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Hematological and Biochemical Reference Values for the Endangered Kiso Horse

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To establish blood and biochemical references for the endangered Kiso horse, blood samples were collected from 111 adult Kiso horses, 74.5% of the existing breed. The samples were analyzed for 23 hematological and biochemical parameters to determine their means and standard deviations (SD). We compared the mean ± 2SD with the reference values cited in one of the most commonly used veterinary textbooks in Japan. The hematology of Kiso horses is characterized by lower erythrocyte count and hematocrit and hemoglobin levels. In addition, their serum biochemistry showed lower levels of aspartate transaminase, alkaline phosphatase, and γ-glutamyl transferase. Whether these propensities are attributed to breed-specific factors or are acquired factors remains unclear. Nevertheless, this study provides useful diagnostic indices for the endangered Kiso horse.

Key words: *biochemistry, hematology, Kiso horse, references*

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The Kiso horse is a breed of Japanese native horse that is raised in the area spanning the Kiso region of Nagano Prefecture to the Higashimino region of Gifu Prefecture [6, 10, 16–18]. It is valuable not only as a Japan-specific genetic resource, but also as a living cultural asset of regional culture. However, the number of these horses has decreased considerably owing to mechanization after the war [4, 5], and it is endangered, with a population of around 150. To properly preserve the Kiso horse, the Kiso Horse Conservation Association and academic institutions have been working together to perform assessments of their genetic diversity and the cryopreservation of reproductive cells [16, 17].

A major challenge in the conservation of rare animals is to conserve the genetic diversity of the population [17]. However, because the population consists of individual animals, proper management of the population requires appropriate care of each individual. Therefore, veterinary clinical research that enables accurate diagnoses and treatments for each individual rare animal is considered to play an important role in the conservation of rare species or breeds.

Because house visits are typical for the primary care of indigenous horses, it is difficult to use large diagnostic devices such as CT and MRI. Therefore, diagnosis of disorders mostly relies on data obtained from blood and urine samples. However, the current standard values for such blood and urine tests are mainly compiled from data obtained from light breed horses, and these cannot always be applied to different breeds or horses with different uses [2, 14, 15, 19]. Thus, if a veterinary practitioner does not have reference laboratory values for the exact horse they are examining, diagnostic errors can occur. Therefore, we collected blood samples from 111 adult Kiso horses aged over 3 years old, accounting for 74.5% of the existing Kiso horse population, and determined the mean and standard deviation (SD) of 23 parameters in order to establish hematological and biochemical references for the endangered Kiso horse.

In this study, we collected samples from 111 Kiso horses aged over 3 years old that were confirmed as Kiso horses by the Kiso Horse Conservation Association (10 males, 15 geldings, and 86 females). Their age rang was 3–29 years

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

Paremeters	Kiso horse* n=116	General horse**
Leukocyte ($10^2/\mu l$)	82.7 ± 21.2	93.0 ± 13.0
Erythrocyte ($10^4/\mu l$)	715.2 ± 105.4	940.0 ± 81.0
Hemoglobin (g/d*l*)	11.6 ± 1.5	15.3 ± 1.4
Heamatocrit (%)	32.9 ± 4.0	42.2 ± 4.0
Total protein (g/d*l*)	6.9 ± 0.6	6.2 ± 0.4
Albumin (g/d*l*)	3.1 ± 0.3	3.6 ± 0.3
Creatine kinase (U/*l*)	305.3 ± 168.8	260.0 ± 186.0
Aspartate aminotransferase (U/*l*)	276.5 ± 62.0	374.0 ± 198.0
Lactate dehydrogenase (U/*l*)	430.5 ± 160.5	550.0 ± 134.0
Alkaline phosphatase (U/*l*)	630.3 ± 301.2	228.0 ± 31.0
Gamma-glutamyl transferase (U/*l*)	15.0 ± 5.4	23.0 ± 12.0
Creatinine (mg/d*l*)	1.2 ± 0.3	1.4 ± 0.2
Urea nitrogen (mg/d*l*)	12.2 ± 4.8	12.7 ± 2.9
Glucose (mg/d*l*)	83.7 ± 13.2	104.0 ± 26.0
Triglyceride (mg/d*l*)	16.4 ± 11.1	24.0 ± 11.0
Total choresterol (mg/d*l*)	76.6 ± 14.2	83.0 ± 14.0
Sodium (mEq/*l*)	136.7 ± 2.4	140.0 ± 3.0
Potassium (mEq/*l*)	4.6 ± 0.7	3.9 ± 0.5
Chloride (mEq/*l*)	102.6 ± 2.8	99.0 ± 4.0
Magnesium (mg/d*l*)	2.0 ± 0.3	2.0 ~ 2.8
Calcium (mg/d*l*)	12.0 ± 0.9	11.8 ± 0.5
Phosphorus (mg/d*l*)	3.1 ± 0.7	3.7 ± 0.6
Total bilirubin (mg/d*l*)	0.7 ± 0.2	1.0 ~ 2.0

*Data of the Kiso horse are shown as mean ± 2SD. **Excerpted from Textbook of Veterinary Internal Medicine [7]. The data are shown as mean ± SD.

old with an average of 16.4 ± 6.7 years old. We selected Kiso horses aged over 3 years because some blood biochemistry markers such as alkaline phosphatase vary between young and adult animals. The animals were kept by zoological parks and equestrian clubs in herds of several animals, which were fed with a mixture of roughage and concentrate feed, and horses kept by private owners, singly or in pairs, which were mostly fed with weed–based roughage.

Blood samples (approximately 10 m*l* each) were collected from the jugular vein. Of this, 2 m*l* was placed into an EDTA blood collection tube, 1 m*l* was placed into a sodium fluoride tube, and the rest was placed into a serum separation tube. These samples were used for analysis of complete blood count, plasma glucose measurement and biochemical tests. The hematological parameters, including the leucocyte count, erythrocyte count, and hemoglobin and hematocrit levels, were measured using a Celltac Auto Analyzer (Nihon Koden, Tokyo, Japan). Biochemical parameters, including total protein, albumin, creatine kinase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, γ-glutamyl transferase, creatinine, urea nitrogen, glucose, triglyceride, total cholesterol, sodium, potassium, chloride, magnesium, calcium, phosphorus and total bilirubin levels, were measured using a Hitachi 7700 Auto Analyzer (Hitachi High Technologies Co., Tokyo), and the mean and SD were calculated for each parameter (Table 1). The mean ± 2SD were used as reference values for the Kiso horse, and they were compared with the standards for horses listed in the Textbook of Veterinary Internal Medicine [7], a textbook commonly referred to by large animal veterinarians. We chose this textbook for comparison since most Kiso horses are treated by large animal veterinarians, who usually treat cows.

The hematological profiles of the Kiso horse are characterized by low erythrocyte count, hemoglobin levels, and hematocrit levels (715.2 ± 105.4 × 10^4 erythrocytes/μl, 11.6 ± 1.5 g/d*l*, and 32.9 ± 4.0%, respectively) compared with the standard values for general horses (940.0 ± 81.0 × 10^4 erythrocytes/μl, 15.3 ± 1.4 g/d*l*, and 42.2 ± 4.0%, respectively). In addition, the biochemical profiles of Kiso horses are characterized by lower levels of hepatobiliary enzymes: the levels of aspartate transaminase, alkaline phosphatase, and γ-glutamyl transferase (276.5 ± 62.0, 630.3 ± 301.2 and 15.0 ± 5.4 U/*l*, respectively) were lower than the standards for general horses (374.0 ± 198.0, 228.0 ± 31.0 and 23.0 ± 12.0 U/*l*, respectively).

Judging from the reference values of the general horses in the textbook [7], the hematology of the Kiso horses could

be considered an anemic state. However, none of the horses examined showed any anemic symptoms. It is a reasonable supposition that the erythrocyte count and hemoglobin and hematocrit levels as well as hepatobiliary enzyme levels of the Kiso horse should be lower than those of Thoroughbreds and other horses, since the latter types of horse are bred for the purpose of racing and competitions and are likely to consume nutritious feed. Thus, the results for the Kiso horse might be attributable to their weed-based roughage feeding. However, a previous report indicated that the erythrocyte count of the Kiso horse is lower than those of Thoroughbred, Arabian, Korean native, and Hokkaido native horses. The authors of that report concluded that these lower values are normal for the Kiso horse [12], and that these profiles might be specific characteristics of the Kiso horse, which has been raised on poor land for a long time. Whether these characteristics, which differ from those of general horses, are attributable to breed-specific factors or are acquired factors remains unclear, but we were at least able to identify the hematological and biochemical traits of the Kiso horse.

Considering the laboratory values obtained in this study and the clinical conditions assessed during the investigation, the reference values obtained were in agreement with actual clinical conditions. For example, there were no horses with anemia-related conditions such as collapsing; severe abraded wounds due to summer itch were found in horses with higher leucocyte counts than the reference value; and a horse with higher blood urea nitrogen and creatinine levels than the reference level, which indicated polyuria/polydipsia, died a few months after the investigation. These findings suggest that the reference values obtained in this study, together with the standards for general horses in the literature, can be used for Kiso horses in clinical settings as certain criteria for diagnosis.

Various factors, including breed, sex, age, and state of training, affect the hematological and biochemical traits of horses [1, 3, 8, 9, 11, 13]. Racehorses and riding horses have been mainly improved for their ability to adapt to races and competitions, and they have been specially trained and maintained for this purpose. Athlete horses such as light breed horses, may be unique compared to native horses. Therefore, it is unlikely that the hematological and biochemical standards that have been mainly obtained from data collected from light breed horses are applicable to all native horses.

In this study, we established specific hematological and biochemical reference values for the Kiso horse. These reference values are different from the reference values given in the literature. It is difficult to determine whether the values for the Kiso horse that are different from the standards for general horses are attributable to fixed breed-specific factors, or are factors acquired through feed or training. However, this study undoubtedly developed useful clinical diagnostic indices for the Kiso horse. The use of these reference values in veterinary practice and proper management of Kiso horses will contribute to their survival, enabling an indigenous genetic resource that bears regional culture to be passed down to future generations.

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