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ORIGINAL ARTICLE

Haematology, cytochemical and ultrastructural characteristics of blood cells in leopard (*Panthera pardus*)

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Abstract The leopard (*Panthera pandus*) is an endangered Asian big cat found in Thailand and also listed in the CITES, Appendix 1. Blood samples from 17 leopards (six males and 11 females) were collected from the cephalic vein for haematology, cytochemical and ultrastructural studies. Red blood cells (RBCs) were slightly anisocytosis, ranged 5–6.5 μm with 5.6 μm mean diameter. They were easy to form rouleaux, blunt end crenation and some RBCs contained Heinz bodies. The male leopards had a significantly higher packed cell volume (39.3±7.5%) values and absolute eosinophil number ($1.658 \pm 1.483 \times 10^9$/l) than the females (30.7±3.2%; $0.965 \pm 0.611 \times 10^9$/l). The cytochemical results were: sudanophilia and myeloperoxidase in neutrophils and some monocytes; nonspecific esterase (alpha-naphthyl acetate esterase, ANAE) in the granules of eosinophils, some lymphocytes, some monocytes and platelets; beta-glucuronidase (βG) in granules of basophils, monocytes and some lymphocytes. The ANAE and βG reaction were detected inter-granular of eosinophils. More detailed morphological aspects of all blood cells were observed by means of scanning electron microscope and transmission electron microscope (TEM). The many round granules and homogeneous content under TEM were characteristics of leopard eosinophils. The engulfed RBCs by neutrophils were detected under TEM in one male leopard. This study provides a guide for the haematology, identification of the morphology, cytochemistry and ultrastructure of blood cells in leopards that is useful for zoological veterinarians in leopard conservation.

Keywords Blood cells · Cytochemistry · Haematology · Leopard · *Panthera pandus* · Ultrastructure

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Introduction

The leopard or panther (*Panthera pandus,* family Felidae), the second largest cat in Southeast Asia, has two phases; pale and dark color phases. In the pale color phase, it has black spots clustered into rosettes on a pale buff coat. In the dark color phase (sometimes called black panthers), it appears all black, but the rosettes are still discernable in good light. The leopard is the most wide-spread of the world's large cats, ranging from Africa and most of southern Asia, including all of mainland Southeast Asia as

well as Java, but not apparently in Sumatra or Borneo (Francis 2001). It is one species of Asian big cat found in Thailand and also on the list of IUCN Red List of Threatened Species and Appendix 1 of CITES (IUCN 2008 www.iucnredlist.org). It is an endangered species in Thailand due to persecution and loss of habitat. Haematology is a common part of the diagnostic evaluation of patients (Latimer et al. 2003). The morphologic characteristics of blood cells from the felids are heterogeneous. Variations in cell characteristics and cell populations exist even between species within the family Felidae. Cytochemical staining is useful for characterizing undifferentiated and acute leukemias in humans (Hayhoe and Quaglino 1980) and animals (Jain 1986). Cytochemical study of normal feline blood and bone marrow cells (Tsujimoto et al. 1983; Jain et al. 1989) and feline leukemic cells (Facklam and Kociba 1986) were reported. Ultrastructural examination of leukocytes may be of benefits in the identification of higher resolution small organelles (Steffens III 2000). However, reports of cytochemical and ultrastructural features of blood cells in leopards are limited.

Morphological work, using light and electron microscopy and cytochemistry, on blood cells of other wild felids in Thailand served as a basis of the present study. They were papers on the fishing cat (*Felis viverrina*; Prihirunkit el al. 2007), the flat-headed cat (*Prionailurus planiceps*; Salakij et al. 2008a) and the clouded leopard (*Neofelis nebulosa*; Salakij et al. 2008b). The aim of this work is to contribute to the knowledge concerning the general morphology and ultrastructure of blood cells, the chemical nature of their cytoplasmic components and to establish a general haematological profile for leopards.

Materials and methods

Leopards and sample collection

During November 2007–March 2008, blood samples were collected from 17 healthy adult leopards (six males and 11 females) located at Khao Kheaw Open Zoo, Chonburi province, Thailand. They were clinically healthy based on clinical examination. Each cat was chemically restrained using Xylazine (Bayer, Puteaux, France; 1 mg/Kg, intramuscular) and Ketamine hydrochloride (Parke-Davis, Morris Plains, NJ, USA; 12 mg/Kg, intramuscular). Blood was collected from the cephalic vein using a 22-ga needle and disposable syringes. Two millilitres of blood samples were collected into tubes containing ethylene diamine-tetra acetic acid (EDTA) for haematologic, cytochemical and ultrastructural analysis of blood cells. The collected blood samples were immediately stored at 4°C and processed within six h.

Haematology, cytochemistry and electron microscopy

The complete blood cell count was performed using an automate cell counter (Abacus Junior Vet®, US Summit, Budapest, Hungary). The packed cell volume (PCV) was determined after the blood had been transferred to microcapillary tubes and centrifuged at 10,000 ×*g* for 5 min. The plasma proteins were measured using an SPR-N refractometer (Atago, Tokyo, Japan). Fibrinogen was calculated as the difference in total solids before and after incubation for 3 min at 56°C and recentrifugation (Sirosis 1995).

Blood smears for Wright–Giemsa (WG) and cytochemical staining were prepared immediately from whole blood and then air dried. Blood smears were fixed in methanol and stained with Wright–Giemsa stain (in-house preparation form Wright's eosin methylene blue and Giemsa Azur eosin methylene blue, Merck KGaA, Damstadt, Germany) for determination of the differential white blood cells (WBC) count and morphologic evaluation of all blood cells. At least 200 (WBCs) were counted for differential WBC determinations. The EDTA blood was used to perform reticulocyte counts by staining with new methylene blue (NMB) using a wet preparation (Sirosis 1995). The percentage of reticulocytes was calculated from the number of reticulocytes per 1,000 red blood cells (RBCs). The reticulocytes that contained distinct aggregated reticulum were described as aggregate reticulocytes, whereas punctate reticulocytes contained a few small dots (Sirosis 1995).

The cytochemical stains, including Sudan black B (SBB), peroxdidase (PO), alpha-naphthyl acetate esterase (ANAE), as described by Jain (1986) and beta-glucuronidase (βG) as described by Hayhoe and Quaglino (1980), were prepared from six leopards (three males and three females). Normal human blood smears were used as controls to ensure the correct staining procedures. Positively and negatively stained cells were differentiated by counting 500 cells on each stained smear.

For scanning electron microscopy (SEM) and transmission electron microscopy (TEM), blood cells from two male and two female leopards were processed. For SEM, a drop of EDTA blood was fixed using 1.5% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.3, at 4°C overnight. Specimens were dehydrated through a graded acetone series, coated with gold and viewed with a JSM-6460 LV scanning electron microscope (JEOL, Japan). For TEM, buffy coats form microcapillary tubes were fixed, immediately after microcentrifuged, in 2.5% glutaraldehyde in PB at 4°C for 24 h, postfixed in 1% osmium tetroxide and embedded in Spurr's epoxy resin. The fixation of buffy coat was performed within 30 min after blood collection. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a JEM 1230 transmission electron microscope (JEOL, Japan). Identification of blood

cells by TEM was based on the relative number, size, shape and distribution of granules and on nuclear appearance.

Results

There was no blood parasite found in any leopards. Haematology from all leopards was tabulated (Table 1). Mean±SD and ranges of blood cell diameter were summarized (Table 2). Cytochemical staining patterns of blood cells were summarized (Table 3). The morphology under light microscope, SEM, TEM and cytochemical characteristics of individual blood cells was evaluated.

Morphology and cytochemistry

RBCs from leopards were homogeneous in color (Fig. 1). They ranged from 5–6.5 μm and were 5.6 μm mean diameter. The Heinz bodies; which shown as a pale area in RBCs; were also recognized in Wright–Giemsa-stained smear (Fig. 1a). Mature RBCs were negative for all cytochemical stains. Platelets were approximately 1/5 to 1/2 of mature RBCs and had prominent reddish-purple granules which were easily seen in Wright's stain (Fig. 1e,i) and Wright–Giemsa stain (Fig. 1m). The platelets count ranged from 1.0–12.4×10^{11}/l. The mean platelets volume was 11.1 fl and 10.3 fl mean diameter in the male and female respectively (Table 1). The giant platelets were easily detected (Fig. 2d). Platelets stained moderately only with ANAE (Fig. 1g,s).

Neutrophils were the most prevalent leucocytes in the leopards (Table 1). With Wright–Giemsa stain, neutrophils were multi-lobed nuclei and faintly pink granules in the cytoplasm (Fig. 1e,i). A well-defined round head appendage of the female sex chromatin was seen in some female chromatin (1% to 6%; Fig. 1a). However, some nodules with shorter stalks were found connected to the nuclei of neutrophils of some male leopards (Fig. 1i). Neutrophils were the smallest granulocytes with a 10.3 μm mean diameter (Table 2). Neutrophils stained strongly positive for SBB (Figs. 1b,f), PO (Fig. 1j,n), while few cells were positive for βG (Fig. 1d) but negative for ANAE (Fig. 1c).

Table 1 Mean±SD of haemotological data in leopards (*Panthera pardus*)

Parameter	Male (n=6)	Female (n=11)	Total (n=17)
PCV (%)	39.3±7.5[a]	30.7±3.2[a]	33.7±6.5
Haemoglobin (g/l)	130.3±13.9	113.8±9.2	119.6±13.4
RBC (×10^{12}/l)	8.739±1.187	7.280±1.071	7.795±1.293
MCV (fl)	44.9±5.8	42.7±5.6	43.5±5.6
MCH (pg)	15.0±1.4	15.9±2.5	15.6±2.1
MCHC (g/dl)	33.7±3.4	37.3±3.5	36.0±3.8
WBC count (×10^{9}/l)	15.008±3.357	11.929±3.287	13.016±3.546
Absolute leukocyte count (×10^{9}/l)			
Banded neutrophils	0.478±0.262	0.285±0.166	0.308±0.213
Neutrophils	10.891±1.655	7.802±2.953	8.892±2.936
Eosinophils	1.658±1.483[a]	0.965±0.611[a]	1.210±1.018
Basophils	Rare	0.129±0.095	0.129±0.095
Lymphocytes	1.665±1.201	2.638±1.555	2.295±1.481
Monocytes	0.389±0.187	0.219±0.163	0.273±0.184
Differential leukocyte count (%)			
Banded neutrophils	2.6±2.4	1.9±1.3	2.4±1.7
Neutrophils	73.8±8.7	64.2±11.5	67.6±11.4
Eosinophils	7.2±5.3	8.2±4.3	8.8±5.3
Basophils	Rare	0.5±0.6	1.0±0.5
Lymphocytes	10.5±6.5	23.3±12.5	18.8±12.3
Monocytes	2.3±1.6	1.9±1.3	2.1±1.4
Platelets (x10^{11}/l)	3.74±2.79	7.22±5.19	5.91±6.05
Mean platelet volume (fl)	11.1±2.3	10.3±1.9	10.6±2.0
Plasma protein (g/l)	87.8±11.0	82.1±16.9	83.1±16.0
Fibrinogen (mg/dl)	167±115	260±183	229±149
Aggregate reticulocytes (%)	1.4±1.4	0.6±0.6	0.9±0.9
Punctate reticulocytes (%)	0.6±0.4	1.2±1.1	1.0±0.8

PCV Packed cell volume, *RBC* red blood cells, *WBC* white blood cells, *MCV* mean cell volume, *MCH* mean cell haemoglobin, *MCHC* mean cell haemoglobin concentration, *fl* femtoliters, *pg* picograms

[a] Significant differences at Ps< 0.05

Table 2 Blood cell diameter in micrometer (mean±SD) and ranges in leopards

Cell type	Number of cells	mean±SD	Ranges (min–max)
Red blood cells	70	5.6±0.4	5–6.5
Neutrophils	50	10.3±0.5	10–11
Eosinophils	46	12.9±0.8	10–14
Basophils	30	13.2±0.9	11–15
Lymphocytes			
Small lymphocytes	32	7.5±0.6	7–9
Medium lymphocytes	8	10.5±0.5	10–11
Monocytes	29	14.5±0.8	13–16

Eosinophils varied from 10–14 μm in diameter with a 12.9 μm mean diameter (Table 2). With Wright–Giemsa stain, eosinophils contained numerous large round granules and lobed-nuclei (Fig. 1e). The granules of eosinophils were negative for SBB (Fig. 1b) and PO (Fig. 1j), but weak ANAE and βG reactions were detected inter-granular (Fig. 1g and h respectively).

Basophils were not frequently observed. With Wright–Giemsa stain, basophils were identified by numerous, lavender cytoplasmic granules (Fig. 1i). Basophils were slightly larger than eosinophils (Table 2). Cytochemically, basophils granules were negative for SBB, PO (Fig. 1n), moderate stained with ANAE and βG (Fig. 1k and l respectively).

Lymphocytes were the second most frequent leukocyte in the leopards (Tables 1). Most of these cells were small and well differentiated (Fig. 1m). Some amounts of azurophilic granules were found in some lymphocytes (1% to 5%). Lymphocytes were negative for SBB (Fig. 1f), PO (Fig. 1r) but had three patterns of cytochemical staining with ANAE (Fig. 1o) and βG (Fig. 1p): negative, fine granules and large focal dots. The fine granular pattern consisted of many small positive granules (Fig. 1o,p), whereas the focal dot pattern was a single large dot.

Monocytes in the leopards varied from 13–16 μm in diameter and were the largest leukocytes (Table 2). The nuclei were extremely variable but usually had lacy chromatin (Fig. 1q). Monocytes were weak positive for

Table 3 Cytochemical staining patterns of white blood cells in leopards as compared to those previous published wild felids and other zoo animals

Cell type	Staining technique	Species					
		Leopard	Clouded leopard (Salakij et al. 2008b)	Flat-headed cat (Salakij et al. 2008a)	Fishing cat (Prihirunkit et al. 2007)	Common palm civet (Salakij et al. 2007)	Asian wild dog (Salakij et al. 2000)
Neutrophils	SBB	+	+	+	+	+	+
	PO	+	+	NR	+	+	NR
	ANAE	−	−	−	+a	−	−
	βG	−	+a	−	+	−	+/−
Eosinophils	SBB	−	−	−	−	+	+
	PO	−	−	NR	−	+	NR
	ANAE	+ (intergranular)	−	−	+ (intergranular)	−	−
	βG	+/−(intergranular)	−	+/−(intergranular)	+ (peripheral granules)	−	+
Basophils	SBB	−	−	−	−	−	+
	PO	−	−	NR	−	−	NR
	ANAE	+	+	+	+	+	+
	βG	+	+	+	+	+	+
Lymphocyte	SBB	−	−	−	−	−	−
	PO	−	−	NR	−	−	NR
	ANAE	+/−	+	+/−	+/−	+/−	+/−
	βG	+/−	+	+/−	+/−	+/−	+/−
Monocytes	SBB	+/−	−	+/−	+/−	+/−	+
	PO	+/−	−	NR	+/−	+/−	NR
	ANAE	+/−	+	+	+	+/−	+
	βG	+	+	+/−	+	+/−	+

Cytochemical staining patterns: − Negative, +/− positive or negative, +a faintly positive, + positive
SBB Sudan black B, *PO* peroxidase, *ANAE* alpha–naphthyl acetate esterase, *βG* beta-glucuronidase, *NR* not reported

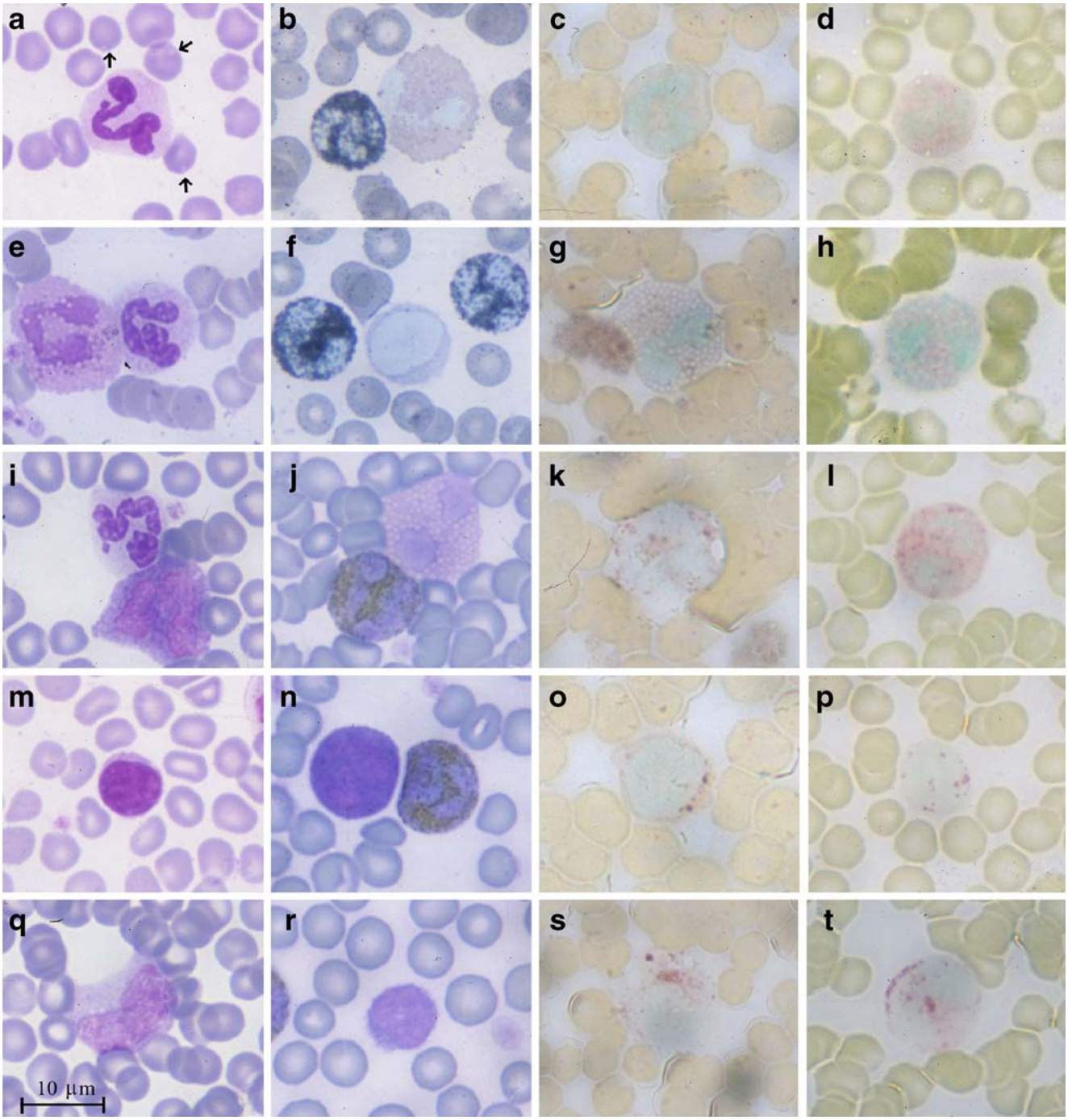

Fig. 1 Morphological and cytochemical characteristics of blood cells in leopards. **a** A neutrophil, Wright's-Giemsa (WG) stain. The pale area of Heinz bodies (*arrows*) was also detected. **b** Neutrophil, positive Sudan black B (SBB) and SBB-negative eosinophil (*right cell*). **c** Neutrophil, negative alpha-naphthyl acetate esterase (ANAE). **d** Neutrophil, weak positive beta-glucuronidase (βG). **e** Eosinophil with numerous round granules (*left*), platelets and a neutrophil, WG. **f** Two SBB-positive neutrophils and SBB-negative lymphocyte (*middle*). **g** ANAE-positive inter-granular of eosinophil. Clump of ANAE-positive platelets (*left*). **h** βG-negative of eosinophil granules while some reaction was detected between their granules. **i** Basophil (*lower cell*) and a neutrophil with sex bud. One platelet attached with the neutrophil, WG. **j** Peroxidase (PO)-positive neutrophil (*lower cell*) and PO-negative eosinophil. **k** ANAE-positive basophil. **l** βG-positive basophil. **m** Small lymphocyte, WG. **n** PO-positive neutrophil (*right*) and PO-negative basophil. **o** Fine granular reaction of ANAE in a lymphocyte. **p** Fine granular reaction of βG in a lymphocyte. **q** A monocyte, WG. **r** PO-negative lymphocyte. **s** ANAE-positive monocyte. **t** βG-positive monocyte

ANAE (Fig. 1s), SBB and PO (Table 3); and moderately stained with βG (Fig. 1t).

Scanning electron microscopy

RBCs were easy to form rouleaux and occasionally blunt end crenation (Fig. 2a). Some defected RBCs (Fig. 2a,b) and echinocytes (Fig. 2a, right cell) were seldom detected. Platelets were discoid cells with smooth surfaces and trended to aggregate (Fig. 2c). Neutrophil surfaces revealed several short microvilli and some micropores (Fig. 3a). Eosinophil surfaces revealed irregular membrane folding of large round granular contour (Fig. 3c,d). Basophil surfaces revealed rod granular surfaces (Fig. 3b). Lymphocytes were the smallest cells and had smooth membranes with some cytoplasmic blebs (Fig. 3e). Monocyte surfaces revealed irregular membranes with deep fissures (Fig. 3f).

Transmission electron microscopy

The mature RBCs contained only haemoglobin (Fig. 2d). Platelets showed dense granules, alpha granules, glycogen granules, mitochondria, clear canalicular structures (Fig. 2d) and microtubules. Neutrophils showed lobed neuclei and many small homogeneous electron-dense specific granules (Fig. 4a). Some neutrophils ingested some RBCs (Fig. 5a,b) was detected in one male leopard but this phenomenon was not found by light microscopy. The majority of granules were in the opposite of the ingested RBCs (Fig. 5b). Basophil granules were larger (Fig. 4b) but less electron-dense than those of neutrophils. The granules in basophils had slightly variably electron density.

Eosinophils presented a homogeneous round shape with small microvilli (Fig. 4c). They contained lobed nuclei, ribosomes and some mitochondria. The numerous well-defined round granules (Fig. 4c,d) with 0.79±0.11 μm in width (n=40) and 1.24±0.17 μm in length (n=40) were observed in the cytoplasm. At higher magnification, the granules showed homogenous electron density and absence of crystalline structure (Fig. 4d).

Lymphocytes contained scant cytoplasm with few mitochondria, endoplasmic reticulum and polyribosomes. The nucleus was round with peripheral clumps of heterochromatin (Fig. 4e). Monocytes showed variable shapes of nuclei with several mitochondria, ribosomes, rough endoplasmic reticulum and pseudopodia (Fig. 4f).

Discussion

The male leopards had significantly higher PCV and absolute eosinophil numbers than the females. The male fishing cat had a slightly higher PCV but lower eosinophil number than the females (Prihirunkit et al. 2007). The red blood cells of leopards were shown to be morphologically similar to those reported in domestic cats (Jain 1986), fishing cats (Prihirunkit et al. 2007), flat-headed cats (Salakij et al. 2008a) and clouded leopards (Salakij et al. 2008b): slightly anisocytosis, easy to form rouleaux, blunt end crenation and contained Heinz bodies. The average size

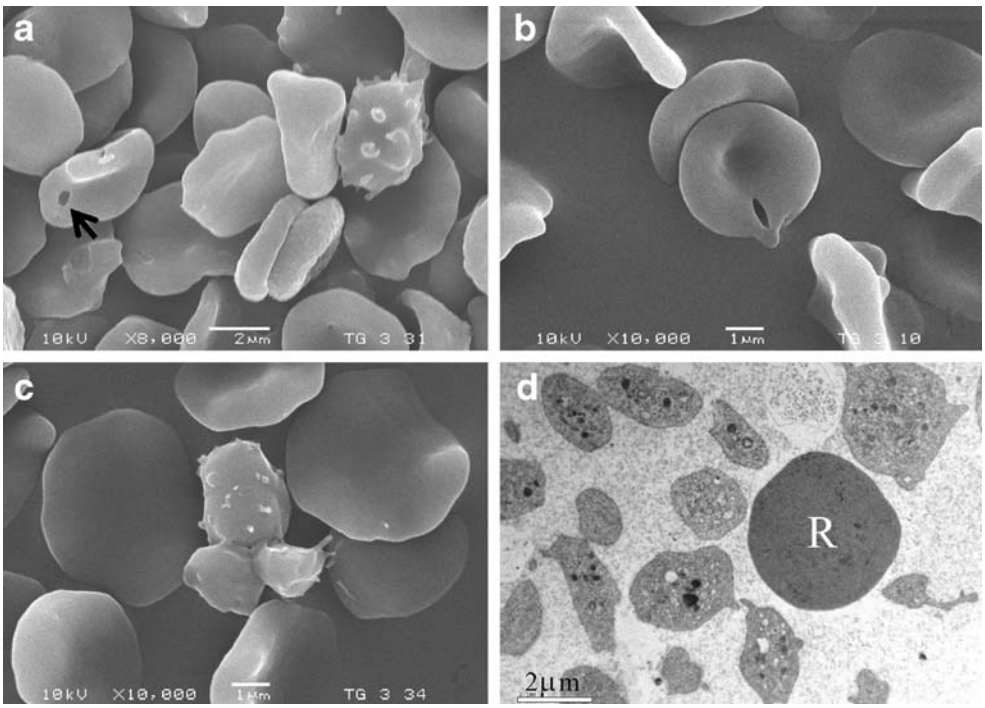

Fig. 2 Scanning electron micrographs of RBCs and platelets in leopards. **a** Groups of RBCs. One RBC showed cytoplasmic hole (*arrow*). **b** RBCs with small cytoplasmic hole. **c** Three platelets aggregated. **d** Transmission electron micrographs of platelets and RBC (*R*). One giant platelet was in *upper right*

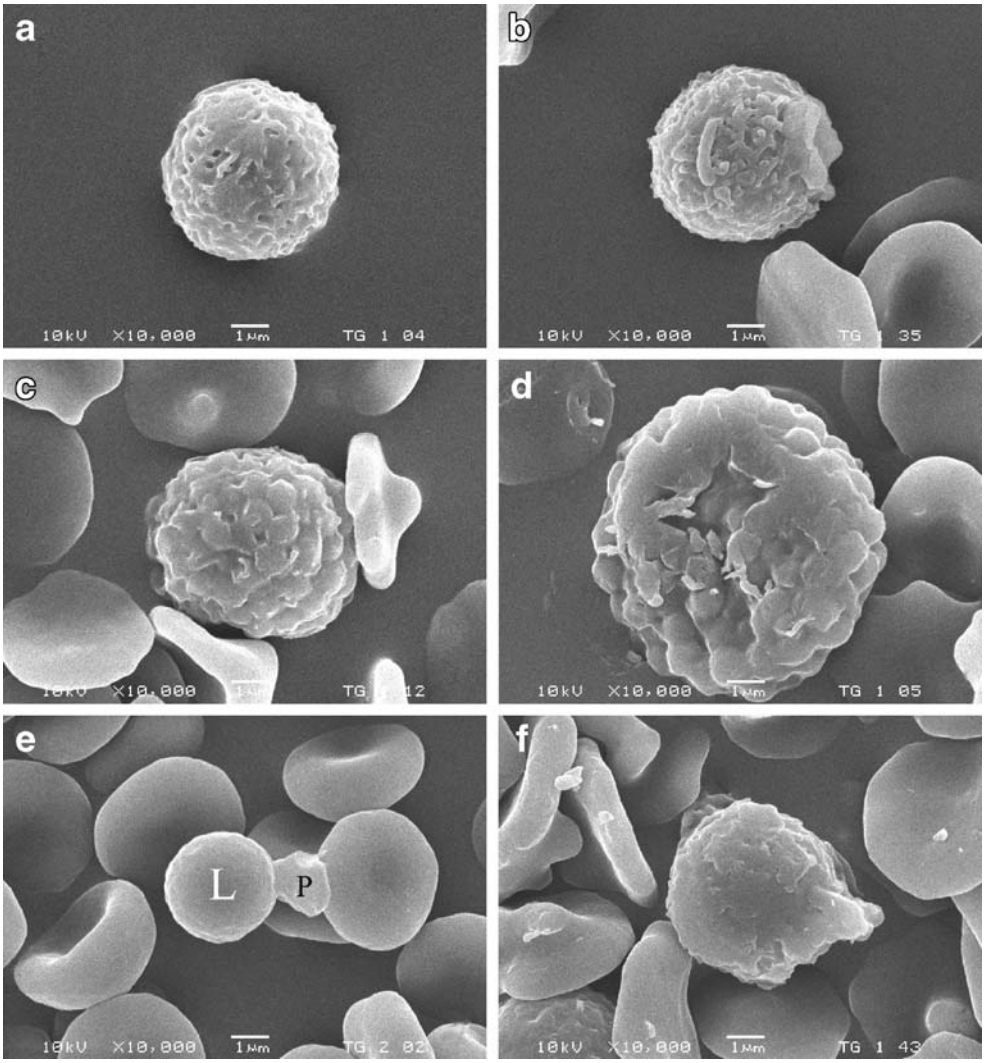

Fig. 3 Scanning electron micrographs of leukocytes in leopards. **a** Neutrophil showing many short microvilli and some micropores. **b** Basophil showing rod granular surface. **c** Eosinophil, showing irregular membrane folding of granular bulking. **d** Ruptured eosinophil with many large round granular surfaces. **e** Lymphocyte (*L*) connects with RBC through a platelet (*P*). **f** Monocyte

of red blood cells in leopards was slightly smaller than those of domestic cats (5.8 μm), fishing cats (5.8 μm) and clouded leopards (6.1 μm); but the same size as those of flat-headed cats (mean=5.6 μm for 80 cells; Salakij et al. 2008a). Neutrophils were the most numerous circulating leukocytes from domestic cats followed by lymphocytes, eosinophils and monocytes (Latimer et al. 2003). Similar patterns of leukocyte differential count were found in the leopards. This might be because they are carnivores (Latimer et al. 2003).

The most characteristic feature of eosinophil was the many prominent large round granules. The morphology of eosinophils in leopard was significantly different from those of domestic cats, fishing cats and flat-headed cats (Latimer et al. 2003; Prihirunkit et al. 2007; Salakij et al. 2008a) but similar to those of clouded leopards (Salakij et al. 2008b). The lavender cytoplasmic granules of basophils in leopards were similar to those of above felids (Jain 1986; Prihirunkit et al. 2007; Salakij et al. 2008a; 2008b).

The ultrastructure of platelets, lymphocytes and monocytes was not different from those of flat-headed cats (Salakij et al. 2008a) and other zoo mammals (Asiatic black bear (Salakij et al. 2005a) and Asian elephant (Salakij et al. 2005b). The ultrastructure of eosinophil granules was different from those of domestic cats (Steffens III 2000) and flat-headed cats (Salakij et al. 2008a) which contained crystalline structure but they were similar to those of fishing cats (Prihirunkit et al. 2007) and clouded leopards (Salakij et al. 2008b). These data support the species heterogeneity of eosinophil morphology among wild felids.

The sudanophilia of all granulocytes were similar to those of domestic cats (Raskin and Valenciano 2000), fishing cats (Prihirunkit et al. 2007) and flat-headed cats (Salakij et al. 2008a). Three cytochemical reactions (SBB, ANAE and βG) in the eosinophils of leopards were similar to those of fishing cats (Prihirunkit et al. 2007). The ANAE reactivity in eosinophils of leopards was detected intergranular while those of flat-headed cats were negative

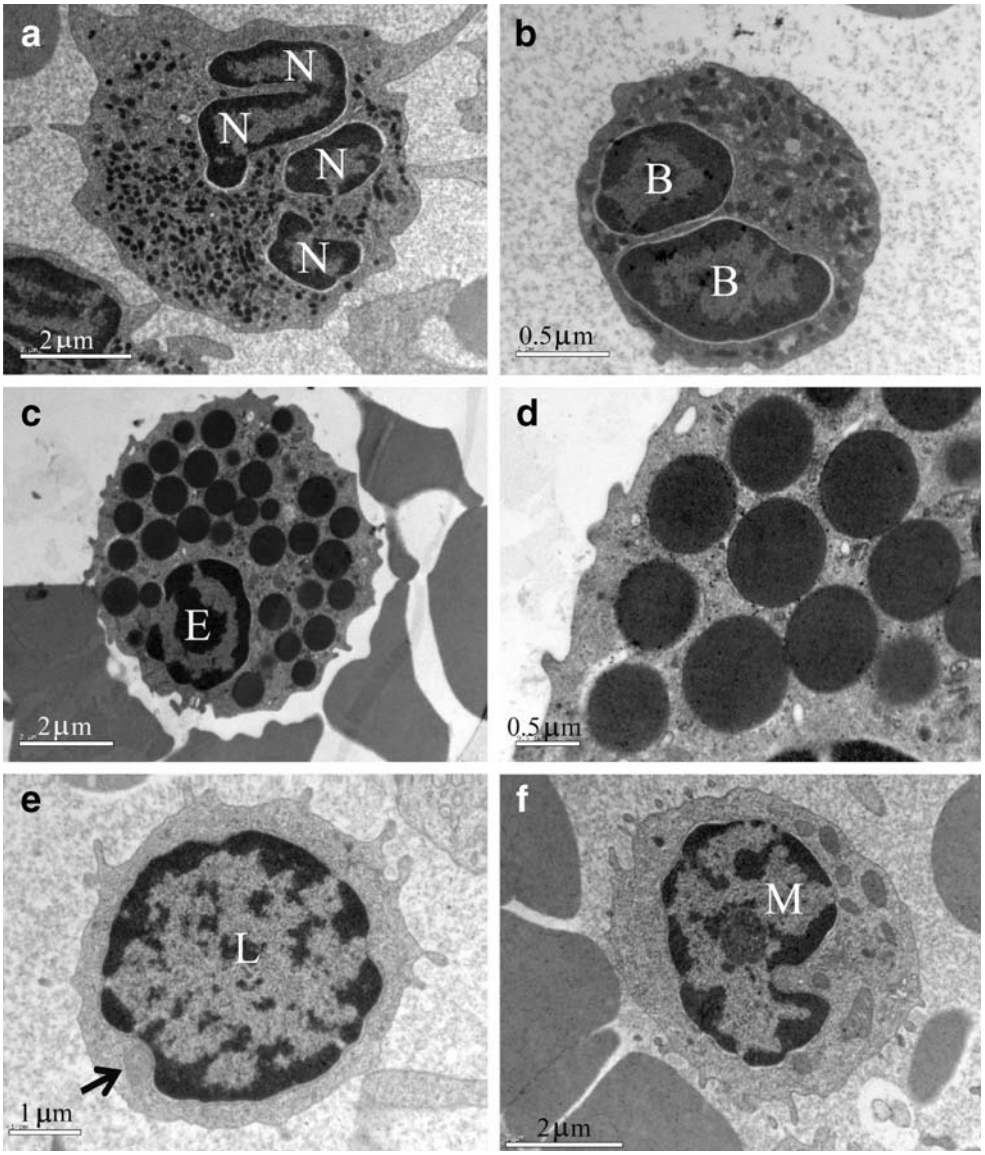

Fig. 4 Transmission electron micrographs of ganulocytes in leopards. **a** Neutrophil with four-lobed nucleus (*N*). **b** Basophil with two-lobed nucleus (*B*) showing granules with less contrast than granules of neutrophil. **c** Eosinophil (*E*) with many large round granules. **d** Higher magnification of eosinophil in **c** showing homogeneous content in granules. **e** Lymphocyte (*L*) with one mitochondria (*arrow*) and cytoplasmic blebs. **f** Monocyte (*M*) with many mitochondria and cytoplasmic process. Uranyl acetate and lead citrate stains

(Salakij et al. 2008a). The eosinophils in leopards were similar to most feline eosinophils which do not stained with either SBB or PO (Raskin and Valenciano 2000). The basophils in normal feline blood and bone marrow cells (Jain et al. 1989) and feline leukemic cells (Facklam and Kociba 1986) were negative for nonspecific esterase but in leopards they were moderately positive for ANAE. The βG reaction of basophils in the leopards were similar to those of fishing cats (Prihirunkit et al. 2007), flat-headed cats (Salakij et al. 2008a), the Asian wild dog (Salakij et al.

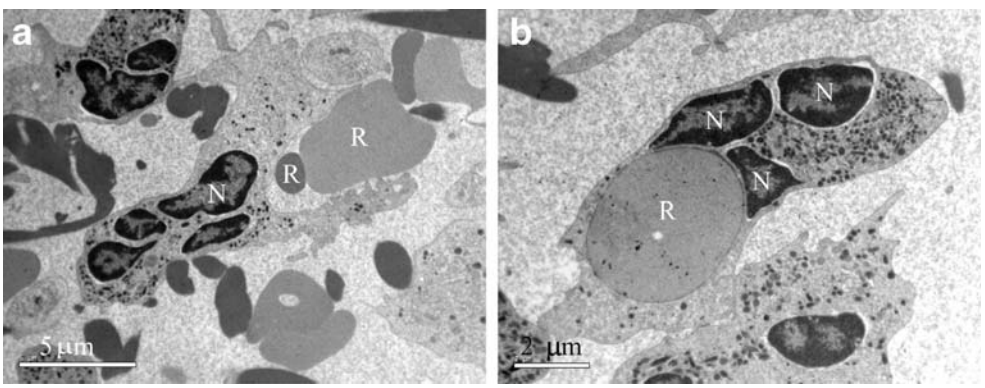

Fig. 5 Transmission electron micrographs of neutrophils in leopard No. 3. **a** A four-lobed nucleus neutrophil (*N*) with cytoplasmic processes surround two RBCs (*R*). **b** Neutrophils (*N*) with ingested RBC (*R*). Note the polarity of specific granules in the opposite site of ingested RBC. Uranyl acetate and lead citrate stains

2000), the Asian elephant (Salakij et al. 2005b) and common palm civets (Salakij et al. 2007). Two pattern of positivity found in lymphocytes of leopards were similar to those of humans (Hayhoe and Quaglino 1980) and the other reported zoo animals (Salakij et al. 2000, 2005a, b, 2007, 2008a, b; Prihirunkit et al. 2007).

The *Hepatozoon* sp. was reported to be common blood parasites in two flat-headed cats in Thailand (Salakij et al. 2008a), Iriomote wild cats (*Felis iriomotensis*) and Tsushima leopard cats (*Felis bengalensis euptilura*) from Japan (Masahito et al. 2006). However, no blood parasite was detected in all studied leopards.

Most erythrophagocytes occur in monocytes (Latimer et al. 2003) but in this study they were found in neutrophils under a transmission electron microscope. They usually indicate the immune-mediated haemolytic anemia (Latimer et al. 2003) but this leopard was not anemic (PCV=47%). The erythrophagocytes detected under TEM was also reported in flat-headed cats infected with *Hepatozoon* sp. (Salakij et al. 2008a).

The results of this study provide information on haematology, the morphology, cytochemical staining and ultrastructural characteristics of blood cells in leopards, which is useful for zoological veterinarians who are involved in leopard conservation.

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