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Morphology and Cytochemistry of Blood Cells from Asian Wild Dog *(Cuon alpinus)*

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ABSTRACT

Blood cells from direct blood smears of four adult Asian wild dogs *(Cuon alpinus)* were examined and measured after stainning with modified Wrights Giemsa stain and cytochemical stains, including Sudan black B (SBB), ∝-naphthyl acetate esterase (ANAE), and β-glucuronidase (β-glu). Red blood cells show uniform in sized, biconcave and prominent central pallor with 6.0 μm mean diameter. Reticulocytes and nucleated red blood cells stained positive for ANAE and β-glu. Neutrophils have tight constricted and multilobulated nucleus which mostly occupied the cells. Neutrophils stain strongly positive with SBB, negative for ANAE and weak positive for β-glu. Eosinophils contain numerous round red refractile granules with some vacuoles. Eosinophils stain moderately positive for SBB and β-glu but negative for ANAE. Basophils have variable numbers of intensely basophilic granules which do not obscure the very long lobulated nucleus. Basophils stain moderately positive for SBB but strongly positive for ANAE and β-glu. Most lymphocytes are small and medium size (65:68). Lymphocytes are negative for SBB but have 3 patterns of reactivity for ANAE and β-glu, including negative, focal dot staining and fine granular staining. Monocytes stain moderately positive for SBB and moderately to strongly positive for ANAE and β-glu. Platelets are negative for SBB and stain moderately to strongly positive for ANAE and β-glu.

Key words: Asian wild dog, blood cell, cytochemistry, morphology

INTRODUCTION

Asian wild dog (*Cuon alpinus*) is one among two species of wild dog that are found in Thailand. Another one is Asiatic jackal (*Canis aureus*). Asian wild dogs differ from the jackals in being more reddish color coat and lacking saddle pattern over the shoulder. They are forest animals, particularly favoring dense montane forest as high as 3,000 meters. They are most active in the early morning and late evening, spending most of the day under a dark shade or in the shallow holes underneath the ground (Lekagul and McNeely, 1988). Because of the massive destruction of the forest, the number of Asian wild dog is decreasing. So this endangered species in the zoo has been studied intensively to determine the health status of the individuals. Veterinary hematology serves as a screening procedure to assess general health, assess the body's ability to fight infection in adjunct to patient

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evaluation or diagnosis (Jain, 1993).

Differential white blood cell count is very useful not only in numbering the white blood cells but also provide evidence of anemic condition or reveal the pathogenesis of the problem. Blood smear examinations provide more information on morphology of red blood cell, white blood cell and platelets (Mills, 1998). Cytochemical method is useful in diagnosis of acute leukemia in human (Apibal, 1987; Khemtonglang *et al*., 1997). The purpose of the present study was to characterize the morphology and cytochemical reaction of Asian wild dog blood cells.

MATERIALS AND METHODS

Peripheral blood samples from four clinically healthy Asian wild dogs in Khao Kheaw Open Zoo, were collected in tubes containing ethylenediamine tetraacetic acid (EDTA) as an anticoagulant. Some of the blood without anticoagulant were direct smeared on the slides. Asian wild dogs A and B were adult males, while C and D were adult females aging between 1-2 years old. Complete blood counts were performed using an automated cell counter (Baker 9110; BioChem ImmunoSystem, U.S, Inc., Allentown, PA) at Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaen, Nakorn Pathom within 24 hours after blood collection. Two direct blood smears from each Asian wild dog were stained with a modified Wrights stain. A minimum of 200 leukocytes were counted for differential leukocyte determinations. EDTA blood was used to perform reticulocyte count; by staining with new methylene blue (Schalm *et al.,* 1975). The percentage of reticulocyte presented in 1,000 red blood cells (RBC) was determined.

The examination of blood cell morphology and measurement of cell diameter were done under light microscope (Olympus® microscope Bx 50, Japan at 1,000x). Nuclear appearance, granule size and granule pattern were differentiated for each white blood cells. Blood cell diameter were randomly measured in all blood samples both in modified Wrights stains and cytochemical stains. Means and standard deviations were generated for each blood cell diameter. Significant differences ($p<0.05$) between means of granulocyte cell diameter were determined using independent sample T-test. All statistical data were calculated using SPSS® for Window^{TM} (Norusis, 1993).

Cytochemical staining procedures for Sudan black B (SBB) was done as described by Sheehan and Storey (1947). ∝–naphthyl acetate esterase (ANAE) was done as described by Yam *et al.* (1971) and β–glucuronidase (β–glu) was done as described by Hayhoe and Quaglino (1980). Cytochemical stainings were performed in department of Pahology, Ramathibodi Hospital, Faculty of Medicine, Mahidol University. Cytochemical positive– and negative-stained cells were differentiated comparing with modified Wrights stained smears.

RESULTS

Differential counts, reticulocyte percentages and absolute number of leukocytes were obtained (Table 1). Mean ± SD and ranges of blood cell diameters and cytochemical staining patterns are summerized in Table 2 and Table 3 respectively. Morphology and cytochemical reaction of individual blood cell were evaluated as follows:

Erythrocytes

Erythorcytes (red blood cell, RBC) of Asian wild dog ranged from 5-7 μm (average 6 μm) in diameter and were uniform in size (Figure 1a). They exhibited biconcave disks and distinct central pallor. In the head of blood smear, rouleaux formation was frequently observed. Reticulocyte

Table 1 Asian wild dog differential leukocyte counts, reticulocyte percentage and absolute number of leukocytes.

Cell type	A	B	C	D	Mean	Dog[1/]
Differential count (%)						
Band neutrophils	0.5	0.5	0.5	0	0.5 ± 0	0 - 3
Segmented neutrophils	56.5	68.5	69	55	62.25 ± 7.5	60 - 77
Lymphocytes	22	13.5	15	22.5	18.25 ± 4.7	12 - 30
Monocytes	2	4.5	5	2.5	3.5 ± 1.5	3 - 10
Eosinophils	17	11	9	18	13.75 ± 4.4	2 - 10
Basophils	2	2	1.5	2	1.88 ± 0.3	rare
Reticulocytes	0	0	0.1	0.1	0.1 ± 0	0 - 1
Total leukocytes ($x10^3/\mu l$)	12.4	19.0	19.5	14.6	16.375 ± 3.445	6.0-17.0
Band neutrophils	0.062	0.095	0.098	0	0.085 ± 0.02	0-0.30
Segmented neutrophils	7.006	13.015	13.455	8.030	10.377 ± 3.331	3.0-11.5
Lymphocytes	2.728	2.565	2.925	3.285	2.876 ± 0.310	1.0-4.8
Monocytes	0.248	0.855	0.975	0.365	0.611 ± 0.358	0.15-1.35
Eosinophils	2.108	2.090	1.755	2.628	2.145 ± 0.360	0.1-1.25
Basophils	0.248	0.38	0.293	0.292	0.303 ± 0.055	rare

[1/] Jain, 1993.

Table 2 Mean ± SD and ranges (minium to maximum) of blood cell diameters (mm) in Asian wild dog.

Cell type	No.	Mean ± SD	Range
Red blood cells	180	5.99 ± 0.44	5 - 7
Segmented Neutrophils	103	11.53 ± 0.91^a	10 - 14
Eosinophils	108	13.21 ± 1.26^b	10 - 16
Basophils	103	13.18 ± 1.08^b	11 - 15
Lymphocytes			
S. Small	65	8.52 ± 0.56	7 - 9
M. Medium	68	10.79 ± 0.98	10 - 13
L. Large	9	14.67 ± 1.12	14 - 17
Monocytes	100	14.28 ± 1.10	12 - 17

Means with different superscript letters are different ($p<0.001$).

percentage varied from 0-0.1% Aggregate reticulocytes were characteristic in the Asian wild dog (Figure 1b). Mature erythrocytes were negative for SBB, ANAE and β-glu but nucleated RBC (Figure 1c) and reticulocyte (Figure 1d) were moderate to strong positive for β-glu.

Table 3 Cytochemical staining patterns in blood cells of Asian wild dog.

Cell type	SBB	ANAE	β-glucuronidase
Neutrophils	+ + +	-	±
Eosinophils	+	-	+
Basophils	+ +	+++	+++
Lymphocytes	-	- /focal dot / fine granular	- / focal dot / fine granular
Monocytes	+	+ +	+ +
Platelets	-	+ +	+ +
NRBC and reticulocytes	-	+	+ +

Note : degree of reaction; - negative, ± weak (few positive cell), + moderate, + + moderate to strong, +++ strong positive.

Neutrophils

With modified Wrights stain, Asian wild dog neutrophils were 10 to 14 μm (average 11.5 μm) in diameter. Neutrophils were the smallest granulocyte (Table 2) which were significantly smaller than eosinophils ($p = 0.001$) and basophils ($p = 0.001$). Neutrophils had faintly stained cytoplasm which contained indistinct pale granules. Neutrophil nuclei were multilobulated with tight constriction and occupied most of the cytoplasm. Some neutrophils (2-5%) of the female Asian wild dog revealed sex chromatin lobe (Figure 2a). Neutrophils stained strongly positive with SBB that contained variable of black granules in the cytoplasm (Figure 2b). Asian wild dog neutrophils were negative for ANAE (Figure 2c) and weak positive or negative for β-glu (Figure 2d).

Eosinophils

Eosinophils varied from 10 to 16 μm (average 13 μm) in diameter (Table 2). Eosinophil granules were red-orange, round and refractile. Some of small vacuoles were oftenly seen in the cytoplasm of eosinophil (Figure 3a). Eosinopil neuclei were less lobulated than those of neutrophils, and usually tetralobed, trilobed or band-shaped. Eosinophils stained moderately positive with SBB and had dark staining at the periphery and were refractile in the center of the granules (Figure 3b). Eosinphil granules were negative for ANAE (Figure 3c). Some granules of eosinophil were moderate positive for β-glu (Figure 3d).

Basophils

Basophils varied from 11 to 15 μm (average 13 μm) in diameter (Table 2), and often angular. Basophil granules stained metachromatically reddish-purple in gray-blue cytoplasm (Figure 4a) or larvender cytoplasm (Figure 8a). The granules varied in number and size. Some basophil contained variable size of clear vacuoles in the cytoplasm (Figure 8b). Basophil nuclei were long polymorphonuclear shaped that tend to coil, with tendendy of long lobe formation. Basophils stained moderately to strongly positive for SBB and strongly positive for ANAE and β-glu. Basophil granules stained with SBB more faintly than those of neutrophil (Figure 4b). Basophil granules appeared red-brown when stained for ANAE (Figure 4c). Basophils had numerous small reddish-pink granules packed in the cytoplasm when stained for β-glu (Figure 4d).

Lymphocytes

Asian wild dog lymphocytes were highly variable in size (7 to 17 μm diameter). Small and

medium lymphocytes were oftenly observed (65:68, Table 2). In modified Wrights stains lymphocytes had high nuclear to cytoplasmic ratio (Figure 2a), with scantly cytoplasm. Cytoplasm was usually pale blue. Occasionally, some lymphocytes had small azurophilic granules in the cytoplasm. Lymphocytes did not stained with SBB (Figure 5a) but had reactivity with ANAE and β-glu stains in 3 patterns; including negative, scattered fine granular (Figure 5b, 5c) and large single dot (Figure 5d).

Monocytes

Monocytes varied from 12 to 17 μm (average 14 μm) in diameter. They are the largest white blood cells with variable in shape. The nuclei are extremely variable but usually have lacy chromatin (Figure 6b, 9a, 9b). The cytoplasm was blue-gray and containing variable size of vacuoles (Figure 9b). Monocytes were moderate positive for SBB with faintly black positive, small granules scattered in the cytoplasm (Figure 6b). They were moderate to strong positive for ANAE, with red-brown fine granular pattern (Figure 6c). Diftuse reddish-pink cytoplasm was observed in β-glu positive monocyte (Figure 6c).

Platelets

Asian wild dog ptatelets size was approximately 1/5 to 1/2 of RBC and had prominent reddish-purple granules which easily be seen in modified Wrights stain (Figure 9b). Plateletes were not stained with SBB (Figure 2b) but were moderate to strong positive for ANAE (Figure 7a) and β-glu (Figure 7b).

DISCUSSION

In this report, the light microscope and cytochemical features of Asian wild dog blood cells were described. Although the Asian wild dog erythrocytes has special features like those of dog, they are smaller in mean diameter (Jain, 1993) and easier in forming rouleaux. β–glucuronidase would be useful to differentiate erythroleukemia in the Asian wild dog when using with the other non specific esterase stains and periodic acid Schiff's reaction like those in human (Apibal, 1987).

Leukocyte morphology was essentially the same for all four Asian wild dogs. Although all the Asian wild dogs in this study were clinically healthy, the high percentage of eosinophils and basophils in all four Asian wild dogs indicates eosinophilia and basophilia. Neutrophilia was found in the Asian wild dogs B and C which were physiologic neutrophilia from the exitement during blood collection (Jain, 1993). Eosinophilia suggests an eosinophilic inflammatory process in the body (Willard *et al.*, 1994) or reflects a chronic disease process (Jain, 1993). When eosinophilia (>750-1250 cells/μl) is present in dog, heartworm examination should be determined (Willard *et al.*, 1994). Asian wild dog B and D were microfilaria positive and had been treated one month before blood collections. At the time of blood collection, microfilaria or other blood parasites had not been detected. However the other causes of eosinophilia (such as insect bite dermatitis or intestinal parasites) would be possible.

Basophils are rare in dog. Basophilia and eosinophilia sometimes may occur concurrently (Jain, 1993). In this study, we found 103 basophils from 8 modified Wrights stained and cytochemical stained smears. These findings suggest the benefit of direct smear without anticoagulant. The storaged blood prior to smearing may affect the degranulation of basophils which may cause an error in differentiating as monocytes (Willard *et al.*, 1994). Characteristic features of Asian wild dog basophils were similar to dog basophils; including a long polymorphonuclear-shaped nucleus that is longer than a neutrophil nucleus and thinner than most monocyte nucleus (Willard *et al.*, 1994). Basophils

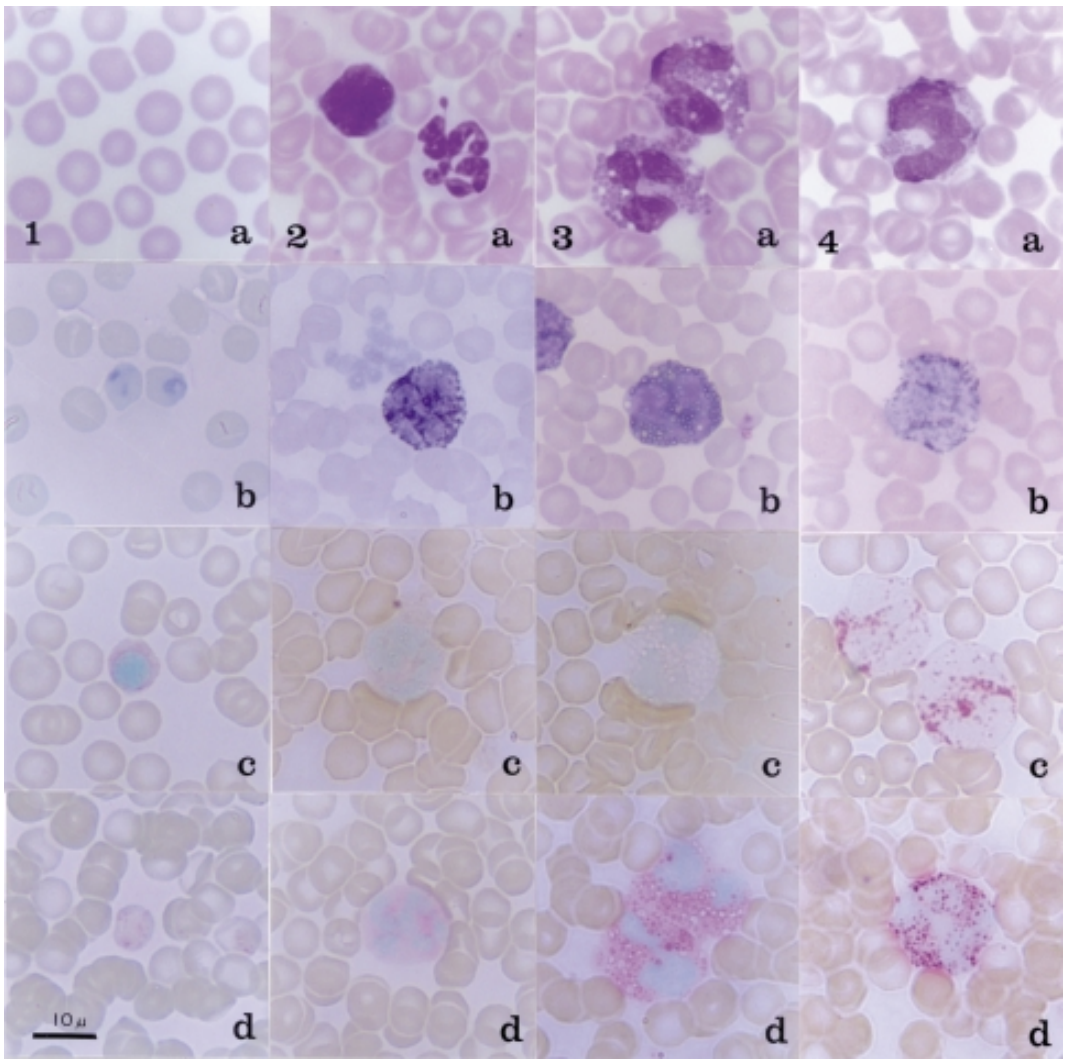

Figure 1 **a.** Uniform in size and central pallor of RBC. Modified Wrights stain. **b**. Two aggregated reticulocytes stained with new methylene blue. **c**. The nucleated RBC and **d.** The reticulocyte shows scattered fine granular positive in the cytoplasm for β-glucuronidase (β-glu). Bar = 10 µm

Figure 2 **a.** Segmented neutrophil with sex bud and 10 µm lymphocyte. Note that high nuclear : cytoplasmic ratio and hyperlobulated nucleus of the neutrophil. **b.** Dark positive (black granule staining) Sudan black B (SBB) in the cytoplasm of 11 µm neutrophil and negative SBB platelets. **c.** Negative α-naphthyl acetate esterase (ANAE) neutrophil. **d.** Faintly positive β-glu in the cytoplasm of the neutrophil.

Figure 3 **a.** Two eosinophils with vacuolated cytoplasm. **b.** SBB positive in the periphery of the granules of the eosinophil. **c**. Negative ANAE eosinophil. **d.** Some granules of two eosinophils are positive for β-glu.

Figure 4 **a.** Basophil with small number of granules in blue cytoplasm. **b.** Positive SBB granules in the cytoplasm of 13 µm basophil. Note lighter stained granules than those of neutrophil. **c.** Intense brown positive ANAE granules in the cytoplasm of two 15 µm basophils. **d.** Strongly pink positive β-glu granules of basophil.

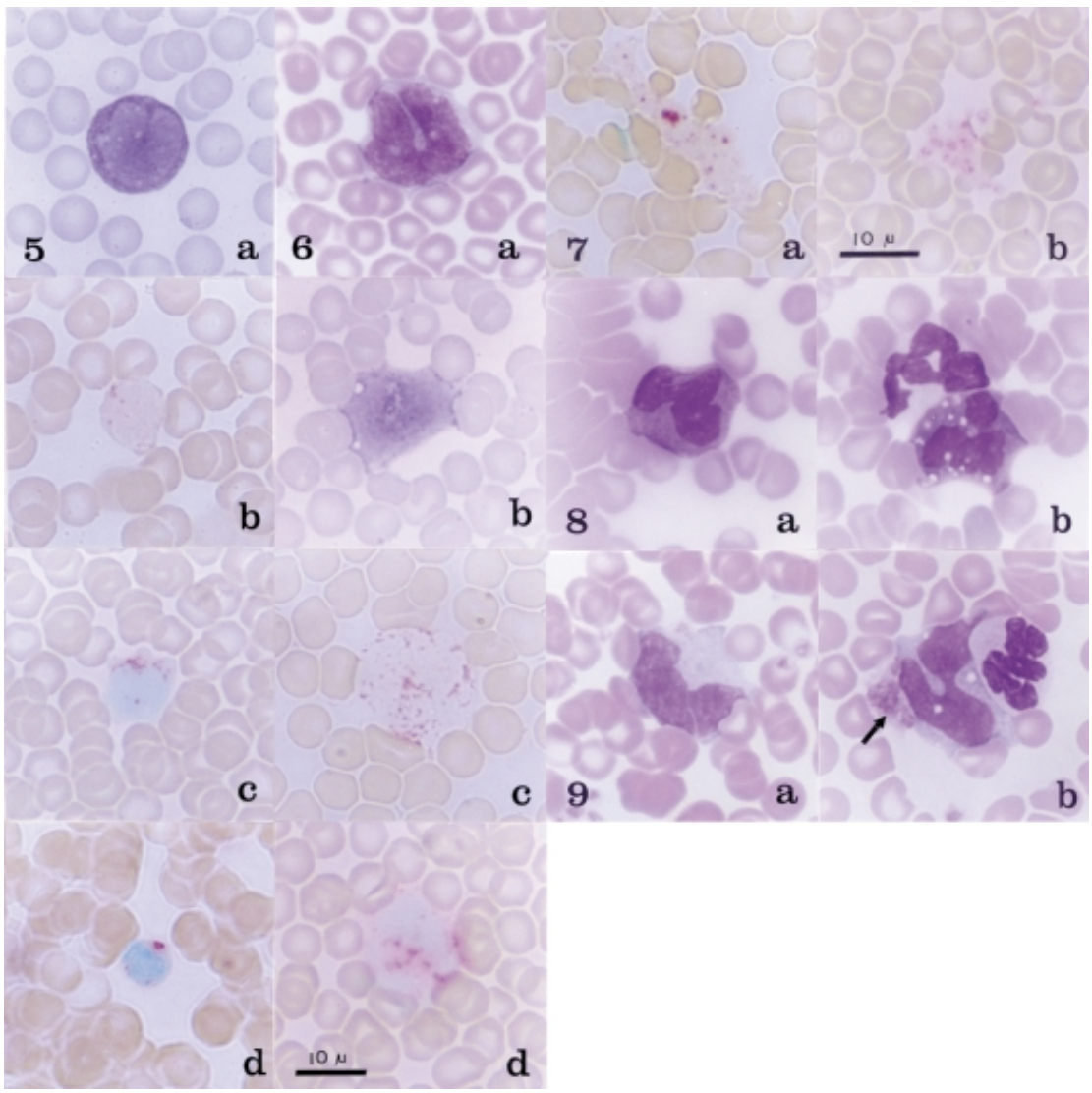

Figure 5 **a.** 14 µm lymphocyte is negative for SBB. **b.** ANAE positive small dot scattered in the cytoplasm of 10 µm lymphocyte. **c**. 9 µm lymphocyte is positive fine granular for β-glu. **d.** 6 µm lymphocyte is positive focal dot for β-glu.

Figure 6 **a.** 15 µm monocyte with U-shape nucleus. **b.** SBB positive monocyte **c**. ANAE positive granules in 15 mm monocyte **d.** β-glu positive 13 µm monocyte. Bar = 10 µm

Figure 7 **a.** ANAE positive platelets. **b.** β-glu positive platelets. Bar = 10 µm

Figure 8 **a.** Baspophil with many dark blue granules that nearly obscure nuclear outline. **b.** Basophil with cytoplasmic vacuoles is next to segmented neutrophil.

Figure 9 **a.** 15 µm monocyte with much volume of cytoplasm. **b.** Monocyte with vacuolated cytoplasm and platelets rosetting (arrow). Segmented neutrophil is next to monocyte.

of Asian wild dog are the same size as the eosinophils but significant larger than the neutrophils (Table 2). These findings may be useful in differentiation of degranulated basophils from netrophils in Asian wild dog.

In the Asian wild dog, SBB stained granulocytes and monocytes. These findings are useful in differentiate acute myelogenous leukemia from acute lymphoblastic leukemia like those in human (Apibal, 1987). ANAE and β-glu staining characteristics of lymphocytes were similar to those reported for human that can differentiate T-lymphocytes (dot staining) from non T-lymphocytes (negative or fine granular staining, Apibal, 1987; Khemtonglang *et al.*, 1997). Asian wild dog basophils were stained strongly positive with all three cytochemical stains. Reindeer basophils were stained with SBB (Henkel *et al.*, 1999) whereas basophils from eastern diamondback rattlesnakes were not (Alleman *et al.*, 1999). The three cytochemical stains in this study are useful in the diagnosis between basophilic leukemia and megakaryocytic leukemia in the Asian wild dog. These results provide more available information on the Asian wild dog blood cells morphology and cytochemical staining.

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