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Hematological and serum biochemical parameters of rescued Sunda pangolins (*Manis javanica*) in Singapore

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ABSTRACT. The Sunda pangolin (*Manis javanica*) population in Southeast Asia faces threats such as poaching and deforestation. Health assessments of rescued individuals including physical examination and blood work are crucial for clinicians to determine the health status of these animals. The establishments of reference intervals of hematology and serum biochemistry are important for identifying clinical abnormalities. The objectives of our study were to establish blood reference intervals for Sunda pangolins, to determine if there are age and sex related differences in hematology and serum biochemistry, and to compare our results with those of a previous study on confiscated Sunda pangolins in Thailand. Fifty-eight Sunda pangolins were rescued between January 2011 and December 2015. The hematology and serum biochemistry results of 51 clinically normal Sunda pangolins were selected for the establishment of the blood reference intervals. No sex related differences were noted in this study. Age-related differences were observed, in which adult Sunda pangolins had a significantly higher mean corpuscular volume than juveniles, and juvenile Sunda pangolins had significantly higher red blood cell counts and hemoglobin levels than those of the adults ($P<0.05$). Age-related differences were also noted in several serum biochemistry parameters: alkaline phosphatase (ALP) was significantly higher in juveniles, and total protein was significantly higher in adult Sunda pangolins. Compared to a previous study the white blood cell counts, neutrophil counts, and ALP were higher, and the lymphocyte counts were lower in the present study.

KEY WORDS: hematology, *Manis javanica*, rescued, serum biochemistry, Sunda pangolin

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Pangolins or ‘scaly anteaters’ belong to the family Manidae in the order Pholidota. There are eight species of pangolins, four of which inhabit sub-Saharan Africa, and the other four are native to Asia [6]. Sunda pangolins (*Manis javanica*) are found in several Southeast Asian countries including Laos, Cambodia, Vietnam, Thailand, Singapore, and on the major islands of Indonesia such as Sumatra, Java, and Borneo [2]. Wild pangolins are illegally hunted because of the high demand of pangolin scales and meat for traditional Chinese medicine [12, 29, 33, 35]. Today, the Sunda pangolin is categorized as ‘critically endangered’ by the International Union for Conservation of Nature (IUCN) with an overall declining population trend [7]. In Singapore, habitat loss due to deforestation is the most serious threat to local wildlife species including Sunda pangolins [4]. This habitat loss leads to displacement of pangolins from their natural home range to nearby urban areas, as observed in other species [4]. Pangolins in urban areas are usually rescued by the public, government agencies, or non-government organizations (NGOs) and delivered to conservation institutions such as zoos and wildlife rescue centers.

The success rates of pangolin rehabilitation are notoriously poor due to little available information on the species’ basic physiology or diseases, which hampers the attempts to rehabilitate animals and increase their survival rate during rehabilitation [13]. High mortality rates of pangolins in captivity are due to a variety of factors, including stress-related complications, parasitic infections, hemorrhagic gastric ulcers, respiratory infections, and poor management [10]. Recent work on Sunda pangolin husbandry including nutrition and enclosure environment has improved their survival rates in captivity [8, 9].

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Table 1. Criteria used to exclude animals from the sample study

Criteria	Description
Poor body condition	Animal appeared to be thin or emaciated. Animal categorized based on the sunken abdomen and obvious appearance of the long bones such as femur or humerus.
High parasite burden	Animal showed high number of ticks on the body. Fecal check revealed large number nematodes ova or coccidian oocyst counted in a single field of microscope on direct and floatation method.
Neonates	Neonates that requires hand rearing.
Diarrhea	Animal that noted to defecated watery, foul smelling, bloody and intestinal mucosa feces.
Lethargy	Animals that are weak and unable to curl itself.
Injuries	Body injury seen on visual examination and also fracture seen on radiograph.
Respiratory difficulties	Animal to have obvious abdominal breathing and blood or mucopurulent discharge seen excreted on respiration.

Hematology and serum biochemical parameters enable veterinarians to confirm tentative diagnoses, define responses to therapy, and determine the severity of diseases [19]. Hematology and serum biochemistry parameters have been reported previously in confiscated apparently clinically normal Sunda pangolins in Thailand [37] and Chinese pangolins [11, 26].

In this study, wild Sunda pangolins that were rescued and delivered to the veterinary department of the Singapore Zoological Gardens (SZG) between 2011 and 2015. The animals had been involved in human-wildlife conflicts due to encroachment of construction sites or urban areas in Singapore. As a part of general health assessment, blood was collected for analyses, and samples from clinically normal pangolins were used to produce hematology and serum biochemistry reference intervals for Sunda pangolins. Potential effects of age and sex on hematology and serum biochemistry were also examined.

MATERIALS AND METHODS

Study animals

From January 2011 to December 2015, 58 Sunda pangolins that were rescued from various parts of Singapore were brought to the SZG veterinary hospital for health assessment and rehabilitation. Animals were rescued either by government agencies, NGOs or members of the public. Each individual was placed in a pet carrier and transported in a vehicle to the SZG, usually within 3–4 hr after capture. Upon arrival, animals were weighed and immediately placed in a quiet and dark environment with little human contact. In the morning of the next day the animals were anesthetized for a health assessment including physical examination, radiography, blood and urine sample collection, and fecal parasite screening. For anesthesia a gas chamber induction with isoflurane (Attane, Piramal Healthcare, Bethlehem, PA, U.S.A.) was used with an oxygen flow rate of 3.0 *l*/min. When the animals began to uncurl and showed no response to stimuli they were fitted with a mask and kept on 3% isoflurane with a flow rate of 1.5 *l*/min.

The general health assessment was conducted under anesthesia once the animals were stabilized. Several criteria for the exclusion of animals from this study were applied a *posteriori* (Table 1) based on the health assessment.

The maturity of apparently clinically normal male and female individuals was estimated during the health assessment based on their body weight. The body weight of mature individuals was compared to previously recorded data of Sunda pangolins at SZG over developmental milestones, *i.e.* at sexual maturity and at the time of mating. Sunda pangolins were considered adult when their body weight was above 4.0 kg in females and above 6.0 kg in males; animals below the respective threshold were considered juvenile.

After the health assessment, anesthetized pangolins were placed on 100% oxygen at a flow rate of 1.5 *l*/min until the animals began to curl. Each pangolin was subsequently placed in an individual pet carrier for recovery. On the respective following day, animals in an ambulant and mentally alert state were released by the National Parks Board into a suitable forested area in Singapore.

This study was conducted according to animal welfare ethics, and was approved by the Animal Welfare & Ethics Committee of Wildlife Reserves Singapore.

Blood sampling

Blood was collected from the coccygeal vein along the ventral midline of the tail (Fig. 1) after disinfecting the skin between the scales with 70% ethanol. A volume of 7–9 m*l* of whole blood was collected using a 10 m*l* syringe (Terumo Co., Tokyo, Japan) with a 21 G × 1.5" needle (Terumo Co.). Blood for hematology (500 μl) and serum biochemistry studies (3 m*l*) was collected in MiniCollect® blood collection tubes (Greiner Bio-One, Kremsmünster, Upper Austria, Austria) containing ethylene diaminetetraacetic acid (EDTA) and plain blood collection tubes, respectively. Immediately after collection a blood smear was produced, air-dried, and stored for analysis. Sample quality was recorded at the time of sampling and before analyses. Samples with signs of lipemia or hemolysis were excluded from the study. All samples were processed within 3 hr of collection, apart from blood smears which were stained and analyzed the following day.

Laboratory analyses

Analyses carried out on EDTA blood included white blood cell (WBC) counts, red blood cell (RBC) counts, hemoglobin (HGB) measurement, erythrocytic indices (i.e. mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular

hemoglobin concentration [MCHC]), platelet counts (PLT), and leukocytes differential (i.e. lymphocytes [LYM], monocytes [MON], neutrophils [NEU], eosinophils [EOS], basophils [BASO]). All hematological studies were performed using a VetScan HM5 (Abaxis, San Francisco, CA, U.S.A.) automated hematology analyzer with the canine setting. Packed cell volume (PCV) was determined by micro-hematocrit centrifugation at 14,000 rpm for 5 min (Hettich Centrifuge Mikro 200, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany).

EDTA blood samples from 20 Sunda pangolins rescued between January 2014 and November 2015 were used for both automated analyses and manual count of RBC and WBC to determine if there were any significant differences between the 2 methods. For the RBC count EDTA blood was diluted in a ratio of 1:200 of blood to isotonic saline. A blood counting chamber (Neubauer Improved blood counting chamber; Boeco, Hamburg, Germany) was used to carry out RBC counts with a light microscope (Nikon Eclipse E100; Nikon Corporation, Tokyo, Japan). WBC counts were carried out by diluting EDTA blood with Turk's stain (Turk's solution; Merck KGaA, Darmstadt, Germany) in a 1:20 ratio, also using a Neubauer hemocytometer chamber and a light microscope.

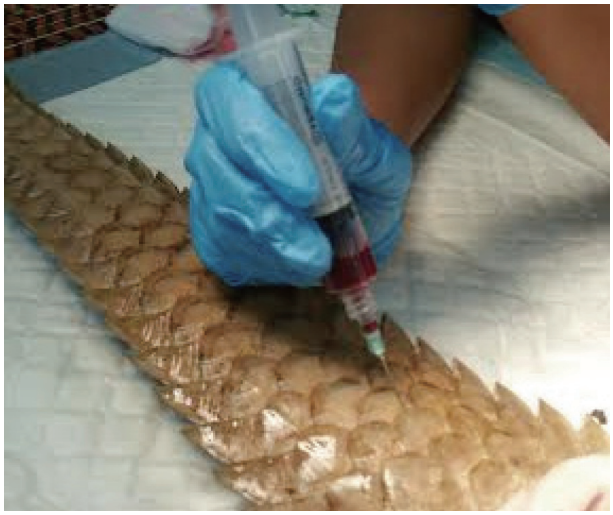

Fig. 1. Blood collection from the coccygeal vein on the ventral aspect of the tail of a Sunda pangolin under general anesthesia.

On the following day the blood smears were stained using Wright's Stain (Wright's Eosin Methylene Blue Solution; Merck KGaA) and a buffer to adjust the pH to 6.8 (Puffertabletten pH 6.8; Merck KGaA). Then, a manual leukocyte differential count was carried out using a light microscope at 1000X magnification to assess the respective proportion of each leukocyte type per 100 leukocytes. The total quantity of each leukocyte type was determined by multiplying the respective percentage from the manual differential count by the total manual leukocyte count.

Blood samples in plain tubes were left for a minimum of 1 hr to clot and were subsequently centrifuged at 3,750 rpm (4222 MKII, ALC International, Cologno Monzese, Italy) for 5 min to separate the serum. All serum biochemical analyses were performed using the VetScan 2 analyzer (Abaxis, Union City, CA, U.S.A.) with 100 μl of serum. The following parameters were measured: total protein (TP), globulin (GLOB), albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMY), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (CREA), glucose (GLU), calcium (Ca), phosphate (PHOS), sodium (Na^+), and potassium (K^+).

Statistical analyses

Manual count and automated count data of 20 Sunda pangolin samples were analyzed using Passing-Bablok regression and cumulative sum control chart (CUSUM) tests to determine if statistical differences were present between these two methods. Hematology and serum biochemistry data were analyzed in separate subsets categorized by age (juveniles and adults) and sex. Normal distribution of each independent variable was tested with the Reference Value Advisor Excel macroinstruction using the Anderson-Darling test, histograms, and Q-Q plots [15]. Outliers were identified and eliminated from the initial dataset using a Smirnov-Grubbs test; this procedure was then repeated on the reduced dataset. To determine the presence of statistically significant differences by age and sex, a Welch's unpaired *t*-test was used for normally distributed data, and a Mann-Whitney *U* test was used for non- normally distributed data. Statistical significance is reported at $P<0.05$. Descriptive statistics for parametric analyses included mean ($\bar{x}$) and standard deviation (SD), and for non-parametric analyses median (Mdn) values are reported. Minimum and maximum values are presented for both statistical approaches. All statistical analysis methods were performed using SPSS v. 19.0 (IBM Corporation, Armonk, NY, U.S.A.). The hematology and serum biochemistry values of this study were also compared to those of a previous study on confiscated Sunda pangolins in Thailand [37].

RESULTS

A total of 58 wild Sunda pangolins were rescued and presented to SZG between January 2011 and December 2015. Of these, 51 individuals (12 female juveniles, 6 female adults, 23 male juveniles and 10 male adults) that were clinically normal upon clinical examination were included in this study. Animals that were excluded from this study due to clinical illness were treated until they were fit for release.

Manual and automated RBC and WBC

Manual and automated RBC and WBC were conducted on 20 rescued Sunda pangolins (6 adults and 14 juveniles) between January 2014 and November 2015. No significant difference was observed between manual and automated RBC, total WBC, neutrophil count, eosinophil count, and monocyte count based on the Passing −Bablok regression graph (Fig. 2). Values from automated counts are shown as reference intervals in Tables 2–4.

The reference intervals of this study were compared to the results of a previous study on confiscated Sunda pangolins [36]

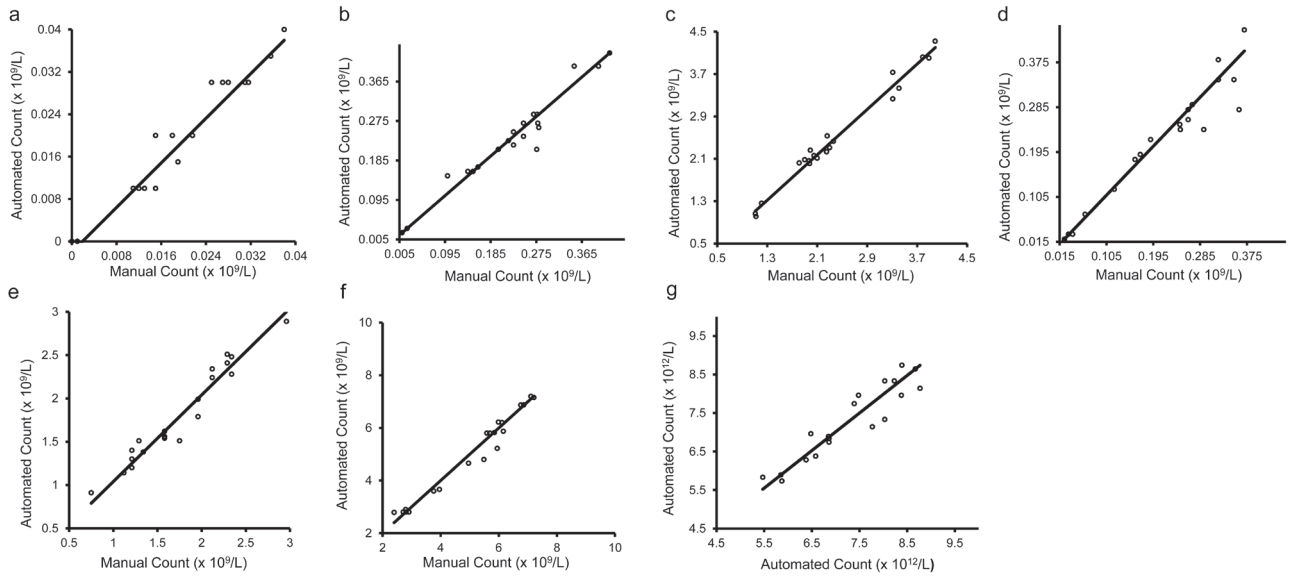

Fig. 2. Comparison of manual count (x) and automated count (y). The Passing−Bablok regression analysis: a. basophil (y=0.9752x + 0.1852), b. eosinophil (y=1.0000x + 0.0100), c. neutrophil (y=1.0714x−0.0746), d. monocyte (y=1.0924x−0.0063), e. lymphocyte (y=1.0000x + 0.0400), f. white blood cell count (y=0.9988x + 0.0056) and g. red blood cell count (y=0.9752x + 0.1852).

Table 2. Hematology and serum biochemistry parameters of rescued Sunda pangolins (*Manis javanica*) in Singapore

Parameters	N	Mean ± SD	Reference range	Distribution
PCV (%)	51	41.26 ± 6.61	25.00−55.00	NG
Hemoglobin (g/*l*)	51	140.20 ± 26.80	61.00−194.00	G
RBC $\times 10^{12}/l$	51	6.60 ± 1.60	1.92−9.65	G
MCV (f*l*)	51	65.00 ± 3.40	56.00−75.00	NG
MCH (*p*g)	49	20.99 ± 1.85	17.30−29.50	NG
MCHC (g/*l*)	50	322.90 ± 22.00	289.00−426.00	NG
WBC Count ($\times 10^9/l$)	51	7.82 ± 3.13	1.86−17.86	G
Lymphocytes ($\times 10^9/l$)	49	1.29 ± 0.69	0.30−3.00	NG
Monocytes ($\times 10^9/l$)	51	0.43 ± 0.41	0.01−2.50	NG
Neutrophils ($\times 10^9/l$)	50	5.70 ± 2.85	1.29−13.96	NG
Eosinophil ($\times 10^9/l$)	50	0.14 ± 0.19	0.00−0.97	NG
Basophil ($\times 10^9/l$)	50	0.01 ± 0.02	0.00−0.08	NG
Total Protein (g/*l*)	51	73.80 ± 9.30	50.00−93.00	NG
Globulin (g/*l*)	51	34.90 ± 12.90	11.00−66.00	G
Albumin (g/*l*)	51	39.00 ± 9.20	27.00−63.00	NG
ALT (U/*l*)	47	140.10 ± 87.80	71.00−569.00	NG
ALP (U/*l*)	44	482.50 ± 192.30	156.00−903.00	G
Total Bilirubin (μmol/*l*)	51	9.80 ± 3.60	6.00−22.00	NG
Glucose (mmol/*l*)	51	5.25 ± 1.43	2.60−9.70	NG
Blood Urea Nitrogen (mmol/*l*)	49	9.64 ± 4.47	3.70−20.60	NG
Creatinine (μmol/*l*)	43	37.20 ± 25.70	4.00−104.00	NG
Sodium (mmol/*l*)	51	144.40 ± 4.70	135.00−160.00	G
Potassium (mmol/*l*)	50	4.59 ± 0.56	3.70−6.20	NG
Calcium (mmol/*l*)	51	2.46 ± 0.16	1.96−2.78	G
Phosphorous (mmol/*l*)	51	2.47 ± 0.51	1.52−4.23	NG
Amylase (U/*l*)	49	351.40 ± 104.70	114.00−653.00	G

N indicates the number of animals used for the establishment of reference; G, Gaussian; NG, Non Gaussian.

(Table 3). WBC, neutrophil counts, ALP, and glucose levels were higher, and lymphocyte counts were lower in the present study, compared with the results of Thomas *et al* [37].

Table 3. Hematology and serum biochemistry values for Sunda pangolins (*Manis javanica*) compared to previously published results

Parameters	Rescued Sunda pangolins (Present study)			Confiscated Sunda pangolins (Previous study) [36]		
		n	Mean ± SD		n	Mean ± SD
PCV (%)		51	41.26 ± 6.61		39	41.67 ± 6.85
Hemoglobin (g/*l*)		51	140.20 ± 26.80		39	120.80 ± 19.10
RBC $\times 10^{12}/l$		51	6.60 ± 1.60		39	6.06 ± 1.05
MCV (fl)		51	65.00 ± 3.40		39	66.10 ± 2.82
MCH (*pg*)[a)]	Male	31	21.60 ± 4.74	Male	24	20.83 ± 1.01
	Female	18	22.73 ± 6.63	Female	15	21.77 ± 1.24
MCHC (g/*l*)[a)]	Male	31	336.00 ± 71.00	Male	24	317.90 ± 45.00
	Female	19	324.10 ± 27.70	Female	13	325.20 ± 91.00
WBC Count ($\times 10^9/l$)		51	7.82 ± 3.13		38	5.28 ± 1.96
Lymphocytes ($\times 10^9/l$)[a)]	Male	30	1.47 ± 0.91	Male	23	1.84 ± 0.54
	Female	19	1.36 ± 0.94	Female	15	4.44 ± 2.45
Monocytes ($\times 10^9/l$)		51	0.43 ± 0.41		38	0.09 ± 0.12
Neutrophils ($\times 10^9/l$)[a)]	Male	31	6.02 ± 2.90	Male	23	2.70 ± 1.32
	Female	19	5.18 ± 2.59	Female	15	4.44 ± 2.45
Eosinophil ($\times 10^9/l$)		50	0.14 ± 0.19		37	0.09 ± 0.10
Basophil ($\times 10^9/l$)		50	0.01 ± 0.02		37	0.02 ± 0.03
Platelet ($\times 10^9/l$)		47	139.60 ± 62.30		39	215.15 ± 100.96
Total Protein (g/*l*)		51	7.59 ± 0.96		39	8.13 ± 0.87
Globulin (g/*l*)		51	34.90 ± 12.90			ND
Albumin (g/*l*)		51	39.00 ± 9.20			ND
ALT (U/*l*)		47	140.10 ± 87.80		39	134.74 ± 57.48
ALP (U/*l*)		44	482.50 ± 192.30		25	374.28 ± 123.79
Total Bilirubin (µmol/*l*)		51	9.80 ± 3.60			ND
Glucose (mmol/*l*)		51	5.25 ± 1.43		39	3.88 ± 0.95
Blood Urea Nitrogen (mmol/*l*)		49	9.64 ± 4.47		39	11.26 ± 17.18
Creatinine (µmol/*l*)		43	37.20 ± 25.70			ND
Sodium (mmol/*l*)		51	144.40 ± 4.70			ND
Potassium (mmol/*l*)		50	4.59 ± 0.56			ND
Calcium (mmol/*l*)		51	2.46 ± 0.16			ND
Phosphorous (mmol/*l*)		51	2.47 ± 0.51			ND
Amylase (U/*l*)		49	351.40 ± 104.70			ND

a) Significant differences ($P<0.05$) were present on the previous study on confiscated Sunda Pangolins in Thailand [36] for these analytes between male and female Sunda pangolins. Values from previous study were converted from conventional units to SI units. ND=not done.

Effects of sex and age

No statistically significant difference between male and female Sunda pangolins was observed in any of the hematology and serum biochemistry parameters. Age, however, had a significant effect on several parameters (Table 4): ALP, RBC, and hemoglobin values were significantly higher in juvenile than in adult Sunda pangolins, and MCV and TP values were significantly lower in juvenile than in adult Sunda pangolins.

DISCUSSION

This is the first evaluation and establishment of hematology and serum biochemistry parameters of rescued wild Sunda pangolins in Singapore. Blood parameters are crucial data for health assessments of rescued animals and are also important for clinical decision making [1, 16, 24]. Several factors can affect hematological and serum biochemical values such as age, environment, diet, physiological stress, and immobilization [5, 30]. In this study, there are two different age groups of pangolins recorded. Blood samples were collected within 24 hr after the rescue of the Sunda pangolins. Several hematological and serum biochemistry parameters were observed to be significantly affected by the factors mentioned above.

Age group differences were observed on parameters of noted in both hematology and serum biochemistry. RBC and hemoglobin values were significantly higher ($P<0.05$) in juvenile than in adult pangolins. Similar findings such as higher RBC were previously observed in younger animals [31, 32, 38]. The MCV was significantly higher in adults than in juvenile Sunda pangolins. Similar findings have been reported in Chinese Pangolins [11, 26] and in southern white rhinos [31]. The lower MCV values in juvenile pangolins are possibly due to the gradual replacement of fetal hemoglobin by adult hemoglobin. It is possible that the high RBC and hemoglobin in juvenile Sunda pangolins could be due to increased excitement in juvenile animals prior to the rescue event. In

Table 4. Hematology and serum biochemistry parameters of rescued juvenile and adult Sunda pangolins (*Manis javanica*) in Singapore

Parameters	Juvenile			Adult			*P* value
	n	Mean ± SD	Reference range	n	Mean ± SD	Reference range	
PCV (%)	35	41.65 ± 7.75	20.00–58.00	16	38.75 ± 6.55	25.00–48.00	0.23
Hemoglobin (g/*l*)[a)]	35	143.63 ± 28.6	61.0–194.0	16	128.1 ± 18.37	92.00–153.00	0.33
RBC ($\times 10^{12}$/*l*)[a)]	35	6.81 ± 1.66	1.92–9.65	16	5.81 ± 1.13	3.22–7.39	0.04
MCV (fl)[a)]	35	63.85 ± 4.23	51.00–71.00	16	66.43 ± 3.76	61.00–75.00	0.04
MCH (*p*g)	34	21.81 ± 5.05	17.30–48.50	15	22.93 ± 6.84	18.70–46.20	0.45
MCHC (g/*l*)	35	318.00 ± 55.35	29.10–426.00	15	325.60 ± 143.40	33.70–747.00	0.19
WBC Count ($\times 10^9$/*l*)	35	7.49 ± 3.09	2.66–17.86	16	8.77 ± 3.36	1.86–14.32	0.21
Lymphocytes ($\times 10^9$/*l*)	35	1.45 ± 0.98	0.30–4.70	14	1.32 ± 0.82	0.46–2.62	0.77
Monocytes ($\times 10^9$/*l*)	35	0.44 ± 0.46	0.02–2.50	16	0.40 ± 0.27	0.01–0.89	0.70
Neutrophils ($\times 10^9$/*l*)	35	5.41 ± 2.65	1.8–13.96	15	6.62 ± 3.41	1.29–13.07	0.21
Eosinophil ($\times 10^9$/*l*)	35	0.14 ± 0.21	0.00–0.97	15	0.14 ± 0.15	0.02–0.57	0.42
Basophil ($\times 10^9$/*l*)	35	0.04 ± 0.13	0.00–0.80	15	0.01 ± 0.02	0.00–0.04	0.88
Platelet ($\times 10^9$/*l*)	34	145.41 ± 67.2	42.00–314.00	14	116.60 ± 37.81	63.00–177.00	0.88
Total Protein (g/*l*)[a)]	35	72.54 ± 9.25	50.00–93.00	16	76.50 ± 8.20	61.00–92.00	0.04
Globulin (g/*l*)	35	34.03 ± 12.58	4.00–66.00	16	37.90 ± 13.20	11.00–59.00	0.42
Albumin (g/*l*)	35	38.49 ± 9.12	24.00–62.00	14	38.20 ± 8.10	28.00–63.00	0.44
ALT U/*l*	33	194.46 ± 221.95	71.00–1116.00	15	126.60 ± 51.50	78.00–569.00	0.70
ALP (U/*l*)[a)]	31	656.73 ± 423.38	227.00–2719.00	13	444.30 ± 172.30	196.00–962.00	0.03
Total Bilirubin (μmol/*l*)	35	10.27 ± 3.89	6.00–22.00	16	8.90 ± 2.80	6.00–16.00	0.14
Glucose (mmol/*l*)	35	5.04 ± 1.17	2.60–9.40	16	5.54 ± 1.70	3.80–9.70	0.26
Blood Urea Nitrogen (mmol/*l*)	34	11.60 ± 10.87	3.70–60.50	15	9.81 ± 5.18	3.70–20.60	0.90
Creatinine (μmol/*l*)	31	49.38 ± 72.06	0.00–362.00	12	34.20 ± 19.40	9.00–149.00	0.61
Sodium (mmol/*l*)	35	144.03 ± 5.03	135.00–160.00	16	146.10 ± 5.30	135.00–150.00	0.44
Potassium (mmol/*l*)	35	4.59 ± 0.57	3.70–6.20	15	4.56 ± 0.46	3.90–9.20	0.82
Calcium (mmol/*l*)	35	2.45 ± 0.17	1.96–2.78	16	2.46 ± 0.13	2.30–2.63	0.61
Phosphorous (mmol/*l*)	34	2.56 ± 0.52	1.89–4.23	16	2.348 ± 0.38	1.52–2.78	0.15
Amylase (U/*l*)	34	344.79 ± 107.79	114.00–653.00	15	340.00 ± 49.60	272.00–616.00	0.56

a) Means between adult and juvenile animals were significantly different ($P<0.05$).

this state, catecholamines are released by the adrenal medulla due to sympathetic stimulation and cause smooth muscle contraction in the splenic capsules which leads to a release of RBC from the spleen into the blood stream [5, 25]. This can cause an increase in RBC and hemoglobin concentrations [25]. Home ranges have been reported in the Sunda pangolins and Cape pangolins [21], and based on these results it is possible that juvenile Sunda pangolins rescued in this study were recently weaned and in search of new home ranges in which to establish territories [25, 28]. Therefore, the exploration of novel habitats such as urban environments may have led to a physiological excitement response; the subsequent rescue by members of the public or by government agencies may have elicited a further catecholamine release that caused the observed difference in hematological parameters between age groups.

The ALP concentrations were significantly higher in juvenile than in adults in this study. Similar findings such as higher ALP were previously observed in younger animals [18, 22, 23]. Various isoenzymes of ALP are present in tissues such as liver, bone, intestines, kidney, and placenta [17]. In young animals, high ALP values are typically caused by increased osteoblastic activity resulting in high bone ALP isoenzyme release that leads to high ALP levels [17].

Serum protein of the adult pangolins was also significantly higher than that of juvenile pangolins. Similar findings were also reported in Formosan pangolins [26], other wildlife species [2, 23, 34], and domestic animals [18, 25]. The high serum protein in adult animals can be attributed to the increase of immunoglobulin concentrations with age, as well as to the accumulation of other proteins such as transferrin, complements, lipoproteins, fibrinogens, and haptoglobin [18].

The sex ratio of rescued Sunda pangolins in this study was about two males per one female. The higher number of male rescued pangolins could be related to higher activity of male pangolins in search for new home ranges or territories [28]. The ongoing habitat destruction and urbanization in Singapore causes male Sunda pangolins to move into existing or developing urban areas, which likely explains the higher proportion of males in the rescued population [10].

In a previous study on confiscated Sunda pangolins in Thailand the animals were kept in captivity for two to three weeks during which blood was sampled in order to produce hematological reference values. In our study blood samples were collected from rescued pangolins within 24 hr of rescue. The results of the present study showed higher WBC, neutrophil counts, ALP, and glucose levels, and lower lymphocyte counts than found by Thomas *et al* [37] (Table 3). The observed elevation of these parameters and low lymphocyte counts were likely due to physiological excitement following capture and transport. In the physiological excitement state, the increased endogenous release of glucocorticoids can affect the blood parameters by increasing the release of neutrophils from storage pools and from the marginal neutrophil pool into the circulating neutrophil pool [3, 20]. This subsequently reduces endothelial adherence which results in prolonged circulation time, causing leukocytosis and neutrophilia

[3] and extending the half-life of neutrophils [14, 27]. The elevation of endogenous glucocorticoids also results in the increased apoptosis of lymphocytes at the thymus cortex and sequestration of lymphocytes in lymphoid organs including bone marrow [3, 20, 27]. The differences in the values of WBC, neutrophils and lymphocyte counts in this study compared with the previous study were likely due to a physiological excitement state in the rescued Sunda pangolins that resulted in the endogenous glucocorticoid release.

In this study, ALP levels were higher than those found in the previous study on confiscated Sunda pangolins in Thailand. Higher ALP values may also be attributed to increased levels of endogenous glucocorticoids [17]. A correlation of increased ALP and the physiological excitement has been reported in dogs [17, 36]. Glucocorticoids may induce ALP isoenzyme release from tissues such as bone, intestines, and liver, which may explain elevated serum ALP levels [17].

No commercial blood analysis machine calibrated specifically to pangolin hematology is available. In this study, manual WBC counts, RBC counts, and WBC differentials were compared to those produced by an automated hematology analyzer to validate the use of the 'canine' settings of the Abaxis HM5 in for the analysis of pangolin WBC and RBC. Our results showed no significant differences between manual and automated counts for any of the parameters except for the basophil counts. Based on these results the 'canine' settings of the Abaxis HM5 machine can be used for automated counts of Sunda pangolin blood, apart from basophil counts which should be performed using a manual differential count method.

Hematology and serum biochemistry parameters examined in this study are important measures for clinical assessments of rescued Sunda pangolins. So far, there are few studies on hematology and serum biochemistry in this species, thus the reference intervals obtained in this study will be important for health evaluations of both rescued and captive Sunda pangolins in rescue centers, zoos, and other facilities. Moreover, it is important for clinicians to be aware that factors such as age, capture, and restraint can affect hematology and serum biochemistry values in pangolins.

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