

## Hematological Characteristic of the Female Asian Vine Snake (*Ahaetulla prasina* Boie, 1827)

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**Abstract**—There is less information about the hematological characteristic of snake in Indonesia. Morphology and morphotic elements size of erythrocyte and leukocyte of the female Asian Vine Snake (*Ahaetulla prasina*) in North Purwokerto, Indonesia, were studied. Blood was sampled by cardiac puncture and blood cells morphology and size were observed and measured from prepared blood smears. Erythrocyte was nucleated and oval in shape. Leukocyte were lymphocytes, heterophils, eosinophils, basophils and monocytes. The erythrocyte was slightly differ from those of other squamates. Morphology and morphotic size of blood cells provided information about the physiological character of Asian Snake Vine. More comprehensive study is necessary to understand the correlation of blood cells characteristic of snakes with other characteristic.

**Keywords**—erythrocyte, leucocyte, asian vine snake, *Ahaetulla prasina*, Purwokerto.

### INTRODUCTION

The study of reptiles in Indonesia is quite popular in recent years, however the study about reptile hematological characteristic is still low. Lack of reference about reptiles in Indonesia caused different interpretation of the hematological data. The result of this study would serve as a reference about Asian vine snake blood cells characteristic. Asian vine snake (*Ahaetulla prasina* Boie, 1827, is a member of family Colubridae and widely distributed in Asia. It has type locality in Java, thus common in Java, Sumatera, Sulawesi, Kalimantan and Lombok. It has extremely slender body with long and projecting snout with horizontal eye pupil. It is mildly venomous snake with rear fang, however it is considered harmless to human. Body coloration is various, usually bright green, some are light brown to dull yellow-green [1].

Reptile blood has slightly different composition compared to the mammalian blood[2]. It is consisted of erythrocytes, thrombocytes, heterophils, eosinophils, basophils, azurophils, monocytes, and lymphocytes[3]. Erythrocytes are elliptic to oval shaped and nucleated, with centrally located basophilic nucleus. It comes in various size, depend on the species.[2][3] Sauria has relatively small erythrocytes compare to Chelonia and Crocodilia[2][4]. Thrombocytes are round to oval with oval to round nucleus and smaller than erythrocytes[2][3]. It is often confused with small lymphocytes and usually clump in smears[5]. Leucocytes in reptiles are inconsistent because of variable criteria to categorize these cells[5][6]. Instead of neutrophils, reptiles have heterophils. Heterophils may vary among groups, genera and species[6]. It is round shaped larger than erythrocytes, with needle-like acidophilic cytoplasmic granules. The nucleus is round and may have lobes in some lizard[6][7]. Azurophils are similar to monocytes but with prominent azurophilic granules in the cytoplasm[2][7]. Eosinophils is quite controversial in Squamata for some are described in one species and not in other species[4][7][8]. Eosinophils have round acidophilic-staining granules with round nucleus, the granules in Iguana are stained blue[2][7]. Basophils are recognized by the purple granules with round nucleus and similar in size with lymphocytes[2]. Lymphocytes are the major circulating leucocytes in reptiles, small to large, round or irregular cells with a round nucleus. Monocytes are the largest cell, round shaped, kidney-shaped nucleus[6]. The cytoplasm is wide and may have the same size as the nucleus, and stained deep blue or grayish blue[2].

### MATERIALS AND METHODS

#### a. Animal

The snake was wildy captured around the eastern area of Faculty of Biology, University Jenderal Soedirman, Purwokerto. The morphometric and meristic of the snake then measured for identification purpose.

#### b. Sample Collection and Processing

The snake was euthanized with chloroform, and dissected to obtain the organs for other research purpose. Blood was drawn by cardiac puncture, and then prepared for the blood smears. Three to six prepared smears were fixated with methanol and stained with Giemsa staining. Blood cells were observed and measured with light microscope. Morphology of the blood cells were described by the shape, cytoplasm, nucleus, and other specific characteristics. The size of the cells were measured by the long and short axis. Data then analyzed the average and the ratio of long axis:short axis were counted.

### RESULTS AND DISCUSSION

The blood cells morphology of the Asian Vine Snake were similar with other reptiles. However, it was difficult to categorize the leucocytes since the data obtained slightly different with the references compared.

#### a. Morphological characteristic of the Asian Vine Snake Blood Cells

Asian vine snake erythrocytes were oval to round shape with a nucleus centrally located. The nucleus was sperical or elliptical shape and stained purple. The cytoplasm were generally stained uniformly pink-purple, but still brighter than the nucleus.

The leucocytes observed were granulocyte cells, heterophils, eosinophils and basophils; and the agranulocyte cells, monocytes and lymphocytes. Azurophils were not identified in the blood of this snake.

The eosinophils were so much alike with the heterophils. There were small round purple granules in the purple stained cytoplasm, and the nucleus were stained pale purple. The size of the eosinophil were as the same size of heterophils thus hardly differentiate each other. The basophils were smaller than erythrocytes, with deep purple granules. The size of the basophils were about the same size with the lymphocytes and were abundant in number like the lymphocyte. The lymphocyte were round shape with round nucleus located centrally. The nucleus were almost as the same size of the cytoplasm and stained purple to dark purple. The monocyte were the least leucocyte observed, based on the reference used. The characteristic of the monocyte in reference didn't match with some cells in question observed. There were also possibility of the presence of azurophils on the blood smears, but then again those cells didn't fit the categorize in the reference. There were still many aspects to consider related with the morphology of the asian vine snake. It is said that sex, habitat, season, parasites could influence the morphological feature of the snake.

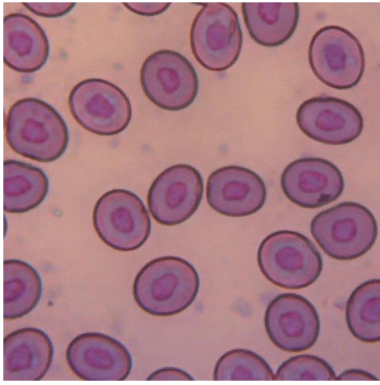


Fig 1. Erythrocytes of Asian vine snakes (Giemsa staining)

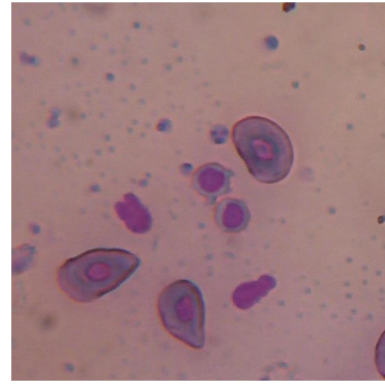


Fig 5. Lymphocytes of Asian vine snakes (Giemsa staining)

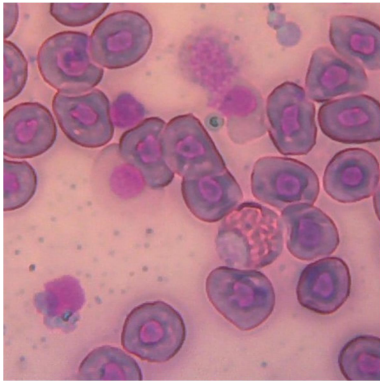


Fig 2. Heterophil of Asian vine snakes (Giemsa staining)

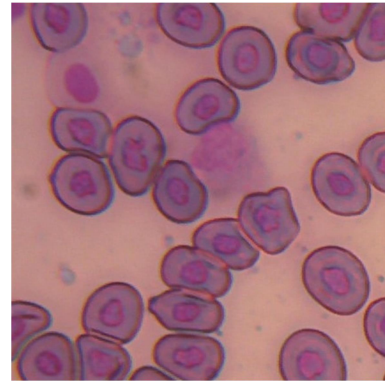


Fig 6. Monocyte of Asian vine snakes (Giemsa staining)

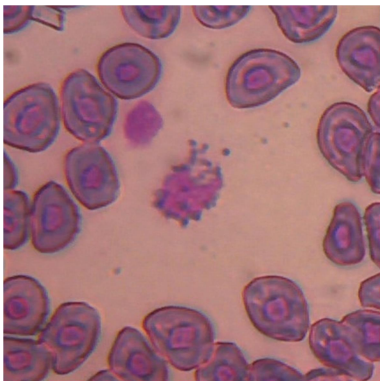


Fig 3. Eosinophil of Asian vine snakes (Giemsa staining)

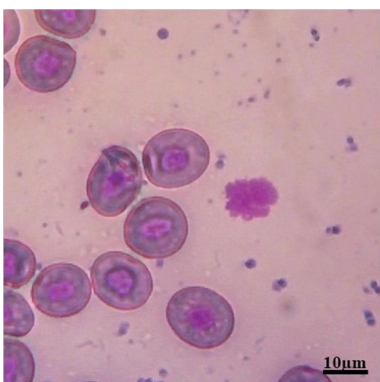


Fig 4. Basophil of Asian vine snakes (Giemsa staining)

#### b. Morphotic size of the Asian Vine Snake blood cells

The average size of the erythrocyte cell was 15,43 x 12,74 µm, and the average size of the nucleus was 6,07 x 4,99 µm. The size range of the erythrocytes was considerable, from 12,50 – 18,75 µm. The size range of the nucleus was from 5 – 7,5 µm. The erythrocyte cells and its nucleus were likely the same shape, oval to round. This was based on the long axis:short axis ratio of the cells and the nucleus were 1,21 and 1,22, respectively. The measurement of the long axis of leucocytes: heterophils, eosinophils, basophils, monocytes and lymphocytes were shown on the Table below. The average size of the heterophils was 13,83 µm, with the size range from 11,25 – 16,25 µm. The average size of eosinophils was 12,17 µm, with the size range from 8,75 – 15 µm. The average size of basophils was 7,46 µm, with the size range from 6,25 – 8,75 µm. The average size of monocytes was 9,38 µm, with the size range from 7,5 – 11,25 µm. The average size of the lymphocyte was 5,8 µm, with the size range from 5 – 7,5 µm. The various measurement might be indicator of the physiological state of the snake. Further comprehensive researches were needed to understand the correlation of the morphological and morphotic characteristics of snake blood cells to physiological state or other characteristics.

Table 1. Size characteristics of the asian vine snake

	Erythrocyte		Nucleus Size	
	Length (L)	Width (W)	Length (L)	Width (W)
Average	15,43	12,74	6,07	4,99
SD	±1,19	±0,86	±0,76	±0,48
Range	12,50-18,75	11,25-16,25	5-7,5	3,75-6,25

Table 2. Ratio of the erythrocyte and Nucleus

	Erythrocyte	Nucleus Size
	L/W	L/W
Average	1,21	1,22
SD	±0,09	±0,14
Range	1-1,5	1-1,67

Table 3. Size characteristic of leucocytes (long axis)

	Leucocyte				
	Heterophil	Eosinophil	Basophil	Monocyte	Lymphocyte
Average	13,83				
SD	±1,37	±1,86	±1,22	±2,65	±0,89
Range	11,25-16,25	8,75-15	6,25-8,75	7,5-11,25	5-7,5

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