



## NOTE

Wildlife Science

# Anthropometric and blood data on a hand-reared captive Asian elephant (*Elephas maximus*) calf: A retrospective case report

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**ABSTRACT.** The anthropometric and blood data of an unsuccessfully hand-reared Asian elephant (*Elephas maximus*) calf were retrospectively compared with the data for calves raised by their real mothers or allomothers, to identify potential reasons for poor outcomes in the hand-reared case. The hand-reared calf grew normally in terms of body weight and withers height. However, blood biochemical data suggested reduced bone metabolism, low immune status, and malnutrition during its life. Blood bone markers were measured to determine whether a skeletal disorder was present in the Asian elephant calf, which was not clear from the anthropometric data. Monitoring these parameters in hand-reared Asian elephant calves, with the aim of keeping them within the normal range, may increase the success rate of hand-rearing of Asian elephant calves.

**KEY WORDS:** Asian elephant calf, blood chemistry, bone markers, hand-rearing, protein fraction

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The Asian elephant (*Elephas maximus*) is one of the most popular animals at zoological, safari, and national parks worldwide. However, the survival rate of captive elephant calves is lower than that of other captive megavertebrates [4]. The most important factors affecting captive elephant juvenile calf mortality rates are herpesvirus-associated diseases, maternal rejection, trauma, the generally low survivability of hand-reared calves, infections, and gastrointestinal disorders [6]. Historically, the prevalence rates of maternal rejection and infanticide in zoos and circuses have been high, which has been associated with the lack of close contact between the dam and an older female [3, 6]. In such cases, Asian elephant dams attack or neglect their calves and give up rearing them permanently; therefore, infant care by human caretakers or elephant allomothers is required. Successful hand-rearing of elephant calves is very difficult, even for short periods [4]. A survey of European zoos found that 22.9% of elephant cows failed to accept their calves and allow them to feed, and no calves were hand-raised successfully [7]. To resolve this problem, several studies have investigated the composition of elephant breast milk to produce artificial elephant milk for hand-rearing [1, 18]. However, no studies have reported on the factors affecting growth or immunological status in hand-reared Asian elephant calves. Unfortunately, few data are available on the anthropometric and blood parameters of hand-reared Asian elephants, although these would be valuable growth indicators [24]. Recently, we hand-raised an Asian elephant calf for 14 months; here, we report the anthropometric and blood data for this calf, along with the data for other elephant calves cared for by their real mothers or allomothers. This study sought to identify potential reasons for poor outcomes in the hand-reared calf based on the anthropometric and blood data.

A male Asian elephant (calf A) was born at Ichihara Elephant Kingdom Zoological Park in October 2016. This was the second parturition for this dam, who had rejected her first calf at another zoo. The dam neglected her second calf soon after calving, and then behaved aggressively toward it and refused to let it suckle. Because there were no any other dams that could accept this calf in our zoo at that time, our only option was to hand-rear it. On its first day of life, the calf was fed a very small amount (about 12.5 ml in total) of colostrum obtained from its mother's breast by human caretakers, followed by hourly bottled raw goat milk (Kimura goat farm, Oita, Japan). Table 1 shows the details of the feedings. Beginning at 1 month of age, the calf was given a

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**Table 1.** Range of amounts bottle fed in calf A

Age	Milk liters/day		Milk liters/hr		Milk liters/time	Milk detail
	Calf A	Reference [8]	Calf A	Reference [8]	Calf A	Rgm:Aem <sup>a)</sup>
Week						
1	2.45–4.00	5.00–10.70	0.102–0.167	0.208–0.446	0.040–0.200	10:0
2	4.27–6.65	8.75–11.60	0.178–0.277	0.364–0.483	0.240–0.300	10:0
3	5.70–8.00	11.00–13.20	0.238–0.333	0.458–0.550	0.350–0.400	10:0
4	8.60–9.35	11.80–12.00	0.358–0.390	0.490–0.500	0.400–0.450	10:0
Month						
1	2.45–12.50	5.00–13.20	0.102–0.521	0.208–0.550	0.040–0.500	10:0
2	11.00–16.25	12.00–18.00	0.458–0.677	0.500–0.750	0.500–0.650 <sup>b)</sup>	10:0
3	13.07–18.30	10.90–20.00	0.545–0.763	0.456–0.833	0.650–0.750 <sup>b)</sup>	10:0
4	16.30–19.30	12.10–24.00	0.679–0.804	0.504–1.000	0.750–0.800 <sup>b)</sup>	7.5:2.5
5	17.00–20.40	14.50–29.00	0.708–0.850	0.606–1.210	0.800–0.850 <sup>b)</sup>	7.5:2.5
6	19.17–25.38	13.70–31.00	0.799–1.058	0.573–1.290	0.850–0.900 <sup>b)</sup>	7.5:2.5
7	19.92–24.00	10.90–24.60	0.830–1.000	0.456–1.020	0.900–1.000 <sup>b)</sup>	7.5:2.5
8	20.67–27.60	12.70–25.80	0.861–1.150	0.531–1.080	1.000–1.200 <sup>b)</sup>	7.5:2.5
9	20.40–28.80	15.00–28.50	0.850–1.200	0.625–1.190	1.200–1.400 <sup>b)</sup>	7.5:2.5
10	28.00–39.10	10.60–28.30	1.167–1.629	0.441–1.180	1.400–1.700 <sup>b)</sup>	7.5:2.5
11	35.70–39.10	12.70–30.60	1.488–1.629	0.531–1.260	1.700–1.800 <sup>b)</sup>	7.5:2.5
12	36.00–41.40	–	1.500–1.725	–	1.800–1.900 <sup>b)</sup>	7.5:2.5

a) Raw goat milk: Artificial elephant milk. b) Added rice water and boiled bananas mixture to supplement nutrition.

mixed solution of rice water and boiled banana as supplementary nutrition [14], which was added to milk (20% of the total volume of the milk). From 3 months of age, the calf was given a mixture of 75% raw goat milk and 25% artificial elephant milk (Morinyu Sunworld, Tokyo, Japan). Commercial hay, green grass, vegetables, and fruit were also added to its feed gradually. Under the care of mahouts, the elephant calf was kept inside at night and walked outside to exercise and absorb solar ultraviolet in the daytime. The calf was reared with 11 other elephants to promote socialization. The amount of milk fed to this elephant calf was increased gradually following general recommendations [8]. At one year of age, the calf was fed about 58 l of milk per day. At 14 months, it died suddenly of acute hemorrhagic disease caused by elephant endotheliotropic herpesvirus type 1A (EEHV1A), although it had appeared healthy until then.

Selected anthropometric and blood data of calf A were obtained from the zoological park health management records and compared with the records of four other elephant calves (calves 1–4) born in the same park between 2013 and 2019. Of these, one male calf (calf 1) was raised by an elephant allomother and three females (calves 2–4) were raised by their real mothers. All four of these calves have grown healthily until the time of writing. Anthropometric and blood data were recorded for four (A, 1, 2, and 3) and three (A, 3, and 4) calves, respectively.

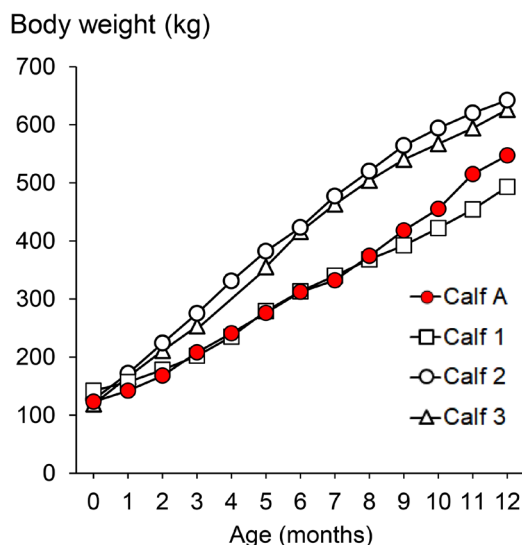
Calf A was compared with calves 1–3 in terms of body weight (BW) and withers height (WH) from the toe of the forelimb to the highest point of the thoracic vertebrae. Gains in BW and WH were calculated using data from the first measurement after birth and at one year.

The complete blood count (CBC) and serum biochemistry were compared between calf A and calves 3 and 4. The CBC included the white (WBC) and red (RBC) blood cell counts and packed cell volume (PCV) and were measured using an automatic cell counter (PCE-210; Erma, Tokyo, Japan). The serum biochemistry included the alanine transaminase (ALT), aspartate aminotransferase (AST), calcium (Ca), inorganic phosphorus (iP), glucose (Glu), total cholesterol (t-Cho), blood urea nitrogen (BUN), total alkaline phosphatase (t-ALP), and total protein (TP) levels, which were determined using a blood chemistry analyzer (DRI-CHEM 4000sV; FUJIFILM Medical, Tokyo, Japan). Additionally, frozen (–20°C) serum samples of calves A, 3, and 4 were used to measure the alkaline phosphatase isoenzyme 3 (ALP3) [20] and tartrate-resistant acid phosphatase 5b (TRAP 5b) activities [19] as bone metabolism markers. Protein electrophoretic analysis was done using a QuickGel SP (J711, Helena Laboratory Japan, Saitama, Japan).

Anthropometrically (Fig. 1), the change in BW of calf A was similar to that of the male elephant (calf 1), whereas two female calves (2 and 3) had greater increases in BW. Daily gains in BW of calves A and 1–3 during the first 12 months of the life were 1.16, 0.96, 1.41, and 1.39 kg, respectively. The WH of calves A and 1–3 at 12 months was 135, 139, 136, and 135 cm, respectively; daily gains in WH during the first 12 months of life were 0.11, 0.08, 0.11, and 0.12 cm, respectively.

Comparison of blood data obtained at three-month intervals during 12 months of life (Table 2) revealed that several parameters differed between calf A and calves 3 and 4. The t-Cho and TP concentrations and t-ALP activity in calf A were lower than those of calves 3 and 4 over the entire period, whereas the serum BUN was higher in calf A. The activities of two bone metabolism makers, ALP3 and TRAP5b, were also low in calf A compared with calves 3 and 4. Protein electrophoresis revealed extremely low  $\gamma$ -globulin ( $\gamma$ -Glb) levels in calf A compared with calves 3 and 4, resulting in a high ratio of albumin to globulins (A/G) in calf A.

Body weight is a simple, understandable indicator of animal growth. A difference in BW gain between male and female Asian



**Fig. 1.** Change in body weight (BW) of a hand-reared Asian elephant calf (calf A) and three allomother or real mother-reared elephant calves (calves 1–3) over 12 months of life. BW was measured using a large floor scale (DLF-3000, Dream Link, Tokyo, Japan).

**Table 2.** Blood laboratory results of selected parameters in a hand-reared Asian elephant calf and two mother-reared elephant calves during 3 to 12-month (mo)-old

Item	Unit	Calf A				Calf 3				Calf 4	
		3-mo-old	6-mo-old	9-mo-old	12-mo-old	3-mo-old	6-mo-old	9-mo-old	12-mo-old	3-mo-old	6-mo-old
Complete blood count <sup>a)</sup>											
WBC	$\mu$ l	13,300	16,700	15,100	22,700	19,000	24,100	28,400	24,700	19,300	24,000
RBC	$\times 10^3/\mu$ l	2,720	2,720	3,270	3,030	2,930	2,670	2,890	3,410	2,770	3,620
PCV	%	35.2	28	25.9	20.4	32.1	24.7	22.7	25.3	29.9	29.7
Serum chemistry											
ALT <sup>b)</sup>	U/l	4	2	4	5	4	5	3	2	5	1
AST <sup>b)</sup>	U/l	21	14	14	17	23	28	17	14	29	16
Ca <sup>b)</sup>	mg/dl	11.9	11.2	11.8	12.5	11.7	11.7	11.3	11.2	11.2	11
iP <sup>b)</sup>	mg/dl	9.9	8.2	8.8	8.9	9	9.2	8.3	7.6	8.8	8.5
Glu <sup>b)</sup>	mg/dl	127	147	157	118	166	149	135	129	139	135
t-Cho <sup>b)</sup>	mg/dl	49	37	38	48	95	94	90	72	92	65
BUN <sup>b)</sup>	mg/dl	11.5	9.9	9.9	13.1	3.9	4.5	4.5	5.2	4.2	5.0
t-ALP <sup>b)</sup>	U/l	1,033	997	1,079	816	3,334	3,168	1,899	1,601	2,250	1,491
ALP3 <sup>c)</sup>	U/l	756	863	671	684	2,977	2,934	1,610	1,403	2,021	1,233
TRAP5b <sup>d)</sup>	U/l	5.3	1.6	2.4	3.2	10.2	11.5	7.0	5.5	9.0	7.4
TP <sup>b)</sup>	g/dl	6.2	6.0	6.0	6.6	8.6	8.0	7.6	7.2	8.6	8.0
Alb <sup>e)</sup>	g/dl	4.19	4.15	4.15	4.12	4.36	4.09	4.39	4.46	4.45	4.35
$\alpha_1$ -glb <sup>e)</sup>	g/dl	0.63	0.61	0.61	0.71	0.40	0.50	0.47	0.48	0.25	0.37
$\alpha_2$ -glb <sup>e)</sup>	g/dl	0.32	0.30	0.30	0.51	0.18	0.30	0.26	0.32	0.08	0.18
$\beta_1$ -glb <sup>e)</sup>	g/dl	0.17	0.20	0.20	0.28	0.31	0.35	0.33	0.24	0.34	0.43
$\beta_2$ -glb <sup>e)</sup>	g/dl	0.58	0.53	0.53	0.61	0.88	0.98	0.85	0.76	1.02	0.97
$\gamma$ -glb <sup>e)</sup>	g/dl	0.31	0.22	0.22	0.37	2.26	1.78	1.30	0.94	2.46	1.70
A/G <sup>e)</sup>		2.1	2.2	2.2	1.7	1.1	1.0	1.4	1.6	1.1	1.2

WBC: white blood cell counts, RBC: red blood cell counts, PCV: packed cell volume, ALT: alanine aminotransferase, AST: aspartate aminotransferase, Ca: calcium, iP: inorganic phosphorus, Glu: glucose, t-Cho: total cholesterol, BUN: blood urea nitrogen, t-ALP: total alkaline phosphatase, ALP3: alkaline phosphatase isoenzyme 3, TRAP5b: tartrate-resistant acid phosphatase 5b, TP: total protein, ALB: albumin, glb: globulin, A/G: ratio of albumin to globulins. a) These parameters of complete blood counts were measured using an automatic cell counter machine (PCE-210, Erma, Tokyo, Japan). b) These biochemical parameters were analyzed using a blood chemistry analyzer (DRI-CHEM 4000sV, FUJIFILM Medical, Tokyo, Japan). c) The ALP3 activity was analyzed by an agarose gel electrophoresis method reported previously [16]. d) The TRAP5b activity was measured by a fluorometric method reported previously [15]. e) The protein fractions were analyzed by an agarose gel electrophoresis method using a Quickgel SP (J711, Helena Laboratory Japan, Saitama, Japan).

elephants has been reported [10]. In this study, the change in BW of the male calves (calves A and 1) differed from that of the female calves (calves 2 and 3); this may have been due to gender or individual differences. The normal range of daily gain in BW in healthy Asian elephant calves is 0.5–1.4 kg [8]. The daily gain in BW of calf A and calves 1–3 was within that normal range. No obvious abnormalities were recognized in calf A or the other calves. Few data on WH and its daily gain in Asian elephant calves are available. Nevertheless, WH and its daily gain in calf A were consistent with those of calves 1–3. Therefore, the growth of calf A during 1 year of life was comparable to the elephant calves that were cared by their real mothers or allomothers.

Circulating bone metabolism markers in Asian elephants are useful indices of bone metabolism [2, 19, 20, 22]. ALP3 is a t-ALP isoenzyme that reflects osteoblast function [5, 17]. TRAP 5b is a bone resorption marker indicating the number of osteoclasts [15]. Calf A had lower serum t-ALP, ALP3, and TRAP5b activities than two other elephant calves, suggesting that both osteoblasts and osteoclasts were inactivated in calf A, although the anthropometric data of this calf were within the normal range. These findings indicate that it is impossible to evaluate bone growth or healthy bone metabolism in calves by measuring BW or WH, confirming the expediency of measuring bone metabolism markers in Asian elephants. The absence of raw elephant milk in the diet of calf A might have affected this result because several dietary factors that affect bone metabolism directly, including Vitamin K<sub>2</sub> and isoflavone, were lacking. Raw milk also affects bone metabolism directly because it plays a functional role in the growth of newborn animals and is an excellent source of nutrients [21]. The contents of raw milk differ depending on the animal species. The goat and artificial elephant milk used here may have lacked nutritional factors that promote healthy bone growth in Asian elephant calves. Therefore, further research is needed to clarify the relationship between raw milk consumption and bone metabolism in Asian elephants and other mammals.

The serum  $\gamma$ -Glb concentration is an index of the immune status of animals and a predictor of passive immune transfer in Asian elephant calves [8]. We used protein electrophoresis to separate the globulin fractions. Calf A had extremely low serum  $\gamma$ -Glb concentrations throughout its life, suggesting insufficient passive immune transfer and reduced immune status. Many animals consume large amounts of immunoglobulins via colostrum [16, 23]. Insufficient colostrum intake results in a failure of passive transfer (FPT) of immunoglobulins, leading to a weakened immune system. Cow calves not fed colostrum (artificial milk) had lower globulin levels than those fed colostrum, although all calves grew well [9]. It has been recommended that newborn elephant calves be given 2–10 l of colostrum within 30 min of birth [8]. In our case, we postulate that insufficient colostrum intake resulted in FPT. However, in Asian elephants, the transplacental transfer of immunoglobulins likely makes a greater contribution to passive immune transfer than the colostrum intake [12]. Additionally, placental transport of immunoglobulins depends on the maternal age or immunization [13]. The low serum  $\gamma$ -Glb concentration in calf A might have resulted from its mother's condition. Further research on passive immune transfer in Asian elephants is needed. However, regardless of the cause of the low serum  $\gamma$ -Glb concentration in this hand-reared calf, the suppressed immune status implies that calf A was at high risk of microbial or viral infection and required supportive therapy. Plasma transfusion or oral administration of immunoglobulins is recommended in suspected cases of FPT in Asian elephants [8]. However, there is no  $\gamma$ -Glb reference value for diagnosing FPT. It will be necessary to collect more data and establish a therapy protocol to counter FPT in Asian elephants.

The serum t-Cho concentration is an indicator of energy intake [11]. Calf A had low serum t-Cho concentrations throughout its life, suggesting insufficient energy intake. Conversely, the serum BUN concentrations in calf A were higher than those in calves 3 and 4. An elevated serum BUN may be due to prerenal causes, such as increased protein catabolism caused by malnutrition [11]. These findings suggest that calf A was malnourished and needed more milk or food. Asian elephant calves feed mainly on breast milk for the first year of life, and it is usually 3 years before the full weaning stage is reached. However, calves older than 3 months can eat solid food, and they gradually increase their roughage intake [14]. Here, elephant calf A was given a mixture of rice water and boiled banana together with milk. After 3 months of age, the calf had free access to green grass, fruits, and vegetables. Nevertheless, it appeared that the calf did not have enough energy to support its large physique and rapid growth. The data presented here suggest that increased amounts of food and frequency of feeding, as well as supplemental nutrients, are necessary in quantities beyond those reported elsewhere [4, 8, 14]. In addition, regular blood biochemistry could be useful for estimating the energy intake of Asian elephants.

In conclusion, three potential issues can occur when hand-rearing Asian elephant calves: abnormal bone metabolism, inactive immune status, and insufficient energy intake. It is very important to identify these potential issues before they become advanced and, potentially, fatal. The anthropometric and blood data monitored herein seem to be useful for assessing the risk of these problems. It will be important to accumulate anthropometric and blood data on captive, hand-reared Asian elephant calves, their real mothers, and allomothers. Further research is needed to improve hand-rearing techniques and thus prevent these problems from occurring in captive Asian elephant calves.

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