefore, it might not be the characteristics in aged horse. Similar tendencies were also observed in Lipizzian horse [3], Lucitano horse [12] and donkey [16].

Mention to biochemical items, lower ALP, Ca and IP in aged horse were also reported in Thoroughbred [5] and donkey [16]. These decreases probably reflect decreased bone metabolism as animals become older [15]. In this study, the concentrations of ALP and IP showed major change by 6-year-old (data not shown). Significantly higher CK in younger horses compared to aged horses were also reported in Murinsulaner horse [1], but not in Thoroughbred [5]. Additionally, absence of age difference with AST is reported in several horse breeds [6, 11]. Significant positive correlations between AST and CK (r=0.561, $P<0.001$) were observed in this study. CK detected in plasma derive mainly from the skeletal muscle, and AST are from both hepatic cell and skeletal muscle [5]. So, these decreases in this study might reflect the decreases in momentums or muscle mass volumes with aging and are more outstanding compared to riding or working horses since Noma horses in this study are not used for these purposes. The age-related increase of γ also reported in racehorses [5]. Fraction of γ commonly increases in response to external antigenic stimuli resulting in a polyclonal gammopathy characterized by broad increases in the γ-fraction [7]. Our result with γ may indicate the increase of opportunity to be exposed to various antigen with aging. The increase of LIPA and Cl with aging were not reported in the previous studies. Silva *et al.* [11] reported that there were not significant differences with Cl between young and old horses of 48 breeds treated in their hospital.

Figure 2 shows relationships between the age and body sizes. The BH, CC and Cannon correlated positively with age. Bone growth developments of horse are thought to continue until 5-year-old [14]. In this study, for adult horses (>6 years), significant correlation was accepted only between age and the Cannon (n=41; r=0.347, $P<0.05$), not between age and the BH or CC. The result may indicate that only cannon bone growth continues after the general period of growth of frame in Noma horse.

The results of this study indicated some characteristics of the relation between hematological or physical examination and aging in Noma horse. However, any individual did not show obviously abnormal test results even in aged horses [13]. We considered that these age-related alterations might not be pathological, but rather physiological accompanied with aging. Furthermore, most age-related changes found in this study were also reported in other horse breeds. It might be considered as a common trend in horse breeds rather than distinctive characteristic in Noma horse. The results such as the decrease of AST and the increase of LIPA, Cl and Cannon seemed unique to Noma horse.

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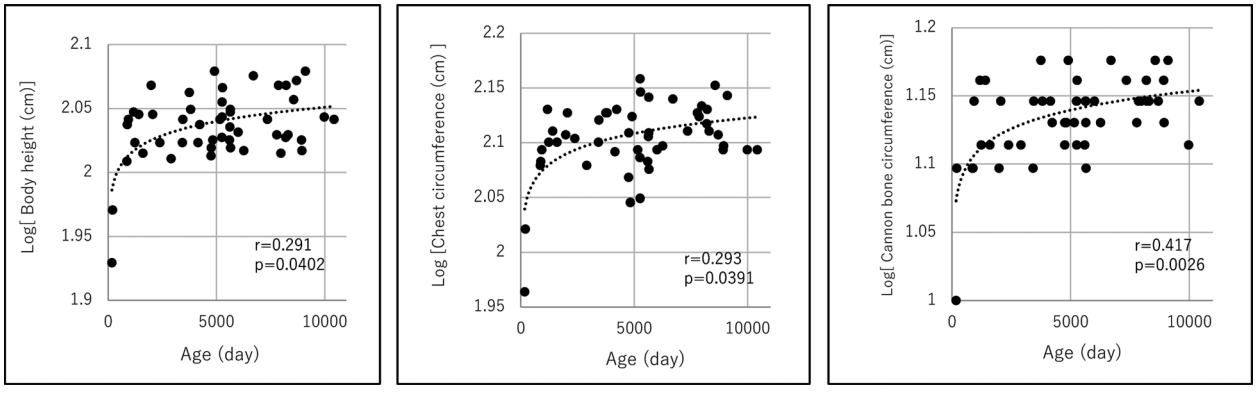

Fig. 2. Correlations between the age and body sizes showing significant difference: n=50 in all items. The data of each physical item is depicted as the logarithm.

References

1. Babic, N.P., Kostelie, A., Novak, B., Salamon, D., Tariba, B., Macesic, N., Karadjole, T., Bacie, G., and Bedrica, L. 2019. Reference values and influence of sex and age on hemogram and clinical biochemistry in protected and endangered Murinsulaner horses. *Vet. Arh.* **89**: 25–42. [CrossRef]
2. Brosnahan, M.M., and Paradis, M.R. 2003. Demographic and clinical characteristics of geriatric horses: 467 cases (1989–1999). *J. Am. Vet. Med. Assoc.* **223**: 93–98. [Medline] [CrossRef]
3. Cebulj-Kadunc, N., Bozic, M., Kosec, M., and Cestnik, V. 2002. The influence of age and gender on haematological parameters in Lipizzan horses. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **49**: 217–221. [Medline] [CrossRef]
4. Dinev, D., and Khubenov, KD. 1986. Normal values of the hematological, biochemical and enzymological indices of the donkey. *Vet. Med. Nauki* **23**: 69–75. [Medline]
5. Equine Research Institute Japan Racing Association. 1995. Normal Values of the Racehorse, 3rd ed., Tochigi.
6. McFarlane, D., Sellon, D.C., Gaffney, D., Hedgpeth, V., Papich, M., and Gibbs, S. 1998. Hematologic and serum biochemical variables and plasma corticotropin concentration in healthy aged horses. *Am. J. Vet. Res.* **59**: 1247–1251. [Medline]
7. Miglio, A., Morelli, C., Maresca, C., Felici, A., Di Gianbattista, A., and Antognoni, M.T. 2019. Serum protein concentrations and protein fractions in clinically healthy Italian Heavy Draft Horses using agarose gel electrophoresis. *Vet. Clin. Pathol.* **48**: 677–682. [Medline] [CrossRef]
8. Ootsuka, K., Hirota, K., Matsumoto, M., and Hshiguchi, T. 1984. The academic report on Noma horse. Addendum. p. 10. *In*: Japanese Native Horses-Their Preservation and Utilization, Japan Equine Affairs Association, Tokyo (in Japanese).
9. Ono, T., Inoue, Y., Hisaeda, K., Yamada, Y., Hata, A., Miyama, T.S., Shibano, K., Kitagawa, H., Ohzawa, E., and Iwata, E. 2021. Effect of seasons and sex on the physical, hematological, and blood biochemical parameters of Noma horses. *J. Equine Sci.* **32**: 21–25. [Medline] [CrossRef]
10. Shawaf, T., Hussen, J., Al-Zoubi, M., Hamaash, H., and Al-Busadah, K. 2018. Impact of season, age and gender on some clinical, haematological and serum parameters in Shetland ponies in east province, Saudi Arabia. *Int. J. Vet. Sci. Med.* **6**: 61–64. [Medline] [CrossRef]
11. Silva, A.G., and Furr, M.O. 2013. Diagnoses, clinical pathology findings, and treatment outcome of geriatric horses: 345 cases (2006–2010). *J. Am. Vet. Med. Assoc.* **243**: 1762–1768. [Medline] [CrossRef]
12. Silvestre-Ferreira, A.C., Cotovio, M., Maia, M., Queiroga, F., Pires, M.J., and Colaço, A. 2018. Reference intervals for haematological parameters in the Lusitano horse breed. *Acta Vet. Hung.* **66**: 530–541. [Medline] [CrossRef]
13. Southwood, L.L. 2013. Appendix C normal ranges for hematology and plasma chemistry and conversion table for units. pp. 339–342. *In*: Practical Guide to Equine Colic, 1st ed. (Southwood, L.L. ed.), John Wiley & Sons, Ames.
14. Tokita, A. 2012. Development of bone. pp. 67–68. *In*: Equine Veterinary Medicine, 2nd ed. (Equine Research Institute, Japan Racing Association, ed.), Midori Shobo, Tokyo (in Japanese).
15. Yashiki, K., Watanabe, H., Takagi, S., and Nitta, M. 1989. Serum alkaline phosphatase isozymes of horse. *Bull. Equine Res. Inst.* **26**: 17–22.
16. Zinkl, J.G., Mae, D., Guzman Merida, P., Farver, T.B., and Humble, J.A. 1990. Reference ranges and the influence of age and sex on hematologic and serum biochemical values in donkeys (*Equus asinus*). *Am. J. Vet. Res.* **51**: 408–413. [Medline]