Original Article

Hematology, Blood Biochemistry and Cytochemical Characteristics of Blood Cells of Captive Aldabra giant tortoise (*Aldabrachelys gigantea*) in Thailand

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Abstract

Aldabra giant tortoises (*Aldabrachelys gigantea*), well-known pet tortoises worldwide, were classified as Appendix II by The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This study aims to investigate the hematological, blood biochemical, and cytochemical characteristics of blood cells in healthy Aldabra giant tortoises in captivity. Blood samples were collected from the dorsal tail vein of 12 tortoises (6 males and 6 females) during May and October 2022. The hematological values showed no statistically significant differences between male and female tortoises. However, in female tortoises, calcium and phosphorus levels were significantly higher than in male tortoises ($P<0.05$). Conversely, uric acid levels in males were significantly higher than in females ($P<0.05$). The staining techniques employed included Wright-Giemsa, Sudan Black B, Acid Phosphatase, Alkaline Phosphatase, Periodic Acid Schiff, and Peroxidase staining. Wright-Giemsa staining is the most suitable stain for basic hematology and differential counts. The morphology of red blood cells was characterized by oval cells with round or oval concentric nuclei. The white blood cells were classified into five categories: heterophils, eosinophils, basophils, lymphocytes, monocytes or azurophils, and thrombocytes. Each type of white blood cell exhibited different staining results, depending on their characteristic or physiological properties. The results of this study can be instrumental for future health care, research in veterinary medicine, and the conservation of Aldabra giant tortoises.

Keywords: Captive farm, Chelonian, Clinical information, Reptile, Vulnerable species

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Introduction

Aldabra giant tortoises (Order: Testudinidae; Family: Testudinidae; Species: *Aldabrachelys gigantea*) are among the largest tortoises in the world. Their average weight ranges from 160 to 250 kilograms. The carapace can measure up to 120 cm in length, exhibiting a dark gray to black color and a high domed shape. Their large size enables them to store substantial food reserves as fat, which are metabolized at an extremely slow rate (Gerlach, 2004). These tortoises inhabit environments such as mangroves, shrublands, and grasslands and are endemic to the islands of Aldabra Atoll in the Seychelles (Walton *et al.,* 2019).

Aldabra giant tortoises in their natural habitat face a significant risk of extinction due to human invasion and illegal trade. According to the International Union for Conservation of Nature (IUCN) Red List, Aldabra giant tortoises are classified as vulnerable. Additionally, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has classified Aldabra giant tortoises under Appendix II. This classification includes species not necessarily threatened with extinction but for which trade must be controlled to avoid utilization incompatible with their survival. International trade in specimens of Appendix II species may be authorized by the granting of an export permit or re-export certificate (IUCN, 1996).

As a pet species, most Aldabra giant tortoises in Thailand are imported from overseas captive breeding farms. They can be purchased legally under the terms of CITES, making them quite expensive, and it takes over 10 years for them to reach reproductive maturity. The limited availability of Aldabra giant tortoise farming in Thailand and globally results in a restricted pet population of the species, as well as limited data for veterinary care and practice. The objectives of this study are to investigate the hematological values and blood biochemistry of Aldabra giant tortoises, as well as to examine the cytochemical characteristics of their blood cells. The aim is to provide valuable information for veterinarians to aid in medication, conservation, experiments, and further studies. The scope of the study was confined to Aldabra giant tortoises raised in captive breeding farms in Thailand.

Materials and Methods

Animals and sample collection: All animal husbandry and experimental conditions were approved by the Chulalongkorn University Animal Care and Use Committee. Protocol number: 2031097. Twelve clinically healthy and mature Aldabra giant tortoises (6 males and 6 females), estimated to be between 20 – 30 years old, were housed in a tortoise holding facility in Prachin Buri province, Thailand. None were seen with nasal and abdominal discharges or skin lesions. External and internal parasites were not found. The enclosure had a garden and fence. The ground area of the enclosure was about 900 m^2, and 20% of the area was concrete with shading for the feeding zone. All tortoises were fed with mainly grass and green leaves. Fruits, such as bananas or mango, were supplemented as a treat. Samples were collected from May to October 2022 (rainy season). The temperature range was between 25 – 35 °C.

The tortoises were restrained on the ground, and their straight carapace length and width, curved carapace length and width, and plastron length and width were measured using a measuring tape and recorded. The tortoises were then suspended on a sling rope and weighed with a hanging scale, with the weights recorded. Blood samples were collected by manual restraint without chemical sedation (Figure 1A, 1B). A total of 3 ml of blood was drawn from the dorsal tail vein using a 21G needle, and the blood was stored in lithium-heparin tubes. Each blood sample was divided into three parts of 1 ml each for hematological testing, blood chemistry, and cytochemical staining. All samples were kept at 4°C and transferred to the Veterinary Medical Aquatic Animal Research Center of Excellence for analysis.

Blood analyses: All hematologic, plasma biochemical, and cytochemical analyses were performed within 24 hours after collection. Red blood cell (RBC) and white blood cell (WBC) counts were determined manually using a hemocytometer (Improved Neubauer, Boeco, Hamburg, Germany) after the blood was diluted 1:200 with Natt and Herrick solution. RBCs were counted in the five RBC squares of the central large square of the grid. Counts were performed in duplicate at 400X magnification and multiplied by 10,000 to calculate RBC/µL. WBCs and thrombocytes were counted together because small lymphocytes and thrombocytes could not be clearly distinguished. Cells were counted in nine large squares in duplicate at 400X magnification and multiplied by 200 to calculate the total WBCs and thrombocytes/µL (Campbell, 2012). Two blood smears were prepared from each sample and stained with Wright-Giemsa; WG (M&P Impex, Bangkok, Thailand). Differential leukocyte counts were performed on 100 WBCs for each animal and classified using a 1000X light microscope. Absolute counts for each leukocyte type were calculated based on percentages of each type and the total WBC count. Types of WBCs were heterophils, eosinophils, basophils, lymphocytes, and azurophils. Pack cell volume (PCV) or hematocrit (Hct) was measured using standard centrifugation of microhematocrit tubes in a micro hematocrit centrifuge (WiseSpin®, Daihan Scientific Co., Ltd., Korea) at 12,000 RPM for 5 minutes. Hemoglobin was analyzed using the cyanmethemoglobin technique, which employs Drabkin's reagent (Sigma-Aldrich, St. Louis, MO, USA) that reacts with all forms of hemoglobin except sulfhemoglobin in the blood. The color development was measured using a UV spectrophotometer at 546 nm (Spectronic 20 Genesys, Thermo Spectronic, Madison, WI, USA).

Blood biochemistry was performed using automated analyzers (Knotková *et al.*, 2002). Blood samples from each tortoise were placed into heparinized tubes and centrifuged to yield plasma for biochemical analyses. The concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), uric acid, Blood Urea Nitrogen (BUN), total protein, albumin, globulin, phosphorus (P), and calcium (Ca) were performed

using a VetTest Chemistry Analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA).

Cytochemical staining of blood cells was performed on two blood smears prepared from each sample. Air-dried blood smears were stained with Sudan Black B; SBB (Procedure No.308), Peroxidase; PO (Procedure No.391), Acid phosphatase; AcP (Procedure No.181), Alkaline phosphatase; ALP (Procedure No.8), and Periodic Acid-Schiff; PAS (Procedure No.395B) according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Positive and negative-stained cells were differentiated by counting 100 cells on each of the cytochemically stained smears (Chansue *et al.*, 2011).

Statistical analysis: All data were analyzed using IBM SPSS Statistics version 28 for Windows. Means, standard deviations (SD), and medians were calculated for both male and female tortoises. The influence of sex on the minimum and maximum lengths of each blood cell type, as well as on hematologic and biochemical variables, was assessed using an independent *t*-test and Mann-Whitney U test, with significance set at $P < 0.05$.

Figure 1 (A) The tortoises were weighed with a hanging scale, and the straight carapace length and width, curved carapace length and width, and plastron length and width were measured using measuring tape. (B) Blood samples were collected from the dorsal tail vein without chemical sedation.

Result

Physical characteristics of Aldabra giant tortoise: The average straight carapace length and width for male tortoises were 94.00 ± 4.56 cm and 65.00 ± 3.56 cm (mean ± SD), respectively. For female tortoises, these measurements were 79.00 ± 3.11 cm and 58.67 ± 3.43 cm, respectively. The average curved carapace length and width, measured with a tape along the curve, were 119.58 ± 4.78 cm and 121.08 ± 8.86 cm for males and 107.92 ± 6.09 cm and 102.58 ± 3.68 cm for females, respectively. The average plastron length and width were 79.42 ± 7.63 cm and 65.27 ± 5.61 cm for males and 72.25 ± 4.67 cm and 60.83 ± 3.84 cm for females, respectively. The mean body weights were 115.77 ± 8.84 kg for males and 94.18 ± 7.87 kg for females.

Hematology: Calculated hematologic data collected from 12 animals are presented in Table 1. No statistically significant differences were observed between male and female tortoises for any of the hematological values ($P > 0.05$).

Blood biochemistry analysis: Clinical blood biochemistry analyses are shown in Table 2. Female tortoises had statistically significantly higher concentrations of calcium and phosphorus compared to males ($P < 0.05$). Conversely, uric acid levels were statistically significantly higher in males than in females ($P < 0.05$). No other blood chemistry values exhibited statistically significant differences between male and female tortoises.

Cytochemical characteristics of blood cells: Blood cells were classified into the following types: RBCs, thrombocytes, lymphocytes, heterophils, eosinophils, basophils, and azurophils. The cytochemical staining reactions of blood smears were comparable for both male and female tortoises.

Red blood cells were oval-shaped, with round or oval nuclei located centrally within the cell. They exhibited variability in both cell and nucleus size. The cytochemical staining characteristics of red blood cells are detailed in Table 3 and illustrated in Figures 2A (SBB), 2B (AcP), 2C (ALP), 2D (PAS), and 2E (WG).

Thrombocytes varied in size and shape, predominantly appearing round to oval. They were smaller than lymphocytes and had a high nuclear-to-cytoplasmic ratio. The cytoplasm of thrombocytes was stained purple. The cytochemical staining characteristics of thrombocytes are provided in Table 3 and depicted in Figures 2F (SBB) and 2G (WG).

Lymphocytes were round, with variable sizes and a large, round, slightly eccentric nucleus exhibiting a high nuclear-cytoplasmic ratio. WG staining revealed that the nucleus was darkly stained with clumped chromatin, while the cytoplasm was basophilic. The cytochemical staining characteristics of lymphocytes are detailed in Table 3 and illustrated in Figures 2S (AcP), 2T (PAS), and 2U (WG).

Heterophils were the most frequent leukocytes. These cells were round to irregular in shape with fusiform granules in the cytoplasm, and nuclei were eccentric of cells. The size of heterophils is equal to or smaller than monocytes. The cytochemical staining

characteristics of heterophils are listed in Table 3 and shown in Figure 2H (SBB), 2I (PAS), and 2J (WG).

Eosinophils were typically round cells with either one or two lobes in the nucleus and occasionally a single lobe. The cytoplasm contained relatively large granules. The cytochemical staining characteristics of eosinophils are detailed in Table 3 and illustrated in Figures 2K (PO), 2L (SBB), 2M (AcP), 2N (ALP), 2O (PAS), and 2P (WG).

Basophils exhibited variable shapes and contained numerous large round granules that often obscured the nucleus. WG staining revealed a purple nucleus with unstained granules (Figure 2R). In PAS staining, the nucleus appeared blue, while the intergranular cytoplasm was dark purple (Figure 2Q).

Azurophils had variable sizes and shapes. These cells were round with nuclei that were either irregular or round and located centrally or eccentrically within the cell. The cytochemical staining characteristics of azurophils are listed in Table 3 and illustrated in Figures 2V (SBB) and 2W (WG).

Table 1 Hematologic values of the Aldabra giant tortoises (*Aldabrachelys gigantea*)

Variable	All tortoises (n=12)	Male (n=6)	Female (n=6)	Range
PCV (%)	16.33 ± 5.85	18.5 ± 5.09	14.67 ± 6.18	9.00 – 25.00
Hb (g/dL)	5.18 ± 2.02	6.29 ± 1.07	4.07 ± 2.21	2.00 – 8.33
RBC (x 10^6 cells/µL)	0.50 ± 0.16	0.59 ± 0.13	0.40 ± 0.13	0.22 – 0.77
WBC (x 10^3 cells/µL)	1.75 ± 0.39	2.00 ± 0.39	1.51 ± 0.19	1.21 – 2.42
Heterophils (%)	41.67 ± 0.43	39.83 ± 2.99	42.5 ± 2.66	36.00 – 46.00
Eosinophils (%)	8.75 ± 2.01	8.67 ± 1.86	8.83 ± 2.31	6.00 – 12.00
Basophils (%)	3.00 ± 1.21	2.83 ± 1.17	3.17 ± 1.33	1.00 – 5.00
Lymphocytes (%)	31.25 ± 1.96	31.50± 2.26	31.00 ± 1.79	28.00 – 34.00
Azurophils (%)	18.83 ± 2.79	20.00 ± 3.29	17.67 ± 1.75	15.00 – 25.00

Data were reported as mean±SD

Table 2 Blood biochemical values of the Aldabra giant tortoises (*Aldabrachelys gigantea*)

Analyte	Tortoises (N = 12)		Male (N = 6)		Female (N = 6)		Range
	Mean±SD	Median (P1, P3)	Mean±SD	Median (P1, P3)	Mean±SD	Median (P1, P3)	
ALT (U/L)	34.33±21.04	19.00 (13.25,33.00)	24.67±10.11	18.00 (17.00,27.00)	28.67±23.19	22.00 (12.00,35.00)	15.00 – 72.00
AST (U/L)	78.58±37.39	61.50 (38.50,103.25)	70.83±37.97	65.50 (37.00,107.00)	71.00±31.88	61.50 (43.00,92.00)	40.00 – 136.00
ALP (U/L)	64.83±31.38	70.50 (34.24,82.50)	56.67±34.63	66.00 (18.00,83.00)	67.33±26.67	76.00 (38.00,81.00)	18.00 -100.00
Uric acid (mg/dL)*	1.31±0.77	1.25 (0.88,1.55)	1.75±0.68	1.50 (1.20,2.50)	0.83±0.57	1.00 (0.20,2.30)	0.10 – 2.70
BUN (mg/dL)	12.00±8.71	11.50 (4.25,18.25)	19.00±15.40	17.50 (9.00,21.00)	8.17±5.38	5.50 (4.00,14.00)	2.00 – 29.00
TP (g/dL)	4.58±1.95	4.35 (2.95,5.07)	4.13±0.82	4.35 (4.00,4.50)	4.47±2.17	4.65 (2.40,6.40)	1.60 – 6.10
Albumin (g/dL)	1.44±0.60	1.30 (1.13,1.75)	1.32±0.25	1.25 (1.20,1.30)	1.38±0.66	1.40 (0.80,2.00)	0.50 – 2.20
Globulin (g/dL)	3.14±1.37	3.10 (1.13,1.73)	2.82±0.66	3.10 (2.80,3.20)	0.66±3.08	3.25 (1.60,4.40)	1.10 – 4.90
Calcium (mg/dL)*	20.65±15.54	11.55 (10.15,25.05)	10.63±1.21	10.30 (9.80,10.60)	25.35±14.37	24.9 (12.50,29.10)	10.30 – 50.40
Phosphorus (mg/dL)*	5.96±4.73	3.65 (2.90,5.08)	3.02±0.61	2.95 (2.90,3.60)	6.67±4.52	4.75 (3.70,9.50)	2.00 – 14.50

*The difference between males and females was significant (P<0.05).
ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP, alkaline phosphatase; BUN, blood urea nitrogen; TP, total protein.

Table 3 Blood biochemical values of the Aldabra giant tortoises (*Aldabrachelys gigantea*)

Cell type	PO	SBB	AcP	ALP	PAS	WG
Red blood cells	-	+	+	+	+	+
Thrombocytes	-	+	-	-	-	+
Heterophils	-	+	-	-	+	+
Eosinophils	+	+	+	+	+	+
Basophils	-	-	-	-	+	+
Lymphocytes	-	-	+	-	+	+
Azurophils	-	+	-	-	-	+

Staining was scored as negative (-) and positive (+).
PO; Peroxidase, SBB; Sudan Black B, AcP; Acid phosphatase, ALP; Alkaline phosphatase, PAS; Periodic Acid-Schiff, WG; Wright–Giemsa

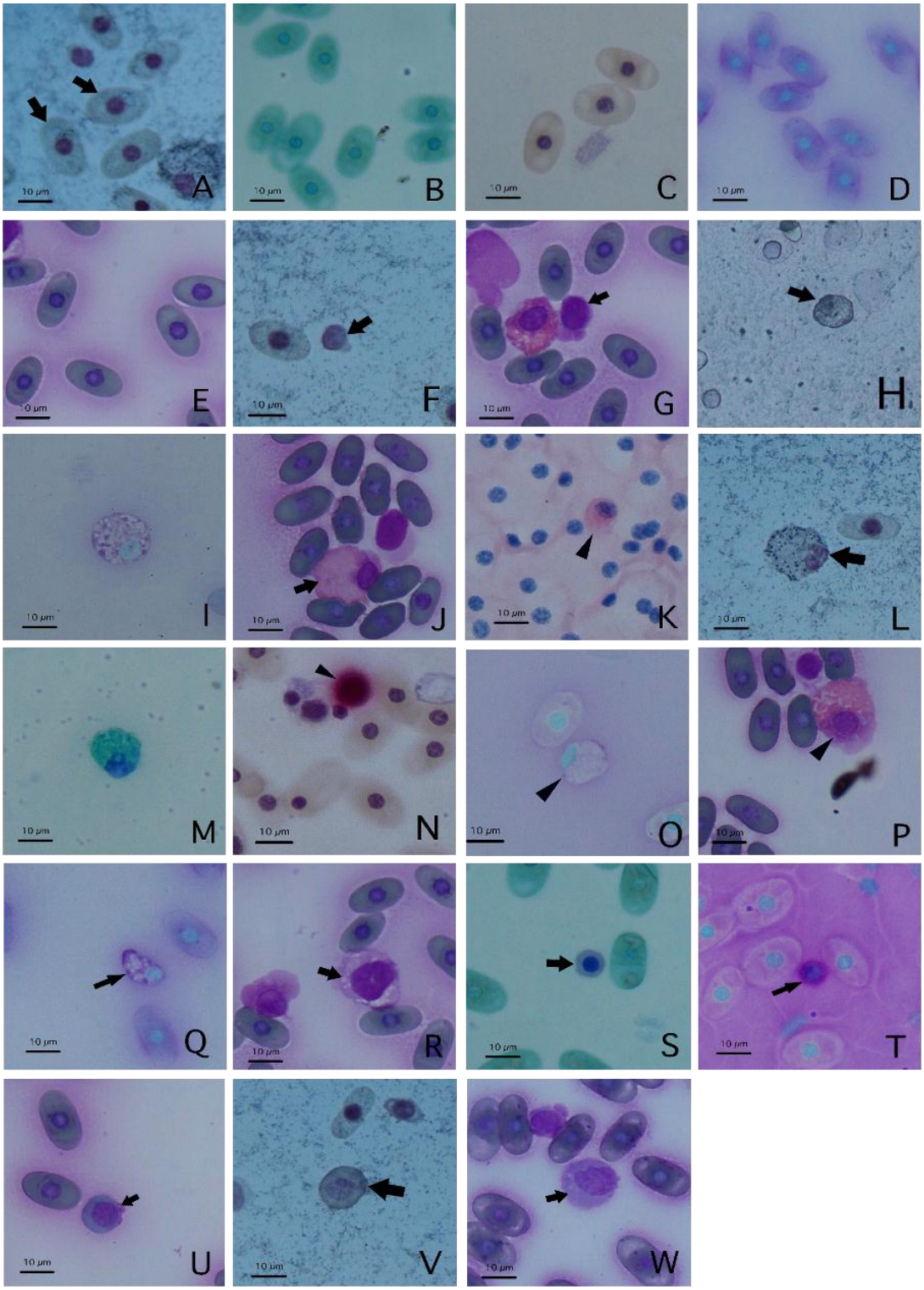

Figure 2 Light microscopic pictures of blood cells of Aldabrachelys gigantea. (A) RBCs (arrow). SBB. (B) RBCs. AcP. (C) RBCs. ALP. (D) RBCs. PAS. (E) RBCs. WG. (F) In thrombocytes, the nucleus was stained purple, and the cytoplasm was stained black with a greater stain in the rim of the cytoplasm. SBB. (G) Thrombocytes. WG. (H) Heterophil has an irregular shape; the nucleus is dark purple, and the intergranular cytoplasm is black. SBB. (I) The Heterophil nucleus was light blue; the intergranular cytoplasm was light purple. PAS. (J) In Heterophil (arrow), the nucleus was dark purple, and the cytoplasm was light purple. WG. (K) Staining of eosinophil granules with pale red color. PO. (L) Eosinophil with positive diffusely black granules. SBB. (M) Eosinophil was positive with greenish-blue granules. AcP. (N) Eosinophil (arrowhead), positive bright red stained cytoplasm. ALP. (O) The eosinophil and intergranular cytoplasm were light purple, and the nucleus was stained blue. PAS. (P) Eosinophil (arrowhead), the nucleus was dark purple, with red granules in the cytoplasm. WG. (Q) Basophil (arrow), the nucleus was blue, and the intergranular cytoplasm was dark purple. PAS. (R) Basophil (arrow), the nucleus was purple, and the granules were not stained. PAS. (S) In the Lymphocyte (arrow), the nucleus was purple to blue, and the cytoplasm was stained pale blue. AcP. (T) In the Lymphocyte (arrow), the nucleus was dark blue, and the cytoplasm was purplish-pink. PAS. (U) Lymphocyte (arrow). WG. (V) Azurophil (arrow). Round shape, the nucleus was dark purple and gray to black cytoplasm. SBB. (W) Azurophil (arrow). WG.

Discussion

This study presents the first comprehensive report on the hematological and biochemical values of Aldabra giant tortoises (*Aldabrachelys gigantea*) in a captive breeding farm setting. A comparison of the hematology values of captive healthy Aldabra tortoises from this study with those from previous studies reveals differences. It appears that Hb and RBC values were higher, and WBC values were lower than those reported for free-living Aldabra tortoises in a previous study (Hart *et al.,* 1991). Hematological data can be used as a rapid assessment tool for evaluating animal health, with variations in blood parameters linked to inflammatory and hemoparasitic diseases in chelonians; additionally, factors such as sex, age, season, geographic location, reproductive stages, and nutritional status also affect for these blood parameter variations (Christopher *et al.,* 1999; Dickinson *et al.,* 2002; Wilkinson, 2003). In this study, we controlled the mature size of the tortoises and collected samples during the rainy season in Thailand. Age differences were no statistically significant differences between the subadult and adult groups in the natural environment (Hart *et al.,* 1991). The higher RBC values in captive-breeding tortoises may result from nutritional and environmental factors (Da Silva Bergamini *et al*., 2017). The most abundant leukocytes were heterophils, followed by lymphocytes, azurophils, eosinophils, and basophils. The proportions of these cells within a specific reptile species vary depending on individual physiological status and the investigation method used (Campbell and Ellis, 2007). WBC values can be high in wild tortoises because of mild parasitic infestation. Heterophils and eosinophils may show a diagnostically useful response to inflammatory disease and parasitic infection, respectively (Hart *et al*., 1991).

The blood chemistry values for Aldabra tortoises differ from those of other tortoise species, reflecting the broad normal range of values in reptiles, which is influenced by their habitats. Notably, female Aldabra tortoises exhibited statistically significantly higher levels of calcium and phosphorus than males. This difference may be attributed to reproductive activities associated with folliculogenesis, eggshell production, and bone calcium mobilization (Clarke, 1967; Dessauer, 1970). Additionally, uric acid levels were statistically significantly higher in males compared to females. This disparity can be linked to physiological factors, particularly the role of estrogen hormone in promoting renal excretion of uric acid (Antón *et al.*, 1986). Similar findings of higher uric acid levels in males *Testudo* spp. (Mathes *et al.*, 2006) and *Geochelone radiata* (Christopher *et al.* , 1999) have also been reported.

A comparison of the blood chemistry values of captive healthy Aldabra tortoises from this study with those from previous studies reveals both similarities and differences. The plasma values of alkaline phosphatase (ALP), calcium, and phosphorus of captive Aldabra were higher than those reported for free-living Aldabra tortoises in a previous study (Ghebremeskel *et al.,* 1991). Calcium deficiency is the most common nutritional disease found in captive reptiles, while nutritional deficiencies are rarely seen in their natural habitats (Wallach, 1977). Effective management practices on captive farms can enhance nutritional intake, promoting better growth and reproduction. *Geochelone pardalis* individuals receiving calcium supplements at three times the recommended dosage exhibited the fastest growth rates. Additionally, tortoises demonstrate improved calcium absorption when their diet contains higher levels of dietary calcium (Fledelius *et al.,* 2005). Seasonal variations in total calcium levels in tortoises have been reported. These fluctuations may partially stem from increased estrogen levels in female reptiles, which can lead to a hypercalcemic effect due to the mobilization of calcium from bone. Such changes occur seasonally, as heat and light levels impact reproductive cycles (Eatwell, 2009). The uric acid levels observed in this study were consistent with previously published findings for free-living Aldabra tortoises. However, the total protein and blood urea nitrogen (BUN) levels were lower in the captive tortoises compared to their free-living counterparts (Ghebremeskel *et al.,* 1991). Increased BUN levels may indicate renal compromise, muscle catabolism, dehydration, or increased protein consumption. However, based on the physical examination and hematological findings, there was no evidence of renal compromise. The variations in BUN levels were suspected to be caused by the differences in diet fed at the locations (Cerreta *et al.,* 2019).

Specific cytochemical stains were utilized to highlight the staining properties of circulating leukocytes, facilitating their identification; these stains can serve as valuable markers for leukocytes in cytological preparations. (Alleman *et al.,* 1992). They contributed to our understanding of the chemical components and functions of different blood cell types in Aldabra giant tortoises. Regarding the cytochemical characteristics of blood cells, SBB and PAS staining were used to detect intracellular glycogen and lipid particles. These components are crucial as they provide sufficient energy for phagocytosis (Ueda *et al.,* 2001). SBB is a lipophilic dye that binds to granule components in granulocytes, eosinophils, and some monocytes of mammals (Bain, 2016). In the SBB staining of Aldabra giant tortoises, eosinophils, heterophils, and azurophils exhibited positive staining, with diffusely black granules in the cytoplasm. Thrombocytes also stained black but lacked granules in the cytoplasm. Basophils and lymphocytes were negative for SBB staining.

PAS staining indicated the presence of intracellular glycogen and neutral mucopolysaccharides (Jamal, 2020). The PAS stain can indicate the presence of neutrophils, eosinophils, basophils, lymphocytes, monocytes, and thrombocytes (Sugiyama, 1955). Periodic acid specifically oxidizes 1-2 glycol groups to produce stable dialdehydes, which yield a red reaction product when exposed to Schiff reagent (Bain, 2016). In the current study, basophils, eosinophils, heterophils, and lymphocytes of captive tortoises were PAS-staining positive, displaying light to dark purple in the cytoplasm and blue-stained nuclei. Azurophils and thrombocytes were negative.

AcP and ALP are lysosomal enzymes involved in the phagocytic process, which is secreted into the phagocytic vacuoles that have ingested bacteria and

other particles (Hirsch and Cohn, 1964). AcP is present in all hematopoietic cells, lymphocytes, monocytic cells, plasma cells, and platelets (Jamal, 2020). In the current study, eosinophils exhibited AcP positivity with greenish-blue granules in the cytoplasm, while lymphocytes were stained pale blue in the cytoplasm. Other white blood cells and thrombocytes were negative. ALP is present in the cytoplasm of neutrophils, eosinophils, and B-lymphocytes (Jamal, 2020). Although ALP appears as a granular reaction product in the cytoplasm, enzyme activity is associated with a poorly characterized intracytoplasmic membranous component distinct from primary or secondary granules (Rosner and Lee, 1965). In this study's ALP staining, eosinophils were positive with bright red cytoplasm, whereas other white blood cells were negative.

PO is found in the primary azurophilic granules of neutrophils, eosinophils, and monocytes in mammals and participates in the defense against bacterial infection. Its activity increases with maturation, and no activity is observed in red cells or lymphocytes (Azevedo and Lunardi, 2003). In the PO staining of this study, eosinophils were positive with red coarse granules, while other WBCs and RBCs were negative.

WG staining is a common procedure routinely performed in hematology laboratories. Consistency in intra-laboratory staining quality is essential for the accurate morphological interpretation of blood smears (Dunning and Safo, 2011). This stain is used for differentiating the nuclear and cytoplasmic morphology of thrombocytes, erythrocytes, and lymphocytes in blood smears or bone marrow aspirates (Biognost, 2018). In the WG staining of this study, RBCs, thrombocytes, and all WBCs were positive, indicating that this stain may be the most suitable stain for routine hematological evaluation in Aldabra giant tortoise.

As the Aldabra giant tortoise is classified as a vulnerable species on the IUCN Red List (IUCN, 2021), limited data were available for veterinary care or health monitoring. Additionally, the trend of raising exotic animals in Thailand has become increasingly popular, with the Aldabra giant tortoise emerging as a favored species. Reptiles are recognized carriers of *Salmonella*, posing a significant risk for human salmonellosis, which typically results in moderate gastrointestinal disorders but can escalate to severe conditions like bacteremia or meningitis. Healthy turtles and tortoises are commonly infected with *Salmonella* and present a risk of possible transmission to people who come in close contact with them. Individuals working with these animals, including owners, keepers, and veterinarians, should be concerned about the risks of zoonosis. To minimize the potential spread of *Salmonella* between reptiles and humans, maintaining strict personal hygiene, particularly thorough handwashing after handling the animals, is essential (Prapasarakul *et al.*, 2012; Sringam *et al.*, 2021: Pees *et al.*, 2023).

The hematological and biochemical blood values obtained from captive Aldabra giant tortoises in this study can serve as valuable reference data for the health management and monitoring of this endangered species. This information will be important for further studies evaluating the effects of other factors, such as sex, age, and environment. It is also expected to be useful in future clinical studies, veterinary care practices, and *ex-situ* conservation efforts.

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