Serum bromine concentrations in horses in Japan

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ABSTRACT. This study investigates bromine (Br) concentration and its relationship with iodine concentration in serum samples of 86 horses. The mean serum Br concentration in horses pastured on green grass near the seashore was significantly higher \((P<0.001)\) than that in horses pastured in a sand paddock. A significantly negative correlation \((r=-0.479, P<0.01)\) between the serum Br and iodine concentrations was evident in the horses that pastured on green grass. The concentrations of several elements such as sodium and potassium were virtually constant in the serum. In addition, there were elements present below the detection limit of the analytical instruments used. In contrast, it was suggested that geological differences have a marked influence on serum Br concentrations in animals. Thus, we hypothesized that serum Br concentration in horses is a possible indicator reflecting geological differences.

KEY WORDS: bromine, horse, iodine, monitoring

The essentiality of bromine (Br) in animals has long been a subject of discussion. Although Br exists in various animal tissues [4], studies have yielded conflicting results regarding its essentiality [13]. In a recent study using drosophila, McCall et al. [7] demonstrated that Br is an essential element in animals. Moreover, Br is a breakdown product of methyl bromide and has been found in imported foods [16]. Owing to its wide efficacy and easy use, methyl bromide has been used in quarantine fumigation and soil disinfection processes. However, its use has a large impact on the ozone layer and therefore was subjected to rigid control by the Montreal Protocol on Substances that Deplete the Ozone Layer [21]. Some chemicals containing Br are gasoline and fire retardant materials. Because several studies have found serum Br concentration to be an indicator of tracing the source of Br compounds [18, 19], Br analysis in animals is supposedly crucial for understanding both its contamination and essentiality. However, sufficient data pertaining to serum Br concentrations in animals remain elusive.

We previously reported that the mean serum iodine concentration in horses living in areas with higher environmental iodine contents [5] was completely higher compared with that in horses living in other areas [9]. Therefore, we suggested that estimating the serum iodine concentration in horses is helpful in environmental monitoring. Single elements in biological samples, including those of the liver, kidney, and hair of animals, were also investigated as a parameter for environmental monitoring [8, 10, 12]. Additionally, the fact that homologous elements exhibit similar chemical properties is well-known and supported by studies describing the relationship among homologous elements in various biological samples. Kuzuhara et al. [6] reported a significant correlation \((P<0.01)\) between the concentrations of the homologous elements cadmium (Cd) and zinc (Zn) in the renal tissue of a human living in an area polluted by Cd. A similar significant correlation \((P<0.01)\) was observed in the renal tissues of humans living in an area not polluted by Cd; however, a significant correlation \((P<0.01)\) between Cd and Zn concentrations in the pancreas was observed only in humans living in the area polluted by Cd. The breeding areas of wild birds were also classified based on the relationship between molybdenum and chromium concentrations in their liver samples [11]. As explained in previous reports, in some cases, geological differences can be elucidated using a combination of homologous elements found in biological samples in some cases. Since Br is a homologous element of iodine, we presumed that the combination of serum Br and iodine concentrations has a potential use as a biomarker of geological differences. To elucidate the efficacy of Br as a biomarker, this study aimed to investigate serum Br concentrations in horses from various areas of Japan.
MATERIALS AND METHODS

Serum samples

Serum samples of a total of 86 horses were used in this study. As presented in Table 1, serum samples were collected from six equestrian clubs in four prefectures located on the main island of Japan. Sixty-nine of the 86 horses in this study are horses from the same five equestrian clubs that we used in our previous study to examine T4 and iodine concentrations [9]. Thus, this study includes further analysis of the horses from the three equestrian clubs in Chiba (Chiba 1, 2 and 3; n=15, 12 and 5, respectively) and from one equestrian club each in Saitama (n=12) and Shizuoka (n=25) described in our previous report [9]. In addition, 17 horses (n=17) from Yamanashi were included in the present study. Among the 25 horses housed at the equestrian club in Shizuoka, 22 were pastured on green grass (Shizuoka-a), while the other three were pastured in a sand paddock (Shizuoka-b) because of surgical lameness. Horses housed at the five equestrian clubs in Chiba, Saitama, and Yamanashi Prefectures were also pastured in sand paddocks. Samples were obtained from horses between December 2011 and September 2013.

Approximately 20 ml of blood samples were transferred into vacuum blood collection tubes (Venoject® II, Terumo Corp., Tokyo, Japan). Blood samples collected from Shizuoka were transported to the Shizuoka Institute of Science and Technology and those collected from Saitama, Yamanashi, and Chiba were transported to the School of Veterinary and Technology, Nippon Veterinary and Life Science University. On the same day of collection, serum was separated by centrifugation (3,000 rpm, 15 min) and was transferred to centrifuge tubes (Eppendorf). Until analysis, these tubes were stored in a deep freezer (Panasonic Healthcare Co., Ltd., Tokyo, Japan) at −30°C. Although horse serum has been studied in a previous dissertation [20], we approached this investigation from a different perspective.

Analytical methods

All serum samples were transferred to plastic tubes and transported on ice to the Tama Laboratory of the Japan Food Research Laboratories for the analysis of elemental concentrations. A volume of 0.1 ml of each sample was diluted using 1% nitric acid (Kanto Kagaku, Tokyo, Japan) to a final volume of 10 ml. The elemental Br and iodine concentrations were determined with a semi-quantitative analysis using an inductively coupled plasma mass spectrometer (ICP-MS, 7500 ce, Agilent Technologies, San Diego, CA, U.S.A.). The operation conditions of ICP-MS were as follows: radio frequency power, 1,600 W; carrier gas flow rate, 0.7 ml/min; plasma gas flow, 15 l/min; sampling depth, 8.0 mm; acquisition mode, spectrum peak hopping; and integration time, 0.1 sec/point. The mass-to-charge ratio of Br and iodine was 79 and 127, respectively, and the recovery rate was 105 ± 5.8% and 92.5 ± 5.8%, respectively (mean recovery value for spikes ± standard deviation, n=3).

Statistical analysis

Lotus 2001 and Excel 2016 software were used for data analysis. The elemental concentrations in the serum are represented as mean ± standard error of the mean. The analysis for significant differences in the elemental concentrations was conducted using the Dunnett’s multiple comparison test (SPSS Statistic 19, IBM Japan, Tokyo, Japan). The statistical significance of a correlation (Spearman’s rank correlation) was analyzed using SPSS Statistics 19 (IBM Japan) software. A P value of <0.05 was considered statistically significant.

RESULTS

The serum Br concentrations in horses are summarized in Fig. 1. As shown in the figure, the mean serum Br concentration (60.36 µg/ml) in horses pastured on green grass in Shizuoka (Shizuoka-a) was significantly higher (P<0.001) than that in other horses. The mean serum Br concentration in horses pastured in sand paddocks (Chiba 1, 2, and 3, Saitama, and Yamanashi) was <15.92 µg/ml. The mean serum Br concentration (11.00 µg/ml) of horses pastured in sand paddocks in Shizuoka (Shizuoka-b) were similar to those of horses pastured in sand paddocks at other locations.

The difference between the results of horses in Shizuoka-a and those of other horses became clearer when the relationship between Br and iodine concentrations was investigated (Fig. 2). A significantly negative correlation (r=−0.479, P<0.01) was found.
between serum Br and iodine concentrations in horses except for those pastured on green grass in Shizuoka-a. The distribution area of the horses in Shizuoka-a was completely different, as shown in Fig. 2. The results of horses in Shizuoka-b in Fig. 2 were similar to those of horses pastured in other sand paddocks.

**DISCUSSION**

In this study, we investigated serum Br concentrations in horses to determine the use of Br concentrations as an indicator in environmental monitoring.

Direct analysis using instruments such as inductively coupled plasma atomic emission spectrometer and atomic absorption spectrometer is possible with diluted serum samples, even though an ashing process is usually necessary for the preparation of tissue samples from the kidney and liver. The use of serum samples can reduce the time taken to prepare samples, with little risk of sample contamination. Sometimes, the study of elements is difficult depending on the selected elements in the serum; the concentrations of various elements such as sodium and potassium are virtually constant in serum, while many elements such as Cd accumulate in the kidney and liver [3]. In particular, Razi *et al.* [14] suggested that serum levels of metals were similar in exposed and unexposed subjects. In contrast, our results showed that the relationship between serum Br and serum iodine concentration in animals are indicative of the environment and feeding aspect of animals.

Although we found few studies describing serum Br concentrations in domestic animals, studies on humans have reported ranges of 3.2–5.6 µg/ml [2]. A similar tendency has been reported in another study too [22]. Sakurai [17] suggested that the mean serum Br concentration in healthy humans is 5 µg/ml; however, Wielopolski *et al.* [23] reported higher Br concentrations (approximately
12 μg/ml). Although serum Br concentrations in horses in our study were slightly higher than those in humans, our results are supported by those of Abuelo et al. [1], who also reported higher serum Br concentrations (range: 2.5–53.1 mg/l) in cattle. In this study, the mean serum Br concentration in horses pastured in sand paddocks was <15.92 μg/ml. A higher serum Br concentration (Shizuoka-a, mean: 60.36 μg/ml) was found in horses from the equestrian club in Shizuoka Prefecture. This result was significantly higher (P<0.001) than that of other horses. In our previous report [9], a higher mean serum iodine concentration was obtained in horses housed in the equestrian club in Chiba 1 (mean: 29.33 μg/l). The mean serum iodine concentrations were found to be in the order of Chiba 1 > Chiba 2 = Chiba 3 > Yamanashi (newly added data in the present study, 23.80 ± 1.01 μg/l) > Saitama = Shizuoka (including Shizuoka-a and Shizuoka-b in the present study) [9]. In contrast, this study showed an opposite trend for Br compared with that for iodine.

Br is ubiquitous in nature and its content in soil and land plants is reportedly 5 and 15 mg/kg, respectively [13]. Furthermore, the club in Shizuoka is located near the seascape and is sometimes enveloped in sea fog. Br content of saltwater is high; that of ocean water is reportedly 65 mg/l, whereas that of salt lakes can be as high as 5,600 mg/l [13]. Yuita [25] reported that Br contents in soils and plants were higher in coastal areas (distance from the sea; 0–25 km) than in inland areas (distance from the sea; >75 km). Yamasaki et al. [24] also suggested that the geometric mean of Br is the highest in coastal marine sediments. Therefore, it is inferred that serum Br concentrations in horses pastured (Shizuoka-a) on green grass may have been influenced by the environment. The mean Br concentration of Shizuoka-b and its relationship with serum iodine in the three nonpastured horses in the Shizuoka Prefecture validated our hypothesis. Alternatively, as discussed in our previously published paper [9], horses housed in Chiba 1 were provided feed supplemented with seaweed. Depending on its type, seaweed is known to contain Br of various concentrations [15]. However, because a significantly negative correlation (r = −0.479, P<0.01) exists between serum Br and iodine concentrations in horses, except for those pastured on green grass in Shizuoka, we suggest an antagonistic action of serum iodine in horses with similar breeding conditions. The present result demonstrates that a difference in serum Br concentrations was reported in horses according to geological differences. Thus, it was assumed that Br itself and the combination of serum Br and iodine concentrations in horses are possible indicators of environmental conditions.

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