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Blood Cell Characteristics and Some Hematological Values of Sand Lizards (*Leiolepis belliana rubritaeniata* Mertens 1961) in Northeastern Thailand

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Abstract: The blood cell characteristics, blood cell dimension and hematological values of sand lizards were studied in eighty sand lizards during March-July, 2006. These lizards were obtained from provinces in the Northeastern part of Thailand. The results gave following information: The blood cell characteristics of sand lizards presented distinct morphology. The blood cell characteristics of sand lizards were similar to those of other reptiles. Red blood cell width and length, diameter of lymphocyte, monocytes and heterophils of the male sand lizard were significantly larger than those of the female. On the other hand, the diameter of male basophil was significantly less than that of the female. Packed cell volume, hemoglobin concentration, total red blood cell count and heterophil of the male sand lizards were significantly higher than those of the female. In contrast, the basophil and eosinophil of the male sand lizards were significantly lower than those of the female. These findings indicated that sex influenced the hematological values of sand lizards.

Key words: Blood cell, dimension, hematological values, sand lizard, *Leiolepis belliana rubritaeniata* Mertens 1961

INTRODUCTION

Sand lizards, *Leiolepis belliana rubritaeniata* Mertens 1961 are classified in kingdom Animalia, phylum Chordata, class Reptilia, order Squamata, family Agamidae, genus *Leiolepis* sp., species *Leiolepis belliana* and subspecies *Leiolepis belliana rubritaeniata* Mertens 1961 (Natalia *et al.*, 2001). Generally, the physical characteristics of sand lizards are slightly flattened dorso-ventrally with a rounded and sharp head and long tail. They are terrestrial lizards, meaning that 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, however, consists of insects. The scales on the body are very small and resemble grains of sand (Ponsen *et al.*, 2007).

At present, the habitats of sand lizards have been on the decrease. The sand lizard is one of many lizards of which very little is known. Data regarding their blood cell characteristics, blood cell dimension and hematological values have not been reported. As such, the objective of this study was

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to establish the blood cell characteristics, blood cell dimension and hematological values of sand lizards. Basic knowledge from this study is important for hematological research, conservation, clinical diagnosis and in depth study of this lizard.

MATERIALS AND METHODS

Animals

Eighty sand lizards (44 males and 36 females) were hand captured in Maha Sarakham and Yasothon province Northeastern, Thailand during March-July 2006 and then taken to the Laboratory of Animal Production Technology, Department of Agricultural Technology, Mahasarakham University.

Blood Sampling

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 inch of length (Aengwanich *et al.*, 2004) then placed in micro tube with EDTA for determining hematological values. The samples were cooled to approximately 4°C (Ritchie *et al.*, 1994) using icepacks and then transferred to the laboratory within 12 h after blood collection.

Hematological Analyses

Blood films were performed and were stained with Giemsa-Wright. The Giemsa-Wright stained blood smears were examined and photos were taken under light microscopy. Blood leukocytes were separated by adapting a previously described method used for garden fence lizard's blood (Aengwanich *et al.*, 2004). Blood cell dimensions were determined by using a stage and ocular micrometer. The PCV was determined after the blood had been transferred to microcapillary tubes and centrifuged at 2500 g for 5 min. Total white blood cell counts were determined manually with the improved Neubauer counting chamber after the blood was diluted with Natt and Herrick's solution. Total red blood cell counts was performed by diluting sand lizard blood with Grower's solution then counted red blood cell in 5 red blood cells square of the improved Neubauer counting chamber (Campbell, 1995). The hemoglobin concentration was determined by the cyanmethemoglobin method (Ritchie *et al.*, 1994).

Statistical Analysis

The results were given as mean±SD, hematological values between males and females were compared by t-test from SAS system (SAS, 1990) and a level of significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Leiolepis 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 (Table 1). The red blood cell length and width of the male sand lizard were significantly larger than those of the female. The diameter of the lymphocytes, monocytes and heterophils of the male sand lizard was significantly greater than that of the female. Contrarily, the diameter of the male sand lizard basophil was significantly less than that of the female (Table 2). Packed cell volume, hemoglobin concentration, total red blood cell count and heterophil of the male sand lizards were significantly higher than those of the female. In contrast, the basophil and eosinophil of the male were significantly lower than those of the female (Table 3).

Table 1: Structural morphology of white blood cell in sand lizards (*Leiolepis deliata rubriventris* Mertens 1961) found in northeastern part of Thailand


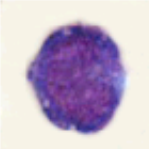



Parameters	Lymphocyte	Monocyte	Heterophil	Basophil	Eosinophil
Blood cell characteristics					
Morphology	Round	Round or uncertain in shape	Round	Round	Uncertain shape
Nucleus characteristics and staining	Large, round and basophilic staining	Large, pea shape and basophilic staining	Large, lobulated and bright-violet staining	Not found	Lobulated and basophilic staining
Cytoplasm color	Blue	Light pale-blue	Pale-blue	Pale violet	Pale violet
Granules	Fine orange dark blue granules	Fine dark blue granules	Spindle or rod shaped orange-dark blue granules	Round dark blue granules	Uncertain in shape of red-violet granules

Table 2: Blood cell dimension of sand lizards (*Leiolepis deliata rubriventris* Mertens 1961) in northeastern part of Thailand

Cell dimension (µm)	Male (n = 44)	Female (n = 36)	p-value	Total (n = 80)	Range
Red blood cells					
Width	9.88±0.97 ^a	9.39±0.53 ^b	0.009	9.74±0.79	8.95-10.53
Length	15.90±1.25 ^a	15.35±0.36 ^b	0.001	15.63±1.00	14.63-16.63
White blood cells					
Lymphocyte	6.43±1.37 ^a	7.41±1.47 ^b	0.001	6.92±1.50	5.42-8.42
Monocyte	8.33±0.10 ^a	9.40±1.33 ^b	0.001	8.87±1.42	7.45-10.29
Heterophil	9.05±1.17 ^a	10.08±1.34 ^b	0.001	9.57±1.35	8.22-10.92
Basophil	7.54±1.48 ^a	6.20±1.43 ^b	0.001	6.87±1.60	5.27-8.47
Eosinophil	8.65±1.36	8.73±1.22	0.662	8.69±1.29	7.4-9.98

^a: Within row, mean with superscripts were significantly different (p<0.01)

Table 3: Hematological values of sand lizards (*Leiolepis deliata rubriventris* Mertens 1961) in northeastern part of Thailand

Hematological values	Male (n=44)	Female (n=36)	p-value	Total (n=80)	Range (mean±SD)	Reference range interval of garden fence lizard ^a	Reference range interval of gnomidana ^b
PCV (%)	27.00±4.27 ^a	20.92±4.50 ^a	0.004	24.41±4.34	18.05-30.77	23.82-29.88	30.80-42.8
Hb (g%)	8.98±2.14 ^a	7.41±1.94 ^a	0.001	8.14±2.12	4.02-10.24	7.07-8.75	7.50-11.9
REBC (×10 ⁶ cells/µL)	0.93±0.30 ^a	0.75±0.23 ^a	0.041	0.84±0.29	0.57-1.15	0.72-1.12	0.82-2.30
TWBC (×10 ⁶ cells/µL)	7.90±0.41	4.50±0.28	0.108	7.30±0.37	3.40-11.0	7.72-14.24	4.58-17.58
Lymphocyte (%)	47.13±10.17	49.17±8.22	0.393	48.10±9.09	39.01-57.19	43.74-43.80	42.14-50.14
Monocyte (%)	10.05±4.05	8.31±3.94	0.438	9.13±4.90	4.23-14.03	8.43-23.45	4.42-5.4
Heterophil (%)	33.32±13.33 ^a	24.81±11.97 ^a	0.001	28.85±13.34	15.49-42.21	15.33-32.21	38.59-44.45
Basophil (%)	7.97±4.04 ^a	14.49±8.83 ^a	0.001	11.44±7.99	3.45-19.43	0.00-5.88	3.23-4.01
Eosinophil (%)	2.00±3.44 ^a	3.24±2.75 ^a	0.009	2.29±2.40	0.00-4.49	0.00-4.24	3.44-5.04

^a: Within row, mean with superscript were significantly different (p<0.01); ^b: Reference range interval of garden fence lizard from Aengwanich et al. (2004); ^c: Reference range interval of gnomidana which adapted from Fear (2002)

The mature erythrocytes of the sand lizards were shown to be morphologically similar to the lizard *Neusticurus bicarinatus* (reptile: Teiidae) (Silva et al., 2005), the lizard *Ameiva ameiva* (Alberio et al., 2005), the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre et al., 2005) and the garden fence lizard (Aengwanich et al., 2004). Lymphocyte characteristics of the sand lizard in this study were similar to the lymphocyte of the giant lizard of El Hierro (*Gallotia simonyi*) (Martinez-Silvestre et al., 2005), the lizard *Ameiva ameiva* (Alberio et al., 2005) and the garden fence lizard (Aengwanich et al., 2004). The monocyte of the sand lizard was similar to the monocyte of the lizard *Ameiva ameiva* (Alberio et al., 2005) and the garden fence lizard (Aengwanich et al., 2004). Almost all granulocytes of the sand lizard (heterophil, basophile and eosinophil) were similar to the granulocytes of the garden fence lizard (Aengwanich et al., 2004). Sex has influenced on blood cell size

and hematological values of the sand lizard. Sturkie (1965) suggested that androgen, which is a male hormone of the avian species, caused an increase in the total red blood cell count, packed cell volume and hemoglobin concentration. This increase was noted in the results of the present study. Due to the close evolutionary relationship between the avian and the sand lizard, this hormone might be causing an increase in some hematological parameters of the sand lizard. The basophil and eosinophil of the male sand lizards were lower than those of the female. However, the mechanism that produces these effects is unknown.

The mean packed cell volume of the sand lizards was lower than the reference range interval of iguana (Tear, 2002), but not different from the reference range interval of the garden fence lizards (Aengwanich *et al.*, 2004). Mean hemoglobin concentration, total red blood cell count and percentage of lymphocytes of sand lizards, garden fence lizards and iguana were not different. The mean total white blood cell count of the sand lizards was lower than the reference range interval of the garden fence lizards, but not different from the reference range interval of iguana. The mean percentage of monocyte, heterophil and eosinophil of sand lizards was lower than the reference range interval of iguana (Tear, 2002), but not different from the reference range interval of the garden fence lizards (Aengwanich *et al.*, 2004). The above documents indicated that the hematological values of sand lizards were partly the same and partly different from other lizards. Therefore, it is important to investigate hematological values of each species in order to interpret the results accurately for a particular individual.

CONCLUSIONS

Blood cell characteristics, blood cell dimension and hematological values of sand lizards were determined. We found that blood cell characteristics of sand lizards were similar to others reptiles. Sex influences blood cell dimension and hematological values of sand lizard. When comparing the mean hematological values of sand lizards with the reference range interval of garden fence lizards and iguana, it was found that the hematological values of the sand lizards were both similar and different than these two lizard species.

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