Journal of Animal and Veterinary Advances 6 (4): 522-527, 2007

Morphological and Ultrastructural Observations on the Blood Cells of Sand Lizards (*Leiolepis belliana* Rubritaeniata) Mertens 1961

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Abstract: The objective of this study was to examine the erythrocytes, leukocytes and thrombocytes of the sand lizard (*Leiolepsis belliana* rubritaeniata Mertens 1961) by light and electron (SEM and TEM) microscopy. Smears were prepared from blood from the heart of ten healthy adult sand lizards (five males and five females). Electron microscopy was also performed on all samples. The results revealed the following information: light microscopy finding; erythrocyte, lymphocyte, monocyte, heterophil, basophil, eosinophil and thrombocyte presented district morphology. Erythrocytes, mononuclear cells, granulocytes and thrombocytes of the sand lizard were nearly similar to those of chickens, snakes, tortoises, turtles and other lizards. SEM *finding*, the lymphocyte showed tiny round cells. The monocyte was larger than the lymphocyte and had a round cell shape and rough membrane. The heterophil was a round or elongated cell with a rough membrane. The basophil was tiny and round with a rough membrane surface. The Eosinophil was a round cell with many spherical spines protruded from their membrane surface. The thrombocyte was a tiny cell with an irregular membrane. *TEM finding*, the nucleus of the lymphocyte was large and round with heterochromatin. The nucleus of the monocyte was mononuclear, kidney shaped with heterochromatin. The nucleus of the heterophil was lobuled with heterochromatin, dense cytoplasm and contained spindle, drum-bell or long shape granules. The nucleus of the basophil was lobuled with heterochromatin and the cytoplasm contained large amount of strip granules. The nucleus of the eosinophil was round and had a dense cytoplasm and contained large bowling pin and round-shape liked granules. The nucleus of thrombocyte was dense with clear cytoplasm and no organelle appearance.

Key words: Sand lizards, *Leiolepsis belliana* rubritaeniata Mertens 1961, blood cell, light microscope, SEM, TEM

INTRODUCTION

Sand lizards, *Leiolepsis belliana* rubritaeniata Mertens1961, are an important protein source for people in the northeastern part of Thailand. The lizards are caught in the forest for making food. They are classified in the kingdom Animalia, phylum Chrodata, class Reptilia, order Squamata, family Agamidae, genus *Leiolepis* sp., species *Leiolepis belliana* and subspecies *Leiolepis belliana rubritaeniata* Mertens 1961 (Natalia *et al*., 2001). General characteristics of the sand lizards include a slightly flattened dorso-ventral rounded and sharp head and a long tail. They are terrestrial lizards, meaning they live on the ground rather than in the trees. They often make their homes in deep burrows, which they dig themselves. Their main diet, consists of insects. The scales on the body are very small and resemble grains of sand.

At present, the habitats of sand lizards are decreasing. The sand lizard is one of many lizards of which very little is known. Data regarding the hematological descriptions of sand lizards are scarce; even simple morphological descriptions of the cell types found in the peripheral blood have not been reported. Therefore, the objective of this study was to establish the blood cell characteristics of sand lizards. Basic knowledge from this study is important for hematological research and further departmental study of this lizard.

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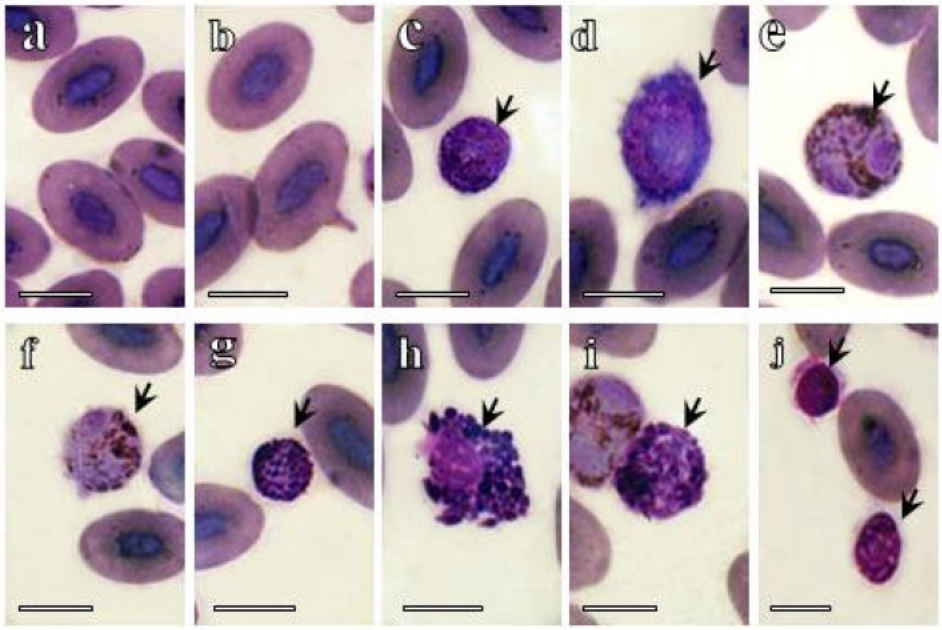

Fig. 1: Wright-Giemsa stained blood smear of sand lizards under light microscope examination (a. normal erythrocytes; b. abnormal erythrocyte; c. lymphocyte (arrow); d. monocyte (arrow); e-f. heterophils (arrow); g. basophil (arrow); h-i. eosinophils (arrow); j. thrombocytes (arrow); bar = 10 μm)

MATERIALS AND METHODS

Animals: Ten sand lizards (5 males and females) were hand captured in Maha Sarakham and Yasothron province northeastern, Thailand during March-July 2006 and taken to the Laboratory of Animal Production Technology, Department of Agricultural Technology, Faculty of Technology and Central Instrumentation Unit, Faculty of Science, Mahasarakham University.

Hematological techniques, blood smear and staining: Lizards were restrained manually and one milliliter of blood sample was collected from the heart using a 1-mL syringe, 26-gauge needle and 1.5 inches of length then placed in microtube with EDTA for determining hematological values. The samples were cooled to approximately 4°C, using icepacks and transferred to the laboratory within 12 h after blood collection. Blood films were performed, fixed in 95% ethyl alcohol for 5 min and were stained with Wright-Giemsa. Giemsa-Wright stained blood smears were examined and photographed under a light microscope using the immersion 100X objective. Blood leukocytes were separated by adapting a previously described method used for garden fence lizards blood (Aengwanich *et al*., 2004).

SEM and TEM techniques: Ultrastructural morphology of the 5 males and females sand lizards were determined at the Central Instrumentation Unit, Faculty of Science, Mahasarakham University. *SEM technique*, blood samples were dropped in 2.5% glataraldehyde in 0.1 M phosphate buffer, pH 7.2 overnight at 4 °C then washed in the same buffer. They were postfixed with 1% osmium tetroxide for 2 h then rinsed by distill water, dehydrated in 20, 40, 60, 80 and 100%, 100% acetone and left to be air dried. Gold coated blood films were examined with a SEM (JSM 6460LV). *TEM technique*, blood samples were centrifuged in capillary tubes at 1,500 rpm for 15 min. Buffy coats were processed in same method as SEM specimens but infiltrated and embedded in Spurr's rein. Lead and uranyl acetate stained ultrathin sections on copper grid were examined with TEM (JEM 1230).

RESULTS AND DISCUSSION

LM finding: *Leiolepsis belliana* rubritaeniata Mertens 1961, sand lizards peripheral blood smear cells were classified morphologically by light microscopy. Erythrocyte, lymphocyte, monocytes, heterophils, basophils, eosinophils and thrombocytes presented a distinct morphology.

The erythrocytes of the sand lizards were homogeneous in size, shape and color. They were typically oval shaped with clear cytoplasm. The nucleus was characterized by lumps of condensed chromatin. (Fig. 1a-b). The mature erythrocytes of the sand lizards were shown to be morphologically similar to the lymphocytes of reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006) the lizard Neusticurus bicarinatus (reptile: Teiidae) (Silva *et al*., 2005) the lizard *Avmeiva ameiva* (Aiberio *et al*., 2005) tortoises and turtles (*Emys orbicularis hellenica*, *Mauremys rivulata*, *Testudo h. hermanni*, *T. graeca ibera*) (Ugurtas *et al*., 2003) the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005) Russian tortoises (*Agrionemys horsfieldi*) (Knotkova *et al*., 2006) king cobra (*Ophiophagus hannah*) (Salakij *et al*., 2002) chickens (Chinrasri and Aengwanich, 2007) and garden fence lizard (Aengwanich *et al*., 2004).

The lymphocyte was typically a round cell shown in the blood film. The nucleus was usually rounded and centrally located in blue cytoplasm. Within the cytoplasm were fine orange-dark blue granules (Fig. 1c). Lymphocyte characteristics of sand lizard in this study were similar to the lymphocyte of the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005) Russian tortoises (*Agrionemys horsfieldi*) (Knotkova *et al*., 2002) chickens (Chinrasri and Aengwanich, 2007) the lizard *Avmeiva ameiva* (Aiberio *et al*., 2005) and the garden fence lizard (Aengwanich *et al*., 2004).

The monocytes are larger than the erythrocytes and lymphocytes, round or uncertian in shape, violet nucleus and a light pale-blue cytoplasm which contains fine dark blue granules and a central kidney shaped nucleus that occupied about 50% of the cell (Fig. 1d). The monocyte of the sand lizard was similar to the monocyte of the reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006) king cobra (*Ophiophagus hannah*) (Salakij *et al*., 2001) chickens (Chinrasri and Aengwanich, 2007) the lizard *Avmeiva ameiva* (Aiberio *et al*., 2005) and garden fence lizard (Aengwanich *et al*., 2004).

The heterophils are typically round in shape with a bright violet lobule nucleus with heterochromatin. Cytoplasm contains many spindle or rod shaped orange-dark blue granules (Fig. 1e-f). The basophils are small and cytoplasm contains many round dark blue cytoplasmic granules (Fig. 1-g). Eosinophils were the rarest cells observed. They are spherical in shape with abundant amo unts of uncertain shape red-violet granules (Fig. 1 h-i). Granulocytes of the sand lizard were nearly similar to

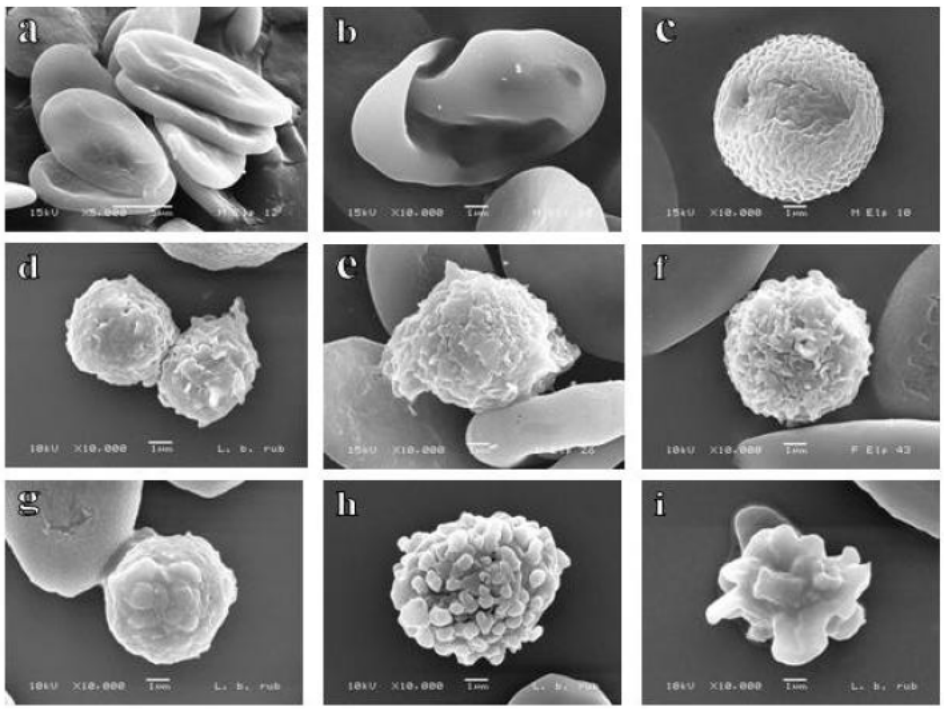

Fig. 2: SEM micrograph of sand lizard blood corpuscles (a. normal erythrocytes; b. abnormal erythrocyte; c. polychromatophilic cell; d. lymphocyte; e. monocyte; f. heterophil; g. basophil; h. eosinophil; i . thrombocytes (a. bar = 5 μm; b-i. bar = 1 μm))

the granulocytes of the reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006) chickens (Chinrasri and Aengwanich, 2007) and garden fence lizard (Aengwanich *et al*., 2004).

Moreover, Thrombocytes were smaller than erythrocytes and often found in clumps. The thrombocytes were typically round in shape having a heterochromatic nucleus in bright pink or hyaline cytoplasm (Fig. 1j). The thrombocyte of the sand lizard was similar to the thrombocyte of the king cobra (*Ophiophagus hannah*) (Salakij *et al*., 2001) the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005) Russian tortoises (*Agrionemys horsfieldi*) (Knotkova *et al*., 2006) chickens (Chinrasri and Aengwanich, 2007) the lizard *Avmeiva ameiva* (Aiberio *et al*., 2005) and garden fence lizard (Aengwanich *et al*., 2004). Finally, azurophils were not found in this study.

SEM finding: Under SEM examination, mature erythrocytes of the sand lizards were flat and had a smooth surface (Fig. 2 a and b). Young erythrocytes were rounder shaped than mature erythrocytes and had a wrinkle surface membrane (Fig. 2c). Lymphocytes showed tiny round cell with rough membrane (Fig. 2d). Monocytes were larger than lymphocyte and had a round cell and rough membrane (Fig. 2e). Heterophils were round or elongated cell with rough membrane (Fig. 2f). Basophils were tiny round and complex cell with large rough surface (Fig. 2g). Eosinophils were round cell with many spherical spines or villus-like protruded from their membrane surface (Fig. 2h). Thrombocytes were a tiny cell with an irregular membrane (Fig. 2i). Finally, the blood cell characteristics of the sand lizards examined by using the SEM in this study are the first report in the suborder Iquaniata.

TEM finding: Under TEM examination, the nucleus of the erythrocyte of the sand lizard was located centrally with the heterochromatin in clear cytoplasm (Fig. 3a-b). The erythrocyte of sand lizards under TEM examination was similar to erythrocyte of the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005).

The nucleus of the lymphocyte was large and round with heterochromatin mainly around the borders (Fig. 3c). The lymphocyte of sand lizard was similar to the lymphocyte of duck, turkey and pigeon (Maxwell, 1974) king cobra (*Ophiophagus hannah*) (Salakij *et al*., 2002) and the lizard *Avmeiva ameiva* (Aiberio *et al*., 2005).

The nucleus of the monocyte was mononuclear, pea shape with heterochromatin mainly around the borders. The cytoplasm of the monocyte was dense and contained microtubule and few vacuoles (Fig. 3d). The monocyte of the sand lizard was similar to the monocyte of the lizard *Avmeiva ameiva* (Aiberio *et al*., 2005) and the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005).

Heterophil presented bilobulated nucleus with heterochromatin mainly around the borders. Cytoplasm of heterophil was dense and contained many different shaped (spindle, drum-bell and long) granules, size and electron density (Fig. 3e-g). The heterophil of the sand lizard in this study differed from the reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006) .

Basophil displayed a bilobulated nucleus with heterochromatin mainly around the borders. The cytoplasm of the basophil was dense, contained vacuoles and a large amount of strip granules which were more electron-dense than heterophil (Fig. 3h-i). Basophil characteristics of the sand lizard differed from the basophil of the king cobra (*Ophiophagus hannah*) (Salakij *et al*., 2001) the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005) and the reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006).

The nucleus of the eosinophil was round with heterochromatin. The cytoplasm of the eosinophil was dense having large bowling pin and round-shaped granules with a double layer membrane. The round shaped granules were more electron dense than bowling pin granules (Fig. 3 j-k). Eosinophil characteristics of the sand lizard in this study were different from the eosinophil of the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre *et al*., 2005) the reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006) duck, goose, quinea-fowl, quail, pigeon and turkey (Maxwell and Siller, 1972) but similar with the eosinophil of the turtle (*Chrysemys dorbignih*) (Azevedo and Lunardi, 2003).

The nucleus of the thrombocytes was dense with heterochromatin. The cytoplasm of thrombocyte was clear and no organelle appearance (Fig. 3l). The thrombocyte of sand lizard was similar to thrombocyte of the duck and pigeon (Maxwell, 1974) but differed from the thrombocytes of the tortoise (*Geoclemys reevesii*).

Finally, the red and white blood cell sizes of the sand lizards were smaller than the lizard *Ameiva ameiva* (Aiberio *et al*., 2005) the giant lizard of El Hierro *(Gallotia simonyi)* (Martinez-Silvestre *et al*., 2005) the reptile *Tupinambis merianae* (Squamata) (Carvalho *et al*., 2006) and the garden fence lizards (Aengwanich *et al*., 2004).

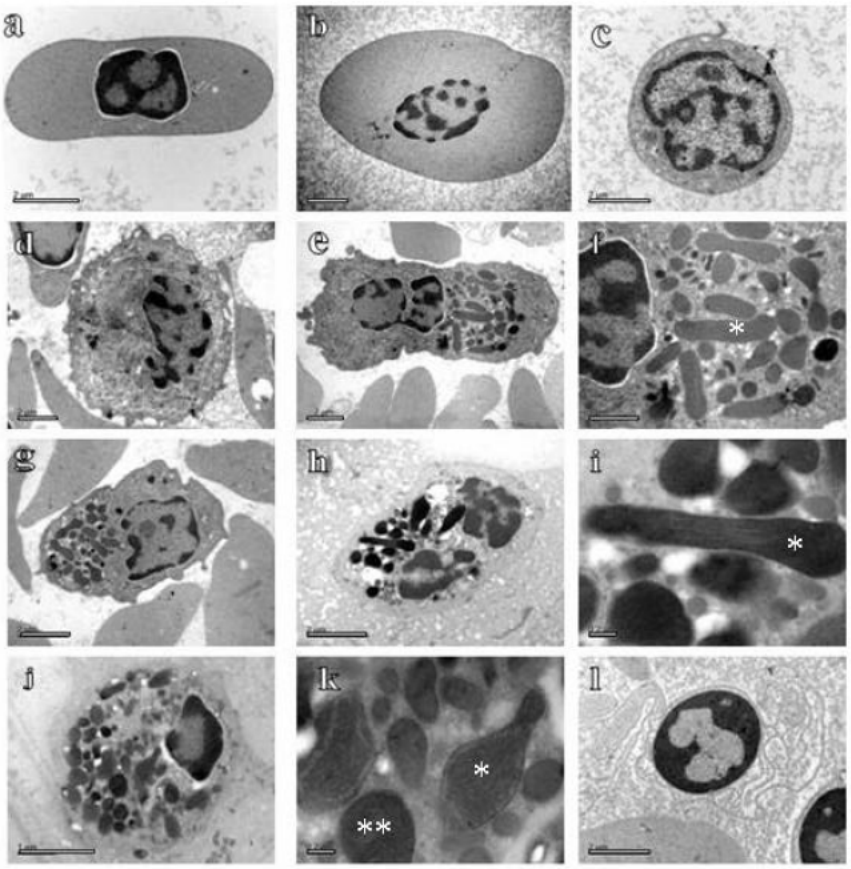

Fig. 3: TEM micrograph of sand lizard blood corpuscles (a. normal erythrocyte; b. abnormal erythrocyte; c. lymphocyted; d.monocyte; e.g. heterophil; f. fine granules in heterophil (*); h. basophil; i. electron dense granules in basophil (*); j. eosinophil; k. granules in eosinophil (* = type 1, ** = type 2); l . thrombocytes (bar = 2 μm; f. bar = 1 μm; i, k bar= 0.2 μm))

CONCLUSION

Blood cell characteristic of the sand lizards, *Leiolepsis belliana* rubritaeniata Mertens 1961 were examined by using light, SEM and TEM microscopy. Under light microscopy examination; erythrocyte, lymphocyte, monocyte, heterophil, basophil, eosinophil and thrombocyte presented district morphology. Erythrocytes, mononuclear cells, granulocytes and thrombocyte of the sand lizard were nearly similar to chickens, snakes, tortoises, turtles and other lizards. Azurophils were not found in this study. Blood cell characteristics of sand lizards, which were examined in this study by using SEM, are the first report in the suborder Iquaniata. Finally, under TEM examination, blood cell characteristics of sand lizards were both similar and different from other lizards such as the lizard *Ameiva ameiva*, the giant lizard of El Hierro *(Gallotia simonyi)*.

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