The Journal of Veterinary Medical Science

FULL PAPER

Wildlife Science

Usefulness of noninvasive blood pressure measurement in captive Red Panda (*Ailurus fulgens*)

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ABSTRACT. The red panda (*Ailurus fulgens*) can be found in zoos around the world, and various behavioral restraint procedures are used as part of their health care. Wild animals that are kept in zoos, including red pandas, are known to have a longer life span than those in the wild. Therefore, the health management of aging animals is considered especially important for zoos that maintain many precious wild animals. Blood pressure measurement is important for determining cardiovascular dynamics, however there are no reports of blood pressure measurements performed in red pandas without anesthesia. In this study, we measured blood pressure in four red pandas, over 4 years to establish a blood pressure measurement method using behavioral restraints. As a result, the blood pressure of red pandas was found to be similar to that of dogs and cats. In addition, in one case of red panda that evaluated high blood pressure during the measurement period, we added the antihypertensive drug and showed good effect for improvement of hypertension on long- term monitoring. Blood pressure values obtained using noninvasive methods were useful for red pandas. Moreover, these data were considered important for animal welfare.

KEYWORDS: blood pressure measurement, health management, husbandry training, noninvasive, without anesthesia

J Vet Med Sci
86(11): 1212–1218, 2024
doi: 10.1292/jvms.24-0060

Received: 8 February 2024
Accepted: 23 September 2024
Advanced Epub:
11 October 2024

The red panda (*Aiurus fulgens*) is an animal native to Sichuan Province, China, and eastern Nepal, and is divided into two subspecies, *Ailurus fulgens fulgens* and *Ailurus fulgens styani* [26, 33]. The red panda is listed as endangered on the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) [21].

In recent years, behavioral restraints for care have been used in many zoos worldwide, including Japan [4, 8, 22, 24, 27]. Weight measurements, crate training in the transport box, palpation, echography, and vaginal smear collection could be performed in the management of red pandas [4, 16]. Behavioral restraint is defined as the use of at least one form of husbandry training, desensitization and operant conditioning to perform or facilitate a procedure [22]. Management of animals using behavioral restraint would reduce hazards and make the process safer for both the animals and keepers. Husbandry training is also considered a method for improving animal welfare [27].

Blood pressure measurement with oscillometric methods were common method for diagnosing any vasocardiac disease in dogs, cats and human [1, 11, 15, 17, 28, 29, 37]. It was useful for the estimation of the vascular and peripheral hemodynamic status that could not be performed by echocardiography and electrocardiogram. Moreover, it was usual in predicting organ damage associated with hypertension and evaluation of treatment efficacy [1, 5, 6, 8]. Additionally, blood pressure measurement was easy to introduce as the measurement technique was simple and there were few differences in values even if the measurer changed. However, blood pressure measurements in zoo animals have rarely been reported. Several of these reports were performed under anesthesia [3, 35]. Anesthetics such as isoflurane and sevoflurane greatly affect blood pressure [5, 30]. Both anesthetics induced tachycardia and a dose-dependent decrease in mean aortic blood pressure [5]. Therefore, blood pressure measurements under anesthesia were useful as intraprocedural monitoring points [1, 3, 5] but did not reflect the true physiological state of the animal [23]. Although measurements under anesthesia could not be performed frequently, measurements with behavioral restraints could be frequently without stressing the animal. Given

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(Supplementary material: refer to J-STAGE: https://www.jstage.jst.go.jp/browse/jvms/)

these considerations, blood pressure measurements with behavioral restraints may be useful in the health care of zoo animals.

To date, blood pressure measurements without the use of anesthesia in zoo animals have been reported only for a limited number of species (such as great apes [8] and lion [34]), and there were no reports for red pandas. In this study, we tried blood pressure measurements of four red pandas under behavioral restraint using husbandry training for over 4 years (Period 2017–2021). In the middle of the measurement period, serum atrial natriuretic peptide (ANP), a diagnostic biomarker of heart disease, was also measured periodically in all animals to clarify the pathophysiology of hypertension [18, 19].

This study adheres to the Japanese Association of Zoos and Aquariums Ethics and Welfare Guidelines and Caring for Wildlife: The World Zoo and Aquarium Animal Welfare Strategy.

MATERIALS AND METHODS

Four red pandas were used in this study. Monthly blood tests, weight measurement, auscultation and palpation using husbandry training were performed from the start of the study. Blood test parameters include Total Protein (TP), Albumin (ALB), Glucose (GLU), Blood Urea Nitrogen (BUN), Creatinine (CRE), Alanine Aminotransferase (ALT), Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Total cholesterol (T-CHO), Calcium (Ca) and Inorganic Phosphorus (IP) used in general health check-ups. In the middle of the measurement period, we performed ANP analysis (Detaminer CLANP, FUJIFILM VET Systems Co., Ltd., Tokyo, Japan) three times approximately every six months. All blood was collected from the lateral saphenous vein. And samples for ANP measurement were frozen quickly. Irregular urinary tests were also performed. There were no current illnesses or notable clinical symptoms of them.

Case A was a male, 8 years old and body weight was 5 kg. Case B was a male, 8 months old and weight of adult was 5.4 kg. Case C was a female, 14 years old and body weight was 6 kg. Case D was a female, 3 years old and body weight was 6.5 kg. Each age was the start of measurement (Table 1). Body weight did not change significantly over the 4 years. Because the amount of food fed was adjusted according to the measurement results except for B who was in the growth phase. The habitat was an indoor room and an indoor/outdoor exhibition area, and the temperatures were controlled according to outside. Blood pressure was measured as a part of health management. Measurements were taken once a month between 9:00 a.m. and 12:00 p.m. [15] in the indoor room, and weather information (e.g. temperature and humidity) was recorded at the time of measurement. Positive reinforcement was used for training for the measurements. In addition, feeding restrictions were not enforced for training. The unconditioned reinforcer during measurement was an apple cut into 1-cm cubes and delivered with tweezers. A dog whistle (ACME Silent Dog Whistles AC-535GP; J. Hudson and Co. (Whistles) Ltd., Birmingham, UK) was used as the conditioned reinforcer, which was paired with the unconditioned reinforcer. The measurement was performed in the standing position, and the measurement site was the region of the tail base, which was also used for dogs and cats [15] to avoid bites and other accidents between the animal and the measurer. Two workers participated in the measurement: a feeder who guided the animal to the platform and fed it, and a measurer who attached the cuff to the region of the tail base and performed the measurement (Fig. 1). The measurement protocol was as follows.

Fig. 1. The measurement using behavioral restraint. Pair measurement stuffs were participated: the feeder, who guided the animal to the platform and fed it, and the measurer, who attached a cuff to tail base and performed the measurement.

Table 1. Blood pressure values, measurement intervals, and physical data for each case (Period 2017–2021)

Case	Sex	Age*	Weight (kg)	Measurement period (month)	Systolic blood pressure (mmHg)	Mean blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Heart rate (BPM)	No. of measurements
A	M	8	5	51	173 ± 16.2	136 ± 12.9	103 ± 10.6	149 ± 21.2	36
B	M	0.8	5.4**	31	147 ± 17.8	118 ± 15.0	89 ± 16.3	144 ± 18.3	27
C	F	14	6	51	164 ± 19.5	135 ± 16.0	105 ± 17.1	158 ± 23.2	36
D	F	3	6.5	51	143 ± 17.5	115 ± 13.5	87 ± 12.5	138 ± 11.7	33
Average					158 ± 21.6	124 ± 16.4	96 ± 16.1	147 ± 20.0	–

*Start of measurement, **weight of adult.

1. Pairing procedure of the unconditioned reinforcer (apples) and the conditioned reinforcer (whistles). 2. Installation of a training-only platform in the indoor room for acclimatization. 3. Using apples to guide to the training-only platform and put on the platform. 4. Touching the region of the tail base. 5. Moistening the region of the tail base with water to brush off hairs. 6. Grasping the region of the tail base with the hand. 7. Maintaining the grip. 8. Wrapping the cuff around the region of the tail base. 9. Maintaining the position of the cuff on the region of the tail base. 10. Applying pressure with the cuff on the region of the tail base. In order to reduce body movements during the measurements, the feeder was required to feed in the same place and in a constant rhythm.

Each red panda allowed the measuring blood pressure in a short period. We used a sphygmomanometer for animals (petMAP graphic II; CardioCommand, Inc., Tampa, FL, USA) to measure blood pressure [2, 7]. The cuff size was 5.5 cm, which was the most stable in terms of generating numerical values at approximately 42–50% the width of the circumference of the region of the tail base. Measurements were taken three times consecutively for approximately 5 min. We obtained four measurement items: systolic blood pressure (SYS), mean blood pressure (MAP), diastolic blood pressure (DIA), and heart rate (HR). For SYS and DIA, we used petMAP's unique function of the Nominal Session Value (NSV). This was not a simple average of the blood pressure measurements but was calculated after eliminating any abnormally high and low values [8, 31].

After all measurements, we used IBM SPSS Statistics version 20 (IBM, Armonk, NY, USA) and Microsoft Excel 2019 (Microsoft, Redmond, WA, USA) for statistics.

RESULTS

All animals could measure blood pressure safety (Table 1, Supplementary Tables 1–4). During the measurement period, the four animals also underwent a veterinary check including a blood test and urinary test for health management. As a result, the general condition of all four animals was stable (Supplementary Fig. 1, Supplementary Table 5).

Cases A and C showed high blood pressure rather than B and D. In particular, Case A continued to have elevated SYS from the middle of the study compared with cases B and D (Table 2, Fig. 2). We suspected atrial capacitance overload, and plasma ANP was measured as 194.4 pg/mL (Table 3). Consequently, we treated telmisartan (Micardis Tablets 40 mg, Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan) 1 mg/kg/sid for A [1, 14]. After treatment, blood pressure values tended to be lower. ANP levels also decreased to the canine reference range [18] (Table 3), and case A remains under observation.

In addition, we examined Correlation coefficients between body weight and blood pressure values. However, all animals showed little variation in body weight, and no correlation was found in the present data (Fig. 3). Furthermore, there was no seasonal variation in the measured values in B and D. B and D were not medicated related to blood pressure during the measurement period. (Fig. 4, Supplementary Fig. 2).

DISCUSSION

Comparing the four cases, the blood pressures of B and D were similar to those reported for dogs and cats with some scattering [11, 15, 17, 20, 28, 29]. Blood pressure values for dogs are as follows: systolic pressure 132.0 ± 17.4 mmHg, diastolic pressure 86.6 ± 14.2 mmHg, and mean blood pressure 106.2 ± 14.5 mmHg [11].

Oscillometric procedures were noninvasive methods [11, 15, 17, 25, 28, 29]. Thus, it was thought that it was easy to take on blood pressure measurements by behavioral restraint as part of the healthcare management for red pandas.

Measuring blood pressure at rest was desirable because blood pressure was easily raised by excitement [11, 28]. However, some agitation was observed because feeding was performed during the measurement. In such cases, we waited until the animals calmed down. Thus, it was thought that the measured values remained almost stable in this study.

In humans, dogs and cats, blood pressure elevated with age [6, 8, 10, 12, 13]. And zoological animals have a longer life span than wild animals [36]. From these findings, routine blood pressure measurement could help maintain the health of older animals. In dogs and cats, routine blood pressure measurement was recommended because it is considered an important parameter for the diagnosis of hypertension [1]. In this study, Case A showed hypertension at the age of 11 years during the measurement period. And case C, who was older from the beginning, showed persistent hypertension. The cause of hypertension in cases A and C was unknown, but they were older than cases B and D [16]. Age-related fibrosis of the cardiac interstitium and atherosclerosis have been reported in

Table 2. Mean blood pressure values for the two cases (Case B and D) assessed as healthy

	Mean ± SD	95% Confidence interval	95% Trimmed average
SYS (mmHg)	145.06 ± 2.27	140.51–149.61	144.34
DIA (mmHg)	87.95 ± 1.84	84.27–91.63	87.25
MAP (mmHg)	116.41 ± 1.83	112.75–120.07	115.38
HR (BPM)	141.15 ± 1.96	137.22–145.07	141.97

SYS: systolic blood pressure, MAP: mean blood pressure, DIA: diastolic blood pressure, HR: heart rate.

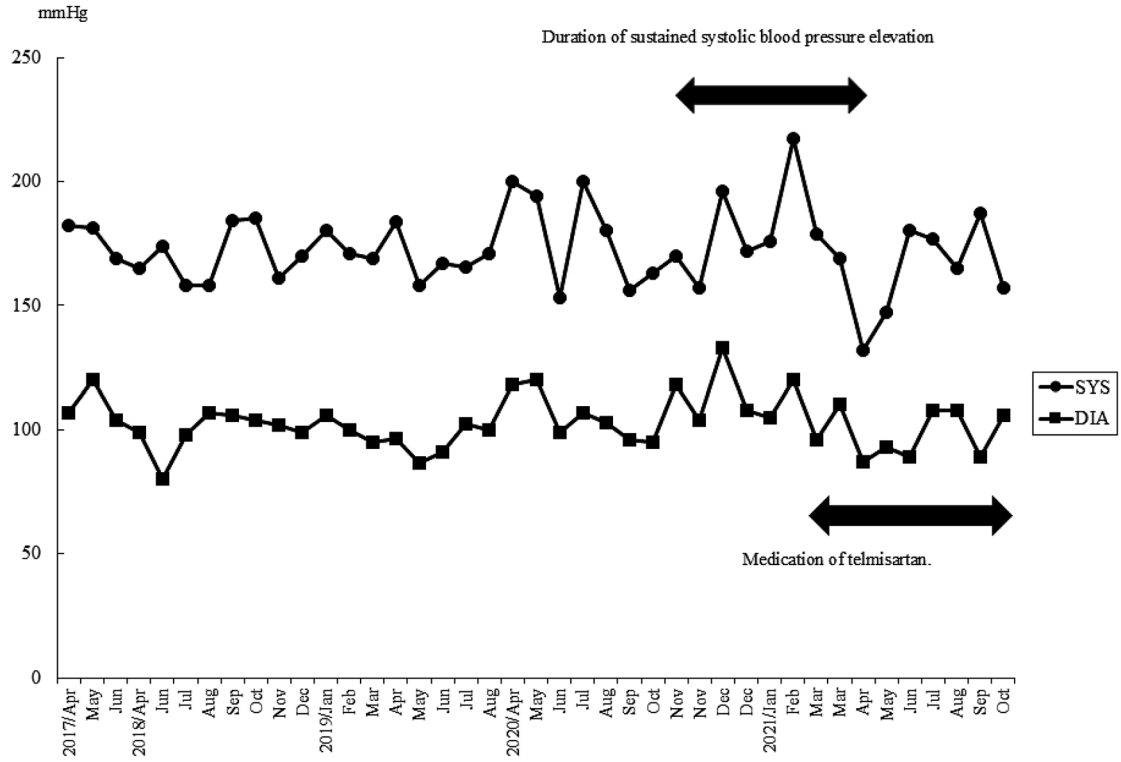

Fig. 2. Changes in blood pressure values in Case A. Case A continued to have elevated SYS from the middle of the study compared with cases B and D. Analyzes were performed using descriptive statistics methods in IBM SPSS (SYS: systolic blood pressure, DIA: diastolic blood pressure).

Table 3. Changes in Serum atrial natriuretic peptide (ANP) after medication in Case A

Case	Day1210 (pg/mL)	Day1391 (pg/mL)	Day1543 (pg/mL)
A	194.4	25	59.3
B	73.2	-	-
C	33.1	-	28.5
D	35.2	-	32.9

Serum atrial natriuretic peptide (ANP) changes before and after medication in case A, who showed an increase in blood pressure from the measurement period, and ANP comparison with the other 3 cases.

humans and dogs [10]. And in autopsies of aged red pandas, fibrosis of the heart and kidney was often observed [32]. Further data were needed to analyze the correlation with age.

According to the International Research Group on Veterinary Nephrology (IRIS) stage classification of chronic kidney disease, a guideline for renal failure in dogs and cats, hypertension was defined as SYS of 160–179 mmHg and severe hypertension as ≥180 mmHg [20]. Therefore, persistent hypertension in A and C may lead to cardiovascular disorders such as heart and kidney damage [14, 20]. However, there were no clinical symptoms or abnormal blood test values in either case during the measurement period. Cardiovascular disease was the leading cause of death in older red pandas [9]. Therefore, blood pressure measurement was also a critical assessment parameter of cardiovascular disease.

The number of captive red pandas in Japan accounts for 3/4 of the world's captive population [26]. Thus, Japanese zoos have an extremely important responsibility and role to play in the conservation of these species. Treatment based on clinical data obtained through behavioral restraint using husbandry training allows for more detailed healthcare management. This is important for the *ex situ* conservation of the endangered red panda and is also expected to make a significant contribution to animal welfare. In the future, we plan to encourage many other zoos to conduct blood pressure measurements, establish blood pressure measurement methods in red pandas, and conduct research on its clinical application.

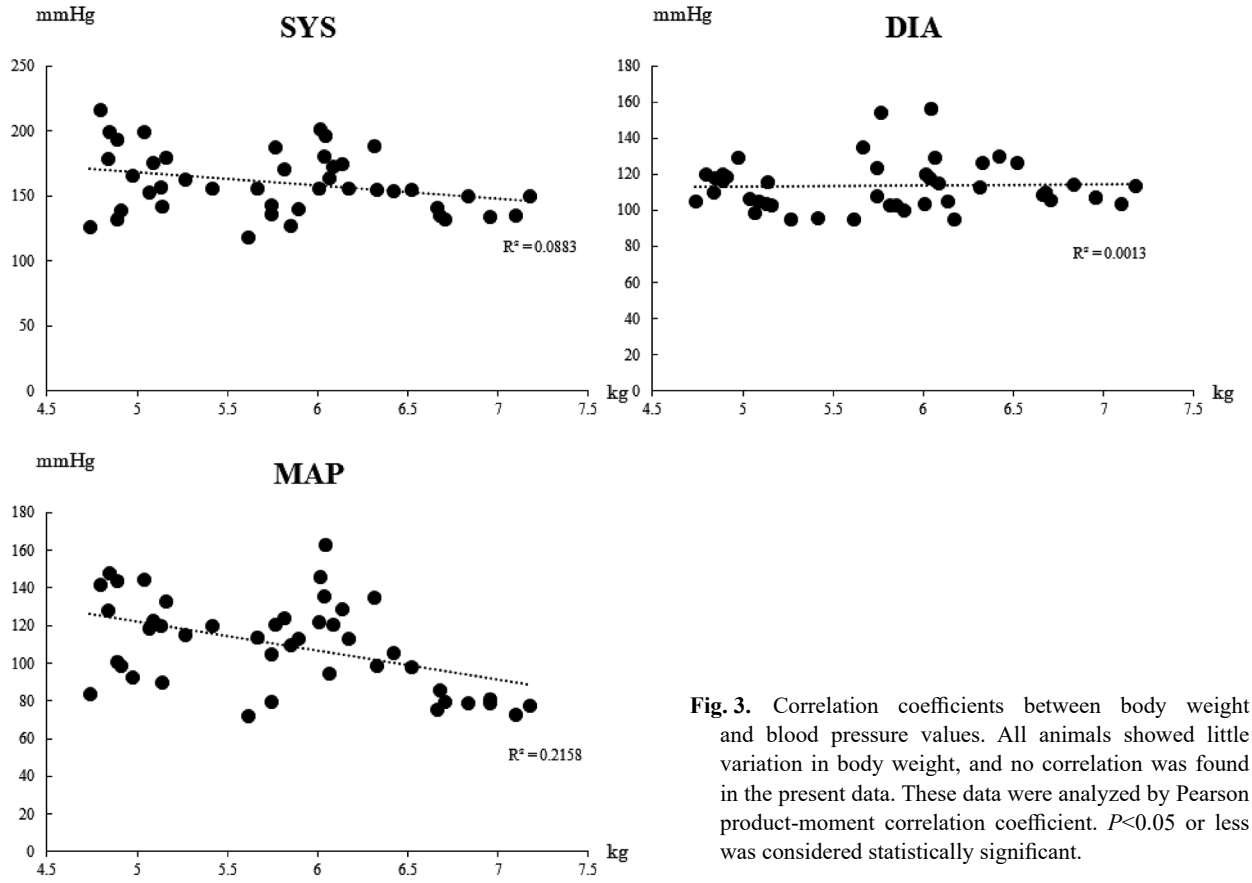

Fig. 3. Correlation coefficients between body weight and blood pressure values. All animals showed little variation in body weight, and no correlation was found in the present data. These data were analyzed by Pearson product-moment correlation coefficient. $P<0.05$ or less was considered statistically significant.

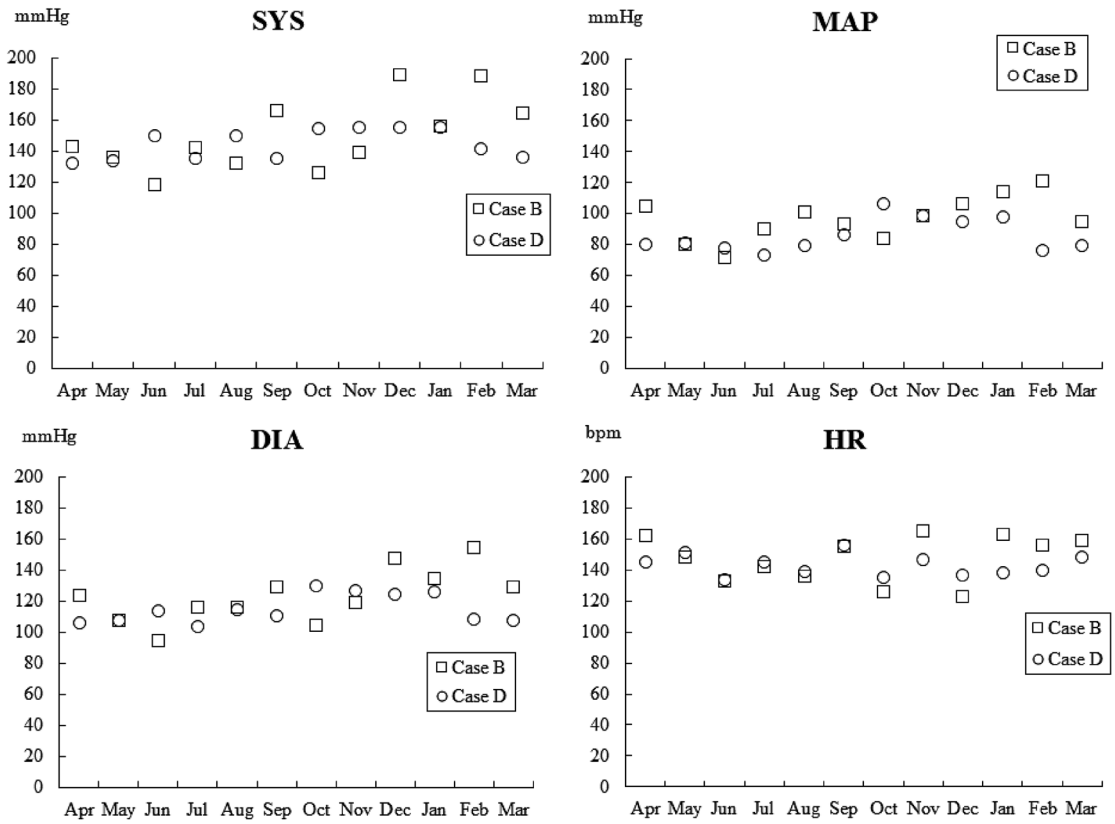

Fig. 4. Seasonal variation in blood pressure in case B and D. Annual blood pressure variability in case B and D considered healthy compared to standard blood pressure values in dogs and cats. Both cases had stable values throughout the year (Period 2020–2021). Analyzes were performed using descriptive statistics methods in IBM SPSS (IBM, Armonk, NY, USA).

POTENTIAL CONFLICT OF INTEREST. All authors have no anything conflict of interest in this article.

ACKNOWLEDGMENT. We thank the staff of the Omuta city zoo, who participated in the clinical examination of four red panda.

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