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Hematology, Morphology and Cytochemistry of Blood Cells in Lesser Adjutant (*Leptoptilos javanicus*) and Greater Adjutant (*Leptoptilos dubius*)

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ABSTRACT

Blood samples of 10 (6 males and 4 females) lesser adjutant (*Leptoptilos javanicus*) and one male greater adjutant (*Leptoptilos dubius*) at Khow Khoew Open Zoo, were collected from the cutaneous ulna vein for basic hematologic, light microscopic and cytochemical studies of blood cell. Heparin was used for anticoagulant in all adjutants. There was no hematozoa detected in all adjutants. There was no significant difference in all hematological values between the male and female lesser adjutants. Lymphocytes were the second commonly observed leukocytes in the lesser adjutant and the male greater adjutant. They averaged 6-8 μm in diameter. By α-naphthyl acetate esterase (ANAE) and β–glucuronidase (β–glu), they were negative and fine granular reactivities. Heterophils were the most commonly observed leukocytes in the adjutants. Heterophils contained many pleomorphic, dull eosinophilic granules and lobed nucleus. Heterophils were the medium-sized cells, averaging 8-12 mm in diameter. Heterophil granules were negative with all cytochemical stainings but pinkish stained with β–glu. The granules of eosinophil in the lesser adjutant revealed many small round granules whereas those of the greater adjutant were pleomorhic granules. They usually were the largest leukocytes, averaging 9-14 μm in diameter. The numbers of eosinophil were quite high in both the male and female lesser adjutants. Eosinophil granules were negative with all cytochemical stainings. Basophils were as small as lymphocytes but revealed many small granules surrounding central round nucleus. Basophils were moderately stained with ANAE and β–glu. These results provided comparative hematologic data, a guide for identification and cytochemical reactions of blood cell in lesser adjutant and greater adjutant.

Key words: cytochemistry, greater adjutant, hematology, lesser adjutant

INTRODUCTION

The lesser adjutant (*Leptoptilos javanicus*) and greater adjutant (*Leptoptilos dubius*) are classified in the genus *Mycteria*, family *Ciconiidae* which includes 19 species in the world but 11 species in Asia. The lesser adjutants have massive dirty yellowish wadge-shaped bill, chiefly glossy metallic black upperparts and white underparts. There are sparse hair-like feathers on naked reddish-yellow head and neck. The greater adjutants are black, grey and dirty-white with naked dull flesh and yellow head and neck, and a huge wadge-shaped bill. Large necked pinkish gular pouch is hanging from base of the neck. Their distributions are in South Asia and South-East Asia but not

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numerous. The greater adjutants are much rarer than the lesser adjutants (Sonobe and Usui, 1993).

Morphologic characteristics of avian blood cells are heterogeneous. Variations in cell characteristic and cell population exist among species within the class Aves (Fudge, 2000). Evaluation of avian hematologram has become a useful tool for the diagnosis of avian diseases (Campbell, 1995). Abnormal complete blood count associated with naturally acquired infections and/or traumatic injury in variety of non-domestic bird species. Heteropenia with left shift and toxicity was an indicator of poor prognosis (Bienzle *et al*., 1997). However, basic hematological values, a guide for identification and cytochemistry of blood cells have not been described in these species. The purpose of this study was to obtain the hematologic data and cytochemistry of blood cells in both the lesser adjutant and greater adjutant.

MATERIALS AND METHODS

In July 2003, blood samples were collected from 10 (6 males and 4 females) mature lesser adjutants and one male greater adjutant located at Khow Khoew Open Zoo, Bangpra, Sriracha. They were captive-bred and were housed freely in large enclosure. Each bird was manually restrained, and blood was collected from the cutaneous ulna vein using a 22-ga needle and disposable syringe. Blood smears were prepared immediately, then air-dried and stained with Wright's-Giemsa (WG) stain and Wright's (W) stain. The whole blood samples were anticoagulated with heparin, stored at 4°C and processed within 2 hours. The complete hematology were performed using the same methods as studied in King cobra (Salakij *et al*., 2002).

Blood smears were fixed in methanol and stained with WG stain (Jain, 1986) for determination of differential leukocyte (WBC) count, identification of hematozoa infection and morphological evaluation of all blood cells. At least 200 WBCs were counted for differential WBC determination. For each parameter obtained, data from each group of the lesser adjutant were calculated for means, variances and standard error (SE) using SPSS® for window™ (Norusis, 1993). Significant differences among means were determined using an independent sample T-test model.

Cytochemical staining characteristics of blood cells were evaluated using air-dried blood smears from 4 lesser adjutants and one greater adjutant. Cells were stained with periodic acid-Schiff (PAS), Sudan black B (SBB), α-naphthyl acetate esterase (ANAE) as described by Jain (1986), and β–glucuronidase (β-glu) as described by Hayhoe and Quaglino (1980). Positive- and negative-stained cells were differentiated by counting 500 cells on each of the cytochemically-stained smears.

RESULTS

There was no hematozoa detected in all adjutants. There were no significant differences in all hematologic values between the male and the female lesser adjutants so their values were pooled (Table 1). One female lesser adjutant was severely infected with lice. Their hematological values are shown in Table 2. Hematology in the male greater adjutant is also included in Table 2. There were no significant differences in nearly all cell diameters except for basophils and lymphocytes where those in the males were larger and smaller, respectively, than those in the female lesser adjutants (Table 3). Cytochemical staining patterns of blood cell in 4 lesser adjutants and one greater adjutant were similar and summarized in Table 4. The morphological and cytochemical characteristics of individual blood cell were evaluated, as described below.

Table 1 Comparative hematology (mean±SE) between the male and the female lesser adjutants.

Hematology	Male (n=6)	Female (n=3)	P value	All lesser adjutants (n=9)
PCV (%)	40.7 ± 2.2	42.7 ± 2.3	0.480	41.3 ± 1.3
Hb (g/dL)	13.9 ± 0.8	13.9 ± 0.3	0.860	13.9 ± 0.3
RBC (10^6/μL)	1.973 ± 0.145	1.750 ± 0.222	0.888	1.892 ± 0.124
MCV (fL)	210.0 ± 2.9	242.7 ± 19.7	0.562	222.8 ± 11.6
MCH (pg)	72.3 ± 4.3	81.6 ± 8.9	0.961	75.4 ± 4.5
MCHC (g/dL)	34.0 ± 1.2	32.7 ± 1.3	0.244	33.8 ± 0.7
WBC (10^3/μL)	9.482 ± 2.255	9.297 ± 0.462	0.389	9.422 ± 1.113
Heterophils (10^3/μL)	5.117 ± 1.187	5.068 ± 0.767	0.499	5.101 ± 0.609
Eosinophils (10^3/μL)	0.729 ± 0.247	0.961 ± 0.262	0.147	0.806 ± 0.179
Basophils (10^3/μL)	0.234 ± 0.048	0.144 ± 0.099	0.210	0.204 ± 0.046
Lymphocytes (10^3/μL)	3.347 ± 1.025	3.059 ± 0.250	0.155	3.251 ± 0.486
Monocytes (10^3/μL)	0.058 ± 0.058	0.033 ± 0.033	0.423	0.049 ± 0.038
Heterophils (%)	55.2 ± 7.1	54.0 ± 5.3	0.926	54.8 ± 3.8
Eosinophils (%)	7.2 ± 1.9	10.7 ± 3.5.	0.241	8.3 ± 1.5
Basophils (%)	2.8 ± 0.6	1.7 ± 1.2	0.155	2.4 ± 0.6
Lymphocytes (%)	34.5 ± 5.6	33.0 ± 2.6	0.926	34.0 ± 2.7
Monocytes (%)	0.3 ± 0.3	0.3 ± 0.3	0.423	0.3 ± 0.2
Plasma protein (g/dL)	4.40 ± 0.38	4.07 ± 0.18	0.765	4.28 ± 0.19
Fibrinogen (mg/dL)	133.3 ± 33.3	167.7 ± 33.3	0.519	144.4 ± 17.6

Erythrocytes

Erythrocytes (RBCs) were homogeneous in color but moderately heterogeneous in size and shape (Figure 1, 2). Nuclei were oval to pleomorphic. The lesser adjutants had shorter width and length of RBCs than those in the greater adjutant (Table 3). RBCs in both adjutants were negative with all cytochemical stainings (Table 4, Figure 3, 4).

Thrombocytes

Thrombocytes in the adjutants were round cells, approximately one-fourth the size of mature RBCs (Figure 1f). Nuclei were round, with dense chromatin. Their morphology were similar to small lymphocyte. However, they were easily differentiated from lymphocytes by a characteristic perinuclear cytoplasmic vacuolation (Figure 1f). Thrombocytes in both adjutants were negative with all cytochemical stainings (Table 4, Figure 4a, 4c).

Leukocytes

Leukocytes (WBCs) of the adjutant were categorized into 5 groups; heterophil, eosinophil, basophil, lymphocyte and monocyte. There were no significant differences in all morphology of WBCs in both lesser adjutants and greater adjutant except the shape of eosinophil granules.

Lymphocytes were the second commonly observed leukocytes in the lesser adjutant (Table 1) and the male greater adjutant (Table 2). They were small, well differentiated averaging 7-10 μm in diameter (Figure 1f, Table 3). Lymphocytes had two patterns of cytochemical staining pattern with ANAE and β-glu: negative and fine granular. The fine granular pattern consisted of many positive granules (Figure 3f).

Table 2 Hematology in the male greater adjutant and the female lesser adjutant.

Hematology	Male greater adjutant	Female lesser adjutant
PCV (%)	38.0	43.0
Hb (g/dL)	13.61	14.42
RBC (10^6/μL)	1.630	2.280
MCV (fL)	233.12	188.59
MCH (pg)	83.49	63.24
MCHC (g/dL)	35.81	33.53
WBC (10^3/μL)	8.720	12.731
Heterophils (10^3/μL)	4.142	4.519
Eosinophils (10^3/μL)	1.177	2.609
Basophils (10^3/μL)	0.567	0.501
Lymphocytes (10^3/μL)	2.747	5.092
Monocytes (10^3/μL)	0.087	0
Heterophils (%)	47.5	35.5
Eosinophils (%)	13.5	20.5
Basophils (%)	6.5	4.0
Lymphocytes (%)	31.5	40
Monocytes (%)	1.0	0
Thrombocytes/100WBC	158	116
Plasma protein (g/dL)	4.2	4.0
Fibrinogen (mg/dL)	200	100

Table 3 Comparative blood cell diameters in micrometer (mean ± SD) in the lesser and greater adjutants.

Parameter	Male lesser adjutant	Female lesser adjutant	Male greater adjutant
Number of cells	32	30	10
RBC (width)	7.5 ± 0.1^a	7.2 ± 0.1^a	8.8 ± 0.2^b
RBC (length)	15.4 ± 0.2^a	15.7 ± 0.2^a	16.4 ± 0.4^b
Heterophils	9.9 ± 0.2^a	10.9 ± 0.4^b	10.9 ± 0.3^b
Eosinophils	10.5 ± 0.3^a	11.2 ± 0.3^b	13.2 ± 0.6^c
Basophils	9.4 ± 0.4	9.7 ± 0.3	10.5 ± 0.6
Lymphocytes	7.9 ± 0.2	7.3 ± 0.2	7.9 ± 0.4
Monocytes	12.8 ± 0.3	12.0 ± 0.3	15

The figures on the same row with the same letters are not different ($p > 0.05$).

Table 4 Cytochemical staining patterns of blood cells from 4 lesser adjutants and one greater adjutant.

Cell type	PAS	Peroxidase	SBB	ANAE	β-glu
Heterophils	-	-	-	-	-
Eosinophils	-	-	-	-	-
Basophils	-	-	-	-	+
Lymphocytes	-	-	-	-/fine granular	-/fine granular
Monocytes	NF	NF	NF	NF	±
Thrombocytes	-	-	-	-	-
RBC	-	-	-	-	-

PAS indicates periodic acid-Schiff; SBB, Sudan black B; ANAE, a-naphthyl acetate esterase; and β-glu, β-glucuronidase. Staining was score as negative (-), weak (±, fews positve cells), moderate (+), moderate to strong (++), or strong (+++). NF indicates not found.

Heterophils were the most prevalent circulating cells in the lesser adjutant and the male greater adjutant (Table 1, 2) but were the second most commonly observed leukocytes in the female lesser adjutant with lice infestation (Table 2). Heterophils contained lobed nuclei and oval, rod to pleomorphic granules which were bright-oranged staining in Wright's stain (Figure 1a, 1b, 2a, 2b). When the heterophils ruptured, they showed central body in each granule (Figure 2c). But with Wright's-Giemsa stain, they had dull and eosinophilic staining (Figure 1d, 1e, 2d). They were round and 8-12 μm in diameter (Table 3). Heterophils stained negative with SBB (Figure 3a, 4a), peroxidase (Figure 3c), PAS (Figure 3b), β-glu (Figure 3f) and ANAE (Table 4). However, their granules stained pink in β-glu reaction (Figure 3f).

Monocytes were not frequently observed and their characters were similar to mammalian monocytes (Figure 1c, 2f). Monocytes in the adjutants stained weakly positive with β-glu (Table 4).

Eosinophils were usually the largest WBCs in adjutants, averaging 9-14 μm in diameter (Table 3). Eosinophils in the lesser adjutant contained lobed nuclei and many round, bright eosinophilic granules (Figure 1a, 1d) while those of the greater adjutant were larger and more pleomorphic to rod-shaped granules (Figure 2a, 2d, 2e). These granules also stained with new methylene blue (NMB) on smear prepared for determination of reticulocytes. Eosinophilic granules were negative with SBB (Figure 3a), peroxidase (Figure 3c), PAS (Figure 4c) and β-glu (Figure 3f). The number of eosinophils was quite high in both male and female lesser adjutants (Table 1).

Basophils were very small, averaging 8-10 μm in diameter (Table 3) which was smaller than heterophils or eosinophils. With Wright's-Giemsa stain, their granules were vacuolated or clear due to the bleaching effect of methanol fixation (Figure 1e, 2e). With Wright's stain, basophil granules were easily identified by their numerous round, intensely dark blue cytoplasmic granules surrounding central round nucleus (Figure 1b, 2b). Cytochemically, basophils stained moderately to strongly with b-glu (Figure 3e, 4f) but were negative with peroxidase (Figure 4d), SBB (Figure 4b), PAS (Figure 3b), and ANAE (Table 4).

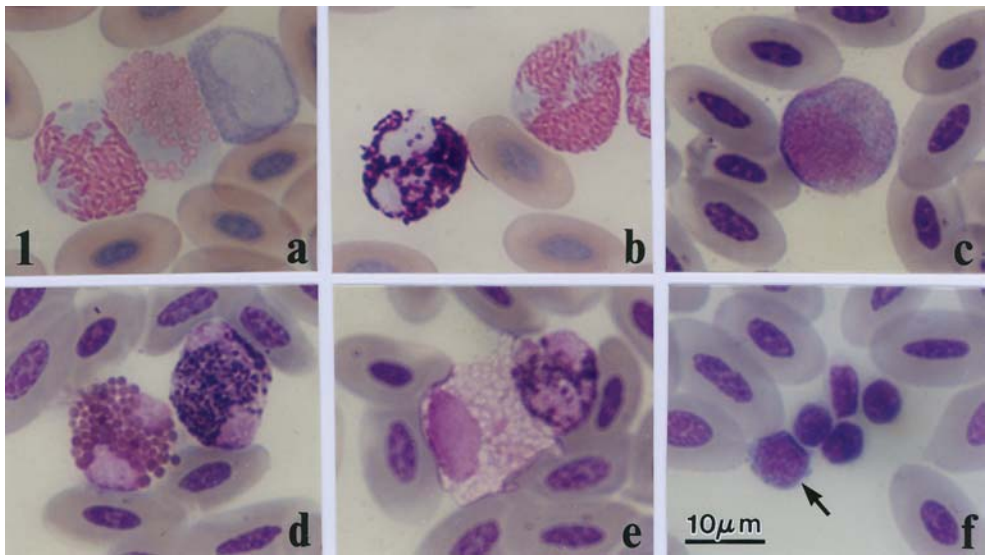

Figure 1 Blood cells in the lesser adjutant. **a.** A heterophil showing rod-shaped granules and an eosinophil with round-shaped granules next to a lymphocyte. Wright's stained (W). **b.** A basophils compared with two heterophil. W. **c.** A 14 μm monocyte. Wright's-Giemsa stained (WG). **d.** An eosinophil and a heterophil. WG. **e.** A vacuolated basophil compared with a heterophil. WG. **f.** A lymphocyte (arrow) and four thrombocytes. Heparinized blood. WG.

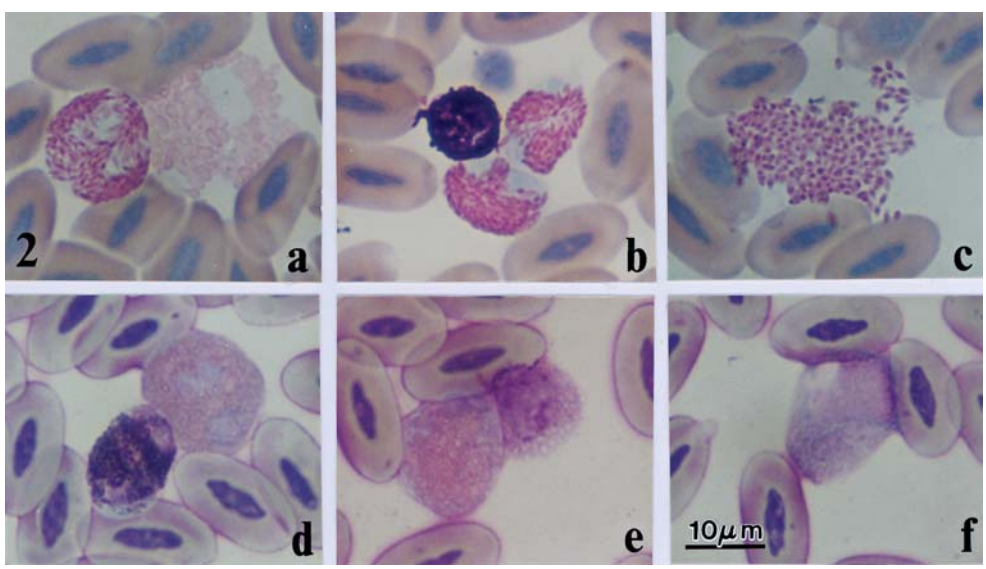

Figure 2 Blood cells in the greater adjutant. **a.** A heterophil showing bright-red granules compared with an eosinophil with pleomorphic pinkish granules. W. **b.** A basophil compared with two heterophils. W. **c.** A ruptured heterophil showing central bodies in the granules. W. **d.** An eosinophil with bright-pink granules compared with dark-rod granules in heterophil from heparinized blood. WG. **e.** An eosinophils and a vacuolated basophil. Heparinized blood. WG. **f.** A monocyte. Heparinized blood. WG.

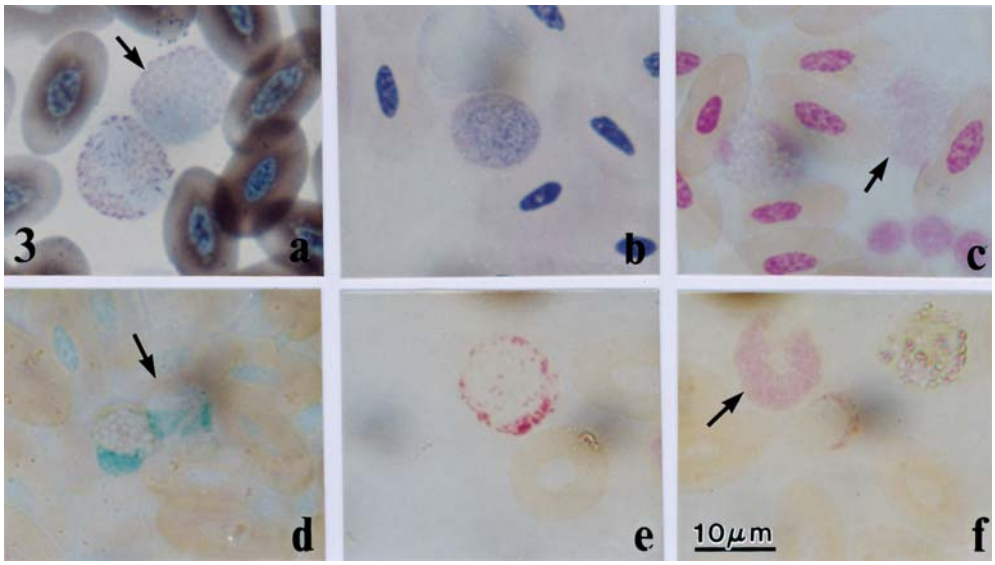

Figure 3 Cytochemical staining of blood cells in the lesser adjutant. **a.** Negative Sudan black B (SBB) staining in an eosinophil (arrow) and a heterophil. **b.** Negative periodic acid-shiff's (PAS) reaction in a basophil. **c.** Peroxidase negative in a heterophil (arrow) and an eosinophil. **d.** Negative α-napthyl acetate esterase (ANAE) activity in an eosinophil and a heterophil (arrow). **e.** β-glucuronidase (β-glu)-positive basophil. **f.** Fine granular patterns of β-glu in a lymphocyte, negative eosinophil and pink granules of heteophils (arrow).

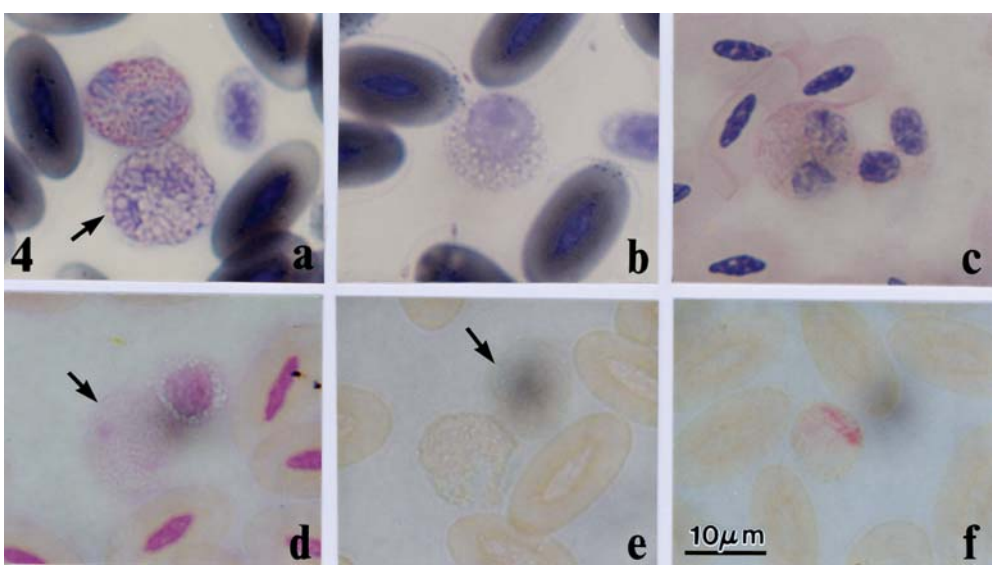

Figure 4 Cytochemical staining of blood cells in the greater adjutant. **a.** Negative SBB staining in a heterophil, an eosinophil (arrow) and a thrombocyte. **b.** SBB negative basophil. **c.** Negative PAS reaction in a heterophil and two thrombocytes. **d.** Peroxidase negative in a heterophil (arrow) and a basophil. **e.** Negative ANAE activity in a heterophil and an eosinophil (arrow). **f.** β-glu activity in a basophil.

DISCUSSION

RBCs in both lesser adjutant and greater adjutant were larger than chicken RBCs (6×12 μm; Bounous and Stedman, 2000) and fighting cocks (Salakij *et al*., 2004a) which corresponded with the MCV (Table 1, 2). All RBC parameters in lesser adjutants were higher than those of chickens except for RBC count which was lower than those in chickens (Bounous and Stedman, 2000). EDTA (ehtylenediamine tetra-acetic acid) was not suitable for anticoagulant in the adjutants even though it could be used in painted storks (Salakij *et al*., 2003), woolly-necked storks and Storm's stork (Salakij *et al*., 2004b) and fighting cocks (Salakij *et al*., 2004a).

Heterophils in both the adjutants were the most prevalent circulating leukocytes which were different from those in chickens (Bounous and Stedman, 2000), painted storks (Salakij *et al*., 2003), woolly-necked storks and Storm's stork (Salakij *et al*., 2004b), and fighting cocks (Salakij *et al*., 2004a). Heterophil number that exceeds lymphocyte number in the adjutants was similar to ring-necked pheasant and black-footed penguin (Stoskopt, 1983). Heterophils in the lesser adjutants were the predominant granulocytes like those in chickens and turkeys (Bounous and Stedman, 2000). They were negative with SBB staining similar to those of chicken (Anderson and Latimer, 1990). They were also negative with all other cytochemical stains (Table 4). The staining patterns of peroxidase were similar to those of SBB (Table 4). Heterophil count in this study was less than $6 \times 10^3/\mu L$. In the study of Bienzle *et al*. (1997) found that the heterophil counts exceeding $100 \times 10^3/\mu L$ were associated with chronic infections.

The small round eosinophil granule characteristic in the lesser adjutants was similar to most of avian species (Campbell, 1995) and fighting cocks (Salakij *et al*., 2004a) but the slightly rod-shaped granules in the greater adjutant was similar to some avian species. The granules of eosinophil in woolly-necked stork (Salakij *et al*., 2004b) were short-rod and smaller than those in the greater adjutant. The high number of eosinophil in the lesser adjutant shown in Table 2 was due to heavy lice infestation which was different from the raptors that were species characteristics (Latimer and Bienzle, 2000). Chicken eosinophils stained positive for SBB (Anderson and Latimer, 1990) but those of the lesser adjutant and greater adjutant were negative for SBB staining (Table 4). Eosinophils in Woolly-necked storks were moderately stained with ANAE and β-glu (Salakij *et al*., 2004b) whilst those of the adjutants were negative.

It was quite difficult to differentiate basophils in WG stained smears because of vacuolated or clear granules that might make it be misidentified as lymphocytes. But they were identified more easily on Wright's stained preparation or in new methylene blue stain. The bleaching effect of basophil granule in both adjutants resembled those of brown boobies (Work, 1999), chickens (Bounous and Stedman, 2000), painted storks (Salakij *et al*., 2003), woolly-necked storks and Storm's stork (Salakij *et al*., 2004b) and fighting cocks (Salakij *et al*., 2004a). Basophils in both adjutants stained only with β-glu which were similar to those in painted storks (Salakij *et al*., 2003), whereas basophils from King cobras stained positively with PAS, SBB, ANAE and β-glu (Salakij *et al*., 2002).

These results provided comparative hematological data, cytochemistry and a guide for identification of blood cells in both lesser adjutant and greater adjutant. These may be useful for health management in the adjutants which are endangered species and is beneficial for further study and related research.

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