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FULL PAPER

Reference data on the anatomy, hematology and biochemistry of 9-month-old silver foxes

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Abstract

Anthropometric, anatomical, hematological and biochemical reference values were estimated in clinically healthy male and female 9-month-old silver foxes.

The coefficients of variation of anthropometric and anatomical measurements for 9-month-old silver foxes were as low as previously reported for adult foxes. However, in relation to body size, all measurements were smaller.

Compared with adult silver foxes, higher values were observed in serum levels of triglyceride, phospholipid, β-lipoprotein, blood urea nitrogen and total protein. Similarly, higher levels were obtained for serum enzymes, especially aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK). The high levels of these serum enzymes may be due to handling stress. Inorganic phosphorus and calcium concentrations in the young foxes were also high. The alkaline phosphatase (ALP) level, reflecting the level of bone growth, was higher than that of adults.

Biochemical values of β-lipoprotein, glucose and calcium in male 9-month-old silver foxes were lower than those of females, whereas those of total cholesterol, total protein, fructosamine, iron, albumin and β-globulin were higher.

Key words: growing silver fox, anthropometry, anatomy, hematology, biochemistry

Introduction

The silver fox is one of the species of red fox (*Vulpes* spp.). Although some studies on the silver fox have been carried out, there is still very limited information concerning their anthropometric, anatomical, hematological and biochemical values[1,20]. A previous report[20] presented anthropometric, anatomical and biochemical reference values for adult silver foxes; however, those of growing foxes are still unknown. In the present paper, the reference values for growing silver foxes are presented and compared with these for adult silver foxes.

Materials and Methods

Eighty-one 9-month-old silver foxes (40 males, 41 females) were obtained from a ranch in Hokkaido, Japan. The animals were clinically healthy. They were raised in wire pens and fed fish and meat by-products. Anthropometric data were obtained from 52 silver foxes (25 males, 9 females). Thirty-six silver foxes (18 males, 18 females) were used for anatomical measurement of the following parameters; the

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weight and size (length, width and thickness) of the main organs and the length of the long bones of the limbs including the humerus, radius, ulna, femur, tibia and fibula. No parasites were found in the digestive and cardiovascular systems.

The packed cell volume (PCV) was determined by the micro-hematocrit method and the hemoglobin (Hb) concentration was measured with Raba-HiSUPER (CHUGAI PHARMACEUTICAL Co., Ltd.). The relative white blood cell (WBC) count in percent was obtained from the WBC differential count from a blood smear examination.

A blood chemical analyzer (COBAS MIRA-S : ROCHE Co., Ltd.) was used to measure the following parameters ; blood urea nitrogen (BUN), creatinine (Crea), uric acid (UA), total cholesterol (T-cho), total bilirubin (T-Bil), triglyceride (TG), phospholipid (Phos-Lip), β-lipoprotein (β-Lipo), fructosamine (FRA), glucose (Glc), free fatty acid (FFA), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, inorganic phosphorus (IP), calcium (Ca), magnesium (Mg) and iron (Fe). Total protein (Tp), sodium (Na), potassium (K) and chloride (Cl) were determined using the methods described previously[20]. Serum protein analysis was performed by the standard electrophoretic method using cellulose acetate membranes[3].

The mean, its standard deviation (SD) and coefficient of variation were calculated for each parameter. Aberrant values were evaluated with Grubbs's method[2] and statistical analyses were reperformed after removal of the outlying values. Statistical differences between the sexes were determined using Student's t-test.

Results

The coefficients of variation for anthropometric parameters were low (3.6%–11.0%). The body length ($p<0.01$), length of the head ($p<0.05$) and depth of the chest ($p<0.05$) of the male were significantly greater than those of the female (Table 1). The coefficients of variation were over 20% for the weights of the lung, spleen, pancreas and prostate in the male, the weight of the pancreas in the female, and thickness of the spleen in both sexes. The others were low (3.6%–19.2%). Significant differences were obtained for weight ($p<0.01$), longitudinal length ($p<0.01$), transverse length ($p<0.05$) of the heart, longitudinal length of the left kidney ($p<0.05$), longitudinal ($p<0.05$) and transverse lengths ($p<0.05$) of the right kidney, and thickness of the spleen ($p<0.05$) (Table 2). The coefficients of variation for all parameters of the long bones were very low (0.8%–2.9%). All

TABLE 1. Reference values of anthropometry for 9-month-old silver foxes

Sex		N	BW (Kg)	BL (cm)	LH (cm)	WH (cm)	DC (cm)	WC (cm)	CC (cm)	LT (cm)
M	Mean	25	5.0	42.6 **	16.2	7.1 **	13.2 *	7.6	37.4	39.7
	±SD		0.55	1.66	0.59	0.34	0.94	0.63	2.02	2.23
	CV (%)		11.0	3.9	3.6	4.8	7.1	8.3	5.4	5.9
F	Mean	27	4.8	41.1 **	15.4 **	7.3	12.6 *	7.5	36.3	39.0
	±SD		0.48	1.41	0.75	0.61	0.89	0.53	1.78	2.26
	CV (%)		10.0	3.4	5.0	8.6	7.0	7.1	4.9	5.8

SD : standard deviation, CV : coefficient of variation N : number of animals, BW : body weight, BL : body length LH : length of the head, WH : width of the head, DC : depth of the chest, WC : width of the chest, CC : circumference of the chest, LT : length of the tail, M : male, F : female.

* ($p<0.05$), ** ($p<0.01$) : parameters for which significant differences were detected between the sexes.

the measurements from the male were significantly larger than those of the female ($p<0.01$) (Table 3).

The values for serum substances, electrolytes and enzymatic activities are shown in Tables 4, 5 and 6, respectively. The concentrations of T-Cho ($p<0.01$), FRA ($p<0.05$) and Tp ($p<0.05$) in the male were significantly higher than those in the female, whereas the concentrations of β-Lipo ($p<0.01$) and Glc ($p<0.01$) were significantly lower in the male than those in the female (Table 4). As shown in Table 5, the concentration of Ca in the male was significantly lower ($p<0.05$), while the concentration of Fe in the male was significantly higher ($p<0.05$) than in the female. No sex difference was found in

TABLE 2. Reference values for main organs of 9-month-old silver foxes

Organ	Sex	N	Organ weight (g)	Longitudinal length (mm)	Transverse length (mm)	Thickness (mm)
Brain	M	18	47.5± 2.3			
	F	18	46.2± 2.1			
Heart	M	18	46.3± 4.5**	49.8± 3.2**	43.9± 2.9*	35.2± 1.3**
	F	18	40.2± 2.6**	47.0± 2.1**	41.7± 2.1*	33.8± 1.4**
Lungs	M	18	52.3±11.1#			
	F	18	51.2± 8.4			
Liver	M	18	167.7±18.2	162.7±11.8	109.8±10.3	18.2± 3.5
	F	18	165.1±14.5	164.7± 7.8	108.1± 6.9	17.5± 2.3
L. Kidney	M	18	13.9± 1.9	42.2± 3.3**	23.9± 2.2	19.3± 1.8
	F	18	13.0± 2.2	39.6± 2.1**	23.9± 1.2	20.3± 2.2
R. Kidney	M	18	13.6± 1.5	43.3± 3.3*	24.3± 2.1*	18.6± 1.2
	F	18	12.8± 1.5	40.4± 3.9*	22.9± 1.3*	19.3± 1.5
Spleen	M	18	6.3± 1.3#	99.5± 9.8	25.7± 3.1	5.3± 1.5*#
	F	18	5.8± 0.9	101.8±11.0	25.4± 2.5	4.7± 1.0*#
Pancreas	M	18	8.1± 1.9#			
	F	18	7.9± 2.3#			
Prostate	M	18	0.6± 0.2#			
Uterus	F	18	8.1± 1.6	100.3± 7.2※	100.3± 9.5※※	

Mean±SD.

※: the length of the corpus uteri.

※※: the length of the cornu uteri.

* ($p<0.05$), ** ($p<0.01$): parameters for which significant differences were detected between the sexes.

#: parameters for which CVs were over 20%.

M: male, F: female, N: number of observations.

TABLE 3. Reference values for the long bones of 9-month-old silver foxes

Sex	N		Humerus (mm)	Radius (mm)	Ulna (mm)	Femur (mm)	Tibia (mm)	Fibula (mm)
M	6	Mean	134.4**	127.7**	148.3**	140.0**	143.0**	152.8**
		±SD	2.3	2.7	2.4	3.3	4.0	4.5
F	5	Mean	124.3**	118.3**	137.4**	132.2**	134.2**	142.5**
		±SD	1.0	2.0	2.1	2.0	3.3	3.3

** ($p<0.01$): parameters for which significant differences were detected between the sexes.

SD: standard deviation.

M: male, F: female, N: number of observations.

TABLE 4. Reference values for serum biochemical components of 9-month-old silver foxes

Parameters	Sex	N	Mean±SD
T-Bil (mg/dl)	M	25	0.46± 0.13
	F	21	0.41± 0.07
T-Cho (mg/dl)	M	25	103.1 ± 13.7 **
	F	20	85.6 ± 8.0 **
β-Lipo (mg/dl)	M	25	387.6 ± 39.2 **
	F	19	405.7 ± 24.7 **
TG (mg/dl)	M	25	91.9 ± 26.1
	F	20	79.7 ± 25.9
FFA (μEq/l)	M	25	303.1 ±128.0
	F	19	332.6 ±132.3
Phos-Lip (mg/dl)	M	25	593.7 ±246.5
	F	19	404.0 ±107.5
UA (mg/dl)	M	25	0.70± 0.11
	F	20	0.64± 0.19
BUN (mg/dl)	M	25	38.7 ± 5.9
	F	20	41.5 ± 9.7
Crea (mg/dl)	M	25	0.7 ± 0.1
	F	21	0.7 ± 0.1
Glc (mg/dl)	M	25	118.6 ± 20.8 **
	F	20	139.8 ± 20.9 **
FRA (μmol/l)	M	25	253.7 ± 19.0 *
	F	20	239.9 ± 19.4 *
Tp (g/dl)	M	25	8.0 ± 0.6 *
	F	20	7.5 ± 0.6 *

* ($p<0.05$), ** ($p<0.01$): parameters for which significant differences were detected between the sexes.
M: male, F: female, N: number of observations.

TABLE 5. Reference values for serum electrolytes of 9-month-old silver foxes

Parameters	Sex	N	Mean±SD
Ca (mg/dl)	M	24	10.4± 0.5*
	F	19	10.8± 0.6*
IP (mg/dl)	M	25	6.6± 0.6
	F	20	6.2± 0.8
Mg (mg/dl)	M	25	1.9± 0.2
	F	19	1.8± 0.1
Fe (μg/dl)	M	25	206.9±42.4*
	F	21	177.1±39.5*
Na (mEq/l)	M	25	140.8± 1.9
	F	22	141.7± 2.5
K (mEq/l)	M	25	4.2± 0.4
	F	22	4.2± 0.3
Cl (mEq/l)	M	25	100.1± 2.5
	F	22	100.7± 3.9

* ($p<0.05$): parameters for which significant differences were detected between the sexes.
M: male, F: female, N: number of observations.

TABLE 6. Reference values for serum enzyme activities of 9-month-old silver foxes

Parameters	Sex	N	Mean±SD
AST (IU/l)	M	25	45.3± 9.0
	F	23	50.5± 16.0
ALT (IU/l)	M	25	101.0± 23.3
	F	23	98.9± 35.1
ALP (IU/l)	M	25	63.4± 11.4
	F	23	61.4± 8.5
LDH (IU/l)	M	25	204.4± 45.8
	F	23	213.8± 79.4
GGT (IU/l)	M	25	6.7± 4.8
	F	23	8.5± 4.2
CK (IU/l)	M	25	171.6± 37.0
	F	23	167.1± 45.5
Amylase (IU/l)	M	25	1437.1±171.7
	F	23	1318.1±245.3

M: male, F: female, N: number of observations.

the activities of the serum enzymes (Table 6).

The electrophoretogram showed five fractions as albumin (Alb) and α1, α2, β and γ globulins (α1, α2, β and γ) (Fig. 1). Significant sex differences ($p<0.01$) were found in the concentrations of albumin and β-globulin (Table 7).

In the differential WBC counts, the proportion of eosinophils in the male was significantly different ($p<0.05$) from that of the female (Table 8). No significant differences were found between the sexes in the other parameters.

Discussion

The present study presents anthropometric

TABLE 7. Reference values for serum protein fractions of 9-month-old silver foxes

Sex	N		Alb (g/dl)	α1 (g/dl)	α2 (g/dl)	β (g/dl)	γ (g/dl)	A/G
M	25	Mean	3.95**	0.43	0.45	1.26**	0.50	1.54
		±SD	0.46	0.11	0.18	0.25	0.15	0.38
F	23	Mean	3.53**	0.45	0.43	1.09**	0.48	1.47
		±SD	0.46	0.10	0.07	0.16	0.13	0.29

** ($p<0.01$): parameters for which significant differences were detected between the sexes.
SD: standard deviation.
M: male, F: female, N: number of observations.
Alb: albumin, α1: α1 globulin, α2: α2 globulin, β: β globulin, γ: γ globulin.

TABLE 8. Hematologic reference values for 9-month-old silver foxes

Parameters	Sex	N	Mean±SD
PCV (%)	M	14	46.6± 2.5
	F	19	48.2± 2.8
Hb (g/dl)	M	20	17.9± 2.2
	F	3	19.6± 0.3
Eos (%)	M	20	11.1± 4.8*
	F	16	7.4± 4.4*
Bas (%)	M	20	0.0
	F	16	0.0
Stab (%)	M	20	0.5± 0.9
	F	16	0.6± 0.7
Seg (%)	M	20	46.5±11.8
	F	16	50.7±10.0
Lymp (%)	M	20	39.6±11.1
	F	16	38.9±10.6
Mono (%)	M	20	2.2± 1.6
	F	16	2.4± 1.5

* ($p<0.05$): parameters for which significant differences were detected between the sexes.
M: male, F: female, N: number of observations.

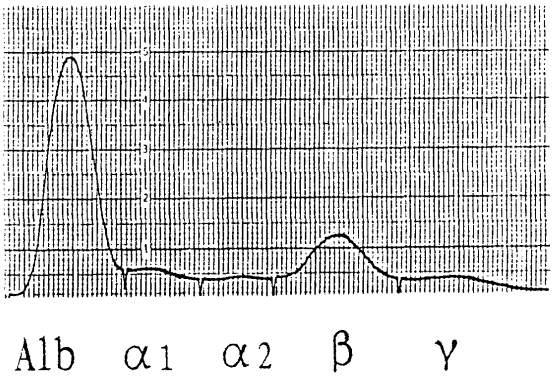

Fig. 1. Typical electrophoretic pattern of serum protein for a 9-month-old silver fox.
Alb: albumin, α1: α1 globulin, α2: α2 globulin, β: β globulin, γ: γ globulin

and anatomical measurements in 9-month-old silver foxes. These values were smaller than those of the adult (2–8 years, mean 4.9 years)[20]. These results may indicate that 9-month-old silver foxes are still in the growing stage, as their bodies were smaller than those of adults. A similar result has already reported for the red fox[7]. Body size and the length of long bones of the limbs in the male at 9-months of age were significantly larger than those in the female as previously reported for the adult[20]. The sex differences in the size of the heart in the young foxes may reflect the body size differences between the sexes.

Some serum biochemical parameters are variable by sex. Uchiyama et al. indicated that the values of T-Cho in 3-month-old and adult male beagles are significantly higher than in the female[17]. A similar result was found in 9-month-old silver foxes, but not in adult ones[20]. A sex difference in the Ca concentration, which is similar to the present results, has been reported in beagles and coyotes[12,13]. Stewart and Longwell[13] suggested that the sex difference in Ca in the beagle is small or may not make a practical difference. A high BUN level was observed in the young foxes. Lane et al.[6] reported that feeding an all-meat diet to dogs produced a BUN level near 40mg/dl. Tyopponen and Valtonen[16] indicated that a low-protein diet causes lower BUN and Crea levels in blue foxes. Based on these findings, the high concentration of BUN observed in this study might

mainly be attributable to the high protein intake, because no data were obtained for renal diseases. In the present and previous[20] studies, the Glc level was high. Capture stress, which elevates serum Glc values has been reported in wolves and coyotes[10,12]. Of note, Glc levels can also be influenced by the duration between the feeding and sampling time. Since blood samples in the present and a previous study[20] were collected in the morning before the first feeding of the day, sampling time may have had less effect on the values. Stress might be the main cause of elevation of Glc values. In the present study, a higher Tp concentration in 9-month-old silver foxes than in the adult was found. This result is inconsistent with the previous finding that the concentration of Tp increases with age in dogs[17]. The value of PCV, a useful indicator of plasma volume, in young silver foxes was similar to those in adults. This suggests that the higher Tp concentration in the young may not be due to dehydration. When compared with adults, 9-month-old silver foxes had higher IP and ALP values in the present study. Higher IP and ALP values in the young animal have been also found in coyotes[12]. A decline in ALP may reflect a decrease in the rate of growth with age. A gradual decrease in serum levels of IP and ALP with age has already been reported in growing dogs[17]. Furthermore, stress from the capturing and handling of animals or from housing conditions may change some biochemical parameters[5,9,19]. Seal et al.[9] reported that post-restraint of white-tailed deer increases in the activities of AST, LDH and CK very markedly, ranging from 3- to 200-fold. Elevated serum AST, LDH and CK levels due to capturing and handling stress were also observed in red foxes and other wild animals[5,19]. High enzymatic activities were also observed in the young foxes, but these data were removed in this study by the statistical analyses.

Since AST and LDH enzymes are distributed inside the cell[15] in the blue fox, elevated activities of the enzymes may represent cell damage, especially to cells of the muscle tissue[5,19]. In this study, markedly higher activities of the muscle type of CK were obtained in the cases with elevated CK activity. This also suggested damage to the muscle cells caused by capturing stress. The effect of stress during the capture and handling of animals is a cause of variation in hematological values[8,9]. Stress can cause splenic contraction, increasing PCV. Seal et al.[11] noted that Hb and PCV values in gray wolves vary seasonally and suggested that the changes are related to the temperature and photo-period cycle. Higher values of Hb and PCV in winter were also observed in dogs[14]. This may explain the high value of Hb obtained since the samples in this study were collected during winter.

McCue and O'Farrell[8] noted that in the wild fox, abundant neutrophils, but very few eosinophils, were observed in blood smears. In the present study and previous studies[1], however, a high ratio of eosinophils was obtained. Of note is the fact that acute stress like immobility can elevate the level of circulating eosinophils[4]. This may explain the difference between the previous study and the present study. Since the animals used in the study by McCue and O'Farrell[8] were captured wild ones, acute stress from the capturing might have prevailed. In the present study, however, the animals used were farm-bred and immobility stress might have been the main stressor.

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