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Effect of seasons and sex on the physical, hematological, and blood biochemical parameters of Noma horses

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This study aimed to evaluate the influence of seasons and sex on body size and hematological and biochemistry parameters of Noma horses, a native Japanese breed. Body size was larger in winter than in summer. Laboratory testing variables, including erythrocytic parameters and urea nitrogen, total cholesterol, and creatinine kinase levels, were higher in winter, while the eosinophil count was higher in summer. These seasonal differences may be related to increased energy consumption of horses due to heat stress. The higher eosinophil counts may have been related to the dermatitis observed in summer. Stallions tended to have smaller bodies compared with mares. Future studies are necessary to investigate the effect of stress in seasonal and sex-based groups.

Key words: *hematological and biochemical parameter, Noma horse, season, sex*

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Noma horses have the smallest body size among the native breeds that currently exist in Japan. They are reared and bred exclusively in the Noma region of Imabari City, Ehime Prefecture, Japan [10]. The current population of Noma horses was created by inbreeding a small number of primogenitors. Their number increased from four animals in 1978 to 84 animals in 2006, but the number has decreased again in recent years. In 2020, 50 horses were housed at the Imabari Nomauma Highland public ranch, which serves as the breeding facility, but the number of fertile horses was even lower, with only 12 mares aged 2–14 years.

Previous studies have focused mainly on the characterization of genetic diversity for the conservation of Noma horses [11, 12, 18]. Appropriate care and management, including regular health examinations for each individual animal, are also necessary to maintain and increase the population of these horses. We previously reported the reference blood profile values for existing Noma horses to determine the baseline data for their healthcare [16]. The hematological and biochemical characteristics of Noma horses differed from those of other breeds; erythrocyte parameters and hepatobiliary enzymes were lower than those of the light horses used for races but similar to those of Kiso horses, one of the Japanese native breeds [16]. However, it is known that the blood profiles of horses vary with sex, age, and season [1–4, 7, 9, 15, 17]. Knowledge of the seasonal alterations and sex-based differences in the blood profiles of Noma horses is essential to facilitate more attentive management of individual horses by veterinarians and horse managers. Therefore, we aimed to elucidate the seasonal alterations and sex-based differences in body size and blood profile in the extant Noma horses using previous and newly collected data obtained during summer and winter.

Physical and blood examinations were conducted on February 4 and 5, 2019 (winter season), and results were compared with those collected on September 18 and 19 (summer season) [16]. During this period, 49 Noma horses were housed at the Imabari Nomauma Highland public ranch, where they were exhibited in outdoor grounds during the day and housed in stables at night. The Noma horses were fed purchased roughage and formula feed twice a day according to the Japanese Feeding Standard for Horses [5], and all horses had free access to roughage and water. There was no difference in the amounts of these feeds during the study period. The monthly values of the daily mean temperatures in Imabari City (Japan Meteorological Agency; http://

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www.jma.go.jp/jma/index.html) in September 2018 and February 2019 were 23.4°C (maximum to minimum daily temperatures, 26.5 to 20.6°C) and 7.2°C (10.7 to 3.3°C), respectively. The maximum temperature in Imabari City reached 30°C or higher on 3 days and 25°C or higher on 19 days in September 2018.

Seasonal variations were examined in 35 horses (14 mares, 11 stallions, and 10 geldings), and blood samples were collected from them during the summer and winter seasons for this purpose. The age of these horses ranged from 3 to 27 years (mean ± standard deviation [SD], 15.6 ± 6.3 years). Data concerning sex-based differences were compared in a total of 76 samples (32 mares, 23 stallions, and 21 geldings) obtained between September 2018 and February 2019. All horses who underwent blood sampling were healthy adult animals that had no apparent symptoms of clinical abnormality and were acclimated to blood sampling with mild restraint. All blood samples were taken at a prescribed morning time, approximately 2 hr after the morning feeds. The physical sizes were examined by measuring their body heights at the withers and chest and cannon circumferences, and body condition scores (BCSs) were estimated using the nine-point scale [8]. Blood samples were collected from the jugular vein and transferred to dipotassium ethylenediaminetetraacetic acid-containing tubes for hematological analyses and plain tubes for biochemical analyses. The hematological and serum biochemical analyses were performed similarly according to the method of our previous report [16]. The experimental protocols were approved by the Animal Care and Use Committee of Okayama University of Science (approval number, 2018-37).

The data analysis of this study was performed using a computer equipped with statistical software (BellCurve for Excel, Social Survey Research Information Co., Ltd., Tokyo, Japan). Data on physical size and hematological and blood biochemical parameters were expressed as the mean ± SD or median and range (from the 1st to 3rd quartiles). Our previous study determined the reference ranges for hematological and biochemical parameters by normalizing summer data [16]. In this study, the data of seasonal and sex-based groups were analyzed by nonparametric tests, respectively. Seasonal data obtained during summer and winter were compared using a Wilcoxon matched-pairs test. Differences among the three sex-based groups were analyzed by multiple group comparison using the Kruskal-Wallis and Steel-Dwass tests. Differences were considered statistically significant if the P-value was less than 0.05 and considered a tendency if the P-value was between 0.05 and 0.1.

The body sizes and laboratory test results for the summer and winter seasons are shown in Table 1. All values of the hematological and blood biochemical parameters of the Noma horses newly examined in winter were within the reference ranges reported previously for summer [16]. However, there were statistically significant seasonal and sex-based differences for some of the variables.

The ratio of chest circumference to height was higher in winter (Table 1), and this may reflect an increase in the thickness of subcutaneous and/or visceral fat in winter. BCSs in winter also tended to be higher than those in summer (P=0.0681).

A higher level of eosinophils was observed in summer (Table 1), and this was most likely associated with allergic dermatitis, as 14 out of 35 horses had mild hair loss and dermatitis with mild pruritus in summer, whereas only one horse had a skin disorder in winter. Peripheral eosinophilia has been reported to be occasionally observed in horses with culicoides hypersensitivity, a type of allergic dermatitis in horses [13].

Low values were observed for erythrocytic parameters (red blood cell counts, hematocrit, hemoglobin), urea nitrogen, albumin, and total cholesterol in summer, and this may have been associated with an increase in the energy and protein requirements of the Noma horses. A previous study reported that the baseline rate of total heat loss from horses remained stable in ambient temperatures ranging from 5 to 25°C [14]. Summer days with a maximum temperature of 25°C or higher in Imabari City may have caused the waste of energy and a decline in the nutritional condition of the Noma horses.

The sex-based body size and laboratory test results are shown in Table 2. Stallions tended to have a smaller body size compared with mares. Noma horses can be traced back to the 1600s, when the local government entrusted horse breeding to breeders to obtain larger military horses [10]. Under this policy, small horses with heights of 121 cm or less were assigned to breeders [10]. The current Noma horses originated from these small horses. Although it remains unclear whether this historical background is related to the breeding of current Noma horses, we presume that stallions with small body sizes may have been selectively used for breeding purposes to maintain the small body size of Noma horses. Moreover, the cannon circumference/body height ratio was smaller in mares. Thinner limbs may be characteristics of mares of Noma horses, although the reason for this difference is unknown.

Slightly higher counts of neutrophils and lower counts of lymphocytes were observed in stallions. Stress and endogenous or exogenous glucocorticoids are known to cause neutrophilia and lymphocytopenia in horses [6]. However, there was no proof that only stallions were exposed to stress, such as undertaking specific exercise or struggles between individual animals during the study period, but a further comparative analysis of the sexes with cortical parameters is required.

Table 1. Seasonal variations in the physical, hematological, and blood biochemistry parameters of Noma horses

Variable	Summer (n=35)			Winter (n=35)			*P*-value
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	
Body condition score	4.9 ± 1.3	5.0	4.0–6.0	5.1 ± 1.1	5.0	5.0–6.0	0.0681
Height (cm)	110.1 ± 4.7	109.0	106.3–114.3	110.2 ± 5.1	110.0	106.8–113.0	0.8726
Chest (cm)	127.8 ± 7.2	127.0	124.5–131.5	133.3 ± 8.2	133.5	128.0–138.0	<0.0001
Cannon (cm)	13.7 ± 0.7	14.0	13.0–14.0	13.9 ± 0.7	14.0	13.5–14.0	0.0127
Chest/height ratio (%)	116.1 ± 4.8	115.1	112.6–119.2	121.1 ± 6.7	120.7	117.0–123.9	0.0005
Cannon/height ratio (%)	12.4 ± 0.5	12.5	12.0–12.9	12.6 ± 0.5	12.7	12.3–12.9	0.0400
Red blood cells ($10^4/\mu l$)	721 ± 76	725	669–773	763 ± 83	764	704–837	0.0013
Hemoglobin (g/d*l*)	12.9 ± 1.3	12.8	11.9–13.9	13.8 ± 1.3	13.8	12.9–14.3	0.0003
Hematocrit (%)	35.8 ± 3.5	35.8	32.9–38.4	38.9 ± 3.8	38.7	36.2–41.3	<0.0001
MCV (f*l*)	49.8 ± 2.6	49.8	48.1–51.9	51.1 ± 2.7	50.9	49.6–52.8	<0.0001
MCH (pg)	17.9 ± 1.0	18.0	17.4–18.6	18.1 ± 1.0	18.0	17.7–18.8	0.0036
MCHC (g/d*l*)	36.0 ± 0.5	36.0	35.7–36.3	35.5 ± 0.5	35.5	35.1–35.8	<0.0001
White blood cells ($10^2/\mu l$)	73.0 ± 11.5	73.0	64.5–78.5	63.6 ± 9.2	64.0	56.0–69.5	<0.0001
Neutrophils ($10^2/\mu l$)	45.1 ± 9.9	43.1	37.8–50.3	37.6 ± 8.2	38.2	31.6–40.7	0.0003
Lymphocytes ($10^2/\mu l$)	22.2 ± 9.2	21.4	14.7–29.6	24.1 ± 7.1	24.9	18.8–28.5	0.0480
Monocytes ($/\mu l$)	81 ± 61	77	31–115	80 ± 72	65	27–111	0.9107
Eosinophils ($/\mu l$)	487 ± 468	405	165–636	101 ± 77	108	55–142	<0.0001
Basophils ($/\mu l$)	0 ± 0	0	0–0	0 ± 0	0	0–0	ND
Platelets ($10^4/\mu l$)	15.2 ± 2.6	14.9	13.8–16.8	14.5 ± 3.9	14.3	13.4–16.8	0.7955
Aspartate transaminase (U/*l*)	370 ± 86	371	307–426	378 ± 80	375	324–410	0.6761
Lactate dehydrogenase (U/*l*)	483 ± 128	474	397–563	428 ± 114	438	331–498	0.0047
Alkaline phosphatase (U/*l*)	460 ± 219	374	317–540	255 ± 55	254	217–282	<0.0001
Gamma-glutamyl transferase (U/*l*)	15.9 ± 5.0	16	13–19	17.9 ± 5.6	17	15–21	0.0116
Creatinine kinase (U/*l*)	182 ± 48	176	151–229	213 ± 65	197	175–240	0.0065
Urea nitrogen (mg/d*l*)	15.2 ± 2.3	14.8	13.4–16.9	18.2 ± 3.3	17.5	15.5–20.0	<0.0001
Creatinine (mg/d*l*)	1.02 ± 0.18	1.01	0.92–1.09	0.93 ± 0.14	0.92	0.845–1.03	<0.0001
Total protein (g/d*l*)	6.50 ± 0.51	6.4	6.2–6.8	6.52 ± 0.4	6.6	6.3–6.8	0.4761
Albumin (g/d*l*)	3.21 ± 0.3	3.2	3.1–3.4	3.74 ± 0.33	3.7	3.5–3.9	<0.0001
Glucose (mg/d*l*)	94 ± 11	95	87–102	97 ± 11	97	91–104	0.2054
Total bilirubin (mg/d*l*)	0.86 ± 0.22	0.82	0.74–0.91	0.80 ± 0.18	0.79	0.68–0.87	0.2281
Triglyceride (mg/d*l*)	37 ±18	31	24–46	38 ±19	33	26–47	0.8832
Total cholesterol (mg/d*l*)	76 ± 13	77	65–86	94 ± 15	94	86–101	<0.0001
Calcium (mg/d*l*)	12.08 ± 0.53	12.20	11.80–12.40	12.71 ± 0.58	12.80	12.45–13.00	<0.0001
Inorganic phosphorus (mg/d*l*)	2.51 ± 0.51	2.50	2.20–2.80	2.67 ± 0.51	2.50	2.40–3.00	0.0257
Magnesium (mg/d*l*)	2.14 ± 0.17	2.10	2.05–2.30	2.00 ± 0.17	2.00	1.90–2.10	0.0004
Sodium (mEq/*l*)	137.6 ± 2.4	137.9	136.4–138.9	141.0 ± 2.2	140.8	140.0–141.9	<0.0001
Potassium (mEq/*l*)	4.7 ± 0.7	4.7	4.2–4.9	4.2 ± 0.6	4.4	3.9–4.6	0.0183
Chloride (mEq/*l*)	101.8 ± 2.7	101.6	100.4–103.1	104.7 ± 1.6	104.5	103.9–105.6	<0.0001

Data are expressed as the mean ± standard deviation (SD), median, and range (from the 1st to 3rd quartiles). The probabilities of significant differences were analyzed using the Wilcoxon matched-pairs test. Differences between the seasons were considered statistically significant if the *P*-values were less than 0.05 and considered a tendency if the *P*-value was between 0.05 and 0.1. Height: height at the withers. Chest: length of chest circumference. Cannon: length of cannon circumference. n, number of horses; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; ND, not detected.

In the present study, we found considerable differences in the physical, hematological, and blood biochemistry parameters in seasonal and sex-based groups of Noma horses. These differences should be accounted for during the diagnosis of various diseases in Noma horses. However, further studies are necessary to investigate the effect of stress in seasonal and sex-based groups.

Table 2. Sex-based differences in the physical, hematological, and blood biochemistry parameters of Noma horses

Variable	Mares (n=32)			Stallions (n=23)			Geldings (n=21)			*P* value of multiple comparisons with the Steel-Dwass test			*P* value of the Kruskal-Wallis test
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mean ± SD	Median	Range	Mares and stallions	Mares and geldings	Stallions and geldings	
Age	17.8 ± 6.9	20	13–23	12.3 ± 5.6	13	10–14	14.8 ± 4.9	14	13–17	0.0223	0.3390	0.1579	0.004975
Body condition score	4.9 ± 1.4	5.0	4.0–6.0	4.7 ± 1.1	5.0	4.5–5.0	5.2 ± 0.9	5.0	5.0–6.0	1.4425	1.6062	0.5451	0.374436
Height (cm)	111.3 ± 4.9	110.0	107.8–116.0	108.1 ± 4.5	107.0	104.0–110.3	110.2 ± 4.6	109.0	108.0–114.5	0.0766	1.3211	0.5391	0.043835
Chest (cm)	132.9 ± 7.2	130.5	126.8–138.0	125.7 ± 6.5	126.0	121.0–130.0	130.9 ± 9.0	131.0	127.0–135.0	0.0064	1.6955	0.2469	0.006454
Cannon (cm)	13.6 ± 0.6	13.5	13.0–14.0	13.7 ± 0.8	13.5	13.0–14.0	14.0 ± 0.7	14.0	14.0–14.5	1.9755	0.1350	0.4428	0.081632
Chest/height ratio (%)	119.6 ± 7.0	119.1	115.5–124.1	116.4 ± 6.4	115.1	112.7–119.3	118.7 ± 4.7	119.1	115.7–121.4	0.3043	1.9767	0.3966	0.121258
Cannon/height ratio (%)	12.2 ± 0.5	12.3	11.9–12.5	12.6 ± 0.6	12.6	12.4–12.9	12.8 ± 0.3	12.8	12.5–13.0	0.0547	0.0003	1.2256	0.000248
Red blood cells ($10^4/\mu l$)	736 ± 89	740	665–772	756 ± 77	763	704–809	718 ± 77	686	661–775	1.0139	1.6085	0.4325	0.247123
Hemoglobin (g/d*l*)	13.3 ± 1.4	13.3	12.2–14.1	13.2 ± 1.4	13.6	12.2–14.3	13.2 ± 1.4	12.8	12.3–13.8	1.9088	1.7996	1.6645	0.824165
Hematocrit (%)	37.2 ± 4.0	37.2	33.9–39.7	37.2 ± 4.0	37.7	34.1–40.0	36.8 ± 4.1	35.8	34.7–38.7	1.9240	1.5033	1.3757	0.668176
MCV (f*l*)	50.7 ± 2.3	50.6	49.3–52.1	49.2 ± 2.8	49.6	46.9–50.7	51.3 ± 2.8	51.9	49.6–53.0	0.2328	0.9909	0.0595	0.023688
MCH (pg)	18.1 ± 0.8	18.0	17.6–18.6	17.5 ± 1.0	17.7	16.7–18.0	18.4 ± 1.0	18.7	17.8–19.2	0.2154	0.4345	0.0236	0.008211
MCHC (g/d*l*)	35.7 ± 0.5	35.7	35.4–36.0	35.7 ± 0.6	35.7	35.3–36.1	35.9 ± 0.6	35.9	35.7–36.3	1.9993	0.5581	0.8184	0.277701
White blood cells ($10^2/\mu l$)	73.3 ± 12.3	73.5	64.8–79.3	67.7 ± 9.1	71.0	60.0–73.5	62.5 ± 8.8	61.0	56.0–69.0	0.5679	0.0077	0.2591	0.004069
Neutrophils ($10^2/\mu l$)	42.1 ± 9.6	41.5	35.8–47.0	45.5 ± 9.8	44.0	38.7–49.9	37.1 ± 8.0	36.0	31.9–40.7	0.9720	0.2967	0.0274	0.018366
Lymphocytes ($10^2/\mu l$)	27.1 ± 6.1	27.1	22.6–30.6	18.3 ± 7.3	15.1	13.0–20.9	22.7 ± 8.1	22.1	18.5–24.9	0.0003	0.0819	0.1725	0.000092
Monocytes ($/\mu l$)	75 ± 71	70	0–93	62 ± 58	70	0–105	90 ± 68	71	54–116	1.8227	1.4953	1.091	0.566937
Eosinophils ($/\mu l$)	341 ± 484	164	71–394	331 ± 296	246	129–486	173 ± 202	112	65–152	1.5250	0.3606	0.1579	0.072598
Basophils ($/\mu l$)	0 ± 0	0	0–0	0 ± 0	0	0–0	0 ± 0	0	0–0	0.9280	ND	1.2076	0.315949
Platelets ($10^4/\mu l$)	15.2 ± 3.5	15.3	13.9–16.8	15.1 ± 2.4	14.7	13.8–17.1	14.7 ± 4.0	15.1	13.0–17.1	1.7624	1.9767	1.9138	0.892531
Aspartate transaminase (U/*l*)	373 ± 88	365	308–418	372 ± 85	365	310–403	373 ± 64	382	334–402	1.9541	1.8848	1.6647	0.861781
Lactate dehydrogenase (U/*l*)	479 ± 128	456	376–561	418 ± 126	438	308–512	490 ± 127	465	423–578	0.5000	1.7501	0.4806	0.174165
Alkaline phosphatase (U/*l*)	369 ± 206	284	257–386	383 ± 184	337	268–401	301 ± 133	267	221–337	1.4951	0.5121	0.2129	0.105242
Gamma-glutamyl transferase (U/*l*)	16.5 ± 5.4	16	13–18	16.8 ± 6.0	17	13–21	16.3 ± 4.7	15	13–20	1.8861	1.9889	1.9332	0.944128
Creatinine kinase (U/*l*)	198 ± 50	197	166–231	200 ± 74	186	161–218	196 ± 48	193	163–233	1.7130	1.9992	1.8836	0.876539
Urea nitrogen (mg/d*l*)	16.1 ± 2.4	15.2	14.8–17.4	19.4 ± 2.9	18.6	17.4–21.8	14.9 ± 2.4	14.3	13.2–15.8	0.0004	0.2962	0.0001	0.000004
Creatinine (mg/d*l*)	0.97 ± 0.18	0.95	0.88–1.09	0.91 ± 0.13	0.92	0.83–1.01	1.02 ± 0.16	1.01	0.91–1.09	0.7617	1.1500	0.1268	0.085557
Total protein (g/d*l*)	6.5 ± 0.5	6.6	6.2–6.8	6.6 ± 0.4	6.6	6.4–6.7	6.3 ± 0.4	6.3	6.1–6.5	1.9333	0.5122	0.1037	0.088852
Albumin (g/d*l*)	3.5 ± 0.5	3.4	3.2–3.8	3.4 ± 0.4	3.5	3.2–3.6	3.5 ± 0.3	3.5	3.2–3.6	1.5945	1.9868	1.6394	0.773169
Glucose (mg/d*l*)	91 ± 9	92	86–96	102 ± 12	99	95–109	97 ± 8	99	95–103	0.0129	0.0576	1.1797	0.00276
Total bilirubin (mg/d*l*)	0.82 ± 0.22	0.78	0.67–0.89	0.81 ± 0.10	0.82	0.75–0.85	0.87 ± 0.24	0.80	0.73–0.94	1.5550	1.4701	1.8399	0.674986
Triglyceride (mg/d*l*)	39 ± 21	34	26–47	36 ± 14	34	25–46	36 ± 17	30	26–45	1.9239	1.6866	1.9781	0.871968
Total cholesterol (mg/d*l*)	88 ± 16	86	79–96	75 ± 15	75	63–88	88 ± 16	89	72–97	0.0379	1.9985	0.0612	0.010983
Calcium (mg/d*l*)	12.4 ± 0.7	12.4	12.1–12.8	12.3 ± 0.7	12.4	12.0–12.9	12.4 ± 0.4	12.2	12.2–12.8	1.9993	1.9889	1.8747	0.988021
Inorganic phosphorus (mg/d*l*)	2.4 ± 0.4	2.4	2.1–2.6	2.7 ± 0.6	2.7	2.4–3.0	2.7 ± 0.5	2.7	2.3–3.0	0.0928	0.1113	1.9133	0.020135
Magnesium (mg/d*l*)	2.1 ± 0.2	2.1	2.0–2.1	2.1 ± 0.2	2.1	1.9–2.3	2.1 ± 0.1	2.1	2.0–2.1	1.6740	1.9999	1.7951	0.840129
Sodium (mEq/*l*)	139.5 ± 3.0	139.8	138.1–140.9	138.9 ± 3.1	138.4	136.8–141.0	139.5 ± 2.2	139.4	138.0–140.8	1.4118	1.9795	1.4769	0.675043
Potassium (mEq/*l*)	4.5 ± 0.6	4.5	4.1–4.8	4.6 ± 0.6	4.6	4.4–4.9	4.2 ± 0.7	4.1	3.8–4.4	1.5053	0.1638	0.0558	0.024612
Chloride (mEq/*l*)	103.9 ± 2.5	104.0	103.1–105.4	102.3 ± 3.0	102.5	100.4–104.0	103.6 ± 1.9	102.9	102.6–104.7	0.1620	1.1966	0.4414	0.063958

The data are expressed as mean ± standard deviation (SD), median, and range. Ranges represent the 1st to 3rd quartiles. Statistical analyses were conducted using multiple group comparisons with the Kruskal-Wallis and Steel-Dwass tests. Differences between the sex-based groups were considered statistically significant if the *P*-values were less than 0.05 and considered a tendency if the *P*-value was between 0.05 and 0.1. Height: height at the withers. Chest: length of chest circumference. Cannon: length of cannon circumference. n, number of horses; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean hemoglobin concentration; ND, not detected.

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